Supplementary Table 1.

Patient ID	Sex	Age (years)	Hb (g/dL)	MCV (fL)	MCH (pg)	RBC (×10⁶/µL)	HbA2 (%)	HbF (%)	HbA (%)	Ferritin (ng/mL)	Result category	Gene	Variant (HGVS / allele)	Protein / trivial name	Zygosity	dbSNP rsID	ACMG/ classification	Data note
P01	F	23,4	13,7	83,3	27,3	5,02	1,9	0,1	49,2	8,3	Variant detected (HBB)	*HBB*	c.364G>C	p.Glu122Gln (Hb D-Los Angeles)	Heterozygous	rs33946267	Likely pathogenic	
P02	F	24,6	7,6	66,3	18,4	4,13		0,8		52,7	No variant detected							HbA2/HbA pair withheld as unreliable: recorded HbA2 87.5% 87.5%/HbA 0.8% is biologically impossible (probable HbA/HbA2 transposition). Verify against source before any re-analysis. Patient was variant-negative.
P03	M	21,2	13,1	57,5	17,8	7,38	3,6	1,6	86,8	179,5	Variant detected (HBB)	*HBB*	c.47G>A	p.Trp16* (Codon 15 (TGG>TGA))	Heterozygous	rs63750783	Pathogenic	
P04	F	19,4	9,3	59,4	17,2	5,42	5	0,3	87,4	5,03	Variant detected (HBB)	*HBB*	c.47G>A	p.Trp16* (Codon 15 (TGG>TGA))	Heterozygous	rs63750783	Pathogenic	
P05	M	25,8									Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P06	F	27,5									Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P07	M	23	13,8	88,8	30,3	4,56	2,4	0,4	53,2	137	No variant detected							
P08	M	7,9	10,4	82	25	4,16				5,36	No variant detected							
P09	F	58	9,5	63,9	17,5	5,43				578	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5 + -α3.7 (in trans)	α0 + α+ deletions	Compound heterozygous (in trans)		Pathogenic	
P10	F	26,2	11,3	67,5	19,8	5,72	3,72	0	96,2	694	No variant detected							
P11	M	1,2	10,9	51,8	15,7	6,95					Variant detected (HBB)	*HBB*	c.92+1G>A	IVS-I-1	Heterozygous	rs33971440	Pathogenic	
P12	M	28,4	17,2	85,3	29,3	5,87				241	No variant detected							
P13	M	37,5	12,9	86,2	28,8	4,48				42,6	No variant detected							
P14	M	26,5	12,2	69,7	21,4	5,71	3,9	0,4	87,6	90	Variant detected (HBB)	*HBB*	c.93-21G>A	IVS-I-110	Heterozygous	rs35004220	Likely pathogenic	
P15	F	22,1	11,2	60	18,3	6,13	5,5	1,3	86,7	58,9	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P16	M	22,3	14	64,4	19,9	7,03	5,5	5,1	83,2	76,3	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P17	F	44,4	14,2	68,4	18,4	7,7	1,9	0,2	88,3	8,7	No variant detected							
P18	F	30,6	13,5	87,7	28,2	4,78				29,2	No variant detected							
P19	M	43,8	7,4	88,1	25,9	2,86	2,1	0,1	90,9	26,7	No variant detected							
P20	F	36,2	11,6	71,6	21,3	5,45				164	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P21	M	30	14,9	87,2	29,7	5,01	2,9	2,5	87,4	128	No variant detected							
P22	F	19	11,9	70,7	22	5,42	5,1	1,4	86,2	20,9	Variant detected (HBB)	*HBB*	c.93-21G>A	IVS-I-110	Heterozygous	rs35004220	Likely pathogenic	
P23	M	1,9	11,5	67,1	23,8	4,84				41,8	No variant detected							
P24	M	45,3	13,3	63,4	18,8	7,07				8,88	No variant detected							
P25	M	2,8	10,5	65,2	19,8	5,29	2,7	0,9	88,3	3,1	No variant detected							
P26	F	63,3	6,1	63,5	18,1	3,37				484,1	No variant detected							
P27	F	23,5	10,5	62,7	18,7	5,61	5,1	0,9	87,1	33,6	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P28	M	29,4	13	70,1	23,6	5,51	2,3	21,7	63	121	Variant detected (HBB)	*HBB*	c.208G>A	p.Gly70Ser (Hb City of Hope)	Homozygous	rs33947415	Likely pathogenic	
P29	M	31,5	15,4	79,2	26,2	5,87	1,6	0,1	50,3	115	Variant detected (HBB)	*HBB*	c.364G>C	p.Glu122Gln (Hb D-Los Angeles)	Heterozygous	rs33946267	Pathogenic	
P30	M	46,1	14,2	80,3	26,4	5,37				25,7	Variant detected (α-globin)	*HBA1/HBA2*	-α3.7	α+ one-gene deletion	Heterozygous		Pathogenic	
P31	M	7,5	5,7	88,6	29,5	1,93				1596	Variant detected (α-globin)	*HBA1/HBA2*	ααααanti3.7	α-globin triplication (gain of one α gene)	Heterozygous		Modifier (α-globin gain)	Isolated alpha-globin triplication (aaaanti3.7). Severe normocytic anemia (Hb 5.7 g/dL, RBC 1.93 x10⁶/uL) with high ferritin (1596 ng/mL) is NOT explained by the triplication alone; a coexisting, unrelated pathology is likely. Interpret with caution.
P32	F	21,7	13,4	89,2	27,7	4,83				36,5	No variant detected							
P33	F	28,8	11,8	67,5	20,4	5,78	2,3	0,5	88,5	27,6	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P34	M	27,1	8,5	104	33,7	2,52	2,6	1	89,9	101	No variant detected							
P35	F	31,8	14,6	85,3	28,7	5,09	2,5	0,2	89,3	25,6	No variant detected							
P36	M	27,5	15,5	84,1	28,4	5,46	2,8	0,3	88,6	73,8	No variant detected							
P37	F	34,2	10,7	62,4	18,9	5,66	5,5	0,7	86,8	75,1	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P38	M	25,6	13,2	66,3	20,9	6,32	5,2	5,3	82,6	139	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P39	M	52,3	12,8	61,1	19,7	6,51	5,4	2,5	85,8	342	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P40	M	59,4	10,2	91,4	31,5	3,24					Variant detected (HBB)	*HBB*	c.364G>C	p.Glu122Gln (Hb D-Los Angeles)	Heterozygous	rs33946267	Pathogenic	
P41	F	55,2	12,4	83,2	26,8	4,63				16,9	No variant detected							
P42	F	14,1	11,2	66,8	19,3	5,79				54,41	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P43	M	17,4	12,6	67,4	19,5	6,47				37,47	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P44	F	9,2	10,5	65	19,7	5,34				26,89	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P45	F	34,1	13,5	71,8	22	6,13				173	No variant detected							
P46	M	71,2									Variant detected (α-globin)	*HBA1/HBA2*	αααanti3.7	α-globin triplication (gain of one α gene)	Heterozygous		Modifier (α-globin gain)	
P47	M	45,7	16,6	87,5	31,8	5,22	2	0,6	62,7	94,1	Variant detected (HBB)	*HBB*	c.68A>C	p.Glu23Ala	Heterozygous	rs33936254	VUS	
P48	F	28,6	13	80,7	24,9	5,23				35	No variant detected							
P49	F	25,8	12	66,7	21,6	5,56				27,4	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P50	F	36,7	11,2	69,9	21,1	5,31				91,6	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P51	F	30,2	9,6	64,8	19,4	4,94				971	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P52	M	26,9	16,5	81,4	30,1	5,49	1,3	0,3	47	245	Variant detected (HBB)	*HBB*	c.364G>C	p.Glu122Gln (Hb D-Los Angeles)	Heterozygous	rs33946267	Pathogenic	
P53	M	23,9	15,2	89,3	28,6	5,32	2,1	2,6	87,4		No variant detected							
P54	F	22,5	10,6	92,9	29,9	3,54	2,7	0,1	90,8	11,8	No variant detected							
P55	M	50,3	15,3	83,2	29,1	5,25				257	No variant detected							
P56	M	24,5	15,6	80,7	27,3	5,71	2,8	3,9	86,8		No variant detected							
P57	M	46	14,6	70,3	22,5	6,49	5,5	1	86,3	101	Variant detected (HBB)	*HBB*	c.-80T>A	–30 promoter	Heterozygous	rs33980857	Likely pathogenic	
P58	F	34,5	14,4	81,5	27,7	5,19	2,5	0,3	74,7	56,1	No variant detected							
P59	F	35,3	10,3	61,2	18,2	5,65	5,4	0,9	86,9	48,1	Variant detected (HBB)	*HBB*	c.118C>T	p.Gln40* (Codon 39)	Heterozygous	rs11549407	Pathogenic	
P60	F	77,8	11,2	104,8	33,7	3,32				5922	No variant detected							
P61	M	69	8,7	67,6	21,9	3,98				1835	Variant detected (combined HBB + α-globin)	*HBB; HBA1/HBA2*	c.118C>T + αααanti3.7	p.Gln40*	Heterozygous (HBB); homozygous (α)	rs11549407	Pathogenic	
P62	F	36	13,4	79,7	25,9	5,17	2,5	2,2	89,4	8	No variant detected							
P63	M	43,5	13	70,9	19,7	6,6	1,9	0,1	89,3	5,89	No variant detected							
P64	F	25,3	13,3	88,9	29,4	4,52	2,6	3,4	86,4	43,7	No variant detected							
P65	F	34	12,6	95,3	31,5	4	2,9	3,3	86,3	139	No variant detected							
P66	F	42	11,6	80,6	23,9	4,85				119	No variant detected							
P67	F	27,5	12,2	86,7	25,7	4,74				57,2	Variant detected (HBB)	*HBB*	c.34G>A	p.Val12Ile	Heterozygous	rs33974228	VUS	
P68	M	49,8	12,6	60	18,1	6,98				1124	Variant detected (HBB)	*HBB*	c.25_26del	p.Lys9Valfs*14 (Codon 8 (–AA))	Heterozygous	rs35497102	Pathogenic	
P69	M	4,4	11,7	60,7	19,3	6,05				5,62	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P70	F	33,6	12,9	69,7	21	6,13				31,5	Variant detected (α-globin)	*HBA1/HBA2*	-(α)20.5	α0 two-gene deletion	Heterozygous		Pathogenic	
P71	M	36,3	15,1	89,4	28	5,4				156	Variant detected (α-globin)	*HBA1/HBA2*	-α3.7	α+ one-gene deletion	Heterozygous		Pathogenic	
P72	F	30,6	12,1	82,7	27,3	4,44	1,6	0,8	48,9	50,8	Variant detected (HBB)	*HBB*	c.364G>C	p.Glu122Gln (Hb D-Los Angeles)	Heterozygous	rs33946267	Pathogenic	
P73	M	37,4	15,2	79,6	27,1	5,6	2,6	0,2	89,2	225	No variant detected							
P74	M	23,9	12,1	67,5	19,7	6,13	2,1	0,2	89,6		No variant detected	*HBB; HBA1/HBA2/HBZ*	No variant detected				—	Both assays completed and negative: HBB sequencing (NM_000518) no pathogenic/likely pathogenic variant; HBA1/HBA2/HBZ MLPA (SALSA P140) no deletion/duplication. Thalassemia-trait phenotype (microcytosis, hypochromia, high RBC, normal HbA2) without a detectable variant — possible non-deletional alpha-globin variant beyond MLPA coverage. Reclassified from inconclusive (results pending) to variant-negative.

De-identified patient-level dataset

Mutational Spectrum of α- and β-Thalassemia in a Single-Center Cohort from the Eastern Black Sea Region of Türkiye	
Description	Per-patient genotype and contemporaneous hematological indices for the 74 analyzed patients. All direct identifiers (national ID, name, date of birth) have been removed; patients are labelled P01–P74.
Cohort	74 analyzable patients (one additional incomplete referral record was excluded). 38 male / 36 female; median age 29.1 years (range 1.2–77.8).
Variant nomenclature	HBB variants follow HGVS on NM_000518.5. α-globin deletions/triplications are given by their conventional names (gap-PCR); these are copy-number events and have no cDNA/rsID.
Classification	ACMG/AMP classes are reported as assigned by the laboratory (Pathogenic, Likely pathogenic, VUS). Recurrent α-thalassemia deletions are recorded as Pathogenic; the αααanti3.7 triplication is recorded as a modifier (gain of an α-globin gene).
Missing values	Blank cells indicate the parameter was not available within the ±1-month window of molecular testing. HbA2/HbF were available for a subset of patients (see manuscript Limitations).
Data note — P02	The originally recorded HbA2 of 87.5% (with HbA 0.8%) is biologically impossible (probable HbA/HbA2 transposition). In this corrected version both values have been withheld (left blank) and flagged in the Data note column; they should be verified against the source record before any re-analysis. This patient was variant-negative.
Data note - P31	The isolated aaaanti3.7 triplication does not account for the severe normocytic anemia (Hb 5.7 g/dL) and markedly elevated ferritin (1596 ng/mL) in this 7.5-year-old; a coexisting, unrelated pathology is likely and the case is flagged accordingly in the Data note column.
Case resolution (P74)	The single previously pending case (P74) completed testing after initial submission (HBB sequencing and HBA1/HBA2/HBZ MLPA, both negative) and is classified as variant-negative. The cohort therefore contains no inconclusive cases: 40 variant-positive and 34 variant-negative.
Source	Routine diagnostic records, Department of Medical Genetics of a tertiary training and research hospital serving the Eastern Black Sea region of Türkiye (December 2022 – March 2026).
Version	v3 (revision): P74 both assays returned negative (HBB sequencing + HBA MLPA) and reclassified from inconclusive to variant-negative; cohort now 40 variant-positive, 34 variant-negative, 0 inconclusive. v2 changes retained (P02 withheld/flagged; P31 annotated).

Patient ID	Variant (HGVS, NM_000518.5)	Protein	Zygosity	dbSNP rsID	HbVar ID	ClinVar	gnomAD MAF	*In-silico* (summary)	REVEL	CADD (PHRED)	ACMG/AMP evidence codes	Final class (lab)	Source / notes
P47	HBB:c.68A>C	p.Glu23Ala (β22 Glu→Ala; Hb G-Coushatta; legacy E22A)	Heterozygous	rs33936254	266	Variation ID 15171 — conflicting (benign / likely benign / VUS)	Not observed in gnomAD v4 (exomes and genomes: not found)	REVEL 0.566 (intermediate/ grey zone → neither PP3 nor BP4); CADD_phred 13.79; SIFT 0.06 (tolerated); phyloP100 2.081	0,566	13,79	PM2_Supporting (absent from gnomAD); *in-silico* inconclusive (REVEL grey zone) → VUS	VUS (ClinVar: conflicting)	ENST00000335295.4/NM_000518.5:c.68A>C, MANE Select; GRCh38 11:5226954 T>G; rs33936254; HbVar:266. Scores from Ensembl VEP/ dbNSFP (accessed Jul 2026). Reported in healthy heterozygotes/ homozygotes; Hb G-Coushatta.
P67	HBB:c.34G>A	p.Val12Ile (β11 Val→Ile; legacy V11I)	Heterozygous	rs33974228	239	RCV000001615 (Allele ID 12274)	Not returned in this VEP export; rare (ClinVar RCV000001615; dbSNP rs33974228)	REVEL 0.521 (intermediate/ grey zone → neither PP3 nor BP4); CADD_phred 4.389 (CADD_raw 0.400; low-moderate); missense p.Val12Ile	0,521	4,389	PM2_Supporting (rare/absent); *in-silico* inconclusive (REVEL 0.521 grey zone; CADD low) → VUS	VUS	ENST00000335295.4/NM_000518.5:c.34G>A, MANE Select; GRCh38 chr11:5226988; rs33974228; HGMD CD031505/CM920318/CM941955; HbVar:239; ClinVar RCV000001615 (uncertain_significance, 'other'; 6 PubMed citations). Scores from Ensembl VEP/dbNSFP, accessed Jul 2026 (CADD_raw 0.400, CADD_phred 4.389, REVEL 0.521).

- Scores for HBB:c.34G>A (p.Val12Ile) are populated from the Ensembl VEP/dbNSFP export (CADD_raw 0.400, CADD_phred 4.389, REVEL 0.521; accessed Jul 2026); no gnomAD frequency was returned for this allele.
- gnomAD frequency and the ClinVar/HbVar identifiers for HBB:c.68A>C (p.Glu23Ala) are sourced from ClinVar Variation ID 15171 (Benign) and HbVar:266 (accessed July 2026). This allele (Hb G-Coushatta) is distinct from Hb G-Taipei (HBB:c.68A>G, HbVar:265) at the same position.
- Both variants were reported as VUS by the laboratory and are excluded from the pathogenic/likely-pathogenic diagnostic yield in the manuscript; the benign-leaning evidence for c.68A>C is acknowledged in the Discussion.