Compound Heterozygosity for KLF1 Mutations Causing Hemolytic Anemia in Children: A Case Report and Literature Review

Linlin Xu1†, Dina Zhu1†, Yanxia Zhang1, Guanxia Liang1, Min Liang1, Xiaofeng Wei1, Xiaqing Feng2, Xuedong Wu2 and Xuan Shang1*

1 Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China, 2 Department of Pediatrics, Nanfang Hospital, Southern Medical University, Guangzhou, China

Background: Anemia is one of the most common diseases affecting children worldwide. Hereditary forms of anemia due to gene mutations are difficult to diagnose because they only rely on clinical manifestations. In regions with high prevalence of thalassemia such as southern China, pediatric patients with a hereditary hemolytic anemia (HHA) phenotype are often diagnosed with β-thalassemia. However, HHA can be caused by other gene defects. Here, a case previously diagnosed with thalassemia in a local hospital was sent to our laboratory for further genetic diagnosis. Preliminary molecular testing did not identify any mutations in globin genes.

Methods: All blood samples were collected after informed consent had been obtained from the proband’s parents. Both clinical and genetic analyses were conducted for the patient and her family members, including clinical data collection and sequencing of the KLF1 gene. Relevant literature was reviewed, including genetically confirmed cases with well-documented clinical summaries.

Results: Based on the detailed clinical data for this case, we diagnosed the patient with severe HHA. Sanger sequencing confirmed that there was a mutation on each KLF1 allele in the proband, which is missense mutation c.892G > C (p.Ala298Pro) inherited from father and frameshift mutation c.525_526insCGGCGCC (p.Gly176Argfs*179) from the mother, respectively. A summary of the KLF1 mutation spectrum and a clarification of genotype-phenotype correlation were performed through a combined analysis of the case and literature studies.

Conclusion: This study corrected the misdiagnosis and identified the etiology in a Chinese patient with HHA. Identification of the disease-causing gene is important for the treatment and care of the patient and prevention of another affected childbirth in her family. In addition, this study provided insight to better distinguish HHA patients with β-thalassemia mutations from those with KLF1 mutations.

Keywords: KLF1, compound heterozygote, hereditary hemolytic anemia, children, chronic non-spherocytic hemolytic anemia, β-thalassemia
INTRODUCTION

Anemia is the most common blood disease worldwide (Kassebaum et al., 2014), which is defined by a hemoglobin (Hb) level below the normal value, leading to decreased oxygen-carrying capacity. Severe anemia impairs growth, cardiac function, and cognitive development in infants. As such, anemia is a leading cause of morbidity and mortality in children in tropical countries (Reyburn, 2010; Khan, 2018).

Anemia is often multifactorial, including genetic and environment factors. Although nutritional-deficiency anemia, due to iron deficiency, is typically assumed to be the most frequent cause in children (Allali et al., 2017), hereditary forms of anemia caused by mutations are not uncommon in the clinic. Moreover, patients with hereditary anemia are often concerned by pediatricians because they are difficult to diagnose and cannot be cured completely with conventional treatment. For such patients, especially those who depend on regular transfusions, a better understanding of etiology and more accurate diagnostic methods are crucial for subsequent treatment and care.

Hereditary hemolytic anemia (HHA) is a group of diseases that result from the destruction of red blood cells (RBCs), and hemolytic anemia shows varied clinical and molecular heterogeneity (Iolascon et al., 2009; Kim et al., 2017). The typical clinical presentation includes pallor, anemia, jaundice, and often enlargement of the liver and spleen. Laboratory results that help confirm HHA include elevated reticulocyte count, increased lactate dehydrogenase, elevated unconjugated bilirubin, and decreased haptoglobin. In southern China, most pediatric HHA cases are caused by β-thalassemia, which is highly prevalent in this region (Shang et al., 2017). β-thalassemia is caused by mutations in the β-globin gene, which results in impaired Hb synthesis (Shang and Xu, 2017). Hematological phenotype analysis, combined with molecular testing for the β-globin gene, provides an avenue for accurate diagnosis. However, HHA can be caused by mutations in other genes. For example, a case of HHA in a Chinese patient was originally misdiagnosed as thalassemia and ultimately diagnosed as hereditary spherocytosis because of mutations in the ankyrin 1 (ANK1) gene (Zhu et al., 2020). More notably, compound heterozygous mutations in the Kruppel-like factor 1 (KLF1) gene, an important erythroid transcriptional factor involved in erythrocyte development, can lead to chronic non-spherocytic hemolytic anemia (CNSHA). The phenotypic traits of CNSHA are very similar to those of β-thalassemia (Viprakasit et al., 2014; Huang et al., 2015; Perkins et al., 2016). Because KLF1 mutations are found significantly more frequently in thalassemia endemic regions such as southern China (Liu et al., 2014), it is important for physicians in these regions to distinguish between HHA caused by β-thalassemia from HHA caused by KLF1 mutations.

Here, we report the case of a Chinese patient with transfusion-dependent hemolytic anemia. The patient was originally misdiagnosed with β-thalassemia by local physicians and was finally diagnosed with CNSHA caused by compound KLF1 mutations. We also present a summary of relevant literature on the mutation spectrum of the KLF1 gene. Moreover, to help clinicians better recognize HHA caused by KLF1 compound heterozygotes, we compared hematological parameters in patients with β-thalassemia and KLF1 mutations.

MATERIALS AND METHODS

Subject

The proband was a 3-year-old girl from Yunfu City in Guangdong Province, southern China. She and her parents were experiencing problems with clinical diagnosis and were referred to a free clinic held by a hospital for “World Thalassemia Day—May 8.” The patient had previously experienced hyperbilirubinemia and severe anemia, and she had received eight blood transfusions before age 3 years. A local hospital initially assumed that she had β-thalassemia because she had microcytic hypochromic anemia and high HbF levels (>5%). Furthermore, she was a native of Guangdong province, which is a region with high prevalence of thalassemia. However, following molecular testing for her and her parents, no known thalassemia mutation was detected. Therefore, the patient was referred to our laboratory for further investigation. Blood samples were collected from the patient and her parents after obtaining informed consent. The pedigree of this family is shown in Figure 1A).

Phenotype Analysis

Fresh peripheral blood samples were collected. The patient had not received a blood transfusion in the 2 months prior to sample collection. The hematological data were analyzed using an automated blood cell counter (Sysmex KX-21; Sysmex Corp., Kobe, Japan). The levels of HbA, HbA2, and HbF were analyzed using the Bio-Rad Variant II HPLC system (HPLC, VARIANTTM, Bio-Rad Laboratories, Hercules, CA, United States). Red cell osmotic fragility test, glucose-6-phosphate dehydrogenase (G6PD) activity, and other immune hemolysis test were performed according to standard operation procedure. Routine biochemical and hemolytic parameters were measured using standard techniques.

Molecular Analysis

Sanger sequencing was used to detect point mutations in the α-globin, β-globin, and KLF1 genes, as previously described (Zhu et al., 2019). Polymerase chain reaction (PCR) fragment were sequenced with BigDye™ Terminators V3.1 Ready Reaction Kit using 3130 Genetic Analyzer (Applied Biosystems). Reference sequences were downloaded from NCBI database1 and used for sequence alignment and analysis. The multiplex ligation-dependent probe amplification assay (MLPA; SALSA P140-C1 HBA or P140-B2 HBB; MRC-Holland, Amsterdam, the Netherlands) was performed to exclude large rearrangements of the α-/β-globin gene clusters.

Literature Review

An electronic literature search of the PubMed database was performed for current and past findings of the molecular genetics

1https://www.ncbi.nlm.nih.gov/gene
of KLF1 from the years 1990 to 2020. The following keywords were used to search: “Kruppel-like factor 1,” “KLF1,” “EKLF,” “hemolytic anemia,” “mutation,” and “variant.” All related publications were reviewed to identify other relevant articles that were not identified in the initial research. The publications were limited to English-language articles. Cases with detailed clinical presentation and KLF1 genotype information were selected.

Statistical Analysis
Statistical differences between groups were determined by one-way analysis of variance, and further comparison across multiple groups was tested by Tukey method. Mean and standard error of the mean were used to describe clinical data in each group. Statistical significance was determined by a P-value of less than 0.05. All statistical analyses were performed using the statistical software GraphPad Prism 6.

RESULTS
Hematological Evaluation and Molecular Analysis of the Patient
Hematological data for this family are summarized in Figure 1A. The father displayed a normal phenotype, except for borderline mean corpuscular Hb (MCH) and HbF level. The mother displayed microcytic and hypochromic features, as well as a slightly elevated HbF level. Patient III1 presented with severe microcytic hypochromic anemia, borderline HbA2 (3.6%) and significantly elevated HbF (16.5%); she has required regular transfusions for survival. This patient was born at full term (38 weeks + 2 days’ gestation) with a birth weight of 2.740 kg. The day after birth, she developed severe jaundice and neonatal hyperbilirubinemia (total and indirect bilirubin were 133.4 and 109.9 μmol/L, respectively), for which she received phototherapy and was referred to a superior hospital. She was followed up as a high-risk baby, at which point she was found to be anemic with hepatosplenomegaly (4 and 3 cm, respectively); she received her first blood transfusion within 1 month after birth. Peripheral blood smear analysis showed increased poikilocytosis and anisocytosis, with hypochromic microcytosis, similar to what is observed with thalassemia. The patient has required regular blood transfusion every 3 months since 4 months of age to keep Hb > 90 g/L, although the interval of blood transfusions was reduced during times when the patient experienced fever or infection. Routine evaluations excluded the possibility of immune hemolysis and RBC membrane disorder. At a local hospital, she was initially diagnosed with β-thalassemia by age 1 year based on her abnormal Hb profiles and the hematological features of microcytic hypochromic anemia. The routine test for common β-thalassemia mutations in Chinese patients by reverse dot blot and gap-PCR methods showed a negative result. Therefore, the patient was thought to have hereditary anemia due to an unidentified thalassemia allele (possible de novo mutation), and she was referred to our laboratory for further study.
### TABLE 1 | Current, in-depth hematological parameters of subject II1.

| White blood cell parameters | Relative values (%) | Reference values (%) | Absolute values (10^6/µL) | Ref. values (10^6/µL) |
|-----------------------------|---------------------|----------------------|---------------------------|----------------------|
| WBC                         |                     |                      |                           |                      |
| NEUa                        | 59.9                | 50.0–70.0            | 6.14                      | 2.00–7.00            |
| LYM                         | 31                  | 20.0–40.0            | 3.17                      | 1.80–4.00           |
| MON                         | 5.9                 | 3.0–12.0             | 0.6                       | 0.12–1.20           |
| EOS                         | 2.7                 | 0.5–5.0              | 0.28                      | 0.02–0.50           |
| BAS                         | 0.5                 | 0.0–1.0              | 0.05                      | 0.00–0.10           |

**Platelet parameters** Values Reference values

| PLT (x 10^9/L) | 192 | 125–350 |
| PCT (%)        | 0.14 | 0.05–0.33 |

| Red blood cell parameters | Values | Reference values |
|---------------------------|--------|------------------|
| RBC (10^12/L)             | 3.13 ↓ | 4.0–5.5          |
| Hb (g/L)                  | 67 ↓   | 110–140          |
| HCT (%)                   | 21.7 ↓ | 35.0–45.0        |
| MCV (fl)                  | 69.3 ↓ | 82.0–100.0       |
| MCH (pg)                  | 21.4 ↓ | 27.0–34.0        |
| MCHC (g/L)                | 309 ↓  | 316–354          |
| RDW-CV (%)                | 26.5 ↑ | 11.5–14.5        |

**Reticulocyte parameters** Relative values (%) Reference values Absolute values (10^6/µL) Ref. values (10^6/µL)

| RET | 16.1 ↑ | 0.5–2.1 | 566.8 ↑ | 40.0–79.0 |

**Hemolysis test** Values Reference values

- Isopropanol test: – –
- Heinz-bodyforming test: – –
- Red cell osmotic fragility test: – –
- G6PD activity test: + +
- Ham test: – –
- Sucrose hemolysis test: – –
- Heat hemolysis test: – –
- Direct Coombs test: – –
- Indirect Coombs test: – –

**Biochemical tests** Values Reference values

| Biochemical tests | Values | Reference values |
|-------------------|--------|------------------|
| Uric Acid (UA; umol/L) | 224 | 120–420          |
| Creatinine (Cr; umol/L)    | 46    | 40–88            |
| Total Protein (TP; g/L)    | 56.4  ↓ | 60–80           |
| Albumins (ALB; g/L)       | 42.3  ↓ | 35–55           |
| Globulins (GLB; g/L)      | 14.1  ↓ | 20–30           |
| Total bilirubin (TBIL; umol/L) | 28.4 ↑ | 5.1–19          |
| Direct bilirubin (DBIL; umol/L) | 6.4 | 1.7–6.8          |
| Indirect bilirubin (IBIL; umol/L) | 22.0 ↑ | 0.0–17.3 |

(Continued)

We have provided an in-depth review of the patient’s hematological parameters in (Table 1). Complete blood cell analysis showed white blood cell, and platelet values were within normal levels. However, RBC parameters suggested moderate anemia with hypochromic microcytosis. Elevated reticulocyte count and red cell osmotic fragility indicated the existence of hemolysis. Immune hemolysis and erythrocyte enzymatic disease (G6PD deficiency) were excluded by related hemolysis tests. Biochemical tests suggested mild damage to liver function with increased levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Elevated bilirubin and decreased globulins further confirmed hemolysis and jaundice. Iron deficiency and megaloblastic anemia were excluded by the normal values of ferritin, folic acid, and vitamin B_{12}, respectively. Therefore, the patient was ultimately diagnosed with non-spherocytic hemolytic anemia.

Based on hematological data from the parents (Figure 1A), it is likely that the patient inherited a recessive disease. However, mutations in the α-, β-, or γ-globin genes that could cause thalassemic phenotypes similar to those seen in the patient were excluded by direct sequencing of above genes and MLPA analysis of α- or β-globin cluster. This indicated that there might be another gene responsible for the observed hemolytic anemia phenotypes. Considering the high HbF levels in the patient, *KLF1* was chosen to be the first candidate gene (Borg et al., 2010). Sequencing of the *KLF1* gene identified a compound heterozygote of missense variant c.892G > A (*p.Ala298Pro*) and frameshift variant c.525_526insCGGCGCC (p.Gly176Argfs*179) in the patient, which she inherited from her father and mother, respectively (Figure 1B). Both variants have been previously reported as pathogenic mutations that affect the biological function of KLF1 (Viprakasit et al., 2014; Huang et al., 2015).

(Continued)
Literature Review of KLF1 Mutation Spectrum and Genotype–Phenotype Correlation in Individuals With KLF1 Mutations

KLF1 (OMIM# 600599) is located at chromosome 19p13.2 and contains three exons that encode 362 amino acids. There are two major functional domains in the KLF1 protein product: two PRA (proline-rich amino) domains located at the N-terminus function as a transcriptional activator and three zinc finger (ZF) domains located on the C-terminus act as a DNA-binding domain (Quaderni and Bieker, 2002).

After searching the literature for KLF1 mutations, 113 mutations were identified (Supplementary Table 1). These

![Diagram of KLF1 gene structure and variants](image)

**FIGURE 2** The literature review of KLF1 mutations and phenotype. (A) Schematic diagram of the KLF1 gene (NM_006563.5 and NP_006554.1) structure and variants, as reported in the literature. The gene structure includes the promoter (small red box) and two introns and three exons (big box). The proline-rich amino domain (PRA) located at the N-terminus is responsible for transcriptional activation (blue box). The zinc finger (ZF) domains (yellow box) located at C-terminus enable KLF1 to bind specific sites on DNA. Mutations are color-coded: promoter mutations and splicing mutations, black; class 1, green; class 2, blue; class 3, purple; class 4, red. The two mutations described in this article are underlined. See Supplementary Table 1 for details and references. (B) Hb level in reported cases carrying KLF1 mutations. All KLF1 alleles were classified into six types: N, normal allele; P, promoter mutations; 1–4, classes 1–4 mutations. The dotted line shows the anemia boundary line. See Supplementary Table 2 for details and references.
mutations can be divided into three groups according to their position on KLF1 gene. Seven mutations are located on the promoter region, and therefore may affect transcription of the KLF1 gene. Additionally, 104 mutations (72 missense mutations, 13 nonsense mutations, and 19 frameshift mutations) are located on the exon region and cause alterations to the primary structure of the KLF1 protein. Although the ZF domains comprise only 20% of KLF1, almost 40% of the functionally effective mutations are found within and between these domains. Two intronic mutations affect splicing. In addition, the 104 mutations in the coding sequence (CDS) region can be divided into four subclasses, based primarily on differing effects on KLF1 protein function (Figure 2A). Class 1 includes 33 mutations with no effects or minor functional effects, most of which are located outside the ZF domains. Class 2 comprises 40 mutations, most of which are missense mutations in ZF domains that interfere with normal function of KLF1. Class 3 includes 30 nonsense or frameshift mutations, which lead to a truncated KLF1 peptide with normal function of KLF1. Class 4 comprises 40 mutations, most of which are located outside the ZF domains. Two intronic mutations can be divided into three groups according to their effects or minor functional effects, most of which are located outside the ZF domains. Class 3 contains only one missense mutation (p.Glu325Lys) (Arnaud et al., 2010), which is a special dominant variant.

According to published literature, 80 cases with detailed hematological phenotype data and KLF1 genotype were collected (Supplementary Table 2). We have presented the genotype-phenotype relationship in Figure 2B. Most cases carrying monoallelic KLF1 mutations do not display anemia. On the contrary, most cases carrying biallelic mutations do exhibit an anemia phenotype, as their Hb level is often less than 110 g/L.

**DISCUSSION**

HHA includes a diverse group of genetically and phenotypically heterogeneous diseases. The major causes of HHA are defects in Hb, the RBC membrane, and RBC enzymes, which are commonly referred to as thalassemia, hereditary spherocytosis, and G6PD deficiency, respectively. However, these common disorders do not represent all HHA patients, and precise diagnosis of HHA remains a challenge for clinical doctors. As clinical presentation often overlaps in cases with different etiologies, it is difficult to make a reliable diagnosis based only on phenotype analysis. Identification of disease-causing mutations can be challenging.

**TABLE 2** | Literature reports of clinical and laboratory characteristics of compound heterozygotes for KLF1 mutations.

| Case | Age/sex | Ethnic | Hb (g/L) | MCV (fl) | MCH (pg) | HbA2 (%) | HbF (%) | Transfusion dependent | α globin | β globin | KLF1 genotype | Description | References |
|------|---------|--------|----------|----------|----------|----------|---------|----------------------|---------|---------|----------------|-------------|------------|
| 1    | 31y-M   | Sardinia | 115      | 82.0     | 27.6     | 2.8      | 30.9    | No                   | αα/αα   | NA      | Ser270*       | Lys332Gln   | HPFH       |
| 2    | 30y-M   | Sardinia | 121      | 73.0     | 24.3     | 3.3      | 22.1    | No                   | αα/αα   | NA      | Ser270*       | Lys332Gln   | HPFH       |
| 3    | NA-F    | Serbia  | 128      | NA       | 22.6     | 2.5      | 11.0    | No                   | NA      | NAα     | −148G > A   | Ser102Pro   | HPFHα      |
| 4    | 56y-M   | India   | 121      | 91.1     | 30.3     | 0.5      | 72.3    | No                   | αα/αα   | kβ/β    | Arg301Cys    | Arg301Cys   | HPFH       |
| 5    | 12y-M   | China   | 97       | 72.4     | 25.1     | 3.9      | 33.2    | Yes                  | αα/αα   | kβ/β    | Gly176Argfs*179 Pro338Ser | CDA IVα |
| 6    | 5y-F    | Thailand | 83       | 70.9     | 21.6     | 3.0      | 50.0    | Yes                  | αCR/aαa | kβ/β    | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 7    | 4y-MF   | Thailand | 79       | 72.4     | 24.9     | 4.8      | 26.6    | No                   | αα/αα   | kβ/β    | Gly176Argfs*179 Ala298Pro | CDA IVα |
| 8    | 1y-M    | Thailand | 77       | 73.0     | 23.0     | 22.7     | 52.8    | Yes                  | αα/αα   | βF/Eβ     | −154C > T   | CNSHA     |
| 9    | 12y-M   | Thailand | 75       | 69.1     | 20.1     | 4.3      | 39.1    | Yes                  | αα/αα   | βE/β     | Gly176Argfs*179 Ala298Pro | CNSHAα |
| 10   | 1y-M    | China    | 67       | 88.9     | 28.5     | 2.0      | 24.6    | Yes                  | αα/αα   | kβ/β    | Gly176Argfs*179 Pro338Thr | CNSHA     |
| 11   | 5y-F    | China    | 67       | 69.3     | 21.4     | 3.2      | 16.5    | Yes                  | αα/αα   | kβ/β    | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 12   | 5y-M    | Thailand | 66       | 69.5     | 21.4     | 9.5      | 2.6     | Yes                  | αα/αα   | kβ/β     | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 13   | 6mon-M  | Australia | 65       | 82.0     | NA       | < 0.1    | 75.0    | Yes                  | αα/αα   | kβ/β    | Arg219Gufs*34 Trp30* | CNSHA     |
| 14   | 7mon-M  | NAa      | 61       | 82.0     | 28.4     | 1.0      | 66.5    | No                   | αα/αα   | kβ/β    | His295Leufs*58 Arg301Leu | CDA IVβ |
| 15   | 2mon-M  | Thailand | 57       | 67.0     | 21.2     | 2.2      | 54.6    | Yes                  | αα/αα   | kβ/β     | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 16   | 4y-M    | Thailand | 54       | 75.0     | 24.7     | 10.0     | 19.0    | Yes                  | αα/αα   | kβ/β     | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 17   | 18y-M   | Thailand | 34       | 81.0     | 24.8     | 4.6      | 29.0    | Yes                  | αα/αα   | kβ/β     | Gly176Argfs*179 Ala298Pro | CNSHA     |
| 18   | 27y-F   | China    | 30       | 123.9    | 37.6     | NAa      | 64.0    | Yes                  | αα/αα   | kβ/β     | Gly176Argfs*179 Pro338Thr | CDA IVβ |
| 19   | 4y-F    | Thailand | 30       | 80.1     | 28.1     | 3.1      | 16.2    | Yes                  | αα/αα   | kβ/β     | Gly335Arg | Arg331Trp   | CNSHA     |

αNA, not available; HPFH, hereditary persistence of fetal hemoglobin; CDA IV, congenital dyserythropoietic anemia IV; CNSHA, congenital non-spherocytic hemolytic anemia.

*Indicates 27 week fetus during pregnancy.

**M and F indicates male and female, respectively.**
at the DNA level can increase the accuracy of HHA diagnosis. Our study provided a new cause of HHA in pediatric patients, KLF1 compound mutations. The patient discussed here had jaundice, hepatomegaly, and microcytic hypochromic anemia with remarkably elevated HbF levels.

KLF1 is known as a hematopoietic-specific transcription factor that regulates erythroid lineage commitment, globin switching, and the terminal maturation of RBCs (Miller and Bieker, 1993; Wijgerde et al., 1996; Hariharan et al., 2019). It is no wonder that KLF1 mutations can lead to shortened

---

**FIGURE 3** | Differential diagnosis of KLF1 anemia and β-thalassemia intermedia. (A) Summary of clinical characteristic features in Chinese patients with β-thalassemia intermedia (China-TI), Indonesian patients with β-thalassemia intermedia (Indonesia-TI) and anemia caused by compound heterozygotes of KLF1 mutations (KLF1-AM). NA indicates not available; + and −, positive and negative phenotypes described in the columns; ++ or +++ degree of symptom. (B–F) Comparisons of Hb, MCV, MCH, HbF, and HbA₂ levels between China-TI, Indonesia-TI, and KLF1-AM. Data are presented as mean ± standard deviation (SD). Reference range values are shown as a dotted line: MCV, 80 fL; MCH, 27 pg; China-TI, β-thalassemia intermedia patients in China; Indonesia-TI, β-thalassemia intermedia patients in Indonesia; KLF1-AM, cases caused by KLF1 compound mutations. Asterisks represent statistically significant differences: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
survival of RBCs, resulting in inherited hemolytic anemias. Currently, there are reports of 19 compound heterozygotes of KLF1 mutations (including our case) with detailed hematological phenotypes (Table 2). Four cases (Table 2, numbers 1–4) showed normal Hb levels with high HbF and were described as hereditary persistence of fetal Hb (HPFH) (Satta et al., 2011; Radmilovic et al., 2013; Rani et al., 2018), a kind of benign hemoglobinopathy. Fifteen cases (Table 2, numbers 5–19) showed mild to severe anemia (Viprakasit et al., 2014; Huang et al., 2015; Magor et al., 2015; Lee et al., 2016; Jiang et al., 2019; Belgemen-Ozer and Gorukmez, 2020). Although some cases carry pathogenic mutations in globin genes that further affect the hematological phenotype, these cases suggest that compound heterozygotes have a high probability of showing a transfusion-dependent anemia phenotype in childhood.

Southern China is a region with a high incidence of β-thalassemia, and local pediatricians receive considerable training in thalassemia through long-term public health education and clinical practices. However, this has raised another problem in that patients in this region with a hemolytic anemia phenotype and recessive inheritance were more likely to be diagnosed with β-thalassemia. According to a previous publication (Liu et al., 2014), southern China is also a region with a high prevalence of KLF1 mutations. Therefore, patients with KLF1 compound mutations should account for a certain proportion of all hemolytic anemia patients. To avoid misdiagnosis, it is necessary to effectively distinguish between anemia caused by KLF1 mutations and anemia caused by β-thalassemia mutations. According to previous reports (Huang et al., 2015), most patients with HHA caused by compound mutations of KLF1 were from Chinese or southeast Asian populations, and they tended to be diagnosed with β-thalassemia intermedia (β-TI) at the local hospital. Here, 44 TI patients from southern China (Chen et al., 2010) along with 30 TI patients from Indonesia (Rujito et al., 2016) (reported previously) were selected for comparison with the 15 previously referenced cases that were caused by KLF1 compound mutations (KLF1-AM; cases 5–19 in Table 2). A summary of typical clinical features is shown in Figure 3A. Some important symptoms were overlapping; for example, both groups presented with hypochromic microcytic anemia, jaundice, splenomegaly, severe iron overload, and elevated HbF levels. The specific symptoms [zinc protoporphyrin, In(Lu) phenotype, and CD44 deficiency] are not routine examination items in clinical practices. Therefore, it is not surprising that misdiagnosis occurs. Because whole-blood cell analysis and Hb electrophoresis are required items for anemia diagnosis and thalassemia screening, the key RBC parameters and the constitution of Hb were compared in patients with TI or KLF1-AM (Figures 3B–F). Hb values and HbF levels were significantly lower in KLF1-AM patients. Additionally, significant differences in mean corpuscular volume (MCV) and MCH values were evident between Chinese TI patients and KLF1-AM patients, suggesting that these two values may be useful in distinguishing KLF1-AM from β-TI in southern China. Patients with an HHA phenotype but borderline-low MCV and MCH values (rather than markedly decreased MCV and MCH values) are more likely to have KLF1 mutations.

In conclusion, our study corrected the misdiagnosis and identified the true cause of disease in an HHA patient. The mutation spectrum of KLF1 and the genotype–phenotype correlation of KLF1 were reviewed. Furthermore, we propose that pediatricians in southern China should be cautious in making a diagnosis in children presenting with HHA. For difficult cases, further molecular testing should be performed, and current clinical and molecular reports should be reviewed to ensure that the final diagnosis is accurate.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethical Committee of Nanfang Hospital, Southern Medical University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LX performed the experiments, analyzed the data, and wrote the manuscript. DZ, YZ, and GL performed the experiments. ML and XFW provided technical support for this work. XF and XDW were responsible for accumulating data and referring patients for the laboratory investigations. XS designed the study and drafted the manuscript. All authors reviewed, edited, and approved the version to be submitted.

FUNDING

This research was supported by the National Natural Science Foundation of China (81870148), the Guangdong Basic and Applied Basic Research Foundation (2019A1515011545), and the Southern Medical University Innovation Training Program for Undergraduate Students (201912121027 and S202012121059).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.691461/full#supplementary-material
REFERENCES

Allali, S., Brousse, V., Sacri, A., Chalumeau, M., and de Montalembert, M. (2017). Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. Expert Rev. Hematol. 10, 1023–1028. doi: 10.1080/17474086.2017.1354696

Arnaud, L., Saison, C., Helias, V., Lucien, N., Stechesko, D., Giarratana, M., et al. (2010). A dominant mutation in the gene encoding the erythroid transcription factor KLF1 Causes a congenital dyserythropoietic anemia. Am. J. Hum. Genet. 87, 721–727. doi: 10.1016/j.ajhg.2010.10.010

Belgemen-Ozer, T., and Gorukmez, O. (2020). 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. Pediatric Hematol.Oncol. 42, e536–e540. doi: 10.1097/MPH.0000000000001727

Borg, J., Papadopoulos, P., Georgitsi, M., Gutiérrez, L., Grech, G., Fanis, P., et al. (2010). Haploinsufficiency for the erythroid transcription factor KLF1 causes hereditary persistence of fetal hemoglobin. Nat. Genet. 42, 801–805. doi: 10.1038/ng.630

Chen, W., Zhang, X., Shang, X., Cai, R., Li, L., Zhou, T., et al. (2010). The Krüppel like factor 1 (KLF1) gene in red blood cell disorders. Genomics 111, 1771–1776. doi: 10.1016/j.ygeno.2018.11.032

Huang, J., Zhang, X., Liu, D., Wei, X., Shang, X., Xiong, F., et al. (2015). Compound heterozygosity for KLF1 mutations is associated with microcytic hypochromic anemia and increased fetal hemoglobin. Eur. J. Hum. Genet. 23, 1341–1348. doi: 10.1038/ejhg.2014.291

Iolascon, A., De Falco, L., and Beaumont, C. (2009). Molecular basis of inherited anemia in children. Blood 113, 395–408. doi: 10.1182/blood-2008-03-143447

Kim, Y., Park, J., and Kim, M. (2017). Diagnostic approaches for congenital dyserythropoietic anemia. Best Pract. Res. Clin. Obstet. Gynaecol. 39, 3–15. doi: 10.1016/j.bpobgyn.2016.10.012

Raji, V., Reuten, K., De Backer, S., and De Waele, T. (2016). Hereditary persistence of fetal hemoglobin: a review. Hematol.Oncol. 34, 224–229. doi: 10.1097/MCO.0000000000000266

Shang, X., and Xu, X. (2017). Update in the genetics of thalassemia: what clinicians need to know. Blood 129, 2546–2558. doi: 10.1182/blood-2017-11-803952

Zeineh, A., Aoun, R., Shih, L., Bladé, J., and Higgs, D. (2019). An open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.