

Unveiling *KLF1* mutations in adult hemolytic anemia

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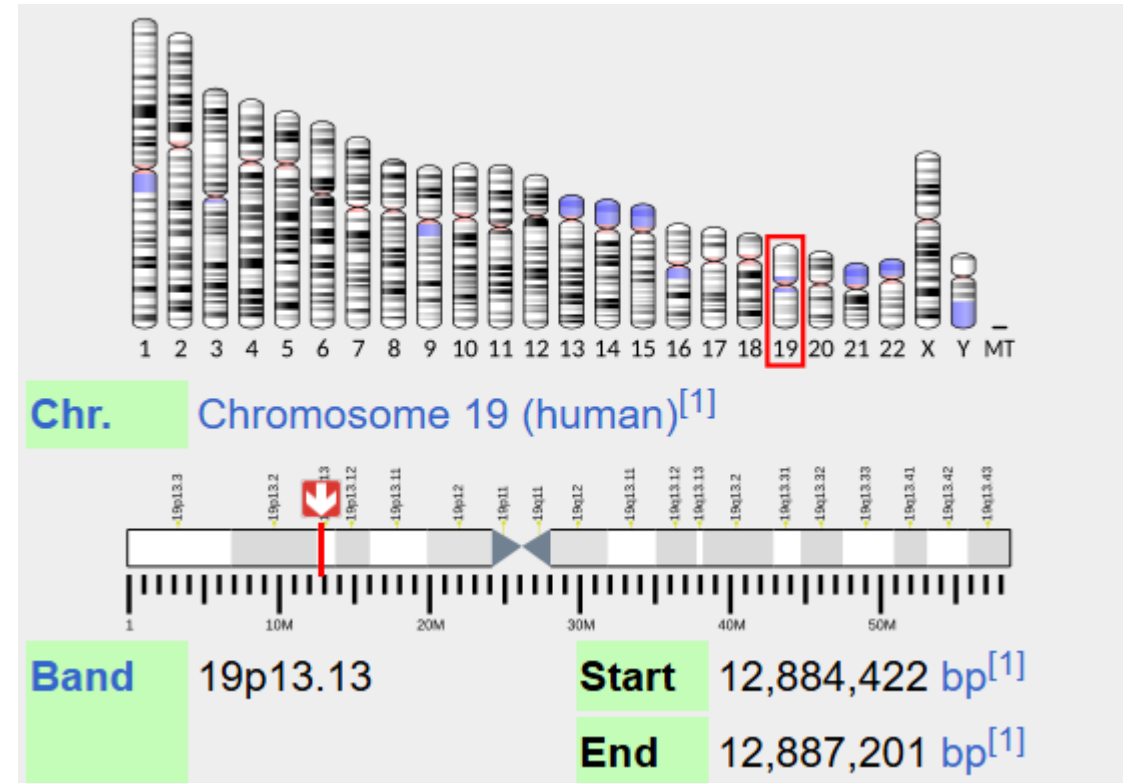
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Outline

- The role of *KLF1* in erythropoiesis
- The types of *KLF1* mutations
- Clinical manifestations
- Diagnosis and treatment strategies

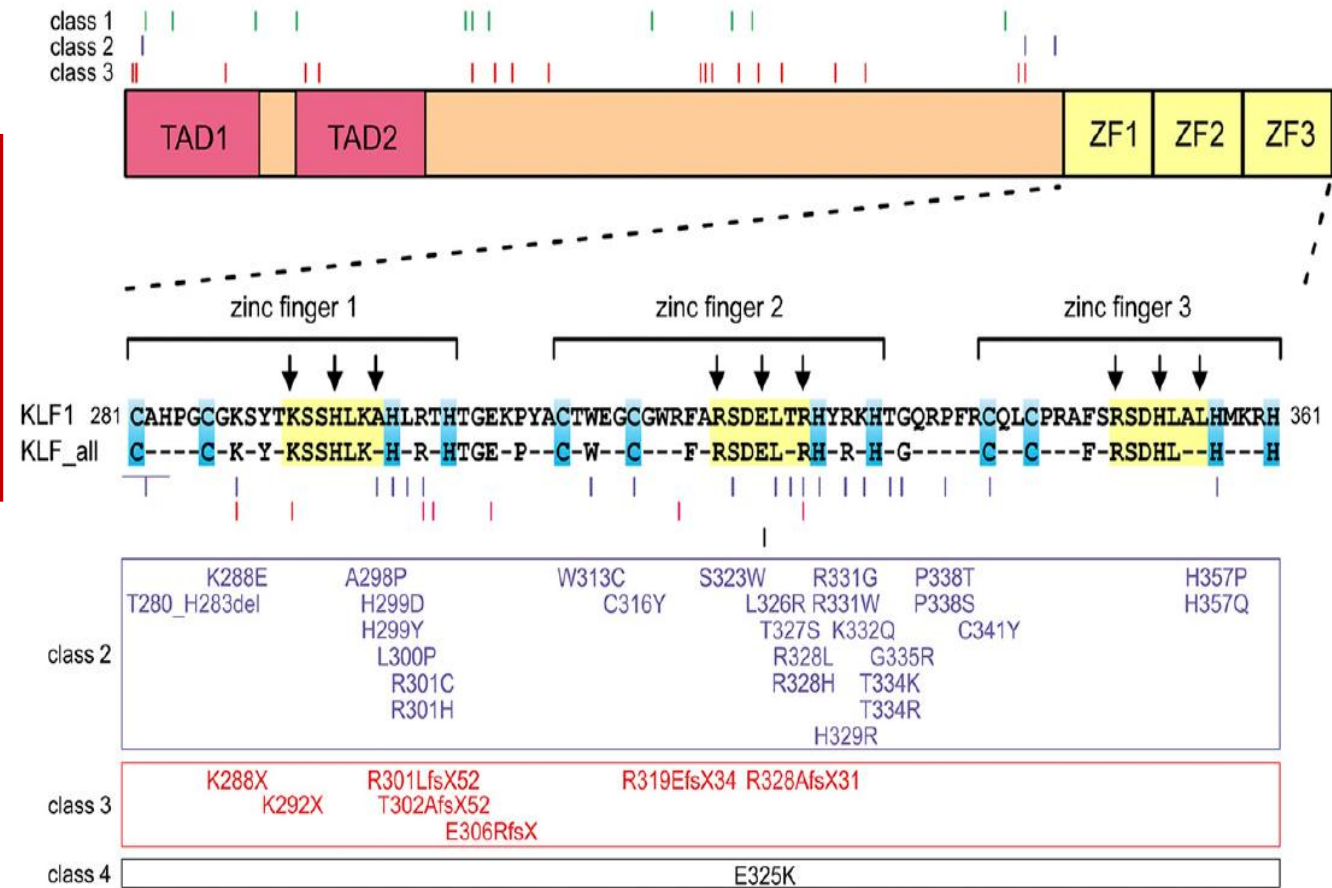
Krüppel-like factor 1 (*KLF1*)

- Erythroid-specific transcription factor
- Located on chromosome 19 (19p13.12-p13.13)
- **Function:** erythropoiesis, globin gene expression (Hb switching), metabolic enzyme, structural protein, RBC Ag, cell cycle regulators
- **Structure:**
 - N-terminal proline-rich transactivation domain
 - C-terminal DNA-binding domain (composed of 3 zinc fingers)



Functional domains of *KLF1* and variants

- **Class 1:** variants with no or minor functional consequences
- **Class 2:** hypomorphic variants with reduced function
- **Class 3:** truncating loss of function variants
- **Class 4:** one variant changing a highly conserved residue in ZF2, p.**E325K**. leading to dominant severe congenital dyserythropoietic anemia (CDA type IV)

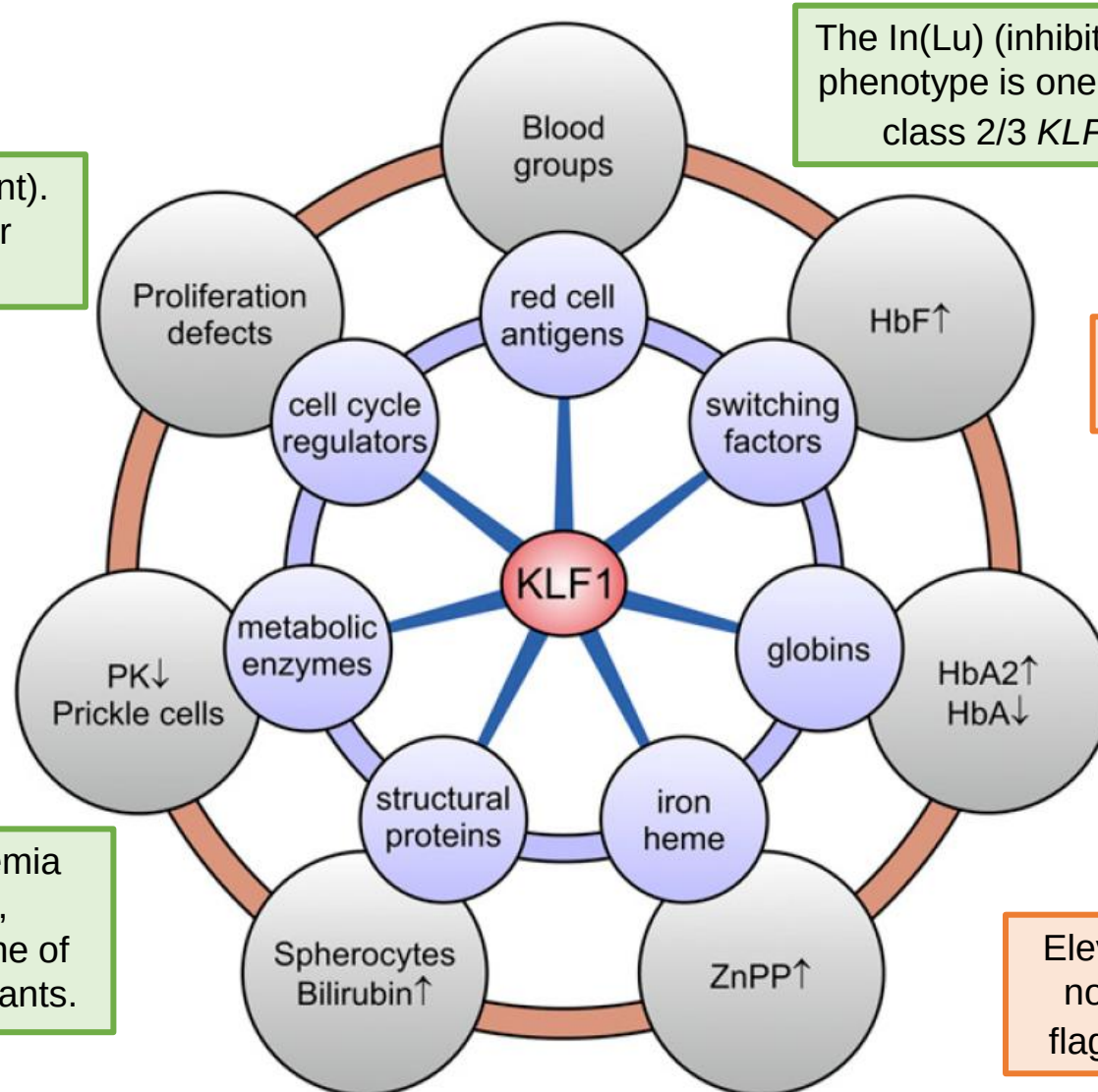


KLF1 target genes and associated clinical phenotypes

- Severe CDA type IV (class 4 variant).
 - Hydrops fetalis in homozygosity for class 3 *KLF1* variant.

Pyruvate kinase deficiency is one of the flags for class 2/3 *KLF1* variants.

Non-spherocytic hemolytic anemia (excluding thalassemia, HS, enzymopathies, and CDA) is one of the flags for class 2/3 *KLF1* variants.



Prevalence of *KLF1* mutation

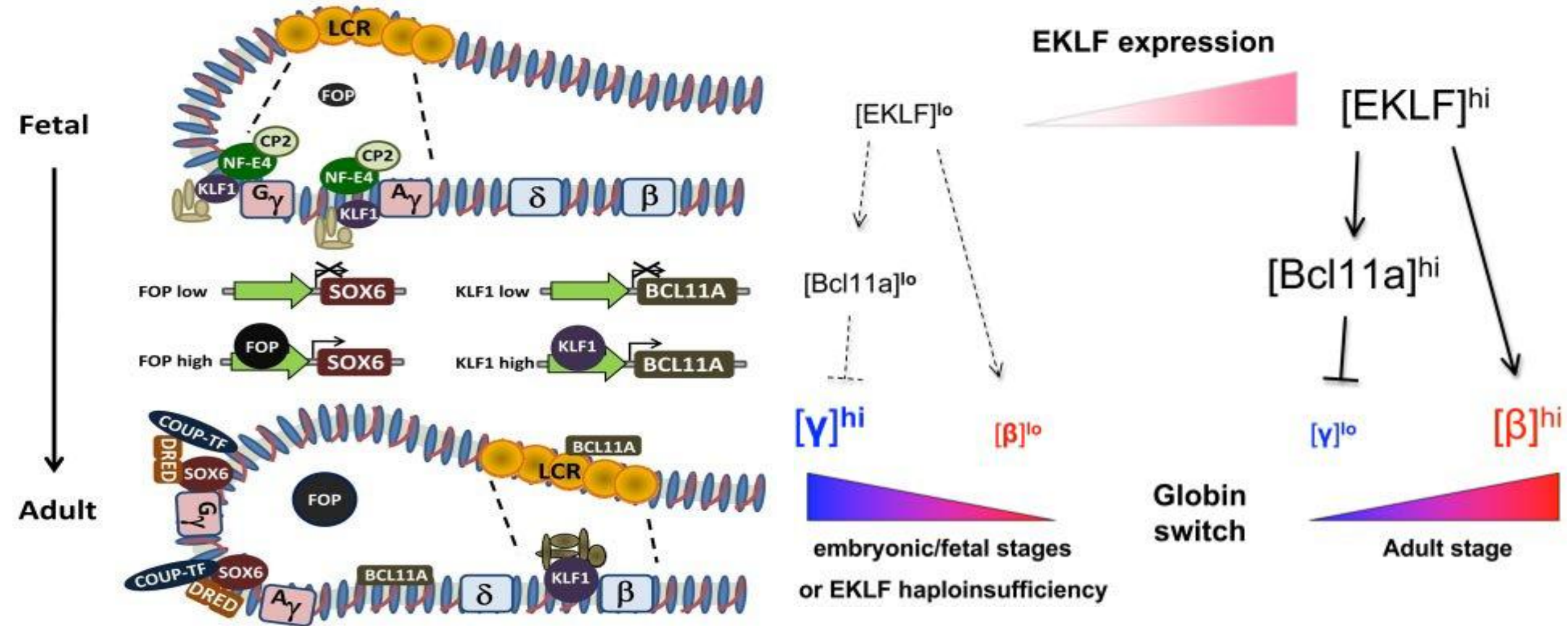
- **Southern China:** The prevalence is 1.25% (thalassemia-endemic region).
- **Northern China:** The prevalence is 0.08%.
- **United Kingdom:** The prevalence is 8.4% (individuals with unexplained elevated HbF, mainly of African, Indian, and Southeast Asian descent).
- **Thailand:** The prevalence is 23.8% (among individuals with borderline HbA₂ levels suspected of thalassemia).

These studies highlight that **regions with a high prevalence of hemoglobinopathies**, such as thalassemia, often **exhibit higher frequencies of *KLF1* mutations**. This correlation suggests a possible adaptive or modifying role of *KLF1* mutations in these populations

KLF1 variants and hemoglobinopathies



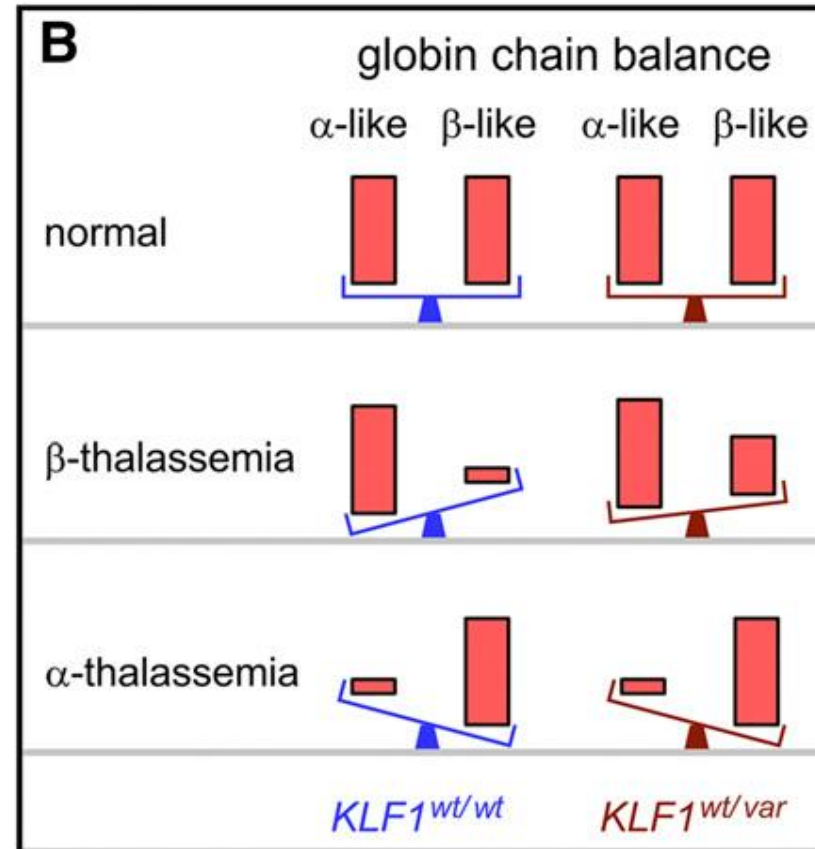
Hb switching



Impact of class 2 or 3 *KLF1* variants on clinical severity of hemoglobinopathies

A hematological parameters

normal	MCV	89.3±4.77	83.3±4.10
	HbA ₂	2.8±0.20	3.4±0.42
	HbF	0.2±0.22	3.0±2.63
β-thalassemia trait	MCV	65.5±4.34	63.5±3.94
	HbA ₂	5.0±0.49	6.5±0.81
	HbF	0.9±0.70	7.7±4.11
α-thalassemia trait	MCV	87.6±7.03	78.0±4.77
	HbA ₂	2.5±0.34	3.3±0.40
	HbF	0.6±0.39	3.1±2.11
		<i>KLF1</i> ^{wt/wt}	<i>KLF1</i> ^{wt/var}



Significant recovery of globin chain balance is observed only when beta-thalassemia is co-inherited with a class 2 or class 3 *KLF1* variant.

Clinical research

Hematology

Thalassemia and erythroid transcription factor *KLF1* mutations associated with borderline hemoglobin A₂ in the Thai population

Hataichanok Srivorakun, Wachiraporn Thawinan, Goonnapa Fucharoen, Kanokwan Sanchaisuriya, Supan Fucharoen

Table I. Hematological parameters, *KLF1* mutations and globin genotypes found in 202 Thai subjects with borderline Hb A₂

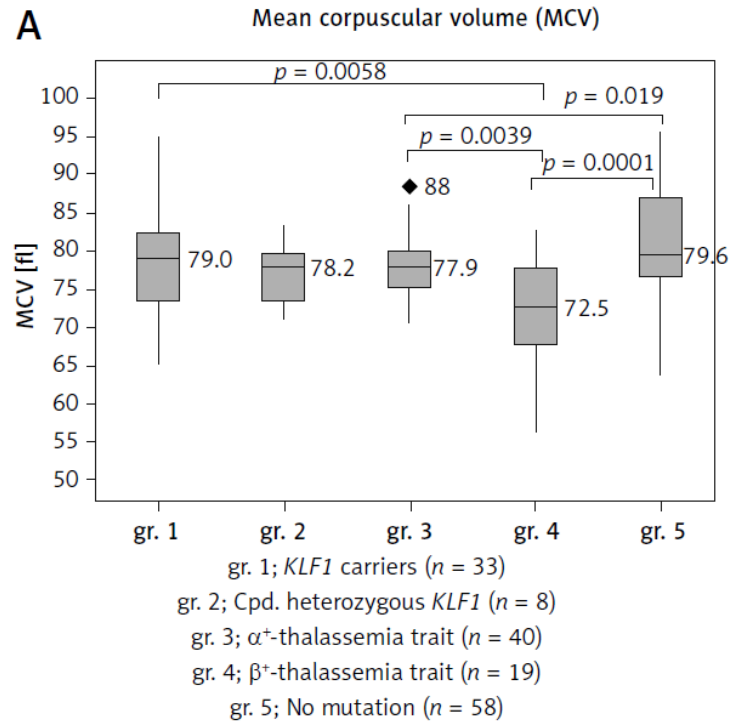
Mutations	n	%	Hb Typing		RBC [10 ¹² /l]	Hb [g/dl]	Hct [%]	MCV [fl]	MCH [pg]	MCHC [g/dl]
			Hb A ₂ (%)	Hb F (%)						
No mutation	84	41.5	3.7 ±0.2	1.1 ±1.8	4.6 ±0.8	12.4 ±1.9	37.6 ±5.9	81.9 ±9.9	27.1 ±3.6	33.1 ±1.6
Globin gene mutations:	70	34.7								
α-thalassemia carriers:	48	23.8								
-α ^{3.7} /αα	34	16.9	3.6 ±0.4	0.4 ±0.4	4.9 ±0.8	12.4 ±1.6	37.8 ±5.1	77.9 ±4.7	25.6 ±1.7	32.6 ±1.3
α ^{CS} α/αα	12	5.9	3.7 ±0.2	1.3 ±1.6	4.8 ±0.6	12.1 ±1.9	38.2 ±5.4	79.2 ±4.7	25.2 ±2.0	31.7 ±1.1
α ^{PS} α/αα	2	1.0	4.0, 3.5	0, 0	4.6	11.4	34.2	70, 75.2	22, 25.1	33.3
β ⁺ -thalassemia carriers:	22	10.9								
β ⁺ -thal trait	17	8.4	3.8 ±0.1	2.4 ±3.4	4.9 ±0.9	11.7 ±2.0	35.1 ±5.3	72.4 ±11.8	23.8 ±5.1	32.6 ±2.0
β ⁺ -thal trait with -α ^{3.7} /αα	2	1.0	3.8, 3.7	0, 0.6	na	na	na	80.7	26.9	na
β ⁺ -thal trait with α ^{CS} α/αα	1	0.5	4.0	0.9	4.5	11.4	35.3	78	25.1	32.2
β ⁺ -thal trait with -- ^{SEA} /αα	1	0.5	3.9	na	5.5	12.6	38	69.2	22.8	33
β ⁺ -thal trait with -- ^{SEA} /α ^{CS} α	1	0.5	3.5	1.9	4.0	6.9	24.2	61	17.5	28.8
KLF1 mutations:	38	18.8								
KLF1 trait	30	14.8	3.7 ±0.2	2.3 ±2.1	5.0 ±0.7	13.3 ±1.5	40.8 ±5.1	80.9 ±8.2	26.5 ±2.5	32.5 ±1.6
compound heterozygous KLF1	8	4.0	3.7 ±0.2	2.7 ±1.9	5.1 ±0.9	12.4 ±2.3	36.8 ±6.0	74.9 ±9.7	24.8 ±3.9	33.9 ±1.1
KLF1 mutations with globin gene mutations:	10	5.0								
KLF1 trait with -α ^{3.7} /αα	7	3.5	3.7 ±0.2	1.1 ±0.9	5.9 ±0.3	13.9 ±1.4	43.5 ±3.8	73.3 ±5.1	23.3 ±2.1	30.4 ±2.7
KLF1 trait with α ^{CS} α/αα	1	0.5	3.5	0	na	na	na	na	na	na
KLF1 trait with -- ^{SEA} /αα	1	0.5	3.6	4.2	6.3	12.3	41	65	19.6	30.3
Compound heterozygous KLF1 with -α ^{3.7} /αα	1	0.5	3.6	0	5.4	12.6	38.4	71	23.4	34

Values are presented as N, mean ± SD or raw data where appropriate. *β⁺-thalassemia mutation; CD19 (A>G) (n = 10), NT -31 (A>G) (n = 5), NT -28 (A>G) (n = 3), CD126 (T>G) (n = 3), NT -50 (G>A) (n = 1). na – not available.

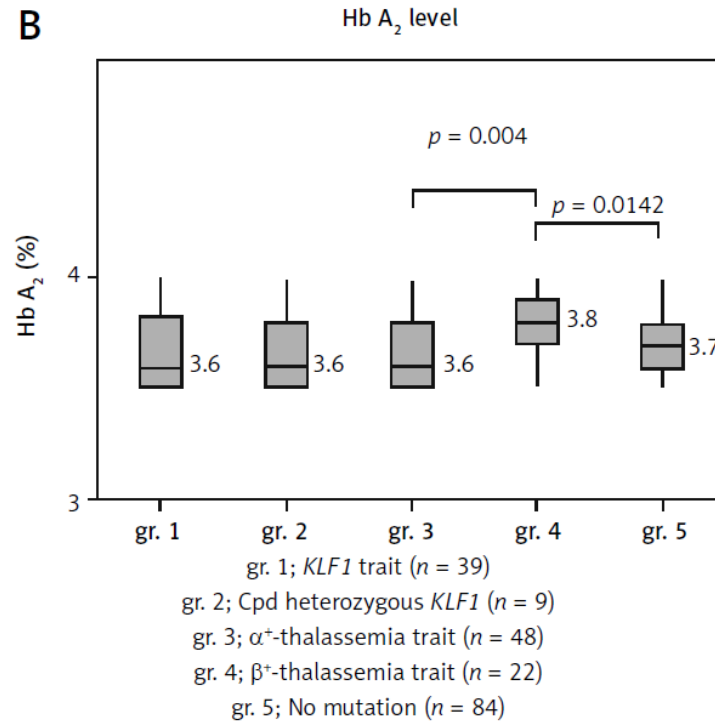
Table II. Hematologic parameters of 48 subjects with *KLF1* mutations and different α -globin genotypes

N	<i>KLF1</i> mutations (n)	α -genotype	Hb analysis		RBC [10 ¹² /l]	Hb [g/dl]	Hct [%]	MCV [fl]	MCH [pg]	MCHC [g/dl]
			Hb A ₂ (%)	Hb F (%)						
17	G176AfsX179 (13)	$\alpha\alpha/\alpha\alpha$	3.6 $\pm$ 0.2	3.3 $\pm$ 2.5	5.4 $\pm$ 0.7	13.4 $\pm$ 1.2	41.5 $\pm$ 4.6	77.7 $\pm$ 6.8	25.4 $\pm$ 2.7	32.2 $\pm$ 1.2
	G176AfsX179 (4)	$-\alpha^{3.7}/\alpha\alpha$ or $\alpha^{CS}\alpha/\alpha\alpha$	3.7 $\pm$ 0.2	1.4 $\pm$ 0.7	5.5 $\pm$ 0.2	13.4 $\pm$ 1.7	41.9 $\pm$ 4.0	73.6 $\pm$ 6.6	23.3 $\pm$ 2.9	29.4 $\pm$ 3.4
7	T334R (4)	$\alpha\alpha/\alpha\alpha$	3.8 $\pm$ 0.2	2.8 $\pm$ 1.2	5.7, 4.1	15.0, 11.9	46.4, 34.3	82.4 $\pm$ 0.7	27.3 $\pm$ 1.1	33.0, 34.6
	T334R (2)	$-\alpha^{3.7}/\alpha\alpha$	3.6, 3.9	0.1, 0.1	6.1, na	14.9, na	47.7, na	78.7, 72.2	24.6, 22.0	31.2, 30.5
	T334R (1)	$--^{SEA}/\alpha\alpha$	3.6	4.2	6.3	12.3	41	65	19.6	30.3
6	S270W	$\alpha\alpha/\alpha\alpha$	3.7 $\pm$ 0.2	1.2 $\pm$ 1.2	4.4 $\pm$ 0.3	12.5 $\pm$ 2.0	39.2 $\pm$ 6.8	88.0 $\pm$ 11.7	28.1 $\pm$ 2.8	31.8 $\pm$ 2.2
4	R328H (3)	$\alpha\alpha/\alpha\alpha$	3.7	2.2, 3.2	5.3, –	14.9	42.8	92.1, 81.3	28.3	34.8
	R328H (1)	$-\alpha^{3.7}/\alpha\alpha$	3.6	0	6.1	15.5	47.8	79.0	25.7	32.4
2	-154 (C-T)	$\alpha\alpha/\alpha\alpha$	3.9, 3.5	0, 0	5.3	13.5	42.2	73.4, 80.1	25.6	32
2	G335R (1)	$\alpha\alpha/\alpha\alpha$	3.6	1.2	na	na	na	73.4	26.2	28.5
	G335R (1)	$-\alpha^{3.7}/\alpha\alpha$	3.8	0	6.3	14.4	44.0	70.0	23.0	32.7
1	Q217X	$\alpha\alpha/\alpha\alpha$	3.7	0.5	na	na	na	na	na	na
6	-154 (C-T) / Q217X	$\alpha\alpha/\alpha\alpha$	3.6 $\pm$ 0.2	2.7 $\pm$ 1.4	5.3 $\pm$ 0.9	12.5 $\pm$ 2.5	37.5 $\pm$ 6.4	72.6 $\pm$ 10.2	23.8 $\pm$ 4.0	33.6 $\pm$ 0.8
2	G176AfsX179 / S270W (1)	$\alpha\alpha/\alpha\alpha$	3.6	0	na	na	na	83.6	26.8	31.2
	G176AfsX179 / S270W (1)	$-\alpha^{3.7}/\alpha\alpha$	3.6	na	5.4	12.6	38.4	71.0	23.4	34.0
1	T334R/R328H	$\alpha\alpha/\alpha\alpha$	4.0	5.4	4.2	11.9	33.4	80.0	28.5	35.6

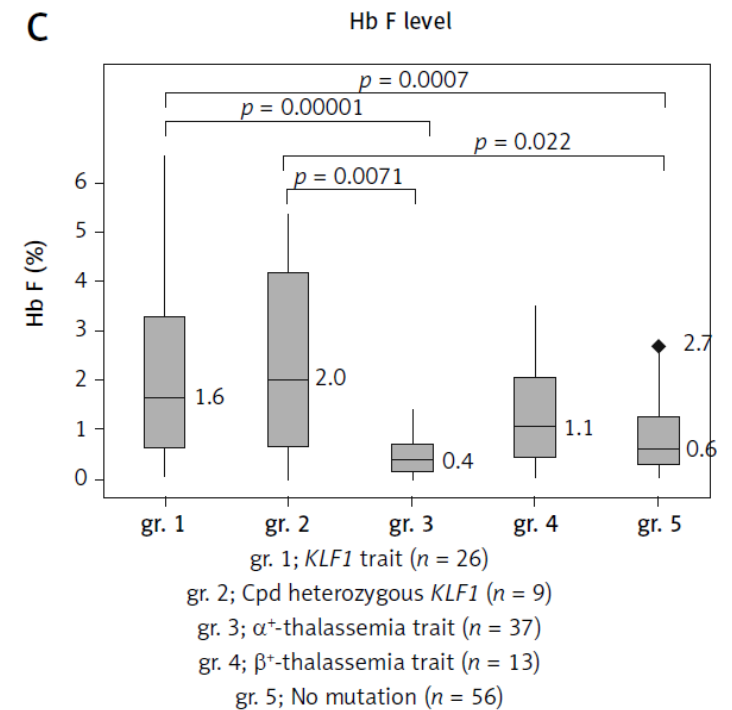
Values are presented as mean $\pm$ SD or as raw data where appropriate. N – total number of subjects for each *KLF1* mutation, (n) – number of subjects for each *KLF1* mutation and corresponding α - and β -globin genotypes.



***KLF1* mutation**
 Higher MCV than Beta-thal trait



***KLF1* mutation**
 Borderline Hb A2 level



***KLF1* mutation**
 Significantly higher Hb F

Molecular and haematological characterisation of haemolytic anaemia associated with biallelic KLF1 mutations: a case series

Kritsada Singha,^{1,2} Nattiya Teawtrakul,³ Goonnapa Fucharoen,² Supan Fucharoen ²

Groups	n	KLF1 mutation
No KLF1 mutation		
Hb E trait	20	—
Normal β -globin gene	13	—
β -thalassaemia trait	5	—
Homozygous Hb E	1	—
Total	39	
Heterozygous KLF1 mutation		
Hb E trait	2	KLF1:c.638T>C (F182L) (n=1), KLF1:c.983G>A (n=1)
Homozygous Hb E	2	KLF1:c.-154C>T (n=1), KLF1:c.902G>A (n=1)
Normal β -globin gene	1	KLF1:c.519_525dupCGGCGGCC (n=1)
Total	5	
Biallelic KLF1 mutation		
Normal β -globin gene	7	KLF1:c.519_525dupCGGCGGCC/c.892G>C (n=5), KLF1:c.892G>C/c.983G>A (n=1), KLF1:c.1001C>G/c.1003G>A (n=1)
Hb E trait	6	KLF1:c.519_525dupCGGCGGCC /c.892G>C (n=3), KLF1:c.892G>C/c.1001C>G (n=1), KLF1:c.-154C>T;643C>T/c.809C>G (n=1), KLF1:c.-154C>T;643C>T/c.892G>C (n=1)
Total	13	

Hb, haemoglobin; KLF1, Krüppel-like factor 1.

- A total of 57 subjects with a thalassemia-like syndrome and unusually elevated HbF levels were investigated for *KLF1* mutations.
- *KLF1* mutations were identified in 18 subjects (31.5%); five had heterozygous *KLF1* mutations, and 13 had biallelic *KLF1* mutations.

Table 2 Haematological parameters, Hb patterns, thalassaemia genotypes and KLF1 functional classes of 13 subjects with biallelic KLF1 mutations

Subject no: sex/age group	Hb (g/L)	MCV (fL)	RDW (%)	Hb E+A ₂ (%)	Hb F (%)	Hb Bart's (%)	Thalassaemia genotypes	KLF1 mutations (class)
P1: F/adulthood	79	84.0	21.9	4.4	23.8	1.1	$\alpha\alpha/\alpha\alpha, \beta^A/\beta^A$	c.892G>C/c.983G>A (2/2)
P2: F/adulthood	87	69.8	20.2	19.7	25.5	Na	$-\alpha^{3.7}/\alpha\alpha, \beta^E/\beta^A$	c.892G>C/c.1001C>G (2/2)
P3: M/middle childhood	Na	Na	Na	2.4	19.9	0.2	$-\alpha^{3.7}/\alpha\alpha, \beta^A/\beta^A$	c.1001C>G/c.1003G>A (2/2)
P4: F/infancy	45	66.4	21.8	3.4	32.1	0.7	$\alpha^{WM}/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P5: M/adulthood	61	80.8	23.9	5.2	18.7	0.2	$\alpha\alpha/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P6: F/early adolescence	68	61.3	30.1	13.5	21.4	0.2	$\alpha\alpha/\alpha\alpha, \beta^E/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P7: F/toddler	74	76.7	25.0	2.5	33.9	0.4	$\alpha\alpha/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P8: F/toddler	77	73.9	27.3	2.6	47.4	0.5	$\alpha\alpha/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P9: M/adulthood	78	77.2	21.7	19.5	24.5	0.1	$\alpha\alpha/\alpha\alpha, \beta^E/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P10: M/early adolescence	84	74.5	24.0	3.9	28.2	0.6	$\alpha\alpha/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P11: F/early childhood	97	62.7	25.5	2.7	41.8	3.0	$\alpha^{CS}/\alpha\alpha, \beta^A/\beta^A$	c.519_525dupCGGCGCC/c.892G>C (3/2)
P12: F/early childhood	96	74.1	21.6	12.1	17.9	Na	$\alpha\alpha/\alpha\alpha, \beta^E/\beta^A$	c.-154C>T;643C>T/c.892G>C (3/2)
P13: F/adulthood	110	69.0	Na	24.3	21.1	0	$\alpha\alpha/\alpha\alpha, \beta^E/\beta^A$	c.-154C>T;643C>T/c.809C>G (3/2)

CS, Hb Constant Spring; Hb, haemoglobin; KLF1, Krüppel-like factor 1; MCV, mean corpuscular volume; Na, not available; RDW, red cell distribution width; WM, Hb Westmead.

Case 1

Patient characteristics

Age	34
Sex (M/F)	F
Thalassemia type	History of HbHCS
Age at start of RBC transfusion	1-year-old

Medical history

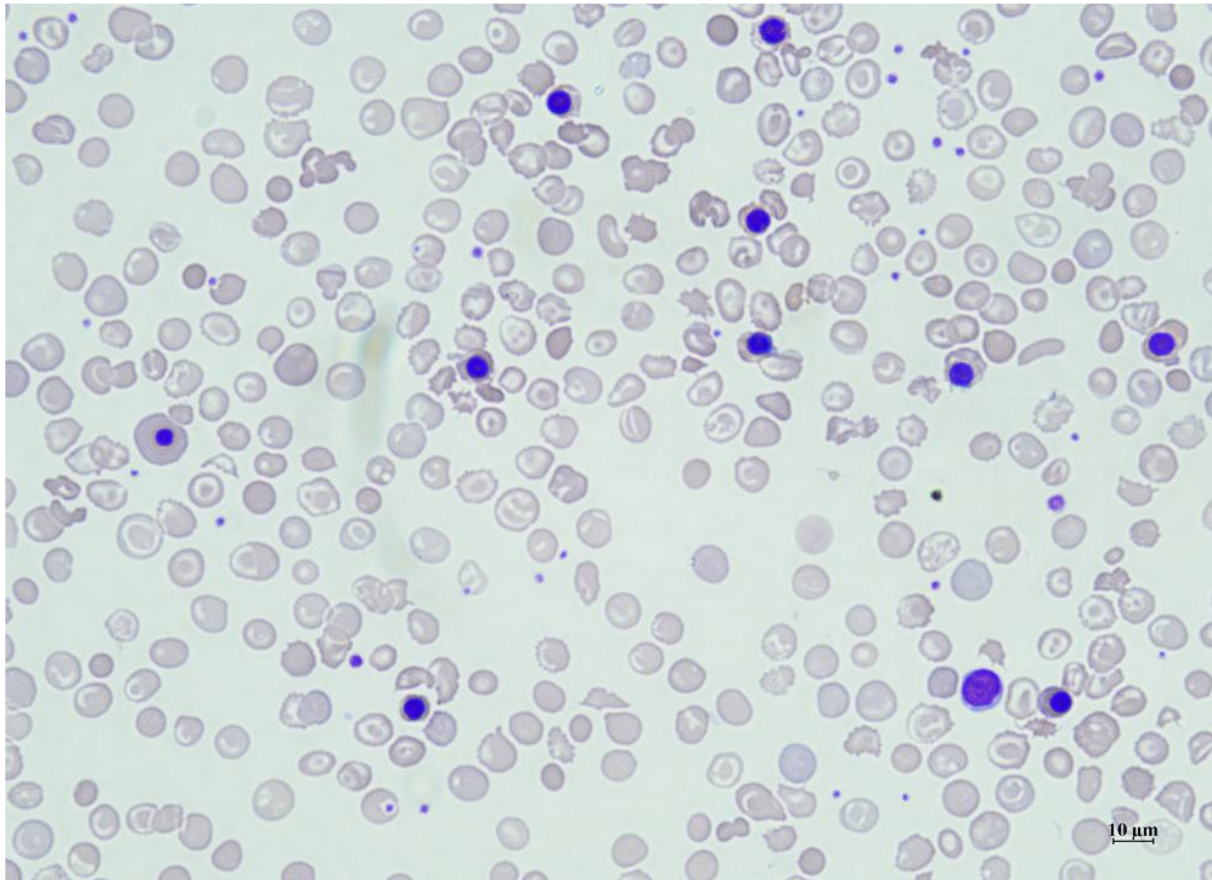
Transfusion regimen	LPB 2 units every 4 weeks
Splenectomy	Yes, at the age of 7
Active morbidities	- Gallstones

E-SAAN study
Median NRC (post-splenectomy)
= **250** (150-350)

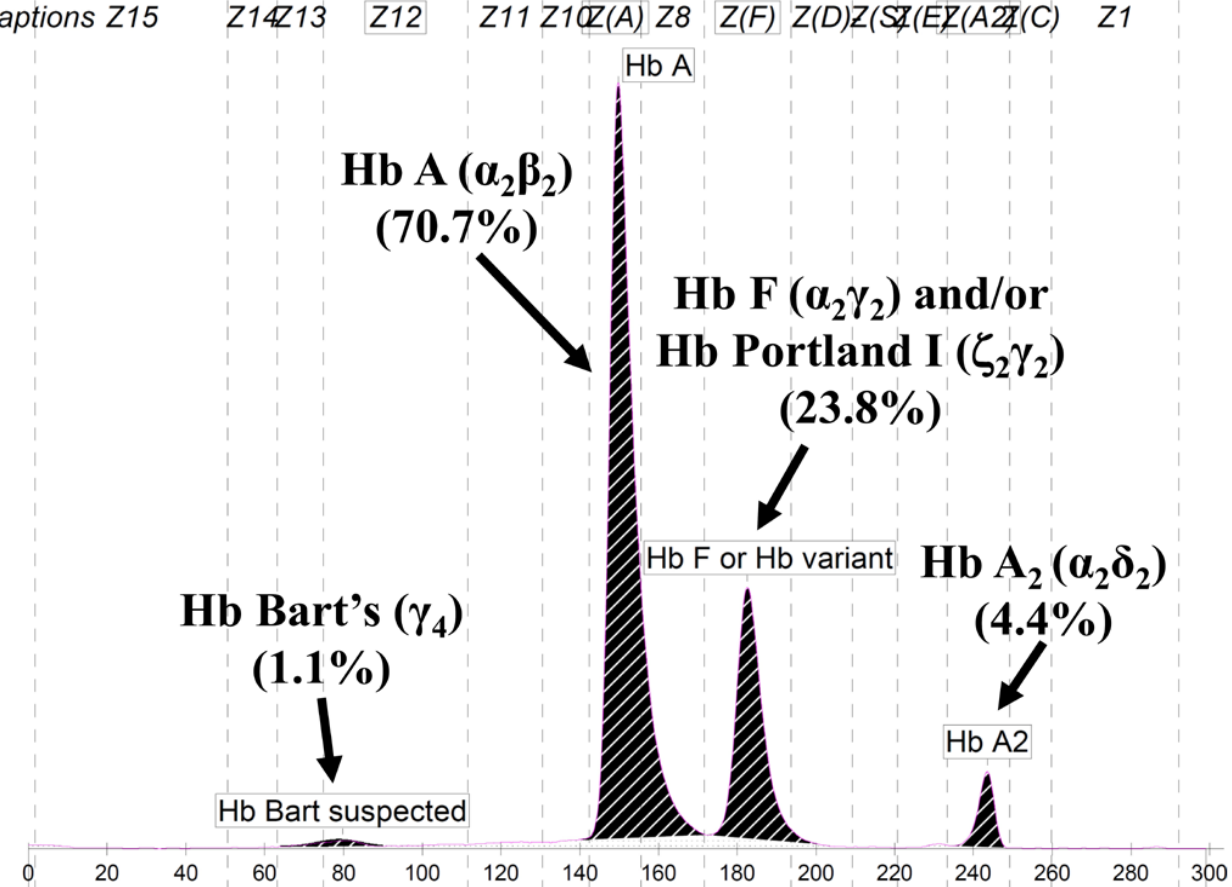
Physical exam and laboratories

Physical exam	mild pale conjunctivae, marked icteric sclerae, no hepatomegaly, no pitting edema, and no leg ulcers
CBC and RBC indices	Hb 7.9 g/dL, Hct 25.1%, MCV 84 fL, MCH 27.2 pg, MCHC 32.2 g/dL, RDW 21.9%, Corrected WBC 7,700 cells/ μ L NRC 877/100 WBC
Hb typing	A2FA(Bart's?) (A2 = 4.4%, F=23.5%)
DNA analysis for beta and alpha thalassemia	$\alpha\alpha/\alpha\alpha$ and β^A/β^A
Serum ferritin (ng/mL)	1,580 ng/mL
Bilirubin	TB 8 mg/dL, DB 0.6 mg/dL, indirect bilirubin 7.4 mg/dL

Peripheral blood smear and Hb typing



Captions Z15



Case 2

Patient characteristics

Age	28
Sex (M/F)	M
Thalassemia type	beta-thalassemia/Hb E
Age at start of RBC transfusion	6-month-old

Medical history

Transfusion regimen	LPB 2 units every 4 weeks
Splenectomy	Yes, at the age of 3
Active morbidities	- Gallstones - Endocrinopathies: hypothyroidism, hypogonadism

Physical exam and laboratories

Physical exam	hyperpigmentation, pale conjunctivae, icteric sclerae, an enlarged liver measuring 4 cm below the right costal margin
CBC and RBC indices	Hb 7.8 g/dL, Hct 25.1%, MCV 77.2 fL, MCH 24 pg, MCHC 31.1 g/dL, RDW 21.7%, Corrected WBC 5,835 cells/ μ L NRC 1,036/100 WBC
Hb typing	EFA (E= 19.5%, F=24.5%)
DNA analysis for beta and alpha thalassemia	$\alpha\alpha/\alpha\alpha$ and β^E/β^A
Serum ferritin (ng/mL)	3,372 ng/mL
Bilirubin	TB 3.8 mg/dL, DB 0.9 mg/dL, indirect bilirubin 2.9 mg/dL

Case 3

Patient characteristics

Age	38
Sex (M/F)	M
Thalassemia type	beta-thalassemia/Hb E
Age at start of RBC transfusion	1-year-old

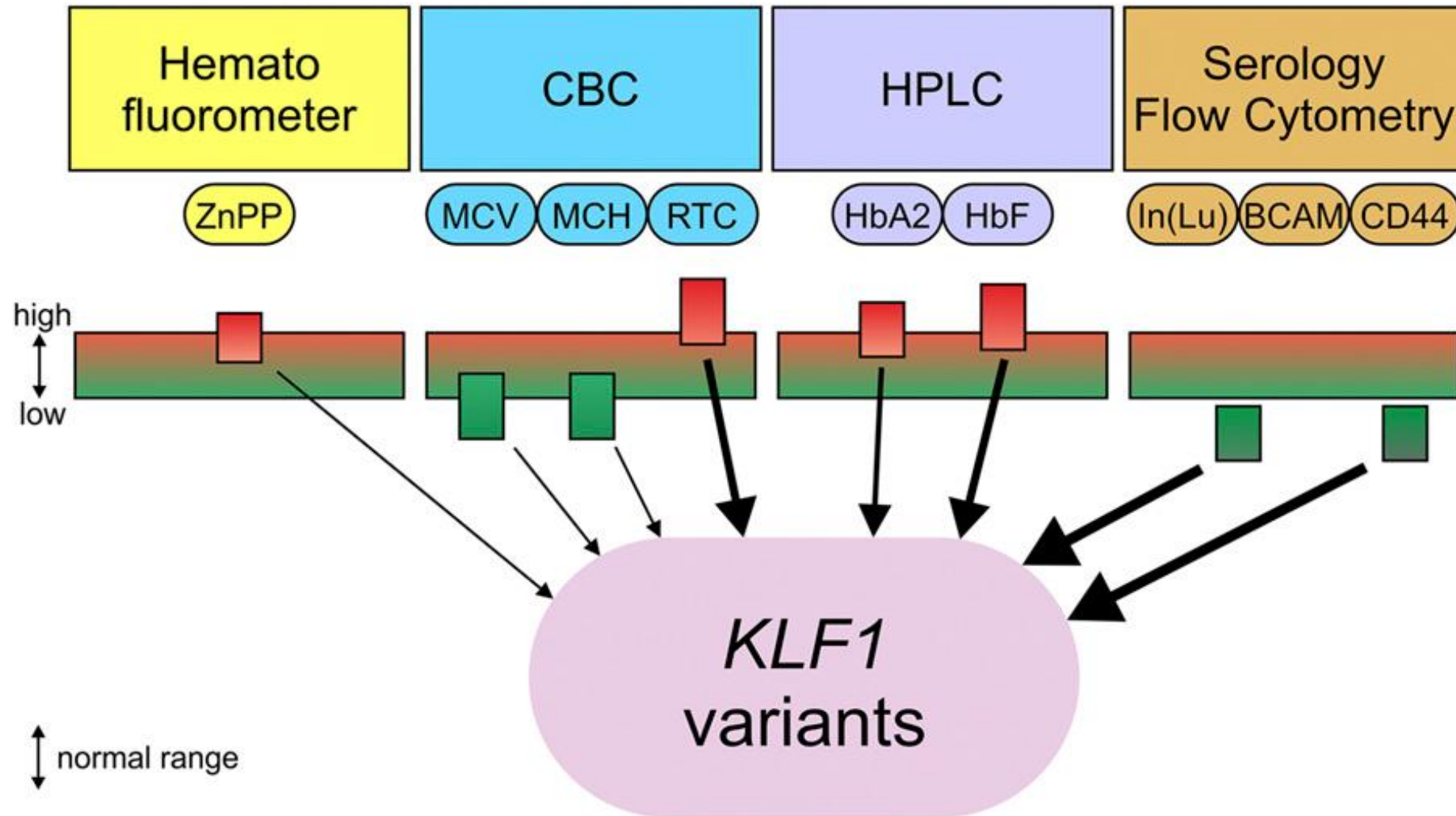
Medical history

Transfusion regimen	LPB 2 units every 6 weeks
Splenectomy	No
Active morbidities	- Gallstones - Osteoporosis - EMH

Physical exam and laboratories

Physical exam	pale conjunctivae, icteric sclerae, massive splenomegaly (24 cm below the left costal margin)
CBC and RBC indices	Hb 6.1 g/dL, Hct 21.4%, MCV 80.8 fL, MCH 23.8 pg, MCHC 29.4 g/dL, RDW 23.9%, Corrected WBC 2,810 cells/ μ L NRC 164/100 WBC
Hb typing	EFA (E = 5.2%, F=18.7%)
DNA analysis for beta and alpha thalassemia	$\alpha\alpha/\alpha\alpha$ and β^A/β^A
Serum ferritin (ng/mL)	342 ng/mL
Bilirubin	TB 3.1 mg/dL, DB 0.6 mg/dL, indirect bilirubin 2.5 mg/dL

Flags indicating carrier status of class 2 or 3 *KLF1* variants



Clinical manifestations

- Hypochromic microcytic anemia, borderline elevated Hb A2
- Elevated Hb F, Hereditary persistence of fetal hemoglobin (HPFH)
- Transfusion-dependent hemolytic anemia + high NRBC + small amount of Hb Bart's
- Hydrops Fetalis
- Congenital dyserythropoietic anemia type IV (class 4 variant)
- Elevated zinc protoporphyrin
- Pyruvate kinase deficiency
- Decreased expression of RBC Ag: Inhibitor of lutheran blood group - In(Lu), CD44 (Indian blood group), etc.

When to suspect *KLF1* mutation?

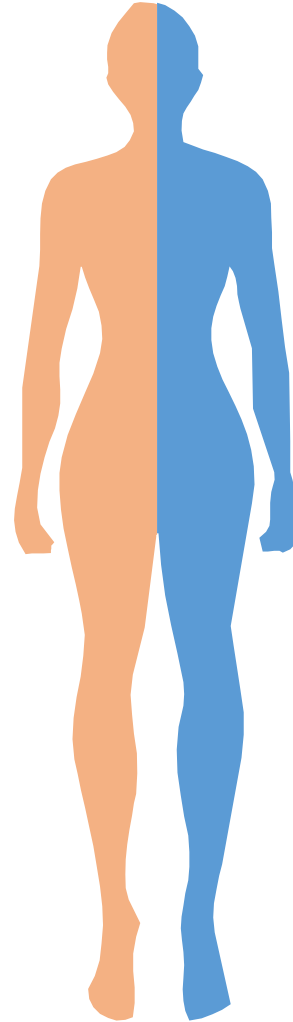
- Hemolytic anemia

Massive splenomegaly

- Hypochromic microcytic RBC

Aniso-poikilocytosis with
numerous NRBC

- Negative for thalassemia disease



- Borderline Hb A2 level

Hb A2: 3.5-4.0%

- **Elevated Hb F level**

- Presence of small amount of Hb Bart's

Treatment Options for Patients with *KLF1* Mutations

1. **Blood transfusion:** indicated in symptomatic anemia or severe hemolysis.
2. **Folic acid supplementation:** due to increased red cell turnover.
3. **Splenectomy:** may be beneficial in severe cases with significant splenomegaly or symptomatic hemolysis.
4. **Chelation therapy:** indicated if iron overload occurs.
5. **Hydroxyurea or other HbF-inducing agents:** considered in cases with severe hemolysis to increase fetal hemoglobin and alleviate symptoms.
6. **Hematopoietic Stem Cell Transplantation (HSCT):** considered in selected severe cases (CDA type IV).

Take Home Messages

1

KLF1 mutations should be suspected in adult patients presenting with unexplained **hemolytic anemia**, especially with **elevated HbF** and **borderline HbA₂ levels**.

2

Associated clinical flags: In(Lu) phenotype, **high NRC**, small amount of Hb Bart's, PK deficiency, elevated ZnPP with normal iron stores

3

Prevalence is high in regions endemic for hemoglobinopathies and may be found co-inherited with hemoglobinopathies

4

Treatment remains supportive (transfusion, folate, splenectomy, iron chelation), with novel therapies under investigation



THANK YOU