


CASE REPORT

A case of congenital dyserythropoietic anemia type IV

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Introduction

Congenital dyserythropoietic anemias (CDAs) are inherited disorders that result from anomalies during the final stages of erythropoiesis and consequently present defective production of red blood cells [1]. CDAs are characterized by chronic hyporegenerative anemia with inadequate reticulocyte count for the degree of anemia, except for CDA type IV, and mild hemolysis. The most common complications associated with CDAs are iron overload, cholelithiasis, and splenomegaly [2, 3]. Dyserythropoiesis and morphological features of erythroblasts are commonly identified in other related anemias (hereditary hemolytic anemia, hereditary stomatocytosis, Diamond-Blackfan anemia, Fanconi anemia, and other inherited bone marrow failure syndromes) that should be excluded in clinical practice. Moreover, the overlapping phenotypes shown in CDAs, even in patients with the same genetic variation, can make differential diagnosis difficult [1]. The identification of the causative genes of CDAs allowed the classification of these diseases, as well as increasing understanding of pathogenesis and clinical

Key Clinical Message

Congenital dyserythropoietic anemias (CDAs) are displayed by ineffective erythropoiesis. The wide variety of phenotypes observed in CDA patients makes differential diagnosis difficult; identification of the genetic variants is crucial in clinical management. We report the fifth case of a patient with unclassified CDAs, after genetic study, with CDA type IV.

Keywords

Congenital dyserythropoietic anemia, *KLF1* gene, next generation sequencing.

management of CDAs [4–8]. Three classic types of CDAs are distinguished by morphological abnormalities in erythroblasts; the most recent classification recognizes six different types of CDAs: Ia, Ib, II, III, IV, and thrombocytopenia X-linked with or without dyserythropoietic anemia, resulting from variants of *CDAN1*, *c15orf41*, *SEC23B*, *KIF23*, *KLF1*, and *GATA-1* genes, respectively [1].

Congenital dyserythropoietic anemias are a rare group of anemias, and their estimated frequency in Europe is variable, the lowest 0.08 cases/million in Scandinavia to the highest 2.60 cases/million in Italy [9]. To date, more than 100 cases of CDA type Ia have been reported [3], several members of a family with CDA type Ib [6], more than 400 cases of CDA type II [10], members of two families with CDA type III [7], and only four patients with CDA type IV [8, 11]. The different genetic variants causing disease are compiled in The Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/>): thirty-four in the *CDAN1* gene, eighty-eight in the *SEC23B* gene, and sixty-three in *KLF1* gene but only one variant responsible for dyserythropoietic anemia.

We report the case of a patient diagnosed with unclassified CDA; genetic study allowed the identification of the pathogenic variant c.973G>A, p.Glu325Lys, in the heterozygous state in the *KLF1* gene, this being the fifth case reported with CDA type IV.

Case History

A female patient aged 13 exhibited systolic murmur, hepatosplenomegaly, polyarthralgia, weight loss as result of high temperature, and signs of anemia onset, dyserythropoiesis, and mild hemolysis. In the neonatal period, she did not have anemia or jaundice, or the need for transfusion. Her twin brother and other siblings have no signs of anemia, her mother has iron deficiency anemia, and her father and grandparents have no known diseases. Her parents are first cousins, and grandparents are cousins.

The blood count data of this patient at 14 years of age were indicative of normocytic hyperregenerative anemia: decreased red blood cells, hemoglobin and hematocrit, increased red distribution width, and reticulocytes; other parameters were within the normal range (Table 1). Serological data were distinctive from hemolysis: increased lactate dehydrogenase, hyperbilirubinemia due to indirect fraction, and haptoglobin was undetectable (Table 1). The autoimmune hemolytic anemia was discarded as the direct Coombs test was negative. There were no signs of iron overload, transferrin saturation, and iron and ferritin values were within the normal range (Table 1). The hemoglobin electrophoresis test was normal except for increased fetal hemoglobin, 12% (normal range of HbF 0.8–2). The tests for glucose-6-phosphate dehydrogenase, HAM, and sucrose were negative.

Data morphology of peripheral blood cells of the patient aged 13 was as follows: presence of poikilocytosis, anisocytosis, basophilic stippling, polychromia, and mild macrocytosis with 10% erythroblasts. Bone marrow morphology aspirated from the patient aged 18 showed intense hyperplasia of red cell series mostly at the last maturation stages, myeloid cells/nucleated erythroid cells ratio of 0.1 (M/E normal range in adults from 1.2 to 5). Binuclearity appears in 15% of orthochromatic erythroblasts, multinuclearity, and abundant iron in mononuclear

phagocyte cells with a small number of sideroblasts (<5%) (Fig. 1A). Analysis by electron microscopy revealed ultrastructural abnormalities of erythroblasts, nuclear and chromatin alterations: pyknosis, karyorrhexis, sponge nuclei, euchromatin areas connecting nuclear membrane, pores in the nuclear membrane through which cytoplasm seems to penetrate into nuclei and intercellular bridges. No double nuclear membrane was observed (Fig. 1B). Given all the laboratory tests and morphological findings observed, this patient was diagnosed with unclassified CDA.

At 24, the patient had a premature twin birth, one twin died at 72 h and the other at 40 days because of *hydrops fetalis*, respiratory failure and sepsis. The patient had jaundice, gallstones, and splenomegaly (18 cm). She underwent cholecystectomy at 25 and splenectomy at 36.

At present, this 44-year-old patient needs occasional blood transfusions, whenever infectious processes (urinary infection, at the initial treatment for virus C hepatitis) but is not transfusion dependent. She presents hyperferritinemia (797 ng/mL) although iron (77 µg/dL) and transferrin saturation (30%) are within the normal ranges.

The patient has been diagnosed with CDA type IV after a genetic study by Ion Torrent™ Next Generation Sequencing Ion AmpliSeq™ (Life Technologies) with a customized panel for CDAs (*CDAN1*, *c15orf41*, *SEC23B*, *KIF23*, and *KLF1* genes). A pathogenic allelic variant in the heterozygous state was identified in the *KLF1* gene (c.973G>A, p.Glu325Lys). No pathogenic variants were found in the other genes analyzed. This study was approved by the local research ethics committee. Written informed consent was obtained from the patient.

Discussion

Congenital dyserythropoietic anemias are rare inherited disorders characterized by a reduced reticulocyte production and hyperplasia in bone marrow. The anemia and hyperbilirubinemia are usually observed in childhood and young adults. Anisocytosis, poikilocytosis, and basophilic stippling are detected in the blood smear. The clinical presentation in these patients could lead to misdiagnosis

Table 1. Hematological and serological data.

| RBC (10 ⁶ /µL) | Hb (g/dL) | Ht (%) | RDW | Retic. (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | PLT (10 ³ /µL) | Total bilirubin (mg/dL) | LDH (U/L) | Tf sat. (%) | Iron (µg/dL) | Ferritin (ng/mL) | Hepcidin (ng/mL) |
|------------------------------|--------------|-----------|------|---------------|-------------|-------------|----------------|------------------------------|----------------------------|--------------|----------------|-----------------|---------------------|---------------------|
| 2.69 | 6.8 | 19 | 24.2 | 7 | 89.9 | 29.6 | 32.9 | 352 | 1.5 | 272 | 18.4 | 36.5 | 44 | 16 |

Data of patient aged 14. Normal ranges in brackets: red blood cells (RBC 3.5–5), hemoglobin (Hb 12–15.5), hematocrit (Ht 36–46), red distribution width (RDW 10.5–14.5), reticulocytes (Retic. 0–1.5), median corpuscular volume (MCV 80–98), median corpuscular hemoglobin (MCH 27–33), median corpuscular hemoglobin concentration (MCHC 32–35), platelet (PLT 120–450), total bilirubin (0–1.2), lactate dehydrogenase (LDH 10–250), transferrin saturation (Tf sat. 20–45), Iron (37–130), Ferritin (10–160, females), Hepcidin (2–26).

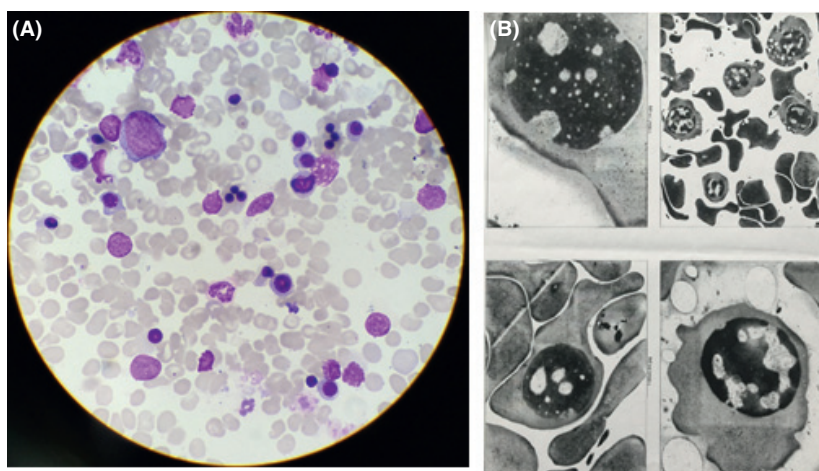


Figure 1. (A) May–Grünwald stain of bone marrow aspirate (40x): erythroid hyperplasia, dyserythropoiesis, erythroblastic multinuclearity. (B) Ultrastructural abnormalities of erythroblasts, nuclear and chromatin alterations: pyknosis, karyorrhexis, sponge nuclei, euchromatin areas connecting nuclear membrane, pores in the nuclear membrane through which cytoplasm seems to penetrate into nuclei, intercellular bridges were also observed but no double nuclear membrane.

such as hemolytic anemia, thalassemia, hereditary spherocytosis, or iron deficiency anemia, and as consequence, to inappropriate therapies.

Therapies for CDA patients are red cell transfusions, iron chelation to prevent organ damage, splenectomy to abrogate transfusion requirements, or special therapies such as interferon- α in CDA I patients [12] or stem cells transplantation in severe cases of CDA [13–15].

The morphological abnormalities of bone marrow erythroblasts support the differential diagnosis of CDAs, mainly represented by CDA types I and II. CDA type I presents megaloblastic binucleated erythroblasts (2–5%), chromatin bridges between nuclei, spongy, or “Swiss-cheese” appearance of heterochromatin and invagination of cytoplasm into the nucleus. CDA type II presents normoblastic binucleated and multinucleated erythroblasts (10–35%) and peripheral double plasma membranes. CDA type III presents giant multinucleated erythroblasts, intranuclear clefts into heterochromatin, autophagic vacuoles, and karyorrhexis. CDA type IV presents dyserythropoietic morphology similar to CDA types I and II: binucleated erythroblasts, rare immature erythroid cells with marked heterochromatin [2, 3]. The analysis of erythrocyte membrane proteins facilitates the diagnosis given that the hypoglycosylation of band 3 is the diagnostic hallmark of CDA type II [16].

The CDA type IV (OMIM 613673) is an autosomal-dominant disorder caused by variants in the *KLF1* gene (chr 19p13.2) which encodes for the Krüppel-like factor 1 (KLF1), an essential erythroid-specific transcriptional factor member of the Krüppel-like family. KLF1 can act as a transcriptional activator and repressor of erythroid gene expression, an activator of genes for heme and globin

synthesis, blood group antigens, and other erythroid factors [17, 18]. Sixty-three variants on *KLF1* gene have been described so far and result in different phenotypes such as: hereditary persistence of fetal hemoglobin, borderline elevated levels of hemoglobin A₂, microcytosis and/or hypochromia, Lutheran blood type, congenital hemolytic anemia, congenital dyserythropoietic anemia type IV [19, 20]. Recently, homozygosity for null variants has been associated with embryonic lethality [21].

To date, four patients with CDA IV have been reported, all of them exhibiting the same autosomal-dominant variant c.973G>A, p.Glu325Lys, in the heterozygous state in the *KLF1* gene, [4, 10], the same genetic variant as the case reported in the present work. This pathogenic variant is found in the second zinc finger of KLF1, an essential region for binding to DNA motif in the regulatory regions of many erythroid genes.

All of the patients reported as CDA type IV, and the patient in this work, showed normocytic anemia, normal or slightly increased reticulocyte count, elevated values of HbF, elevated LDH, hyperbilirubinemia, and reduced haptoglobin. In two cases, anemia had been found at birth and required repeated blood transfusions. In another case, anemia was associated with *hydrops fetalis* and treated with intrauterine transfusions; thalassemic facies and female genitalia with a male karyotype were also presented [8]. Almost all patients exhibit hepatomegaly and splenomegaly, one was splenectomized aged 4 [8], and the present patient was splenectomized aged 36. In one case, the liver showed a moderate iron overload, not caused by blood transfusion therapy [11]. These patients showed similar features in peripheral blood smear and in bone marrow aspirate: anisopoikilocytosis, polychromasia, erythroid

hyperplasia, dyserythropoiesis signs, basophilic stippling of erythroblasts and erythrocyte, bi- and multinucleated erythroblasts with internuclear bridges and enlarged nuclear pores. Moreover, erythrocytes of patients reported had low protein expression of CD44 and AQP1 water channel, both expression genes regulated by KLF1 [8, 11].

As KLF1 is a transcriptional regulator of the switch from fetal to adult hemoglobin, and of many red blood cell membrane proteins, the clinical characteristics of CDA type IV are a combination of hemoglobinopathy, red blood cell defect, and hereditary persistence of fetal hemoglobin features. These conditions together distinguish CDA type IV from other diseases such as β -thalassemia, hereditary spherocytosis, and hereditary persistence of HbF [11].

The wide variety of phenotypes observed in CDA patients makes differential diagnosis difficult; the identification of the genetic variants plays a crucial role in the clinical management of patients. Genetic diagnosis would contribute to classify CDA patients, and even to identify genetic factors that modify phenotype severity. The detection of a genetic variant in misdiagnosed or unclassified CDA patients would mean these inherited disorders were more frequent than at present.

Authorship

SDLII: acquired, analyzed, and interpreted the clinical data and morphological images, and revised the manuscript. MIMC: performed the technical work of the genetic study. ALC and TML: performed and analyzed the bone marrow morphology. MM: contributed to the design and interpretation of the genetic study. MJMJ: designed the genetic study, analyzed and interpreted genetic variants, and wrote the article. All authors: have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interests.

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