bjh research paper

Severe neonatal haemolytic anaemia caused by compound heterozygous *KLF1* mutations: report of four families and literature review

Tanu Tangsricharoen,[1] Rungrote Natesirinilkul,[1] Arunee Phusua,[1] Kanda Fanhchaksai,[1] Chupong Ittiwut,[2,3] Wanna Chetruengchai,[2,3] Monthana Juntharaniyom,[4] Pimlak Charoenkwan,[1] Vip Viprakasit,[5] Chureerat Phokaew[2,3] and Vorasuk Shotelersuk[2,3]

[1]Department of Pediatrics, Division of Hematology and Oncology, Faculty of Medicine, Chiang Mai University, Chiang Mai, [2]Department of Pediatrics, Center of Excellence for Medical Genomics, Medical Genomics Cluster, Faculty of Medicine, Chulalongkorn University, [3]Excellence Center for Genomics and Precision Medicine, King Chulalongkorn Memorial Hospital, The Thai Red Cross Society, Bangkok, [4]Department of Pediatrics, Division of Hematology and Oncology, Khon Kaen Regional Hospital, Khon Kaen, and [5]Department of Pediatrics, Division of Hematology and Oncology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Received 12 April 2021; accepted for publication 13 May 2021

Correspondence: Associate Professor Pimlak Charoenkwan, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, 110 Intawarorot road, Sriphum, Muang, Chiang Mai 50200, Thailand.
E-mail: pimlak.c@cmu.ac.th

Summary

Mutations in the *KLF1* gene, which encodes a transcription factor playing a role in erythropoiesis, have recently been demonstrated to be a rare cause of hereditary haemolytic anaemia. We described the genotypic and phenotypic spectra of four unrelated families with compound heterozygous class 2/class 3 *KLF1* mutations. All patients had p.G176RfsX179 on one allele and either p.A298P, p.R301H or p.G335R on the other allele. All presented on the first day of life with severe haemolytic anaemia with abnormal red blood cell morphology, markedly increased nucleated red blood cells and hyperbilirubinaemia. Three patients later became transfusion-dependent. All parents with heterozygous *KLF1* mutation without co-inherited thalassaemia had normal to borderline mean corpuscular volume (MCV) and normal to slightly elevated Hb F. Fifteen previously reported cases of biallelic *KLF1* mutations were identified from a literature review. All except one presented with severe haemolytic anaemia in the neonatal period. Our finding substantiates that compound heterozygous *KLF1* mutations are associated with severe neonatal haemolytic anaemia and expands the haematologic phenotypic spectrum. In carriers, the previously suggested findings of low MCV, high Hb A_2 and high Hb F are inconsistent; thus this necessitates molecular studies for the identification of carriers.

Keywords: haemolytic anaemia, *KLF1*, Krüppel-like factor 1, Newborn, transcription factor.

Introduction

Krüppel-like factor 1 (KLF1), also known as erythroid Krüppel-like factor (EKLF), is an erythroid-specific transcription factor that plays an important role in the terminal differentiation of erythroid cells.[1,2] KLF1 activates genes that are involved in the expression of globins, zinc protoporphyrin, structural proteins, metabolic enzymes, cell cycles regulators, red blood cell antigens and haemoglobin (Hb) switching factors.[3,4] The *KLF1* gene is located on chromosome 19p13·12-p13·13.[5] KLF1 protein is composed of two transactivation domains (TAD1 and TAD2) at the N-terminus and three zinc finger (ZF) domains at the C-terminus. The TADs and zinc finger domains are encoded by exons 1–2 and exons 2–3 respectively.[3,6,7]

Mutations in *KLF1* cause a spectrum of phenotypes. To date, more than 50 mutations of the *KLF1* gene have been

First published online 5 July 2021
doi: 10.1111/bjh.17616

reported.[3] *KLF1* mutations are classified into four functional classes. Class 1 variants are missense mutations that cause a benign phenotype. Class 2 variants are hypomorphic alleles that comprise missense variants or small in-frame deletions that affect the functions of KLF1 protein. Class 3 variants are null mutations resulting from non-sense or frameshift mutations. The class 4 variant is caused by the E325K mutation, which is located in a highly conserved ZF domain. The mutation has a strong dominant negative effect and has been reported as a *de-novo* mutation. The condition, classified as congenital dyserythropoietic anaemia (CDA) type IV, is characterized by severe ineffective erythropoiesis and haemolysis.[3,8,9]

KLF1 controls the *BCL11A* gene that encodes a suppressor of Hb F expression and also controls globin gene switching.[10,11] Haploinsufficiency for *KLF1* due to loss-of-function mutations causes hereditary persistence of fetal haemoglobin (HPFH).[12–15] *KLF1* mutations can cause borderline high Hb A_2 levels.[16–18] *KLF1* has also been reported to ameliorate the severity of beta-thalassaemia disease.[19–21] The findings were demonstrated in a pair of twins who had beta-thalassaemia major with discrepant clinical severity. The twin who harboured a co-inherited *KLF1* mutation was non-transfusion-dependent.[21]

Singleton *et al.* firstly reported in 2008 that heterozygous *KLF1* mutation causes a rare In(Lu) (inhibitor of Lutheran antigen expression) blood group and low levels of red cell membrane proteins BCAM and CD 44.[22] The changes are benign as all individuals with heterozygous *KLF1* mutation and In(Lu) blood group are asymptomatic. Several reports confirm the association of the In(Lu) blood group with the heterozygous *KLF1* mutation.[23–25]

Recently, Viprakasit *et al.* reported for the first time that compound heterozygosity of *KLF1* mutations was a cause of severe transfusion-dependent haemolytic anaemia.[26] The haematologic phenotypes were extensively characterized. All eight reported patients presented with neonatal hyperbilirubinaemia and early onset of anaemia with characteristics of increased nucleated red blood cells, reticulocytosis, high Hb F, reduced pyruvate kinase activities and In(Lu) blood group.[26] After the initial report, there were further reports of seven cases with compound heterozygous *KLF1* mutations-associated haemolytic anaemia with clinical manifestation ranging from hydrops fetalis to mild non-transfusion-dependent anaemia.[27–32] As the condition is rare and may mimic other more common hereditary haemolytic anaemias, knowledge of clinical manifestations and haematologic characteristics should be useful for understanding the *KLF1* mutations and establishing a diagnosis of new cases. Herein, we described the genotypes and haematologic phenotypes in cases of compound heterozygous *KLF1* mutations-associated haemolytic anaemia and their parents who were carriers. Previously reported cases of compound heterozygous *KLF1* mutations-associated haemolytic anaemia were reviewed and summarized.

Patients and methods

The study protocol was approved by the institutional ethics committee. Paediatric patients, aged from birth to 18 years old, who were diagnosed with *KLF1*-associated haemolytic anaemia at Chiang Mai University Hospital, Faculty of Medicine, Chiang Mai University, Thailand and their parents were enrolled. All parents gave informed consent and patients older than seven years old gave their assent to the study. *KLF1* genotypes, clinical presentation and haematologic data were analyzed. Blood samples were collected from all patients and parents to be tested for common thalassaemia mutations and common mutations on *SPTB* that cause hereditary elliptocytosis and hereditary pyropoikilocytosis.

The *KLF1* mutations were detected by whole-exome sequencing (WES) and confirmed by direct Sanger sequencing in families 1 and 2 and by direct Sanger sequencing of the *KLF1* gene in families 3 and 4. The WES and direct Sanger sequencing methods were as described previously.[33,34] The southeast Asian, Thai, 3·7 kb and 4·2 kb deletional alpha-thalassaemias were analyzed as per gap-PCR-based methods as described previously.[35,36] Mutations causing Hb Constant Spring (*HBA2*: c.427 T > C) were searched for by the high-resolution melting analysis method.[37] The beta-thalassaemia mutations were analyzed by high-resolution melting analysis.[38] Three common mutations on *SPTB,* Providence (*SPTB*: c.6055 T > C), Buffalo (*SPTB*: c.6074 T > G) and Chiang Mai (*SPTB*:c.6224 A > G) were analyzed by Sanger DNA sequencing.

Clinical and haematological parameters were summarized in the patients who had biallelic *KLF1* mutations and in the parents who had a monoallelic *KLF1* mutation. Co-inherited mutations of other relevant genes were described. Hb analysis was performed by high-pressure liquid column chromatography (HPLC) using the Variant II HPLC system (Bio-Rad Laboratories, Hercules, CA, USA) in families 1, 2 and 4, and by capillary electrophoresis using the Capillarys 2 automated analyzer (Sebia, Lisses, France) in family 3.

We performed a literature review of biallelic *KLF1* mutations-associated haemolytic anaemia, using the keywords "*KLF1*" or "*EKLF*" or "Krüppel-like factor 1" and "CDA" or "congenital dyserythropoietic anemia" or "congenital dyserythropoiesis" or "congenital hemolytic anemia" or "congenital non-spherocytic hemolytic anaemia" or "CNSHA" or "anemia" to search PubMed for articles published up until January 2021. Only articles in English were included. This search revealed 156 articles. TT and PC reviewed the titles and abstracts for reported cases of compound heterozygous *KLF1* mutations-associated haemolytic anaemia. The reports of the dominantly negative *KLF1* E325K mutation causing CDA type IV were excluded. Seven articles reporting a total of 15 cases were selected for review.

Table I. Clinical characteristics and outcomes of patients with biallelic mutations of *KLF1* genes.

Family number	Sex	Gestational age (weeks)	Birth weight (g)	Age at diagnosis (hours)	Clinical presentation	Treatment	Outcome
1	Female	37	3,030	15	Neonatal anaemia and hyperbilirubinaemia (Hct 36%, MB 12·1 mg/dl at 15 h of age)	Phototherapy for neonatal hyperbilirubinaemia, received one RBC transfusion at six months old	Required occasional transfusion
2	Female	39	2,470	13	Neonatal anaemia and hyperbilirubinaemia (Hct 41%, MB 10·5 mg/dl at 13 h of age)	Phototherapy for neonatal hyperbilirubinaemia, received first RBC transfusion at two months old then every 3-4 weeks	Transfusion-dependent
3	Male	37	2,490	5	Neonatal anaemia and hyperbilirubinaemia (Hb 119 g/l, MCV 98 fl, RC 4·9% nRBC 243/100 WBC, TB 7·1, DB 1·3 mg/dl at 5 h of age)	Phototherapy for neonatal hyperbilirubinaemia, RBC transfusion three times during neonatal admission, at three months and then every 3–4 weeks	Transfusion-dependent
4	Male	40	2,250	20	Neonatal anaemia and hyperbilirubinaemia (Hb 78 g/l, MCV 88 fl, nRBC 1,812/100 WBC, TB 15·3, DB 1·9 mg/dl at 20 h of age)	Phototherapy for neonatal hyperbilirubinaemia, RBC transfusion three times during neonatal admission, at three months and then every 3–4 weeks	Transfusion-dependent

DB, direct bilirubin; Hb, haemoglobin; Hct, haematocrit; MB, microbilirubin; MCV, mean corpuscular volume; nRBC, nucleated red blood cell; RBC, red blood cell; RC, reticulocyte count; TB, total bilirubin; WBC, white blood cell.

Table II. Haematological and molecular characteristics of patients with biallelic mutations of *KLF1* genes.

Family number	Age at blood count	RBC parameters									Hb analysis			*KLF1* mutation	Other mutations
		Hb (g/l)	Hct (%)	RBC ($x10^6/mm^3$)	MCV (fl)	MCH (pg)	MCHC (g/l)	RDW (%)	RC (%)	nRBC/ 100WBC	Age at Hb analysis	Hb A (%)	Hb A_2 (%)	Hb F (%)		
1	5 d	101	31·8	3·7	85·1	27·0	317	22·7	9·4	138	10 m	56·0	3·9	32·0	G176RfsX179/A298P	None
	6 m	72	21·6	3·3	65·9	22·1	335	28·9	–	14						
	14 m	75	25·5	3·7	69·1	20·3	294	25·0	2·0	–						
2	2 m	63	19·8	2·6	76·6	24·5	320	19·9	5·4	56	ND	–	–	–	G176RfsX179/R301H	*G6PD* Kaiping (het)
3	5 h	119	40·0	3·9	98	31	320	25·1	4·9	243	ND	–	–	–	G176RfsX179/G335R	None
	1 d*	133	42·0	4·8	88	28	320	20·9	–	237						
	1 w	97	30·0	3·8	77	25	330	19·1	–	57						
4	20 h	78	23·9	2·7	88·6	28·9	326	34·9	–	1 970	3 m	68·6	2·9	23·1	G176RfsX179/A298P	*HBA* −3·7 kb del (het)
	3 m	78	25·5	3·6	71·4	21·7	304	24·1	6·0	–						

Hb, haemoglobin; Hct, haematocrit; het, heterozygous; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin level; MCV, mean corpuscular volume; nRBC, nucleated red blood cell; RBC, red blood cell; RC, reticulocyte count; RDW, red cell distribution width; WBC, white blood cell.
*Recent post-transfusion sample.

Results

Four unrelated paediatric patients, aged from two to nine years old, who were diagnosed with *KLF1*-associated haemolytic anaemia and their parents were enrolled. The *KLF1* genotypes and clinical presentation from the patients are summarized in Table I. Two patients were male. All were born at term with a birth weight from 2 250 to 3 030 g. All presented within the first day of life (onset 5–20 h of age) with neonatal anaemia and hyperbilirubinaemia. All received phototherapy and two patients received their first transfusion within the neonatal period. Three patients later became transfusion-dependent and one required occasional transfusion.

The genetic and haematologic data are summarized in Table II. All eight mutant alleles comprising four different mutations were successfully identified. All patients had p.G176RfsX179 (c.525_526insCGGCGCC) on one allele, and p.A298P (c.892G>C), p.R301H (c.902G>A) or p.G335R (c.1003G>A) on the other allele. A patient had co-inherited heterozygous *G6PD* Kaiping and another had heterozygous 3·7 kb deletional alpha$^+$-thalassaemia. All patients presented with moderate-to-severe microcytic anaemia with increased nucleated red blood cells. Red blood cell morphology of all patients was noted with anisocytosis and poikilocytosis. Peripheral blood smears from patient no. 1 and her parents are shown in Fig 1. Hb analysis from patient no. 1 and patient no. 4 (post-transfusion sample) showed increased Hb F levels.

All eight parents harboured monoallelic *KLF1* mutations. One mother also had a heterozygous *G6PD* Kaiping mutation. Three other parents each harboured a heterozygous beta-thalassaemia (*HBB*:c.52A>T), Hb Pakse (*HBA2*: c.429A>T) and 3·7 kb deletional alpha$^+$-thalassaemia mutation respectively. All were asymptomatic without a previous history of anaemia. All five parents without co-inherited thalassaemia had normal Hb levels and normal to borderline mean corpuscular volumes (MCV) and mean corpuscular Hb (MCH), while the three parents with heterozygous thalassaemia mutations had low MCV and MCH. One parent who was also a beta-thalassaemia carrier had high Hb A_2 5·5% and Hb F 6·6%, while the other seven parents had normal to high Hb A_2 2·9–3·8% and three had high Hb F 2·0–2·2%. A summary of the haematological characteristics of parents with a heterozygous *KLF1* mutation is presented in Table III.

Table IV shows a summary of 15 previously reported cases of biallelic *KLF1* mutations that were identified from literature review.[26–32] Fourteen cases presented with severe haemolytic anaemia in the fetal or neonatal periods. One case with compound heterozygous null mutations presented with severe anaemia and kernicterus.[31] A case with a homozygous R301C mutation presented with mild anaemia and splenomegaly in the adult period.[32]

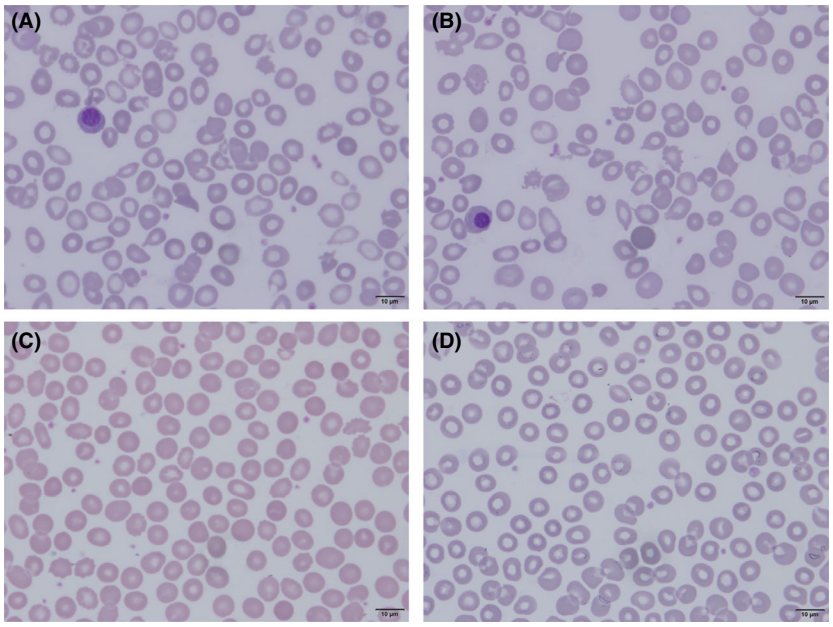

Fig 1. Peripheral blood smear (Wright-stained) from family 1. (A, B) Patient's blood smear at six months of age showed hypochromic, microcytic and anisopoikilocytotic red blood cells. There were elliptocytes, teardrop cells, acanthocytes and schistocytes. Nucleated red blood cells were noted. (C) Maternal blood smear showed mild microcytosis, a few elliptocytes, microspherocytes and acanthocytes. (D) Paternal blood smear showed normochromic and normocytic red blood cells. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

We report the *KLF1* genotypes, clinical and haematologic characteristics of four paediatric cases of congenital haemolytic anaemia due to compound heterozygous *KLF1* mutations and of their parents. Four different *KLF1* mutations were detected. All have been reported previously. All patients harboured a compound heterozygosity of class 2/class 3 *KLF1* mutations. The p.G176RfsX179, a class 3 mutation, is caused by an insertion of seven base pairs in the N-terminal domain. The mutation results in a disruption of the protein before the zinc finger DNA-binding domain.[3] The other three mutations are class 2 mutations that are located in the zinc finger 1 and zinc finger 2 regions. These mutations alter an amino acid in the zinc fingers and result in a disruption of expression of the associated *KLF1* transcript.[3] The combination of the mutations could explain the severe and early-onset haemolytic anaemia in the patients.

All patients with compound heterozygous *KLF1* mutations presented within the first day of life with moderate-to-severe haemolytic anaemia and hyperbilirubinaemia. All patients were treated with phototherapy and two required red blood cell transfusions during the neonatal period. Three patients later became transfusion-dependent. Of note, there was a clinical discrepancy between two patients with the same p.G176RfsX179/A298P mutations. The patient who had a co-inherited heterozygous alpha^{+}-thalassaemia had a more severe disease. He required red blood cell transfusion during the newborn period and later became transfusion-dependent, while the other patient without a co-inherited thalassaemia required the first transfusion in infancy and was non-transfusion-dependent. Red blood cell parameters of the patients showed common characteristics of low MCV and MCH, increased red cell distribution width (RDW), and increased nucleated red blood cells/reticulocytes. The markedly increased nucleated red blood cells reflect the important role of KLF1 in the regulation of cell cycle exit and enucleation.[39] Red blood cell morphology in all patients was noted with anisocytosis and poikilocytosis. In two cases with available Hb analysis results, high Hb F levels were observed.

All patients in this report had compound heterozygous class 2/class 3 *KLF1* mutations. The clinical and haematological characteristics of our patients were comparable to those with similar genotypes previously reported. Most reported cases presented with fetal or neonatal severe haemolytic anaemia and later became transfusion-dependent.[26–31] A case of homozygous null *KLF1* mutations (class 3) presented with the more severe disease of hydrops fetalis characteristics at birth and later became transfusion-dependent.[31] On the other hand, a case with homozygous R301C mutation, which is a class 2 mutation, presents in adulthood with mild anaemia and splenomegaly.[32]

Apart from the findings of severe haemolytic anaemia and abnormal red cell morphology, pathologically reduced pyruvate kinase (PK) levels are seen in patients with compound heterozygous class 2/class 3 *KLF1* mutations.[26] The low PK levels in *KLF1* mutations may result in a misdiagnosis of congenital PK deficiency.[26] The In(Lu) phenotype is observed in both patients and parents who carry *KLF1* mutations as shown in Table IV.[26,28,29,31] A limitation to our study is that

Table III. Hematological and molecular characteristics of parents with monoallelic mutation of *KLF1* genes.

Family number	Age (years)	RBC parameters							Haemoglobin analysis			*KLF1* mutation	Other mutations
		Hb (g/l)	Hct (%)	RBC ($\times 10^6$/mm^3)	MCV (fl)	MCH (pg)	MCHC (g/l)	RDW (%)	RC (%)	Hb A (%)	Hb A_2 (%)	Hb F (%)	
1 Mother	32	129	39·3	4·9	79·9	26·2	328	13·7	1·63	83·9	3·0	2·2	A298P	None
1 Father	33	140	42·6	4·9	87·1	28·6	329	12·4	ND	85·5	3·6	0·3	G176RfsX179	None
2 Mother	30	130	41·2	4·9	83·6	26·4	316	13·5	0·72	84·1	3·4	2·0	G176RfsX179	*G6PD* Kaiping (het)
2 Father	34	123	38·4	5·8	66·0	21·1	320	18·2	0·88	77·7	5·5	6·6	R301H	*HBB*:c.52A>T (het)
3 Mother	24	124	40·0	5·6	71·5	22·5	308	17·1	ND	96·2	2·9	0·5	G176RfsX179	*HBA2*: c.429A>T (het)
3 Father	21	133	39·6	4·8	81·8	27·5	336	13·3	ND	96·9	3·1	0·0	G335R	None
4 Mother	26	119	39·5	5·5	72·2	21·8	301	16·8	0·60	87·6	3·1	0·2	A298P	*HBA* −3·7 kb del (het)
4 Father	27	145	44·8	5·2	87·0	28·2	324	13·6	1·2	84·1	3·8	2·0	G176RfsX179	None

Hb, haemoglobin; Hct, haematocrit; het, heterozygous; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin level; MCV, mean corpuscular volume; nRBC, nucleated red blood cell; RBC, red blood cell; RC, reticulocyte count; RDW, red cell distribution width; WBC, white blood cell.

other associated findings of a decreased PK enzyme activity and In(Lu) blood group were not tested for in our study due to unavailability of the tests.

The *KLF1* G335R mutation detected in family 3 in this study (G176RfxX179/G335R) and one family (R331W/G335R) in the study by Viprakasit *et al.*, is similar in position to a murine H350R mutation described recently by Sorolla *et al.*[26,40] The murine *KLF1* H350R was an *N*-ethyl-*N*-nitrosourea (ENU)-generated mouse mutation detected in a screen for modifiers of expression of an alpha-globin-GFP transgene. The murine H350R mutation locates in the conserved linker peptide between zinc fingers 2 and 3 of KLF1. The mice with homozygous H350R mutation were noted with mild compensated anaemia and mild red blood cell microcytosis although they had normal KLF1 protein level and normal embryonic development. The alpha- and beta-globin gene expression was also normal. The findings were different to those in mice with a homozygous non-sense *KLF1* mutation that led to absent KLF1 protein and embryonic lethality. The authors suggest that the conserved linker peptide plays a role in KLF1 function. Both patients who harboured the G335R mutation along with another *KLF1* mutation presented with an early-onset severe haemolytic anaemia while the heterozygous parents had normal red blood cell parameters. The clinical characterization of the two patients supports that the G335R mutation is a hypomorphic allele and also supports the important role of the linker peptide in KLF1 function.

All parents with the heterozygous *KLF1* mutation were asymptomatic. Parents without co-inherited thalassaemia had normal Hb levels and normal to borderline MCV and MCH. Three parents with co-inherited heterozygous thalassaemia had normal to low Hb level, low MCV, MCH and increased RDW. The parent with co-inherited beta-thalassaemia had high Hb A_2 and Hb F, while the other seven parents had normal to high Hb A_2 and three had high Hb F. Therefore, we conclude that the changes in haematological parameters in individuals with heterozygous *KLF1* mutation are subtle or mild, and *KLF1* mutation likely contributes to anaemia in thalassaemia carriers and high Hb F in beta-thalassaemia carriers. The findings of subtle or mild changes in MCV, Hb A_2 and Hb F levels in carriers without thalassaemia are consistent with the previously reported cases as summarized in Table IV.[26,28–31]

Our findings support that compound heterozygous class 2/class 3 mutations in *KLF1* are associated with severe neonatal haemolytic anaemia. Based on our findings and previous reports, *KLF1*-associated haemolytic anaemia should be in the differential diagnosis of cases with moderate-to-severe haemolytic anaemia in the fetal or neonatal period. The clues to diagnosis are the early onset of severe haemolytic anaemia, abnormal red cell morphology and markedly increased nucleated red blood cells. The findings of low MCV, high Hb A_2 and Hb F in the parents who are carriers are not consistent. Therefore, upfront molecular characterization of *KLF1* is

Table IV. Summary of previously reported cases of compound heterozygous *KLF1* mutations.

References	Number of patients	Age	*KLF1* genotypes	Clinical presentation and haematological characteristics
Viprakasit *et al.*[26]	8	2 months–22 years	R331W/G335R (1) G176RfsX179/R301H (1) -154C/T/A298P (1) Q58X/A298P (1) G176RfsX179/A298P (4)	All presented with neonatal hyperbilirubinaemia on day 1–2 of life. Five patients received red blood cell transfusion during neonatal period. Two patients with G176RfsX179/A298P mutations had a later onset of anaemia at 2 and 9 months. All patients except one with G176RfsX179/A298P mutations later became transfusion-dependent. Three underwent splenectomy with good to fair outcome. All had borderline to low MCV and increased nucleated red blood cells. All had elevated Hb F level of 5·5–54·6%, reduced levels of pyruvate kinase enzyme activity and In(Lu) blood group. Hb Portland was present in all patients. All parents were asymptomatic. Parents without co-inherited thalassaemia had normal to borderline MCV. Parents with heterozygous Hb E had elevated Hb F level of 1·8–4·7%. Others had normal or elevated Hb A_2 level of 2·0–3·8% and Hb F level of 0–3·6%. All except one had In(Lu) blood group. One had a weakly positive b antigen of the Lutheran blood group.
Huang *et al.*[28]	2	4 and 12 years	G176RfsX179/A298P G176RfsX179/P338S	Both presented with early onset of haemolytic anaemia that was suspected of thalassaemia intermedia. The patient with G176RfsX179/P338S mutations was transfusion-dependent and the patient with G176RfsX179/A298P mutations required occasional transfusion. Both had low MCV, elevated Hb F level of 26·3% and 33·2% and In (Lu) blood group. The parents had normal Hb level and MCV, normal or elevated Hb A_2 level of 2·9–3·5% and Hb F level of 1·8–1·9%, and In(Lu) blood group.
Magor *et al.*[31]	1	1 year	R319Efs34X/W30X	The patient was born at 38 weeks of gestational age with clinical characteristics of hydrops fetalis. He had severe hyperbilirubinaemia requiring exchange transfusion, and later became transfusion-dependent. Anaemia and increased nucleated red blood cells were noted at birth. Hb analysis at 6 h of age showed 100% Hb F and <0·1% Hb A_2. The patient had In(Lu) blood group. The parents had normal Hb level and MCV, normal Hb A_2 level of 3·0% and 3·3% and Hb F level of 2·4%, 3·5% and In(Lu) blood group.
Lee *et al.*[30]	1	10 months	G176RfsX179/P338T	The patient presented with cardiomegaly detected by prenatal ultrasound at gestational age of 21 weeks. Fetal anaemia and hydrops were detected at a gestational age of 27 weeks. Fetal red blood cell morphology showed marked anisocytosis, moderate macrocytosis, marked polychromasia and increased nucleated red blood cells. Hb analysis at 27 weeks comprised Hb F, Hb Bart's and Hb Portland. She received three intrauterine transfusion. After birth, she was transfusion-dependent. The parents had normal Hb level and MCV, normal Hb A_2 level of 3·2% and Hb F level of 1·6%, 2·6%.
Rani *et al.*[32]	1	56 years	R301C/R301C	The patient had mild anaemia (Hb 121 g/l), mild unconjugated hyperbilirubinaemia and splenomegaly detected in adulthood. Red blood cells were normochromic and normocytic with occasional nucleated red blood cells. He had elevated Hb F level of 72·3%. He never received transfusion.

Table IV. (*Continued*)

References	Number of patients	Age	*KLF1* genotypes	Clinical presentation and haematological characteristics
Jiang *et al.*[29]	1	1 year	G176RfsX179/P338T	The patient presented with haemolytic anaemia requiring red blood cell transfusion on day 1 of life. He became transfusion-dependent. Hb analysis at one year of age showed elevated Hb F of 24·5%. The patient had In(Lu) blood group. The father had normal Hb level and MCV. The mother had heterozygous alpha^{0}-thalassaemia. Both parents had normal Hb A_2 level of 2·5% and 3·2% and Hb F level of 1·0%, 2·4%, and In(Lu) blood group.
Belgemen-Ozer and Gorukmez[27]	1	7 months	H295LfsX58/R301L	The patient presented with anaemia since birth requiring occasional red blood cell transfusion. He became transfusion-dependent. Hb analysis at seven months of age showed elevated Hb F of 66·6%. Red blood cells were hypochromic, anisopoikilocytotic with other abnormal red blood cells, polychromasia and nucleated red blood cells.

Hb, haemoglobin; MCV, mean corpuscular volume.

suggested in suspected cases regardless of the absence of these findings in the parents.

Acknowledgements

The authors thank the laboratory staff at the Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, for their assistance.

Funding information

The work was supported by the Faculty of Medicine Research Fund (Grant number 103/2563) from the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, the Health Systems Research Institute, Thailand Science Research and Innovation Fund (CU_FRB640001_01_30_10) and Thailand Research Fund (DPG6180001).

Author contributions

TT and PC conceived and designed the study, interpreted the results, wrote the paper and gave critical comments. AP and KF performed the molecular laboratory assay. TT, RR, MJ and PC collected the data. VV and VS wrote the paper and gave critical comments. CI, WC and CP performed the whole-exome sequencing and data analysis. All authors were involved in the final revision of the article, contributed to the final analysis of the data and gave their final approval for publication.

Conflicts of interest

All authors declare no conflict of interest.

References

1. Miller IJ, Bieker JJ. A novel, erythroid cell-specific murine transcription factor that binds to the CACCC element and is related to the Krüppel family of nuclear proteins. *Mol Cell Biol*. 1993;**13**:2776–86.
2. Siatecka M, Lohmann F, Bao S, Bieker JJ. EKLF directly activates the p21WAF1/CIP1 gene by proximal promoter and novel intronic regulatory regions during erythroid differentiation. *Mol Cell Biol*. 2010;**30**:2811–22.
3. Perkins A, Xu X, Higgs DR, Patrinos GP, Arnaud L, Bieker JJ, et al. Kruppeling erythropoiesis: an unexpected broad spectrum of human red blood cell disorders due to KLF1 variants. *Blood*. 2016;**127**:1856–62.
4. Siatecka M, Bieker JJ. The multifunctional role of EKLF/KLF1 during erythropoiesis. *Blood*. 2011;**118**:2044–54.
5. van Ree JH, Roskrow MA, Becher AM, McNall R, Valentine VA, Jane SM, et al. The human erythroid-specific transcription factor EKLF localizes to chromosome 19p13.12-p13.13. *Genomics*. 1997;**39**:393–5.
6. Borg J, Patrinos GP, Felice AE, Philipsen S. Erythroid phenotypes associated with KLF1 mutations. *Haematologica*. 2011;**96**:635–8.
7. Hariharan P, Colah R, Ghosh K, Nadkarni A. Differential role of Kruppel like factor 1 (KLF1) gene in red blood cell disorders. *Genomics*. 2019;**111**:1771–6.
8. Jaffray JA, Mitchell WB, Gnanapragasam MN, Seshan SV, Guo X, Westhoff CM, et al. Erythroid transcription factor EKLF/KLF1 mutation causing congenital dyserythropoietic anemia type IV in a patient of Taiwanese

origin: review of all reported cases and development of a clinical diagnostic paradigm. *Blood Cells Mol Dis*. 2013;**51**:71–5.
9. Ravindranath Y, Johnson RM, Goyette G, Buck S, Gadgeel M, Gallagher PG. KLF1 E325K-associated congenital dyserythropoietic anemia type IV: insights into the variable clinical severity. *J Pediatr Hematol Oncol*. 2018;**40**:e405–9.
10. Satta S, Perseu L, Maccioni L, Giagu N, Galanello R. Delayed fetal hemoglobin switching in subjects with KLF1 gene mutation. *Blood Cells Mol Dis*. 2012;**48**:22–4.
11. Zhou D, Liu K, Sun CW, Pawlik KM, Townes TM. KLF1 regulates BCL11A expression and gamma- to beta-globin gene switching. *Nat Genet*. 2010;**42**:742–4.
12. Borg J, Papadopoulos P, Georgitsi M, Gutiérrez L, Grech G, Fanis P, et al. Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. *Nat Genet*. 2010;**42**:801–5.
13. Gallienne AE, Dreau HM, Schuh A, Old JM, Henderson S. Ten novel mutations in the erythroid transcription factor KLF1 gene associated with increased fetal hemoglobin levels in adults. *Haematologica*. 2012;**97**:340–3.
14. Radmilovic M, Zukic B, Petrovic MS, Bartsakoulia M, Stankovic B, Kotur N, et al. Functional analysis of a novel KLF1 gene promoter variation associated with hereditary persistence of fetal hemoglobin. *Ann Hematol*. 2013;**92**:53–8.
15. Wang T, He Y, Zhou JY, Xie XM, Li J, Li R, et al. KLF1 gene mutations in Chinese adults with increased fetal hemoglobin. *Hemoglobin*. 2013;**37**:501–6.
16. Lou JW, Li DZ, Zhang Y, He Y, Sun MN, Ye WL, et al. Delineation of the molecular basis of borderline hemoglobin A2 in Chinese individuals. *Blood Cells Mol Dis*. 2014;**53**:261–4.
17. Paglietti ME, Satta S, Sollaino MC, Barella S, Ventrella A, Desogus MF, et al. The problem of borderline hemoglobin A2 levels in the screening for beta-thalassemia carriers in Sardinia. *Acta Haematol*. 2016;**135**:193–9.
18. Perseu L, Satta S, Moi P, Demartis FR, Manunza L, Sollaino MC, et al. KLF1 gene mutations cause borderline HbA(2). *Blood*. 2011;**118**:4454–8.
19. Fanis P, Kousiappa I, Phylactides M, Kyrri A, Hadjigavriel M, Christou S, et al. A novel mutation in the erythroid transcription factor KLF1 is likely responsible for ameliorating beta-thalassemia major. *Hum Mutat*. 2019;**40**:1768–80.
20. Liu D, Zhang X, Yu L, Cai R, Ma X, Zheng C, et al. KLF1 mutations are relatively more common in a thalassemia endemic region and ameliorate the severity of beta-thalassemia. *Blood*. 2014;**124**:803–11.
21. Xie XM, Liu YN, Li J, Jiang F, Li DZ. A Kruppel-like factor 1 gene mutation ameliorates the severity of beta-thalassemia: a case report. *Hemoglobin*. 2019;**43**:137–9.
22. Singleton BK, Burton NM, Green C, Brady RL, Anstee DJ. Mutations in EKLF/KLF1 form the molecular basis of the rare blood group In(Lu) phenotype. *Blood*. 2008;**112**:2081–8.
23. Chen S, Hong X, Xu X, He J, Zhu F. Two novel mutations in KLF1 were identified in Chinese individuals with In(Lu) phenotype. *Transfusion*. 2018;**58**:271–2.
24. Kawai M, Obara K, Onodera T, Enomoto T, Ogasawara K, Tsuneyama H, et al. Mutations of the KLF1 gene detected in Japanese with the In(Lu) phenotype. *Transfusion*. 2017;**57**:1072–7.
25. Keller J, Vege S, Horn T, Keller MA, Leger RM, Aeschlimann J, et al. Novel mutations in KLF1 encoding the In(Lu) phenotype reflect a diversity of clinical presentations. *Transfusion*. 2018;**58**:196–9.
26. Viprakasit V, Ekwattanakit S, Riolueang S, Chalaow N, Fisher C, Lower K, et al. Mutations in Kruppel-like factor 1 cause transfusion-dependent hemolytic anemia and persistence of embryonic globin gene expression. *Blood*. 2014;**123**:1586–95.
27. Belgemen-Ozer T, Gorukmez O. A very rare congenital dyserythropoietic anemia variant-type IV in a patient with a novel mutation in the KLF1 gene: a case report and review of the literature. *J Pediatr Hematol Oncol*. 2020;**42**:e536–40.
28. Huang J, Zhang X, Liu D, Wei X, Shang X, Xiong F, et al. Compound heterozygosity for KLF1 mutations is associated with microcytic hypochromic anemia and increased fetal hemoglobin. *Eur J Hum Genet*. 2015;**23**:1341–8.
29. Jiang H, Jiang F, Li J, Tang F, Li DZ. Congenital nonspherocytic hemolytic anemia caused by kruppel-like factor 1 gene variants: another case report. *Hemoglobin*. 2019;**43**:292–5.
30. Lee HH, Mak AS, Kou KO, Poon CF, Wong WS, Chiu KH, et al. An Unusual Hydrops Fetalis Associated with Compound Heterozygosity for Kruppel-like Factor 1 mutations. *Hemoglobin*. 2016;**40**:431–4.
31. Magor GW, Tallack MR, Gillinder KR, Bell CC, McCallum N, Williams B, et al. KLF1-null neonates display hydrops fetalis and a deranged erythroid transcriptome. *Blood*. 2015;**125**:2405–17.
32. Rani N, Jamwal M, Kaur J, Sharma P, Malhotra P, Maitra A, et al. Homozygous KLF1 mutation c.901C>T (p.Arg301Cys) resulting in mild thalassemia intermedia in an Indian: A next-generation sequencing diagnosis. *Blood Cells Mol Dis*. 2018;**72**:19–21.
33. Ittiwut C, Natesirinilkul R, Tongprasert F, Sathitsamitphong L, Choed-Amphai C, Fanhchaksai K, et al. Novel mutations in SPTA1 and SPTB identified by whole exome sequencing in eight Thai families with hereditary pyropoikilocytosis presenting with severe fetal and neonatal anaemia. *Br J Haematol*. 2019;**185**:578–82.
34. Khamphikham P, Sripichai O, Munkongdee T, Fucharoen S, Tongsima S, Smith DR. Genetic variation of Kruppel-like factor 1 (KLF1) and fetal hemoglobin (HbF) levels in beta(0)-thalassemia/HbE disease. *Int J Hematol*. 2018;**107**:297–310.
35. Tan AS, Quah TC, Low PS, Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for alpha-thalassemia. *Blood*. 2001;**98**:250–1.
36. Mankhemthong K, Phusua A, Suanta S, Srisittipoj P, Charoenkwan P, Sanguansermsri T. Molecular characteristics of thalassemia and hemoglobin variants in prenatal diagnosis program in northern Thailand. *Int J Hematol*. 2019;**110**:474–81.
37. Seeratanachot T, Sanguansermsri T, Shimbhu D. Detection of Hb H disease genotypes common in northern Thailand by quantitative real-time polymerase chain reaction and high resolution melting analyses. *Hemoglobin*. 2013;**37**:574–83.
38. Charoenkwan P, Sirichotiyakul S, Phusua A, Suanta S, Fanhchaksai K, Sae-Tung R, et al. High-resolution melting analysis for prenatal diagnosis of beta-thalassemia in northern Thailand. *Int J Hematol*. 2017;**106**:757–64.
39. Gnanapragasam MN, McGrath KE, Catherman S, Xue L, Palis J, Bieker JJ. EKLF/KLF1-regulated cell cycle exit is essential for erythroblast enucleation. *Blood*. 2016;**128**:1631–41.
40. Sorolla A, Tallack MR, Oey H, Harten SK, Daxinger LC, Magor GW, et al. Identification of novel hypomorphic and null mutations in Klf1 derived from a genetic screen for modifiers of alpha-globin transgene variegation. *Genomics*. 2015;**105**:116–22.