

Original Article

Mutational Spectrum of α - and β -thalassemia in a Single-Center Cohort from the Eastern Black Sea Region of Türkiye: A Cross-sectional Study

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ABSTRACT

Aim: Türkiye shows marked regional heterogeneity in α - and β -thalassemia mutation spectra, yet the Eastern Black Sea remains poorly characterized. We aimed to describe hemoglobin subunit beta gene (HBB) and HBA1/HBA2 variants in patients referred for thalassemia testing at a tertiary center in the Eastern Black Sea region of Türkiye.

Methods: This cross-sectional retrospective study included 74 consecutive patients (38 men, 36 women; median age 29.1 years, range 1.2-77.8) who underwent α -globin deletion/duplication analysis by multiplex ligation-dependent probe amplification and/or HBB analysis by targeted next-generation sequencing between December 2022 and March 2026. Hematologic indices were retrieved from hospital records. Variant nomenclature followed HGVS recommendations and pathogenicity was assigned using ACMG/AMP-modified criteria with HbVar annotations.

Results: Forty patients (54.1%) carried at least one pathogenic (P), likely pathogenic (LP) or variant of uncertain-significance (VUS) allele; excluding two VUS, the yield was 51.4% (38/74). Among 26 HBB-positive cases, the codon 8 (–AA) frameshift was the dominant β^0 allele (10/26, 38.5%), followed by Hb D-Los Angeles (5/26, 19.2%). Rare findings included a homozygous Hb City of Hope carrier with elevated fetal hemoglobin (21.7%) and a heterozygous β^0 (codon 39) carrier co-inheriting a homozygous $\alpha\alpha^{\text{ant}13.7}$ triplication (Hb 8.7 g/dL, ferritin 1,835 ng/mL). Among 12 deletional α -thalassemia cases, the α^0 deletion (– α)^{20.5} predominated (10/12, 83.3%).

Conclusion: In contrast to most Turkish cohorts where IVS-I-110 predominates, the Eastern Black Sea HBB pool is enriched for codon 8 (–AA) and Hb D-Los Angeles, and the α -globin pool is dominated by –(α)^{20.5}, with direct implications for strip-assay design, genetic counseling and risk-couple identification in regional premarital screening.

Keywords: Thalassemia, hemoglobinopathies, HBB protein human, HBA1 protein human, mutation, genetic testing, Türkiye

Introduction

The thalassemias are the most common monogenic disorders worldwide and are characterized by reduced or absent synthesis of one of the globin chains of adult hemoglobin (HbA, $\alpha\beta_2$). Quantitative defects of the β -globin chain are caused by pathogenic (P) variants in hemoglobin subunit beta gene (HBB) on chromosome 11p15.4, while α -thalassemia results predominantly from large deletions involving HBA1

and HBA2 on chromosome 16p13.3 [1,2]. Although the carrier state is usually asymptomatic, biallelic involvement of either gene cluster can lead to transfusion-dependent disease, ineffective erythropoiesis, secondary iron overload and end-organ damage [1,3].

Türkiye lies along a historical migration corridor that links the Mediterranean, the Caucasus and Central Asia, and the country has long been considered a hot-spot of hemoglobinopathy diversity [4,5]. The mean nationwide β -thalassemia carrier

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rate is approximately 2.1%, but regional rates exceed 10% in parts of the Mediterranean and Aegean coasts [4]. Although a national hemoglobinopathy control program with mandatory premarital screening was launched in 2003 and has reduced the annual number of thalassemia-major births by approximately 90%, residual affected births still occur in regions with low premarital screening uptake [4,6]. The molecular spectrum of HBB has been extensively characterized in Istanbul, the Aegean and the Çukurova regions, where IVS-I-110 (HBB:c.93-21G>A) is consistently the most frequent allele, followed by codon 8 (–AA) and IVS-I-6 [5-7]. More recent work in Konya has further documented heterogeneity, with notable differences between Turkish and Syrian populations served by the same center [7]. Data from the Eastern Black Sea region remain comparatively sparse: published premarital data are limited to one large Samsun survey (β -thalassemia trait prevalence 1.37%, HbS 0.04%) [8] and there is no published HBB sequencing-based mutation spectrum from this region.

Knowledge of the local mutation spectrum has direct clinical consequences. First-line strip-assay panels marketed in Türkiye are designed for the most common Mediterranean alleles, and an unanticipated regional founder effect can lower their negative predictive value. Beyond β -thalassemia, the α -globin contribution to the regional anemia burden is even less well defined. The α -globin chains are encoded by two paralogous genes, HBA2 and HBA1, present in duplicate on each copy of chromosome 16 ($\alpha\alpha/\alpha\alpha$), so that most individuals carry four functional α -globin genes [2,9]. Approximately 80–90% of α -thalassemia alleles are large deletions that remove one (α^+ ; e.g., $-\alpha^{3.7}$ and $-\alpha^{4.2}$) or both (α^0 ; e.g., $-(\alpha)^{20.5}$, $--MED$ and $--SEA$) α -globin genes in cis, while the remainder are non-deletional point variants such as Hb Constant Spring [9]. Clinical severity tracks the number of residual functional genes: loss of one or two genes is usually silent or causes only mild microcytosis; co-inheritance of an α^0 and an α^+ allele ($--/\alpha$) produces HbH disease; and loss of all four genes ($--/--$) causes Hb Bart hydrops fetalis, which is generally lethal in utero [2,9]. In Türkiye, the single-gene $-\alpha^{3.7}$ deletion is consistently the most frequent α -globin allele, whereas α^0 deletions are comparatively uncommon and display pronounced regional variation [9,10]. Because α^0 carriers cannot be reliably distinguished from α^+ or silent carriers on red-cell indices alone, and because only couples in which both partners carry an α^0 (or an α^0 and an α^+) allele are at risk of a severely affected pregnancy, the regional α^0 deletion burden is the principal determinant of α -thalassemia risk in premarital counseling—yet no molecular α -globin data have been reported for the Eastern Black Sea region.

The aim of this single-center, cross-sectional study was therefore to describe the molecular and hematological spectrum of α - and β -thalassemia in patients referred to a tertiary medical genetics service serving the Eastern Black Sea region of Türkiye, with particular emphasis on regionally distinctive findings that may inform local screening strategies and genetic counseling.

Methods

Study Design and Population

This cross-sectional retrospective study was conducted at the Department of Medical Genetics of a Ordu University Training and Research Hospital serving the Eastern Black Sea region of Türkiye. All consecutive unrelated patients who underwent at least one of the following molecular tests for the indication of microcytic anemia, suspected hemoglobinopathy, abnormal high-performance liquid chromatography (HPLC) pattern, family history or premarital risk evaluation between December 2022 and March 2026 were eligible: (i) α -globin deletion/duplication analysis by MLPA, (ii) HBB sequence analysis by targeted next-generation sequencing (NGS), or (iii) both. Repeat samples from the same patient and within-family duplicate referrals were excluded. After exclusion of duplicates, 74 patients were included. Of these, 44 underwent both HBB sequencing and α -globin MLPA, 13 HBB sequencing only, and 17 α -globin MLPA only; the testing flow and molecular findings by assay are summarised in the STARD-style diagram (Supplementary Figure 1). Individual geographic origin (province of residence/referral) was not retrievable from the records and is therefore not reported; the cohort is accordingly described as a referral series from a tertiary centre serving the Eastern Black Sea region rather than a province-resolved population sample.

Hematological Work-up

Routine pre-test workup included a complete blood count, hemoglobin electrophoresis or HPLC for HbA, HbA₂ and fetal hemoglobin (HbF) quantification, and serum ferritin to exclude concomitant iron-deficiency anemia. Hematological indices [Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell (RBC), HbA₂, HbF, HbA, ferritin] recorded at the time of the molecular test were retrieved from the hospital information system. Microcytosis was defined as MCV <80 fL and an HbA₂ $\geq$ 4.0% was considered diagnostic for β -thalassemia trait, in accordance with current consensus [1,3]. This threshold is stricter than the widely used $\geq$ 3.5% cut-off; notably, one molecularly confirmed beta-plus (IVS-I-110) carrier in this cohort had an HbA₂ of 3.9%, within the 3.5-3.9% grey zone, illustrating why molecular testing complements HPLC. HPLC/electrophoresis was not available for every referral; in patients without contemporaneous HbA₂ quantification, the diagnosis of a carrier state rested on the molecular identification of a P or likely pathogenic (LP) HBB or HBA1/HBA2 allele, supported where available by microcytic, hypochromic indices. Carrier status was therefore assigned on a molecular basis and the proportion of cases with confirmatory HPLC is reported explicitly in the results and study limitations.

Molecular Analysis

HBB analysis was performed by targeted NGS. Genomic DNA from peripheral EDTA blood was enriched with laboratory-specific primer/probe sets and the Nextera XT DNA Library Prep Kit (Illumina) and sequenced on an Illumina platform. All

HBB coding exons and the flanking ± 10 bp of intronic sequence were covered (reference transcript NM_000518.5); reads were visualized in the Integrative Genomics Viewer (IGV) and variants with < 20 supporting reads were not reported. Candidate variants were filtered against gnomAD, ExAC, 1000 Genomes and dbSNP (minor allele frequency $< 1\%$) and annotated with Franklin, VarSome, HGMD Public and ClinVar. α -Globin (HBA1/HBA2) deletions and duplications were screened by multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA Probemix P140-HBA (MRC Holland, Amsterdam, the Netherlands), which detects the common α^+ ($-\alpha^{3.7}$, $-\alpha^{4.2}$) and α^0 (e.g., $-(\alpha)^{20.5}$, --MED, --SEA) deletions and α -globin triplications ($\alpha\alpha\alpha^{\text{anti}3.7}$). Variant nomenclature followed Human Genome Variation Society recommendations. HBB sequencing (NM_000518) was performed on fragmented genomic DNA enriched with laboratory-specific primer/probe sets and the Nextera XT DNA Library Prep Kit; analysis covered the protein-coding exons and the flanking ± 10 bp intron-exon boundaries, and reads were aligned to the GRCh38/hg38 reference. Variants supported by fewer than 20 reads were not reported. Genomic positions were reviewed in IGV; candidate variants were those reported as disease-causing in Franklin, VarSome, HGMD Public and ClinVar and/or with a minor-allele frequency below 1% in gnomAD, ExAC, the 1000 Genomes Project and dbSNP (instrument model, read length and mean on-target depth were not specified in the diagnostic report). One patient (P74) had a thalassemia-trait phenotype (microcytic, hypochromic, high red-cell count, normal HbA₂: Hb 12.1 g/dL, MCV 67.5 fL, MCH 19.7 pg, RBC $6.13 \times 10^6/\mu\text{L}$, HbA₂ 2.1%) but no variant on either HBB sequencing or deletional α -globin MLPA; this genotype-phenotype discordance is most consistent with a non-deletional α -globin variant beyond the coverage of the MLPA panel, and the case is classified as variant-negative on the assays performed.

Variant Interpretation

Each detected variant was classified following the American College of Medical Genetics and Genomics / Association for Molecular Pathology (ACMG/AMP) framework [11] with hemoglobin-specific modifications, integrating the HbVar database, ClinVar, gnomAD and the published literature. Variants were reported as P, LP or of uncertain significance (VUS).

Statistical Analysis

All analyses were descriptive. Continuous variables were summarized as mean $\pm$ standard deviation or as median and range, and categorical variables as counts and percentages. Hematological indices (Hb, MCV, MCH, HbA₂, HbF, RBC and ferritin) were tabulated for the whole cohort and stratified by molecular result (variant-negative, α -thalassemia, β -thalassemia and compound groups); because some laboratory values were missing, the number of patients with available data is reported for each variable. Mutation-detection yield and allele frequencies were expressed as proportions of the relevant denominator; because these

are proportions within a referral series carrying an HBB variant, they indicate relative allele rank and are not directly comparable with population-based β -thalassemia allele frequencies. Throughout, “ α -thalassemia spectrum” refers to the deletional/duplication spectrum resolved by MLPA, which does not detect non-deletional α -globin point variants. Given the retrospective, descriptive design and the limited subgroup sizes, no formal inferential hypothesis testing was performed, and between-group differences in red-cell indices are presented descriptively. Data were compiled and analyzed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Ethics Committee Approval and Informed Consent

This retrospective single-center study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ordu University Institutional Non-interventional Clinical Research Ethics Committee (decision no: 2026/94, date: 10.04.2026). Because the analysis was based on fully anonymized routine clinical-laboratory data, the requirement for individual informed consent was waived.

Results

Cohort Characteristics

Seventy-four unrelated patients were included [38 men (51.4%), 36 women (48.6%); median age 29.1 years, range 1.2–77.8]. Nine patients were younger than 18 years (12.2%), of whom six were variant-positive (five carrying a thalassemia allele and one an α -globin triplication). The mean Hb was 12.31 ± 2.36 g/dL, mean MCV 75.5 ± 11.8 fL and mean MCH 23.9 ± 4.8 pg. Microcytosis (MCV < 80 fL) was more common in the β -thalassemia (73.9%) and α -thalassemia (76.9%) groups than among variant-negative subjects (33.3%), and mean MCV was correspondingly lower in the two variant-positive groups (69.8 fL and 71.3 fL) than in the variant-negative group (81.6 fL). HbA₂ was elevated ($\geq 4.0\%$) in 10 of 18 HBB-positive subjects with available HPLC (55.6%), consistent with β -thalassemia trait. Cohort demographics and hematological indices stratified by mutation status are shown in Table 1.

Overall Mutation Yield

A P, LP or VUS allele in HBB and/or HBA1/HBA2 was identified in 40 of 74 patients (54.1%); when the two HBB missense VUS were excluded, 38 of 74 patients (51.4%) carried a P/LP allele. Of the 40, 25 (33.8% of the cohort, 62.5% of mutation-positive cases) had isolated HBB involvement, 14 (18.9%/35.0%) had isolated α -globin involvement and one patient (1.4%/2.5%) carried combined HBB plus α -globin defects. Thirty-four patients (45.9%) had no detectable variant on the panels performed ($40 + 34 = 74$); the one case pending at initial analysis (P74) has since returned negative on both HBB sequencing and HBA deletion/duplication analysis and is included here as variant-negative. Because the single combined case carries both an HBB and an α -globin allele, it is counted once in this cohort breakdown but contributes to both the HBB-positive group ($n = 26$) and the α -globin-positive group ($n = 15$) in the

variant-specific tables below.

β -Globin (HBB) Variants

Ten distinct HBB variants were detected across 26 patients. The frameshift codon 8 (–AA) allele HBB:c.25_26del (p.Lys9Valfs*14; rs35497102; β^0) was by far the most frequent and accounted for 10 of 26 HBB-positive cases [38.5%; exact binomial 95% confidence interval (CI): 20.2–59.4%]. The structural Hb D-Los Angeles variant HBB:c.364G>C (p.Glu122Gln; rs33946267; HbVar:509) was the second most common allele (5/26, 19.2%; 95% CI 6.6–39.4%). Two patients each carried the codon 15 (TGG>TGA) nonsense allele HBB:c.47G>A (p.Trp16*; β^0), IVS-I-110 HBB:c.93-21G>A (β^+) and codon 39 HBB:c.118C>T (p.Gln40*; β^0). Single instances of the IVS-I-1 splice donor variant HBB:c.92+1G>A (β^0), the –30 promoter variant HBB:c.-80T>A (β^+), Hb City of Hope HBB:c.208G>A (p.Gly70Ser; HbVar:377), and two missense VUS

(HBB:c.68A>C p.Glu23Ala and HBB:c.34G>A p.Val12Ile) were also observed. The full HBB allele distribution is presented in Figure 1 and Table 2. Expressed per β -thalassemia allele (i.e. excluding the structural variants Hb D-Los Angeles and Hb City of Hope and the two VUS; 18 β -thalassemia alleles in total), codon 8 (–AA) accounted for 10/18 (55.6%; 95% CI 30.8–78.5%), followed by codon 15, IVS-I-110 and codon 39 (each 2/18, 11.1%) and IVS-I-1 and the –30 promoter variant (each 1/18, 5.6%); this allele-based denominator permits direct comparison with population series that report β -thalassemia allele frequencies. Individual genotypes and hematological indices for all 74 patients are provided in Supplementary Table 1.

α -Globin (HBA1/HBA2) Variants

Fifteen patients (one also carrying an HBB variant) had at least one α -globin allele. Twelve of these carried a deletional

Table 1. Demographic and hematological characteristics of the study cohort (n=74), stratified by molecular result

Variable	Variant-negative (n=34)	α -thalassemia (n=14)	β -thalassemia (n=25)	Compound (n=1)	Total (n=74)*
Age, years (mean $\pm$ SD)	32.7 $\pm$ 15.3	30.4 $\pm$ 19.4	30.3 $\pm$ 12.5	69.0	31.9 $\pm$ 15.6
Sex, male/female	17/17	6/8	14/11	1/0	38/36
Hb, g/dL (mean $\pm$ SD; n)	12.6 $\pm$ 2.5 (34)	11.5 $\pm$ 2.3 (13)	12.5 $\pm$ 2.0 (23)	8.7 (1)	12.3 $\pm$ 2.4 (71)
MCV, fL (mean $\pm$ SD; n)	81.2 $\pm$ 10.9 (34)	71.3 $\pm$ 9.1 (13)	69.8 $\pm$ 11.1 (23)	67.6 (1)	75.5 $\pm$ 11.8 (71)
MCH, pg (mean $\pm$ SD; n)	25.9 $\pm$ 4.5 (34)	21.9 $\pm$ 3.7 (13)	22.3 $\pm$ 4.8 (23)	21.9 (1)	23.9 $\pm$ 4.8 (71)
HbA ₂ , % (median; n)	2.6 (20)	2.3 (1)	5.05 (18)	N/A	2.7 (39)
HbF, % (median; n)	0.4 (19)	0.5 (1)	0.9 (18)	N/A	0.8 (39)
Ferritin, ng/mL (median; n)	43.7 (31)	37.5 (13)	90 (21)	1,835 (1)	58 (66)
MCV <80 fL, n (%)	12 (35.3)	10 (76.9)	17 (73.9)	1 (100)	40 (56.3)

*The case pending at initial analysis (P74) returned negative on both assays and is included in the variant-negative group (n=34); some laboratory values were missing for individual patients, so the n contributing to each statistic is given in the row.
Values are mean $\pm$ SD or n (%). Lab values were missing for some patients; n is reported in the row.
SD: Standard deviation, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, Hb: Hemoglobin, HbA₂: Hemoglobin A₂, HbF: Fetal hemoglobin, N/A: Not applicable

Table 2. Distribution of HBB variants in the study cohort (26 patients carrying $\geq$ 1 HBB allele)

HBB variant (HGVS)	Trivial/common name	Type	ACMG class	n	% of HBB-positive
c.25_26del p.Lys9Valfs*14	Codon 8 (–AA)	β^0 frameshift	P	10	38.5
c.364G>C p.Glu122Gln	Hb D-Los Angeles (HbVar:509)	Structural/abnormal Hb	P/LP	5	19.2
c.47G>A p.Trp16*	Codon 15 (TGG>TGA)	β^0 nonsense	P	2	7.7
c.93-21G>A	IVS-I-110 (G>A)	β^+ splice	P/LP	2	7.7
c.118C>T p.Gln40*	Codon 39 (CAG>TAG)	β^0 nonsense	P	2	7.7
c.92+1G>A	IVS-I-1 (G>A)	β^0 splice	P	1	3.8
c.208G>A p.Gly70Ser (homozygous)	Hb City of Hope (HbVar:377)	Missense	LP	1	3.8
c.-80T>A	–30 promoter (T>A)	β^+ promoter	LP	1	3.8
c.68A>C p.Glu23Ala	—	Missense	VUS	1	3.8
c.34G>A p.Val12Ile	—	Missense	VUS	1	3.8
Total				26	100.0

Variant nomenclature follows HGVS using NM_000518.5.

β^0 : no β -globin produced, β^+ : reduced β -globin produced, LP: Likely pathogenic, P: Pathogenic, VUS: Variant of uncertain significance, Hb: Hemoglobin, HBB: Hemoglobin subunit beta gene, ACMG/AMP, American College of Medical Genetics and Genomics/Association for Molecular Pathology

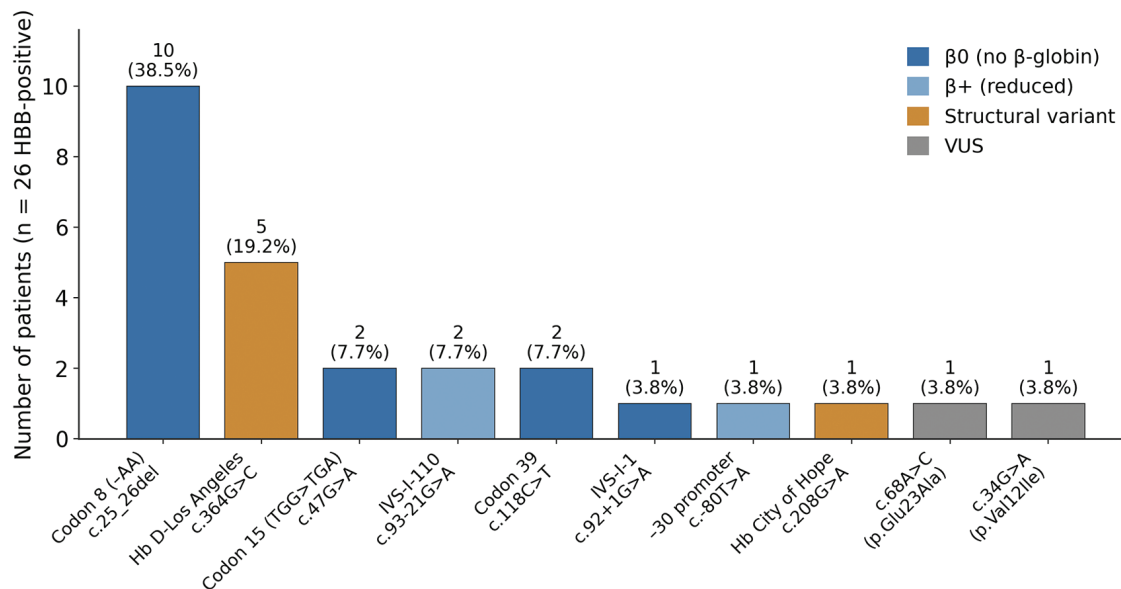


Figure 1. Distribution of hemoglobin subunit beta gene (HBB) alleles among the 26 HBB-positive patients. Bars show the number of patients carrying each allele; percentages are of the 26 HBB-positive cases. Alleles are colored by functional class: β^0 (no β -globin produced), β^+ (reduced β -globin), structural hemoglobin variant, and variant of uncertain significance (VUS). The codon 8 (-AA) frameshift (HBB:c.25_26del) and the structural variant hemoglobin D-Los Angeles (HBB:c.364G>C) together account for more than half of the HBB alleles

α -thalassemia allele, while three carried the non-deletional $\alpha\alpha^{\text{anti3.7}}$ triplication—a gain of an α -globin gene rather than a loss-of-function thalassemia allele—which is therefore reported separately. Among the 12 deletional cases, the α^0 two-gene deletion $-(\alpha)^{20.5}$ was dominant, detected in 10 patients (10/12, 83.3%; 95% CI 51.6–97.9%; heterozygous), one of whom additionally carried the single-gene $-\alpha^{3.7}$ (α^+) deletion in trans, raising the suspicion of HbH disease; the remaining 2 deletional cases carried a heterozygous $-\alpha^{3.7}$ deletion. The deletional subgroup showed a consistent genotype-phenotype gradient: the nine α^0 -trait $-(\alpha)^{20.5}$ heterozygotes were microcytic and hypochromic with near-normal hemoglobin (mean MCV 67.3 fL, range 60.7–71.6; mean MCH 20.4 pg; mean Hb 11.7 g/dL), the two α^+ $-\alpha^{3.7}$ carriers had higher MCV (80.3 and 89.4 fL), and the single patient carrying $-(\alpha)^{20.5}/-\alpha^{3.7}$ in trans (a three-gene deletion) was more anaemic (Hb 9.5 g/dL, MCV 63.9 fL), consistent with HbH disease. Because the α -globin subgroup was ascertained molecularly, HbA₂/HbF quantification was available for only one deletional patient (HbA₂ 2.3%, HbF 0.5%), in keeping with the low-normal HbA₂ expected in α -thalassemia; individual indices are given in Supplementary Table 1. The $\alpha\alpha^{\text{anti3.7}}$ triplication was found heterozygously in two patients and homozygously in one further patient who also carried an HBB variant (detailed below). The α -globin allele distribution is summarized in Table 3.

Notable Individual Cases

Homozygous Hb City of Hope: A 29-year-old man referred for asymptomatic microcytosis was found to be homozygous for the HBB:c.208G>A (p.Gly70Ser) missense variant—the rare Hb City of Hope. His Hb was 13.0 g/dL with mild microcytosis (MCV 70.1 fL, MCH 23.6 pg), HbA₂ was within normal limits (2.3%) but HbF was strikingly elevated at 21.7%. To our knowledge,

this is the first homozygous Hb City of Hope reported from Türkiye—prior Turkish carriers have all been heterozygous or compound heterozygous—and one of only a handful of homozygotes documented worldwide; notably, its markedly elevated HbF distinguishes it from the normal-blood-count phenotype of the few previously reported homozygotes.

Heterozygous β^0 (codon 39) with a homozygous $\alpha\alpha^{\text{anti3.7}}$ triplication: A 69-year-old man presented with longstanding anemia (Hb 8.7 g/dL, MCV 67.6 fL, MCH 21.9 pg) and a markedly elevated ferritin of 1,835 ng/mL. HBB analysis revealed heterozygous codon 39 HBB:c.118C>T (p.Gln40*; β^0) and MLPA confirmed a homozygous $\alpha\alpha^{\text{anti3.7}}$ triplication. The combination of a single β^0 allele with six functional α -globin genes is expected to magnify the α /non- α chain imbalance and to produce a thalassemia-intermedia phenotype, as has been reported in heterozygous β -thalassemia carriers with α -globin gene amplification [1,2,12].

Pediatric IVS-I-1 carrier: The youngest mutation-positive patient was a 14-month-old boy with microcytic anemia (Hb 10.9 g/dL, MCV 51.8 fL, MCH 15.7 pg), heterozygous for the splice-donor disrupting variant HBB:c.92+1G>A (IVS-I-1; β^0). Although clinically a thalassemia trait, this finding is notable because it identified the family for cascade testing and prenatal counseling for any future pregnancy.

Discussion

This is, to our knowledge, the first sequencing-based description of the α - and β -thalassemia mutation spectrum from the Eastern Black Sea region of Türkiye. Three findings deserve emphasis: (i) the codon 8 (-AA) frameshift dominates the HBB allele pool, contrary to the IVS-I-110 dominance reported in most Turkish cohorts; (ii) Hb D-Los Angeles is over-represented relative to nationwide premarital screening data;

Table 3. Distribution of α -globin alleles (15 patients, including 1 with a co-existing HBB variant). Deletional α -thalassemia alleles and the non-deletional $\alpha\alpha^{\text{anti3},7}$ triplication are shown separately

α -globin allele	Class	Description	Zygosity in this cohort	Patients (n)	% within group
Deletional α -thalassemia alleles — denominator: 12 patients with a deletional allele					
$-(\alpha)^{20,5}$	α^0 (two α genes removed)	~20.5 kb	Heterozygous (one case in trans with $-\alpha^{3,7}$)	10	83.3
$-\alpha^{3,7}$	α^+ (one α gene removed)	~3.7 kb	Heterozygous	2	16.7
Subtotal, deletional alleles	—	—	—	12	100.0
α -globin triplication — gain of an α gene; counted separately from the deletional spectrum					
$\alpha\alpha^{\text{anti3},7}$	Triplication (one α gene added)	~3.7-kb-type triplication	Heterozygous (n=2); homozygous with an HBB co-variant (n=1)	3	—
Patient-level summary					
Patients with ≥ 1 α -globin allele	—	One of whom also carries an HBB variant	—	15	—
Values are patient counts. Percentages in the deletional block are proportions of the 12 patients carrying a deletional α-thalassemia allele; the α^0/α^+ compound case ($-(\alpha)^{20,5} / -\alpha^{3,7}$ in trans) is counted once within the $-(\alpha)^{20,5}$ row to avoid double-counting. The three $\alpha\alpha^{\text{anti3},7}$ carriers represent a gain of an α gene and are therefore excluded from the deletional spectrum. In total, 15 patients carried at least one α-globin allele, one of whom also carried an HBB variant. α^0, two-gene (cis) deletion; α^+, single-gene deletion. Deletions are described by their approximate size and the number of α-globin genes removed; α^0 deletions remove both HBA1 and HBA2 in cis, α^+ deletions remove a single α-globin gene. The $\alpha\alpha^{\text{anti3},7}$ triplication adds a third α-globin gene and is a gain, not a loss-of-function thalassemia allele; it is therefore listed and counted separately. HBB: Hemoglobin subunit beta gene					

and (iii) the α^0 deletion $-(\alpha)^{20,5}$ accounts for the majority of α -thalassemia alleles in this referral population.

The historical reference of the codon 8 (–AA) variant is itself a Turkish individual: the original cloning and dideoxy sequencing report of HBB:c.25_26delAA was performed in 1981 in a single patient of Turkish origin and the variant has since been classified as a recurrent Mediterranean β^0 mutation. National data from the Turkish Pediatric Hematology Society registry [6] and the comprehensive Boğaziçi University series [5] consistently rank IVS-I-110 first (~40–47%) and codon 8 (–AA) second to fourth (5–10%). The same pattern holds across the major regional series: IVS-I-110 is the leading allele in the Aegean (41.7%) [13], Çukurova (57.3%) [14] and a national pediatric referral cohort (35.3%) [15], in each of which codon 8 (–AA) ranks well below it (5.7–10.4%). In our Eastern Black Sea cohort the order is reversed, with codon 8 (–AA) accounting for 38.5% of HBB alleles and IVS-I-110 for only 7.7% (Figure 2, Table 4). Although referral bias cannot be excluded and a referral series of 26 HBB-positive patients is too small to establish a founder effect, the direction of this finding mirrors the mutational landscape of the neighboring Caucasus: in a large Azerbaijani genotyping study, codon 8 (–AA) was likewise the single most frequent β -thalassemia allele (34.96%), well ahead of IVS-I-110 (10.12%), with its strongest regional association in Shaki-Zaqatala, immediately across the border from the Eastern Black Sea littoral [20]. Our data therefore raise the possibility of regional enrichment—plausibly reflecting historical Caucasian–Anatolian population mixing along the Black Sea coast [5–7,16]—that should be tested prospectively in population-based premarital screening cohorts rather than inferred from a referral series. Practically,

this means that strip-assays validated for Mediterranean populations will retain high sensitivity for codon 8 (–AA) but might require regional adjustment if cost-effective targeted assays are to be designed for Eastern Black Sea screening.

The second striking feature was the over-representation of Hb D-Los Angeles (HBB:c.364G>C, p.Glu122Gln; HbVar:509). This structural variant is the fourth-most-common abnormal hemoglobin worldwide, with founder enclaves in the Punjab, southern Europe and Türkiye [16,17]. In the largest published Turkish premarital screening series from Antalya (89,981 individuals), Hb D-Los Angeles was detected in only 0.15% of subjects [17]. In contrast, 5 of 26 HBB-positive patients (19.2%) in our cohort harbored this allele—an over-representation that, while potentially influenced by referral bias, is of a magnitude that warrants prospective evaluation through regional premarital screening data. Because Hb D-Los Angeles is silent in heterozygotes but causes severe sickle-like or β -thalassemia-intermedia phenotypes when inherited in trans with HbS or a β^0 allele [16], its high prevalence has clear counseling implications. Combined with the codon 8 (–AA) burden, the at-risk-couple denominator for severe Hb-D / β^0 -thalassemia compound heterozygotes might be larger in the Eastern Black Sea than current national estimates suggest.

The third finding concerns α -globin. The $-(\alpha)^{20,5}$ deletion is a Mediterranean α^0 allele [9] and its prevalence among α -thalassemia carriers is highly population-dependent [10]. In a recent Denizli-province cohort it accounted for only 17.1% of α -globin deletions [18], whereas it represented 10 of 12 (83.3%) of our deletional α -thalassemia cases. Two considerations temper this observation: α -globin MLPA is preferentially requested when an α^0 allele is clinically suspected (marked

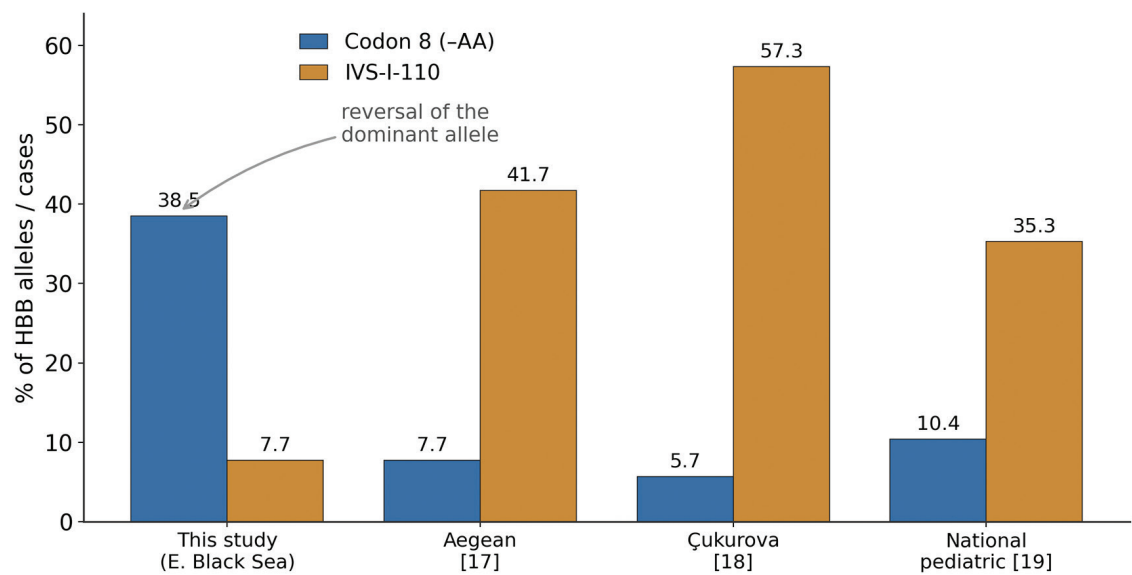


Figure 2. Regional comparison of the two leading hemoglobin subunit beta gene (HBB) alleles. The frequency (%) of codon 8 (–AA) and IVS-I-110 in the present Eastern Black Sea cohort is shown alongside three other Turkish series: the Aegean region [13], Çukurova [14] and a national pediatric referral cohort [15]. The rank of the two alleles is reversed in the present cohort—where codon 8 (–AA) predominates—relative to the IVS-I-110 dominance seen elsewhere in Türkiye. Denominators differ between series (see Table 4 footnote); the figure is intended to show relative allele rank rather than strictly equivalent proportions

Table 4. Frequency of the principal β -globin alleles in the present Eastern Black Sea cohort compared with other Turkish series

β -globin allele	This study, Eastern Black Sea (n=26)	Aegean [13] (n=1171)	Çukurova [14]	National pediatric [15] (n=106)
Codon 8 (–AA) (HBB:c.25_26del)	38.5	7.7	5.7	10.4
IVS-I-110 (HBB:c.93-21G>A)	7.7	41.7	57.3	35.3
IVS-I-1 (HBB:c.92+1G>A)	3.8	8.9	8.3	7.5
IVS-I-6 (HBB:c.92+6T>C)	0	6.6	5.7	NR
IVS-II-1 (HBB:c.315+1G>A)	0	7.2	3.4	8.0
Codon 39 (HBB:c.118C>T)	7.7	4.6	6.4	7.1
Hb D-Los Angeles (HBB:c.364G>C)	19.2	NR	NR	NR
Most common allele	Codon 8 (–AA)	IVS-I-110	IVS-I-110	IVS-I-110

Aegean, Özkinay et al. [13]; Çukurova, Cürük et al. [14]; national pediatric referral cohort, Fettah et al. [15]. NR (not reported) among the common alleles in that series; the structural variant Hb D-Los Angeles was not enumerated in the comparator β -thalassemia mutation series
Values are percentages of HBB alleles (comparator series) or of HBB-positive cases (present study). Denominators are not identical: the comparator studies report alleles among β -thalassemia patients, whereas the present cohort comprises all referrals carrying an HBB variant, including the structural variant Hb D-Los Angeles. The table is therefore best read as a comparison of relative allele rank rather than of strictly equivalent proportions
Hb: Hemoglobin, HBB: Hemoglobin subunit beta gene

microcytosis, HbH/hydrops risk assessment), so ascertainment bias may inflate the apparent $-(\alpha)^{20.5}$ fraction; nonetheless, the 20.5-kb and 3.7-kb deletions together account for ~74% of the α -thalassemia spectrum in neighboring Azerbaijan [20], so a genuine regional signal cannot be excluded and should be confirmed in larger samples. While the modest numerical denominator (n=15) of our α -globin analyses tempers strong conclusions, the implication—if confirmed in larger samples—is twofold: (i) α -globin deletion screening offered in our region—whether MLPA- or gap-PCR-based—should preserve coverage of $-(\alpha)^{20.5}$ alongside the more common $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles; and (ii) at-risk couples for HbH disease ($--/-\alpha$) and Hb Bart hydrops fetalis ($--/--$) may be quantitatively more relevant in Eastern Black Sea premarital counseling than is generally appreciated.

Two individual cases deserve particular emphasis. The first—a homozygous carrier of Hb City of Hope (HBB:c.208G>A, p.Gly70Ser)—is—to our knowledge, though we did not undertake a formal systematic literature search—the first homozygous Hb-COH case reported from Türkiye and one of only a handful documented worldwide. Although Hb-COH has been recognized in the Turkish population since 1989, every previously reported Turkish carrier has been heterozygous or compound heterozygous; homozygosity itself was described for the first time only very recently, in a single multi-ethnic series in which the three homozygous individuals identified all had entirely normal blood counts [19]. Our patient is hematologically distinct, with markedly elevated HbF (21.7%). HbF elevation has been documented in Hb-COH compound heterozygotes with β -thalassemia [19] but is unexpected in an

isolated homozygous Hb-COH carrier; it should therefore be interpreted with caution, as it may reflect the co-inheritance of a $\delta\beta$ -thalassemia, a hereditary persistence of fetal hemoglobin (HPFH) determinant, or another HbF modifier rather than an effect of Hb-COH itself. We were unable to resolve this finding in the present study; targeted sequencing of the β -globin cluster and the BCL11A locus would be required to clarify it, and the case is accordingly presented as an observation requiring further investigation rather than a definitive genotype–phenotype correlation. The second case—heterozygous codon 39 (β^0) on a background of homozygous $\alpha\alpha^{\text{anti3.7}}$ triplication—exemplifies a well-recognized but under-diagnosed mechanism of β -thalassemia intermedia [12]. The accompanying ferritin of 1,835 ng/mL is consistent with iron loading driven by ineffective erythropoiesis [3] and underlines the importance of routinely co-testing for α -globin gene amplification in apparently mild HBB heterozygotes with disproportionate anemia.

Study Limitations

Several limitations merit acknowledgment. First, this is a retrospective single-center referral series and the prevalence estimates obtained should not be extrapolated to the general Eastern Black Sea population. A formal regional premarital screening study using HPLC and reflex sequencing is needed to refine the population-level numbers. Second, contemporaneous HbA₂/HbF quantification by HPLC or electrophoresis was available for only 39 of 74 patients (52.7%) overall and for 18 of 26 HBB-positive cases (69.2%), and for just one patient in the α -globin subgroup; the hematological characterization is therefore incomplete and carrier status was assigned primarily on a molecular basis. Third, the molecular assays have intrinsic coverage limits: MLPA of the α -globin cluster detects deletions and duplications but does not identify non-deletional α -globin point variants (such as Hb Constant Spring), which account for a minority of α -thalassemia alleles and would require HBA1/HBA2 sequencing, whereas the targeted HBB NGS panel covered the coding exons and ± 10 bp of flanking intronic sequence with a 20-read threshold and would therefore miss deep-intronic, regulatory and large structural HBB variants. A few mutation-negative microcytic patients may consequently harbor variants beyond the reach of the assays performed, and orthogonal methods such as α -globin gene sequencing and long-read sequencing are increasingly being adopted to close these gaps. Relatedly, molecular calls were not orthogonally confirmed by an independent method (e.g. Sanger sequencing, HPLC/capillary electrophoresis or mass spectrometry); although the structural variants Hb D-Los Angeles and Hb City of Hope are well characterized and the recurrent deletions are reliably resolved by MLPA, orthogonal confirmation would further strengthen individual genotype assignments in future work. Fourth, the two HBB missense variants classified as VUS (HBB:c.68A>C and HBB:c.34G>A) are reported separately and excluded from the P/LP diagnostic yield; family segregation was not systematically performed, and parental testing combined with HPLC phenotyping and population-frequency/in-silico re-

evaluation will be required for definitive reclassification. Both correspond to previously catalogued beta-globin substitutions: c.68A>C is Hb G-Coushatta (HbVar:266; rs33936254; gnomAD minor-allele frequency $\sim 8.4 \times 10^{-5}$), repeatedly reported in healthy homozygotes and heterozygotes, and c.34G>A p.Val12Ile is a recognized HBB missense variant (HbVar:239; rs33974228). Per-patient genotypes - with HGVS nomenclature (NM_000518.5 for HBB), zygosity, dbSNP identifiers and the laboratory-assigned ACMG/AMP class for every reported variant, together with the contemporaneous hematological indices - are provided in Supplementary Table 1; for the two VUS, gnomAD minor-allele frequencies, in-silico predictions (REVEL, CADD) and the applied ACMG/AMP evidence codes are additionally tabulated. Finally, hemoglobin chain synthesis studies and orthogonal mass-spectrometric confirmation of Hb D-Los Angeles and Hb City of Hope were not available in our setting. Notwithstanding these limitations, the molecular and phenotypic dataset presented here fills an important regional gap and provides a baseline for prospective multicenter studies.

Conclusion

In this 74-patient single-center cohort from the Eastern Black Sea region of Türkiye, the codon 8 (–AA) frameshift was the dominant β^0 allele, Hb D-Los Angeles was the dominant structural HBB variant, and $-(\alpha)^{20.5}$ was the dominant α^0 deletion. The molecular landscape differs substantially from that of the Mediterranean and Aegean coasts, where IVS-I-110 predominates and α^0 deletions are rare. Targeted strip-assays should preserve coverage of these regionally enriched alleles, and at-risk-couple counseling—particularly for compound HbD/ β -thalassemia and for HbH disease—deserves explicit attention in the local premarital screening program. A regional premarital cohort is now under design to validate these single-center findings.

Ethics

Ethics Committee Approval: This retrospective single-center study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ordu University Institutional Non-interventional Clinical Research Ethics Committee (decision no: 2026/94, date: 10.04.2026).

Informed Consent: Owing to the retrospective design and the use of fully anonymized routine clinical-laboratory data, the requirement for individual informed consent was waived by the ethics committee.

Footnotes

Authorship Contributions

Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: All authors contributed equally to this study.

Conflict of Interest: No conflict of interest was declared by the authors.

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Declaration Regarding the Use of AI and AI-Assisted Technologies: During the preparation of this work, the authors used a large language model-based assistant (anthropic claude) for language editing and structuring of paragraphs in the Discussion. All clinical, laboratory, statistical and genotype data were generated, analyzed and verified manually by the authors. After carefully reviewing and editing the content, the authors take full responsibility for the content of the publication. The use of this tool affected only language polishing and did not affect the analysis, interpretation or conclusions of the study.

Data Availability Statement: The de-identified, variant-level dataset supporting the findings of this study (anonymized genotypes and the associated hematological indices) has been deposited in a public repository. Any additional data are available from the corresponding author upon reasonable request. The deposited dataset has been updated: the biologically implausible P02 HbA₂ value (probable HbA/HbA₂ transposition) has been withheld and flagged, and the isolated $\alpha\alpha^{\text{anti3.7}}$ triplication case P31 (severe normocytic anaemia, Hb 5.7 g/dL, ferritin 1596 ng/mL) has been annotated as likely reflecting an additional, unrelated pathology rather than the triplication alone. The corrected dataset will be re-deposited and the repository version/DOI updated accordingly.

Supplementary Figure 1: <https://d2v96fxpocvxx.cloudfront.net/32e1d65a-7ef3-4d28-858e-7da63d316173/content-images/df0c024d-e348-43a1-bdbe-7bbb163ec5b7.pdf>

Supplementary Table 1: <https://d2v96fxpocvxx.cloudfront.net/32e1d65a-7ef3-4d28-858e-7da63d316173/content-images/f856eb90-d98a-4960-b2a0-25e812a20be7.pdf>

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