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Fetal presentation of Congenital Dyserythropoietic Anemia Type 1 with novel compound heterozygous *CDAN1* mutations

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Abstract

The congenital dyserythropoietic anemias are a heterogeneous group of disorders characterized by anemia and ineffective erythropoiesis. Congenital dyserythropoietic anemia type I (CDA1) can present *in utero* with hydrops fetalis, but more often it presents in childhood or adulthood with moderate macrocytic anemia, jaundice, and progressive iron-overload. CDA1 is inherited in an autosomal recessive manner, with biallelic pathogenic variants in *CDAN1* or *C15orf41*. This case report documents a severe fetal presentation of CDA1 where we identified two novel compound heterozygous mutations in *CDAN1* and describes the associated pathologic findings and levels of iron-regulatory proteins hepcidin, erythroferrone, and GDF15.

Keywords

Congenital dyserythropoietic anemia; *CDAN1* mutations; hepcidin; erythroferrone; GDF15

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Declaration of Conflicting Interests

The authors have no conflicts of interest to disclose.

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Introduction

Congenital dyserythropoietic anemia type I (CDA1) is an inherited hematological disorder characterized by ineffective erythropoiesis, distinctive dysmorphic changes in erythroid precursors, and iron overload [1,2]. The majority of CDA1 cases result from autosomal recessive compound heterozygous mutations in the *CDAN1* gene. This gene encodes codanin-1, a 3684 amino acid protein that acts as a negative regulator in chromatin assembly. Defects in codanin-1 may disrupt the connection between cell cycle dynamics and terminal erythroid differentiation. This can be manifested in the typical morphologic findings of double-nucleated erythroblasts, interchromatin bridges between erythroblasts, and hemolytic anemia with relatively low circulating reticulocyte counts due to intramedullary destruction of abnormal erythrocytes [3].

Clinically CDA1 is usually diagnosed in childhood or adolescence, but a fetal sub-type presents with fetal hemolytic anemia, hydrops, hepatosplenomegaly, severe pulmonary hypertension, and significant jaundice [1, 4–7]. CDA1 presenting in the fetal or neonatal period can also demonstrate early iron overload, with very high serum ferritin levels and hepatocyte dysfunction. The more common later-onset variety involves gradual iron overload with secondary hemochromatosis developing over time, even without multiple erythrocyte transfusions. The iron loading in this condition is believed to be related to inappropriately low hepcidin levels signaling the need for increased iron absorption which in the presence of ineffective erythropoiesis results in iron overload, although this has not been tested in the fetal sub-type of CDA1 [8].

Hepcidin binds to ferroportin on the basolateral surface of enterocytes and macrophages driving ferroportin internalization and degradation, which results in a block of iron absorption and iron release from storage [9]. Thus, a lack of hepcidin leaves ferroportin active on the membrane surface as an open port for iron absorption. The cause of the low hepcidin serum levels in some patients with CDA1 is not known. Erythroferrone is the primary inhibitor of hepcidin expression [10]. Growth differentiation factor 15 (GDF15) is another inhibitor of hepcidin expression, and a study of 17 Arab Israeli Bedouins with CDA1, all with the Bedouin *CDAN1* founder mutation (Arg1042Trp), reported higher levels of GDF15 in all [11]. Thus, potential explanations for the low hepcidin levels and unrestricted iron absorption in patients with CDA1 includes over-expression of erythroferrone and/or GDF15.

Much remains to be learned about the pathogenesis and treatment of CDA1. In this report, we describe the diagnosis, clinical course, and pathologic findings of a neonate with typical clinical and laboratory features of CDA1 in which two novel compound heterozygous mutations in *CDAN1* were identified. We now report these novel mutations, as well as associated hepcidin, erythroferrone, and GDF15 measurements and bone marrow pathologic findings, in an attempt to better understand the pathogenesis of the fetal-onset variety of this disorder.

Case Report

This female was delivered by emergent Cesarean section due to non-reassuring fetal heart rate tracings at 37 weeks 3 days gestation. Mother was 28 years old, gravida 4, para 2,0,1,2. A previous pregnancy underwent intrauterine fetal demise for unknown reasons at 35 weeks gestation. Parents were Caucasian, healthy, and non-consanguineous. Mother's blood type was O (+). Her antibody screen was negative, as were VDRL, Hep B, and HIV, and she was rubella immune. The infant's Apgar scores were 7 and 8. Birth weight was 3070 grams (21st percentile), length 45.7 centimeters (16th percentile), and OFC 32.5 centimeters (3rd percentile). Ten minutes after birth she developed cyanosis and progressive respiratory distress requiring endotracheal intubation. Inhaled nitric oxide (iNO) was initiated for presumed pulmonary hypertension, later confirmed by echocardiography. Examination revealed a pale, intubated term female who had normal vital signs on a high frequency infant ventilator, MAP 12 cm H₂O, 80% FiO₂. She had marked hepatosplenomegaly and ascites but no other dysmorphic features and no noted skeletal abnormalities.

The initial laboratory tests indicated anemia with Hgb 7.4 g/dl, Hct 23.7% (both <<95th percentile for age [12], MCV 120.9 fl (>95th percentile for age [13]), MCH 37.8 pg (>95th percentile for age [13]), MCHC 31.2 g/dl (normal), RDW 38.7 % (>>95th percentile for age [14]), reticulocytes "unable to report", NRBC 45/100 WBC (>95th percentile for age [15]). Her blood type was B (+), and Coombs was positive. Kleihauer-Betke test on mother was negative for fetal-maternal hemorrhage. Leukocyte count 28.9 K/μl, 27% segmented neutrophils, 8% band neutrophils. The platelet count was 166 K/μl. Serum ferritin was 40,664 ng/ml (reference interval 14–647 ng/ml). Evidence on the day of birth that the congenital anemia was hemolytic included elevated total bilirubin 6.9 mg/dl (direct 2.1 mg/dl, indirect 4.8 mg/dl) three hours after birth (>95th percentile), elevated carboxy Hgb 2.1 to 3.3% (normal <1.5%), absent haptoglobin (<8 mg/dl), and large hemoglobin, in the absence of red blood cells, in the urine. No blood smear was available for examination from before a packed red blood cell (PRBC) transfusion was administered.

Over the first days of life she developed progressive coagulopathy requiring fresh frozen plasma infusion, thrombocytopenia with platelet count nadir of 42 K/μl, elevated liver function tests (peak ALT 206 U/L, AST 1341 U/L), and direct hyperbilirubinemia (peak total bilirubin 16.5 mg/dl, direct 10.0 mg/dl, indirect 6.5 mg/dl). No evidence of infection was discovered by bacterial and viral culture, antibody titer, or qualitative PCR. No inborn metabolic errors were found using serum and urinary amino acid quantification by LC-MS/MS. Ammonia, urinary organic acids, and very long/branched-chain fatty acids were normal. A normal interleukin-2 receptor on DOL 2 reduced the possibility of hemophagocytic lymphohistiocytosis [16], and a normal salivary gland biopsy on DOL 5 reduced the possibility of this being a case of gestational-alloimmune liver disease [17]. Other diagnostic testing included liver biopsy, which revealed cholestasis and dyserythropoietic normoblasts. A rapid sequencing panel of over 4500 genes was performed (ARUP Laboratories, Salt Lake City, UT), which revealed compound heterozygous variants in *CDAN1* as listed in Table 1. No other pathogenic variants were detected in the American College of Medical Genetics recommended list of genes.

During hospitalization her ferritin levels remained elevated, reticulocytes remained low (<1%), and two additional PRBC transfusions were given because of continued anemia. She was weaned from the ventilator after 16 days, and discharged home on day of life 43 on supplemental oxygen (0.5 L/min by nasal cannula) and sildenafil for pulmonary hypertension.

At most recent follow-up at 18 weeks of age, she has continued to require PRBC transfusions approximately every 4 weeks, and has received a total of 12 PRBC transfusions with total volume of 622 ml. She has weaned off daytime supplemental oxygen but remains on sildenafil. End-tidal CO measurement was <1.1 ppm (normal). She had excellent growth: height 62 cm (24%ile), weight 5.99 kg (21%ile), OFC 39 cm (19%ile) and normal development. Her thrombocytopenia, transaminitis, hyperbilirubinemia, and coagulopathy have resolved. Serum ferritin, hepcidin, erythroferrone, and GDF15 levels (all measured at 11 weeks of age) are shown in Table 2. These levels were obtained three weeks following the most recent PRBC transfusion.

Examination of bone marrow aspirate (Figure 1A and 1B), performed at 18 weeks of age, revealed the bone marrow to be normocellular with a cellularity approaching 100%. Erythroid elements are significantly increased resulting in low M/E ratio. Significant dyserythropoiesis is noted. Most of the erythroid elements exhibit megaloblastic changes with numerous binucleated and occasional trinucleated forms. Frequent intracytoplasmic bridging is noted, with rare erythroblasts showing intranuclear chromatin bridging. These morphologic findings are consistent with CDA1. Megakaryocytes were normal in both numbers and morphology, and the myeloid elements shows all stages of maturation. No tumor cells, granulomata, or abnormal storage cells were seen. Examination of the bone marrow by electron microscopy (EM) revealed nuclei with wide nuclear pores, aggregates of electron-dense heterochromatin with extensive electron-lucent vacuolization, and intranuclear extension of cytoplasm (Figure 2).

Discussion

CDA1 is a rare autosomal recessive disorder typically presenting in childhood, sometimes in adults, and is characterized by dyserythropoiesis and iron overload. Mutations in codanin-1 (*CDAN1*) and more recently *C15orf41* genes have been identified as causative in human CDA1 [18,19]. The fetal and neonatal onset of CDA1 is less common; however, severe manifestations presenting in the intra-uterine and neonatal periods have been described in several cases. In the largest reported series, among 70 Israeli Bedouin patients with CDA1 and homozygous for the Bedouin *CDAN1* mutation R1040W, Shalev *et al* found that 60% (45/70) presented in the neonatal period. All patients had neonatal anemia with 80% (36/45) requiring blood transfusions during the first month of life; however, all but five patients were transfusion-independent by the age of 4 months [1]. Other clinical manifestations described in fetal-onset CDA1 include skeletal abnormalities occurring both in the appendicular and axial skeleton, persistent pulmonary hypertension of the newborn, hepatic dysfunction including direct hyperbilirubinemia and transaminitis, and thrombocytopenia [4–7]. Current management strategies in CDA1 include supportive care with transfusions and chelation for iron overload [18]. Interferon therapy has been utilized with success in several cases of fetal-

onset CDA1 [20–22], and stem cell transplant has been described as a curative therapy in severe cases of CDA1 [23,24].

Several compound heterozygous *CDAN1* variants have been previously described in association with fetal-onset CDA1. El-Sheikh *et al* described a fetal-onset case presenting with PPHN and axial skeleton abnormality and found to be compound heterozygous for *CDAN1* variant c.1796A>G (p.Asn599Ser) and a previously undescribed deletion mutation c.1104_1106delCTT (del of Phe#368/369) [6]. McDaniel and Cramer also reported a case of the fetal-onset variety and found it to be associated with novel compound heterozygous *CDAN1* variants c.2072dupT and c.2093A>T [25]. Recently, a homozygous *C15orf41* mutation was also associated in a severe fetal case of CDA1 presenting with *in-utero* anemia and typical peripheral limb manifestations in addition to multi-system congenital anomalies [21].

In this case, we report two novel compound heterozygous *CDAN1* variants also presenting in the neonatal period with typical clinical, bone marrow, and erythropoietic findings consistent with a diagnosis of CDA1. Our patient presented at birth with transfusion-dependent hemolytic anemia and hyperferritinemia, and rapidly developed pulmonary hypertension, respiratory failure, transaminitis, coagulopathy, hyperbilirubinemia, and thrombocytopenia. Genetic analysis revealed two previously unreported *CDAN1* variants c.2174G>A (p.Arg725Gln) and c.1003C>T (p.Arg335Trp), each inherited from an asymptomatic parent. *In silico* prediction models suggest both *CDAN1* variants are damaging mutations, and thus are the likely explanation for her condition. In this case hyperferritinemia, hemolysis, and anemia have been ongoing and she remains transfusion dependent at 18-weeks of age. Bone marrow morphology was typical of CDA1 with internuclear bridging, megaloblastic changes, and dyserythropoiesis. We also performed EM on the bone marrow to characterize more fully the pathologic changes. In a report of EM findings in four patients with CDA1, Heimpel *et al.* reported normal appearance of proerythroblasts; but with progressive maturation nuclear pores became more numerous and wider, and abundant electron-dense heterochromatin manifested numerous electron-lucent vacuoles. These patients also showed apparent extension of cytoplasm into the nucleus, binucleation, increased cytoplasmic and mitochondrial ferritin, and siderosomes [26]. In a recent report of EM findings in 35 cases of CDA1, Resnitzky *et al.* noted “spongy” (vacuolated) heterochromatin, wide nuclear pores, and apparent invagination of cytoplasm into nuclei in all cases [27]. These authors indicated that those changes were not seen in four patients with CDA, Type II that they had previously reported [28], but EM images were not included in that earlier report. In this patient, we noted similar EM findings with normoblastic nuclei and wide nuclear pores, aggregates of electron-dense heterochromatin with electron-lucent vacuolization, and evidence of intranuclear extension of the cytoplasm.

We also present the findings of iron-regulatory proteins hepcidin, erythroferrone, and GDF15, which have not been previously reported in a fetal onset case. The pathogenesis of the development of iron overload in CDA1 remains enigmatic. As hepcidin is the master regulator of iron levels, its expression is tightly regulated [9]. Previous work has identified the erythropoietin (EPO)-responsive gene *Fam132b/ERFE* or gene product erythroferrone as a suppressor of hepcidin expression [29,30]. When EPO levels are elevated erythroid

precursors in the bone marrow increase *ERFE* transcription and the increased erythroferrone signals to the liver to reduce hepcidin expression, thus allowing for unregulated iron absorption and release into plasma to meet the demands of increased erythropoiesis. In our patient, erythroferrone levels were not elevated as would be predicted during increased iron demand for erythropoiesis [30]. Further, the hepcidin levels were elevated, which would prevent iron absorption from the gut and release from iron stores [31]. These findings suggests that the bone marrow was not responding to the need for increased red cell production. Bone morphogenetic protein family member GDF15 has also been shown to decrease hepcidin expression [11]. Our patient showed high levels of GDF15 relative to the high levels of hepcidin; however, this may be attributed to the age of the infant at the time of blood collection (11 weeks) where the placental contribution may exaggerate the infant generated GDF15 levels. It is important to note that there is very little information on the levels of hepcidin and erythroferrone in infants thus it is unclear if the same regulatory mechanisms act during infant development.

Further investigation is needed to determine how these novel mutations of the *CDAN1* gene result in this clinical phenotype with fetal-onset disease, and to promote better mechanistic understanding of the pathogenesis of this disorder. Additional analyses on infant hepcidin, erythroferrone, and GDF15 levels may also provide further insight into new means for improving treatment for this disorder and other iron-limited anemias in infants.

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Abbreviations

CDA1 Congenital dyserythropoietic anemia type 1

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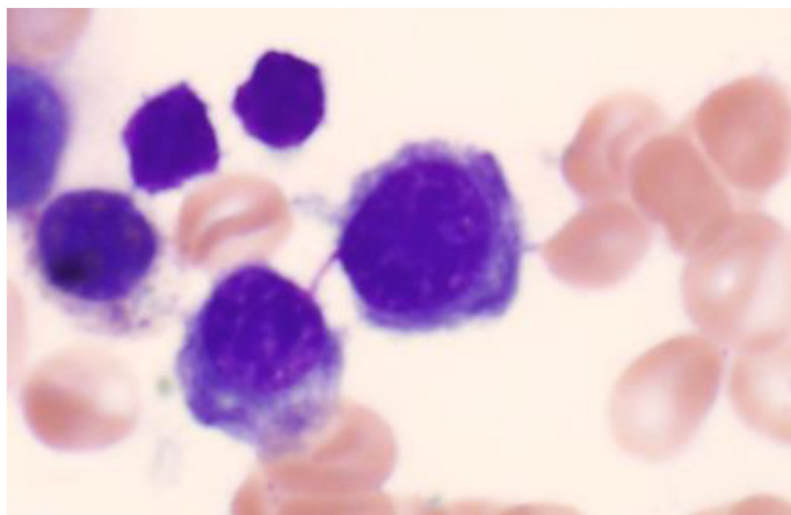
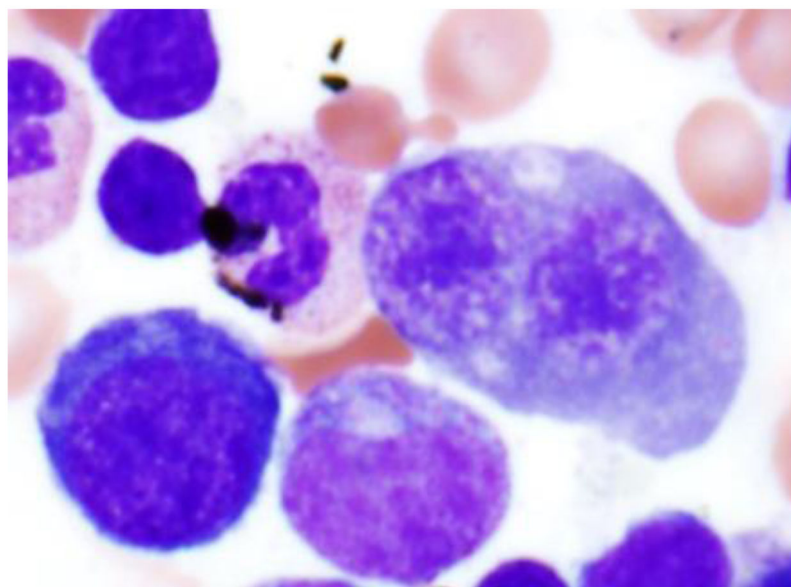
Figure 1A.**Figure 1B.****Figure 1.**

Figure 1A. Bone marrow aspirate smear showing internuclear bridging of erythroblasts. The erythroblasts also show megaloblastic morphology, most prominently in the upper cell. (Wright-Giemsa stain, high power oil immersion)

Figure 1B. Bone marrow aspirate smear showing megaloblastic changes and binucleation in the erythroid precursor cells (Wright-Giemsa stain, high power oil immersion)

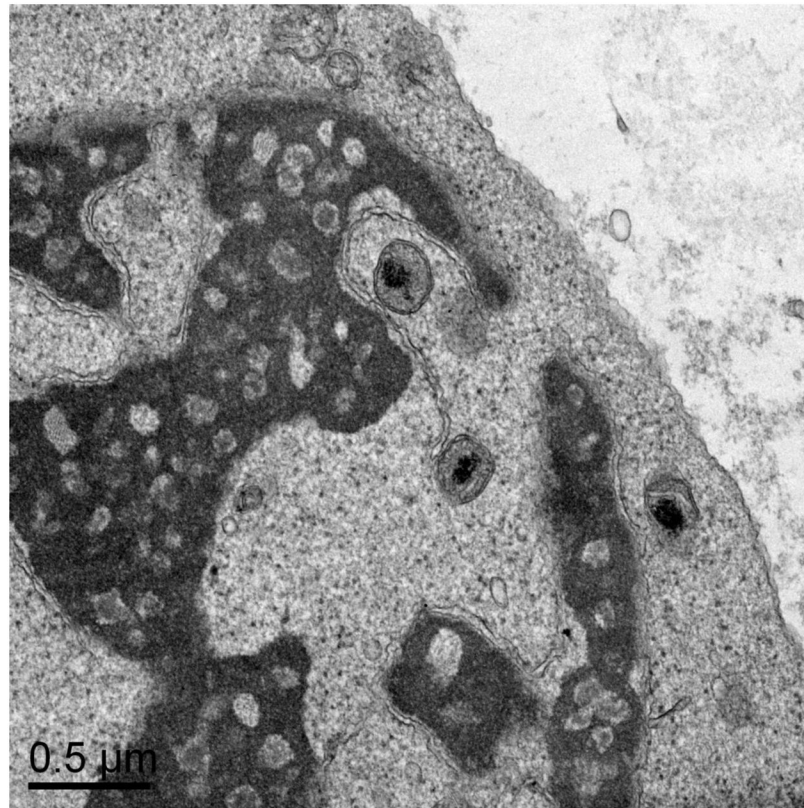


Figure 2.

Electron micrograph showing normoblastic nuclei with wide nuclear pores, aggregates of electron-dense heterochromatin with extensive electron-lucent vacuolization, and apparent intranuclear extension of cytoplasm evidenced by mitochondria with iron inclusions. (Lead citrate, uranyl acetate, original magnification 6500X)

Table 1

Codanin-1 (*CDANI*) variants identified in the father, mother, and proband. The variants were initially identified by massively parallel sequencing and subsequently confirmed by Sanger sequencing. Autosomal recessive inheritance of damaging mutations in *CDANI*, from asymptomatic carrier-parents, is suggested by this compound heterozygous pattern. Severity predictions from three *in silico* programs are shown.

Variant	Father	Mother	Proband
Coding DNA	c.2174G>A	c.1003C>T	Both
Protein	p.Arg725Gln	p.Arg335Trp	Both
Zygosity	Heterozygous	Heterozygous	Compound heterozygous
SIFT prediction	Damaging	Damaging	
PolyPhen-2 prediction	Probably damaging	Probably damaging	
MutationTaster prediction	Disease-causing	Polymorphism	

Table 2

Serum levels of ferritin and iron-regulatory proteins in the proband and her mother (conducted when the proband was 11 weeks old).

Parameter	Patient	Mother	Proper Reference Intervals
Ferritin (ng/ml)	1867	ND	14–647
Hepcidin (ng/ml)	196.7	10.1	10–60
Erythroferrone (ng/ml)	15.5	undetected	<25
GDF15 (pg/ml)	1627.6	362.8	263±141

ND, not done