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Congenital dyserythropoietic anemia type I: First report from the Congenital Dyserythropoietic Anemia Registry of North America (CDAR)

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Abstract

Congenital dyserythropoietic anemias (CDAs) are characterized by ineffective erythropoiesis and distinctive erythroblast abnormalities; the diagnosis is often missed or delayed due to significant phenotypic heterogeneity. We established the CDA Registry of North America (CDAR) to study the natural history of CDA and create a biorepository to investigate the pathobiology of this heterogeneous disease. Seven of 47 patients enrolled so far on CDAR have CDA-I due to biallelic *CDAN1* mutations. They all presented with perinatal anemia and required transfusions during infancy. Anemia spontaneously improved during infancy in three patients; two became transfusion-independent rapidly after starting interferon-α_2; and two remain transfusion-dependent at last follow-up at ages 5 and 30 y.o. One of the transfusion-dependent patients underwent

splenectomy at 11 y.o due to misdiagnosis and returned to medical attention at 27 y.o with severe hemolytic anemia and pulmonary hypertension. All patients developed iron overload even without transfusions; four were treated with chelation. Genetic testing allowed for more rapid and accurate diagnosis; the median age of confirmed diagnosis in our cohort was 3 y.o compared to 17.3 y.o historically. In conclusion, CDAR provides an organized research network for multidisciplinary clinical and research collaboration to conduct natural history and biologic studies in CDA.

Keywords

congenital dyserythropoietic anemia; CDAN1; anemia; erythropoiesis; rare disease registry

Introduction

Congenital dyserythropoietic anemias (CDAs) are rare inborn errors of erythropoiesis. The principal mechanism shared by all types of CDA is ineffective erythropoiesis, leading to varying degrees of anemia and progressive iron overload.[1] CDA is defined by the presence of distinct morphologic abnormalities of the erythroblasts in the bone marrow.[2] Patients present typically with hemolytic anemia and inappropriate reticulocyte response, jaundice, poikilocytosis, and frequently, splenomegaly.[3] The clinical presentation overlaps with more common hematologic diseases, such as red blood cell (RBC) membrane disorders and thalassemias, which often complicates and delays accurate diagnosis. Classification of CDA has mainly been morphologic, based on the classification proposed by Heimpel and Weindt in 1968, in which three major subtypes of CDA were identified based on morphologic similarities.[2] Recently, more subgroups and variants of CDA have been identified and added to the classification.[4,5] CDA type II (CDA-II) is the most common type followed by CDA type I (CDA-I).[6] The phenotypic and genetic variability of CDA is remarkable, including intra-family variability, suggesting a role for other modifiers in determining disease phenotype.[7,8]

Pathogenetic mutations causing the common CDA types have been identified. CDA-I is a recessive disease caused by biallelic mutations in either *CDAN1* or *CDIN1* (CDAN1-interacting nuclease 1, previously known as *C15orf41*); the molecular cause is still unidentified in 10–20% of cases.[9,10] CDA-II is an autosomal recessive disease caused by mutations in *SEC23B* gene; however, in several patients, only one *SEC23B* variant is identified, suggesting the presence of as yet unidentified, non-coding mutations that result in reduced *SEC23B* expression.[3] Dominant mutations in *KIF23* underlie familial CDA-III; the *KLF1* mutation p.E325K cause dominant CDA-IV, while mutations in *GATA1* cause X-linked thrombocytopenia with dyserythropoietic anemia.[5,6,11–13] These genetic discoveries have improved our understanding of CDA; however, more work is needed to elucidate the molecular pathogenetic mechanisms and the basis for the significant heterogeneity seen in the different types of CDA.

CDA-I is characterized clinically by macrocytic anemia. Bone marrow studies characteristically demonstrate binucleated erythroblasts with chromatin bridges by light microscopy, and spongy heterochromatin in intermediate and late erythroblasts

ultrastructurally.[14] Non-hematological features, especially skeletal features affecting distal extremities, have been reported in 4–25% of CDA-I patients.[15,16] Because of its remarkable clinical variability and its overlap with other hematologic conditions, misdiagnosis or delayed diagnosis of CDA-I was common, with a median age of 17.3 years at the time of diagnosis, historically.[7]

The rarity of CDA is a significant impediment to continued investigation of this group of diseases. Findings from the European registries of patients with CDA-I and II have expanded our knowledge about the natural history of these subtypes of CDA.[5,7,17,18] Nonetheless, there are still significant gaps in our understanding of CDA pathophysiology, clinical heterogeneity, natural history, optimal treatment, and genotype-phenotype correlations.[14] We established the CDA Registry (CDAR) in North America in 2016 (NCT02964494) to provide a platform for the systematic study of CDA in patients in the United States, Canada, and Mexico. The objectives of CDAR are twofold: 1) to longitudinally collect demographic, clinical, and treatment data on patients with CDA, and 2) to create a biorepository of de-identified samples that can be used as a tool to investigate the biology and molecular pathology of CDA. This study describes CDAR's structure and current findings and focuses on the clinical data and disease course of participants with CDA-I due to *CDAN1* mutations, enrolled to date in CDAR.

Methods

Study design and patient enrollment

We established CDAR in August 2016 at Cincinnati Children's Hospital Medical Center (ClinicalTrials.gov Identifier: NCT02964494) as a collaborative effort between the investigators and the referring physicians. Participants were identified by their treating physician and referred to CDAR for enrollment. Patients or family members can also contact CDAR directly for enrollment. Patients of any age who have a phenotypic or genotypic diagnosis of CDA are eligible for enrollment.[1] Family members of patients with CDA are also eligible to enroll to facilitate genetic studies. Exclusion criteria include the presence of cancer or myelodysplastic syndrome diagnosis, explaining a new onset of dyserythropoiesis, or other causes of acquired dyserythropoiesis, e.g., vitamin B12 deficiency or medications. The current status of CDAR and the data of participants with CDA-I, including two previously reported cases,[19,20] are presented in this study.

Procedures, data, and sample collection

The study was approved by the Institutional Review Board (IRB) at Cincinnati Children's Hospital Medical Center. Adult participants and parents/legal guardians of minors provided written informed consent and permission, respectively, and minors 11 years of age or older provided written assent. Patients or legal guardians and family members were consented to participate in person or by teleconference by the investigators and a clinical research professional as a phone-consent witness. After informed consent is obtained, participants and their physicians are invited to complete standardized clinical forms or share pertinent medical records to capture demographic information, medical history, family pedigree and history, diagnostic test results, treatments, and complications. Annual follow up information

is solicited from patients and physicians. Pathology reports and bone marrow slides, when available, are centrally reviewed by an expert hematopathologist for confirmation and classification of the diagnosis.

Participants may elect to provide biological samples to the CDAR biorepository, such as blood, bone marrow, or tissue samples collected only when clinically indicated procedures are performed. Written informed consent is sought to collect DNA and genetic studies, and the generation of induced pluripotent stem cells (iPSC) and immortalized B-cells for biological studies.

Genetic studies

For participants without a genetic diagnosis at time of referral, high throughput next-generation sequencing and deletion/duplication panels for the known causative CDA genes (*CDAN1, CDIN1 [or C15orf41], SEC23B, KIF23, KLF1, GATA1*) are used to identify pathogenic or potentially pathogenic variants. In addition, genes known to be associated with other hereditary hemolytic anemias that may cause reactive dyserythropoiesis were also investigated. All substitution and small indel mutations were subsequently confirmed by Sanger sequencing. If a causative mutation is not identified, whole-exome or genome sequencing (WES or WGS) is performed to identify candidate genes for further validation and investigation of CDA pathogenesis.

Results

General Characteristics of CDAR Participants

Between August 2016 and July 2020, 84 individuals (47 affected and 37 asymptomatic family members, from 42 different families) were enrolled on CDAR (Figure 1A). Nine individuals who had been previously diagnosed with CDA based on bone marrow morphology were excluded because pathogenic mutations for other diseases were identified per CDAR protocol's genetic evaluation: unstable hemoglobin [n=4], pyruvate kinase deficiency [n=2], hereditary spherocytosis due to biallelic *SPTA1* mutations [n=1], Diamond-Blackfan anemia due to *RPL35A de novo* variant [n=1], and primary myelofibrosis due to compound heterozygosity of *MPIG6B* mutations [n=1]. Among the 38 patients remaining, the pathologic diagnosis was confirmed genetically for 18 participants: 7 (18%) with CDA-I, 8 (21%) with CDA-II, 1 (2.5%) with CDA-III, and 2 (5%) with CDA-IV. Twenty (53%) have untypable CDA, i.e., pathologic diagnosis of CDA but no known CDA-causing pathogenic variant (Figure 1B). In three patients with untypable syndromic CDA associated with severe neurodevelopmental disorder, we identified mutations in *VPS4A*, a gene encoding an ATPase that regulates the ESCRT III machinery in a variety of cellular processes, including abscission during cytokinesis and endosomal vesicle trafficking.[21] We also identified a heterozygous missense variant in *PRDX2* gene that encodes the antioxidant enzyme peroxiredoxin II in a family with atypical CDA.[5,22]

Characteristics and Presentation of CDA-I Participants

Seven participants had a diagnosis of CDA-I due to biallelic *CDAN1* mutations. The median age of genetic diagnosis was 3 y.o (range 0.1 to 27 years).

All participants had characteristic blood and bone marrow findings. A representative example of these findings in patients with CDA-I is demonstrated in figure 2. Blood smears showed anisopoikilocytosis, RBC fragmentation, and basophilic stippling (Figure 2A). Bone marrow examination by light microscopy showed erythroid hyperplasia (Figure 2B), binucleated erythroid progenitors in ≤10% of erythroblasts, chromatin bridges between nuclei, and nuclear lobation (Figure 2C). Ultrastructural evaluation of erythroblasts confirmed the presence of nuclear heterochromatin with a characteristic "spongy" appearance of the nuclear heterochromatin and widening of pore membrane dilations (Figure 2D).

All CDA-I patients presented early in life with varying degrees of non-immune hemolytic anemia. One was diagnosed prenatally with fetal anemia and started intrauterine transfusions at 24 weeks of gestation; 3 presented with severe neonatal anemia and signs of hydrops, transient pulmonary hypertension, transaminitis, severe hyperbilirubinemia, and thrombocytopenia; and 3 presented with neonatal jaundice and only moderate anemia (Table 1). Two had family history of stillbirth or fetal demise in older siblings due to hydrops fetalis.

Transfusion and Treatment History

All participants required blood transfusions in the neonatal period. Three had spontaneous improvement of anemia and did not require transfusions beyond infancy. Two patients became transfusion-independent after initiation of interferon-alpha (IFN-α_2): patient #4 became transfusion-independent after starting IFN-α_2 at 1 year of age and remained transfusion-independent after discontinuation at age 3 y.o., while patient #6 was chronically transfused up to 19 years of age when she was started on IFN-α_2 resulting in transfusion-independence for the last year. Two patients (#5 and #7) remain transfusion-dependent at last follow up at ages 5 and 30 y.o, respectively. Patient #7 was initially misdiagnosed with hereditary spherocytosis and underwent splenectomy at 11 y.o. He was lost to follow up and returned to medical care in adulthood, presenting with hemolytic anemia and pulmonary hypertension and was diagnosed with CDA-I by genetic sequencing.[20]

Iron overload

Serum ferritin was elevated in all participants at the last follow-up (range from 361 to 2012 ng/mL), and 4 received iron chelation. Chelation was discontinued in one patient after one year due to improved liver iron concentration and serum ferritin (Table 1). All four participants who had hepatic iron assessment by MRI (range 2–30 years of age) showed hepatic hemosiderosis (liver iron concentration range: 6 to 33.5 mg/g dry liver weight), and the oldest patient, who underwent splenectomy and had an extended lapse in medical care until 27 years of age, had evidence of myocardial hemosiderosis (T2* 17.8 ms; normal >20 ms). Notably, serum ferritin elevation was progressive over time even in the absence of transfusions. For example, patient #2 received only four transfusions in the neonatal period; however, her serum ferritin increased from 408 ng/dL at 1 y.o to 809 ng/dL over 4 years despite not receiving any blood transfusion beyond infancy, consistent with spontaneous iron-loading independent of blood transfusions.

Non-hematological features

All participants had one or more non-hematological manifestations including curved toenails, syndactyly, café-au-lait spots, skin pigmentation, macrocephaly, dolichocephaly, spinal fusion, scoliosis, and short stature. Three participants developed transient neonatal pulmonary hypertension in the setting of severe anemia, requiring mechanical ventilation and inhaled nitric oxide; one of them also suffered a thalamic stroke during this period. One participant developed pulmonary hypertension post-splenectomy in adulthood (Table 1).

Discussion

The registry for patients with CDA in North America, CDAR, provides a collaborative platform to study the natural history, genetics, and biology of CDA. Registries facilitate the study of rare diseases and can improve clinical outcomes when carefully planned and implemented. In this first report from CDAR, CDA-I cases due to biallelic *CDAN1* mutations represented approximately 40% of genetically confirmed cases of CDA. CDA-I patients presented with early-onset anemia, even prenatally, with varying hemolysis severity ranging from hydrops to mild neonatal jaundice and anemia. Non-hematological manifestations, mainly distal skeletal, nail, and skin abnormalities, were more common in our study than previously reported. Importantly, molecular testing expedited the definitive diagnosis of CDA in this cohort of patients.

The characteristics of successful registries and the main challenges facing hematology registries were recently discussed in a comprehensive review.[23] The registries for CDA-I and CDA-II in Europe and Israel have been instrumental in delineating these diseases' clinical characteristics, identifying causative genes (*CDAN1* for most patients with CDA-I[7] and *SEC23B* for CDA-II[18,24]), and establishing phenotype-genotype correlations.[25,26] Learning from those successful precedent CDA registries and similar registries for rare hematologic disorders,[27,28] we established CDAR with two specific objectives: 1) to collect longitudinal data that allow us to study the natural history of CDA, and 2) to establish a biorepository that will allow for novel candidate gene discovery and mechanistic biologic studies. We have intentionally designed CDAR with broad inclusion criteria to capture a diverse CDA population and not miss eligible candidates before thorough evaluation. Concurrently, we instituted a central review process to ensure the accuracy of the diagnosis and the quality of data. Maintaining a cooperative effort between CDAR investigators, the referring and collaborating physicians, and the patients will be essential for the success of long-term natural history studies in CDAR. The elective CDAR biorepository is one of the main strengths of our registry. It provides a tool for physicians and scientists to collaborate on biologic and genetic studies in CDA. Results of such studies can improve CDA treatment and expand our understanding of erythropoiesis in general. Using the CDAR biorepository, novel candidate genes have been identified in participants who do not conform to a known form of CDA and are being validated by multidisciplinary teams, such as the *VPS4A* variants, which were recently shown to cause syndromic CDA associated with neurodevelopmental disorder.[21]

Due to the phenotypic heterogeneity of CDAs and their clinical overlap with other more common hematologic diseases, delayed diagnosis or misdiagnosis is not infrequent.[7] In

CDAR, 9 out of 47 enrolled patients (19%) carried a misdiagnosis of CDA, sometimes for decades. Misdiagnosis in such cases likely reflects the prominent dyspoiesis that may be a feature of the stress erythropoiesis associated with severe hemolytic anemias due to globin, erythrocyte membrane or enzyme disorders that can be confused for CDA. Besides, one patient with CDA-I (patient #7) and another with CDA-II enrolled in CDAR were initially misdiagnosed with hereditary spherocytosis, before genetic testing revealed biallelic *CDAN1* and *SEC23B* variants, respectively. The importance of early use of genetic testing in diagnosing CDA was recently emphasized.[5,29–31] In our study, genetic testing significantly expedited the accurate diagnosis compared to historical data (median age of 3 y.o vs. 17.3 y.o).[7] A timely, accurate, genetically confirmed CDA diagnosis has several advantages. First, It can obviate the need for invasive studies such as bone marrow aspiration and biopsy. In a recent study, up to 55% of patients were estimated to have avoided unnecessary testing if gene testing was employed early enough.[31] Second, an accurate diagnosis informs counseling about the disease and its complications and impacts treatment decisions such as the use of IFNα_2, avoidance of splenectomy in patients with CDA-I, and monitoring and management of iron overload. Finally, as shown in this study, accurate diagnosis based on phenotypic evaluation alone may not be possible, especially in transfusion-dependent patients. Our results underscore the importance of early genetic testing and support recently proposed diagnostic algorithms for hereditary hemolytic and dyserythropoietic anemias.[5,32,33]

Consistent with previous studies,[8] we observed significant variability in the clinical course of patients with CDA-I enrolled in CDAR. All patients presented with prenatal or neonatal macrocytic anemia of varying severity ranging from hydrops fetalis to neonatal jaundice and anemia. All of them required blood transfusions during infancy. Neonatal or perinatal anemia is reported in up to 60% of CDA-I patients with most requiring blood transfusion in the neonatal period.[16,34] Four out of seven patients had improvement in anemia during infancy or childhood, while the remaining three manifested a more severe disease course requiring ongoing transfusions or treatment with IFN-α_2, consistent with the known phenotypic heterogeneity of CDA-I.[6,7,35]

IFNα_2 has been shown to be an effective treatment for CDA-I. It was first noted to normalize hemoglobin in an adult with transfusion-dependent CDA-I and chronic hepatitis C.[36] Since this serendipitous discovery, several reports have confirmed the efficacy of IFNα_2 in ameliorating anemia and reducing iron overload in patients with CDA-I.[7,37–39] The mechanism of this salutary effect of IFNα_2 in CDA-I is unclear; however, IFN appears to attenuate the nuclear structural abnormalities and ineffective erythropoiesis characteristic of CDA-I.[40] EBV-transformed B-cells derived from CDA-I patients produced less IFNα *in vitro*,[41] and erythroblasts cultured in IFNα demonstrated significant reduction in the “swiss-cheese” nuclear appearance evident by EM.[42] One patient in our study received IFNα_2 for ~ 2 years with amelioration of anemia within three months of starting treatment and another patient who was transfusion-dependent for 19 years achieved transfusion-independence after starting IFNα_2. Importantly, IFNα_2 should be used cautiously in infancy due to the risk of spastic diplegia.[43] Some studies suggest that this devastating complication is secondary to benzyl alcohol, a preservative in some preparations of IFNα_2, and that alcohol-free preparations are safer.[44,45] IFN may also have inherent toxicities including peripheral

neuropathy. Considering these concerns, initiation of IFN therapy should be delayed, when possible, beyond early childhood, especially since many patients may spontaneously improve after the first few years of life. Recently, long-acting pegylated-IFNα_2 was used successfully in patients with CDA-I. In a recent study, five out of seven patients became transfusion-independent after receiving pegylated-IFNα_2. However, one patient had to stop treatment due to side-effects ("moon face" appearance, abdominal distention, weight gain and generalized weakness), and a sixth patient had partial response with decreased transfusion requirement.[46]

Iron overload is a recognized, progressive complication of CDA and is the most common cause of early mortality in CDA-I.[7] Iron overload was thought to appear in the second or third decade of life; however, evidence of iron overload, including hepatic and cardiac siderosis, may be observed in younger patients.[47,48] In our cohort comprised of relatively young patients, high serum ferritin or evidence of hepatic hemosiderosis, was detected in all patients as early as 14 months of age. Three patients are receiving ongoing chelation while a fourth was treated for 2 years and was able to discontinue treatment for now. These results confirm that iron overload, due to ineffective erythropoiesis and enhanced iron absorption,[49] develops early in patients with CDA-I. Therefore, all children with CDA-I should be vigilantly monitored for evidence of iron overload, including transfusion-independent patients, to guide appropriate initiation of chelation therapy.

Unlike CDA-II, where splenectomy may be effective in reducing transfusion requirement although not iron overload,[17] splenectomy is contraindicated in CDA-I due to poor hematologic efficacy and the potential for significant morbidity. In one study, splenectomy was ineffective in patients with CDA-I.[7] In another study, five of six patients with CDA-I reportedly had improvement in hemoglobin concentration following splenectomy (but with no specifics about response or duration), while the sixth remained transfusion-dependent.[8] Importantly, however, significant morbidity and early mortality were noted in this study and attributed to complications of splenectomy (pulmonary hypertension and sepsis).[8] Similarly, patient #7 in our study who underwent splenectomy at a young age developed pulmonary hypertension in his 3rd decade of life,[20] a known complication of splenectomy,[50] and continues to require transfusions and chelation for iron overload. This high rate of complications and low efficacy highlight the importance of diagnostic accuracy, especially if splenectomy is contemplated.

The outcome of stem cell transplant as a curative therapy for transfusion-dependent patients with CDA is guarded. In a recent report of the outcome of stem cell transplant in 39 patients with CDA, including 10 patients with CDA-I, the overall survival and event-free survival at 36 months were 71% and 45%, respectively, and 12% experienced graft failure. Iron overload was a poor prognostic factor on outcome of stem cell transplant in this study.[51]

Non-hematologic features are reported in 4–25% of CDA-I patients.[15,16] In our study, non-hematologic findings were common including skin and skeletal features, especially in distal extremities, and short stature. Additionally, findings likely due to complications of severe anemia rather than *CDAN1* defects included transient pulmonary hypertension which

was noted in 3 of our cohort, as previously reported,[16,52–54] and perinatal ischemic stroke (thalamic infarction), a complication not previously reported with CDA-I.

The cause of skeletal features in CDA-I is unknown. *CDAN1* mutations in CDA-I result in defects in Codanin-1 protein whose function is not yet well-understood. Codanin-1 is known to interact with the histone chaperone Asf1 (anti-silencing function 1) which provides histones during the S-phase for replication-coupled nucleosome assembly. Codanin-1 depletion disrupts chromatin assembly and accelerates DNA replication disrupting chromatin assembly and DNA synthesis,[55] which may interfere with the normal silencing and packing of large parts of the erythroblast nucleus during the late terminal erythropoiesis. While Codanin-1 is ubiquitously expressed in most tissues, its expression is cell-cycle-dependent, which could explain the predilection of the rapidly dividing late erythroblasts to Codanin-1 abnormalities in CDA-I.[31] Alterations in cell division may also affect the rapidly dividing cells of the developing limb buds causing the characteristic limb abnormalities of CDA-I.[31,56] The high rates of neonatal pulmonary hypertension and non-hematologic features in CDA-I, especially limb and skin abnormalities, should provide a clue to the diagnosis.

In summary, CDA-I due to *CDAN1* mutations presents with early-onset macrocytic anemia and high rates of distal skeletal and skin dysmorphic features, with or without pulmonary hypertension of the newborn. Anemia improves in about half of the patients over time, but the rest remain in need of treatment, which may include chronic transfusions, iron chelation, or IFNα. Historically, CDA was frequently misdiagnosed, or the accurate diagnosis was significantly delayed. Genetic testing improved the accuracy and speed of diagnosis in our study and should be considered a first-line test as it may obviate the need for invasive diagnostic procedures and could impact treatment decisions, prognosis, and family counseling. CDAR allows for a systematic study of this rare disease and provides a collaborative platform for physicians, scientists, and patients to study the natural history of CDA and explore molecular and genetic pathways of CDA and erythropoiesis.

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Highlights

- The CDA registry (CDAR) is an organized network for the collaborative study of CDAs
- Genetic testing allows for a rapid and accurate diagnosis of CDA type I (CDA-I)
- Anemia severity in CDA-I is variable and iron overload develops even without transfusions
- Non-erythroid features, especially skeletal features affecting distal extremities, point to CDA-I diagnosis
- Interferon-α_2 improves anemia and iron overload in at least some patients with severe CDA-I

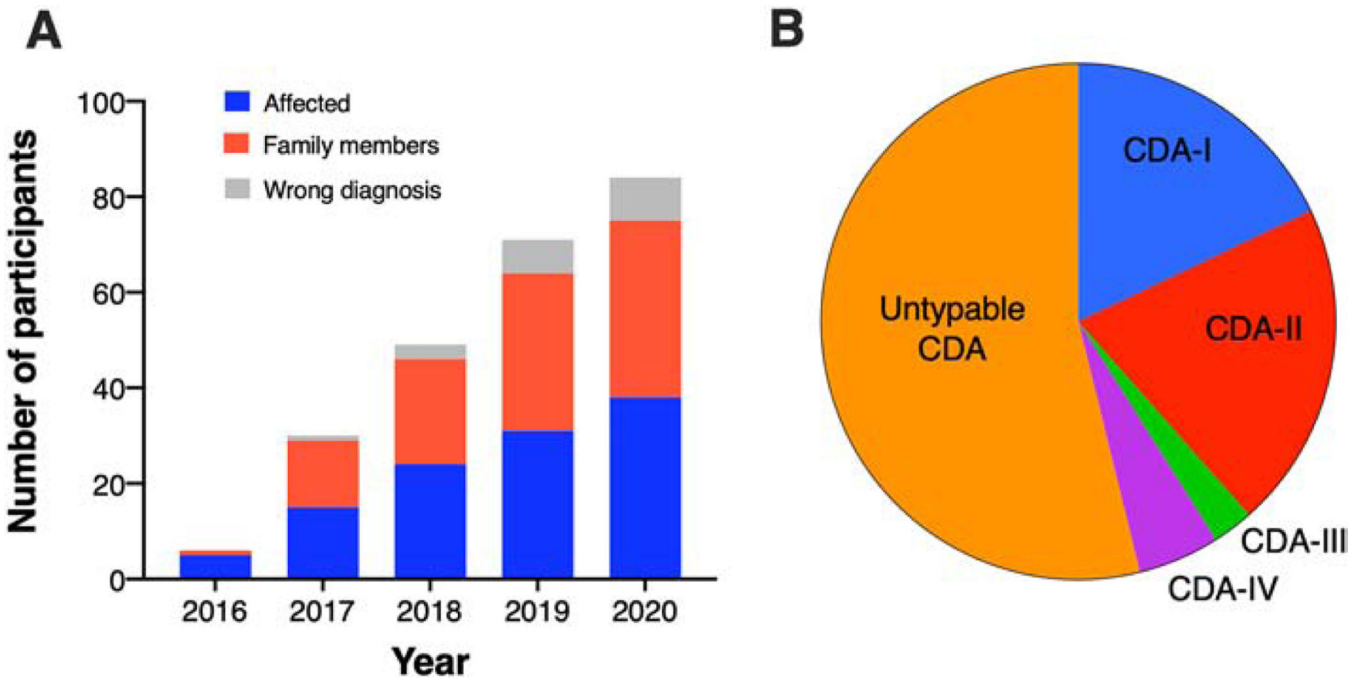

Figure 1. The general characteristics of CDAR participants.

(A) Cumulative enrollment on CDAR by year. The proportion of affected individuals, family members, and misdiagnosed participants is shown in each year. (B) The distribution of CDA subtypes based on genetic results in the 38 affected individuals with CDA in CDAR.

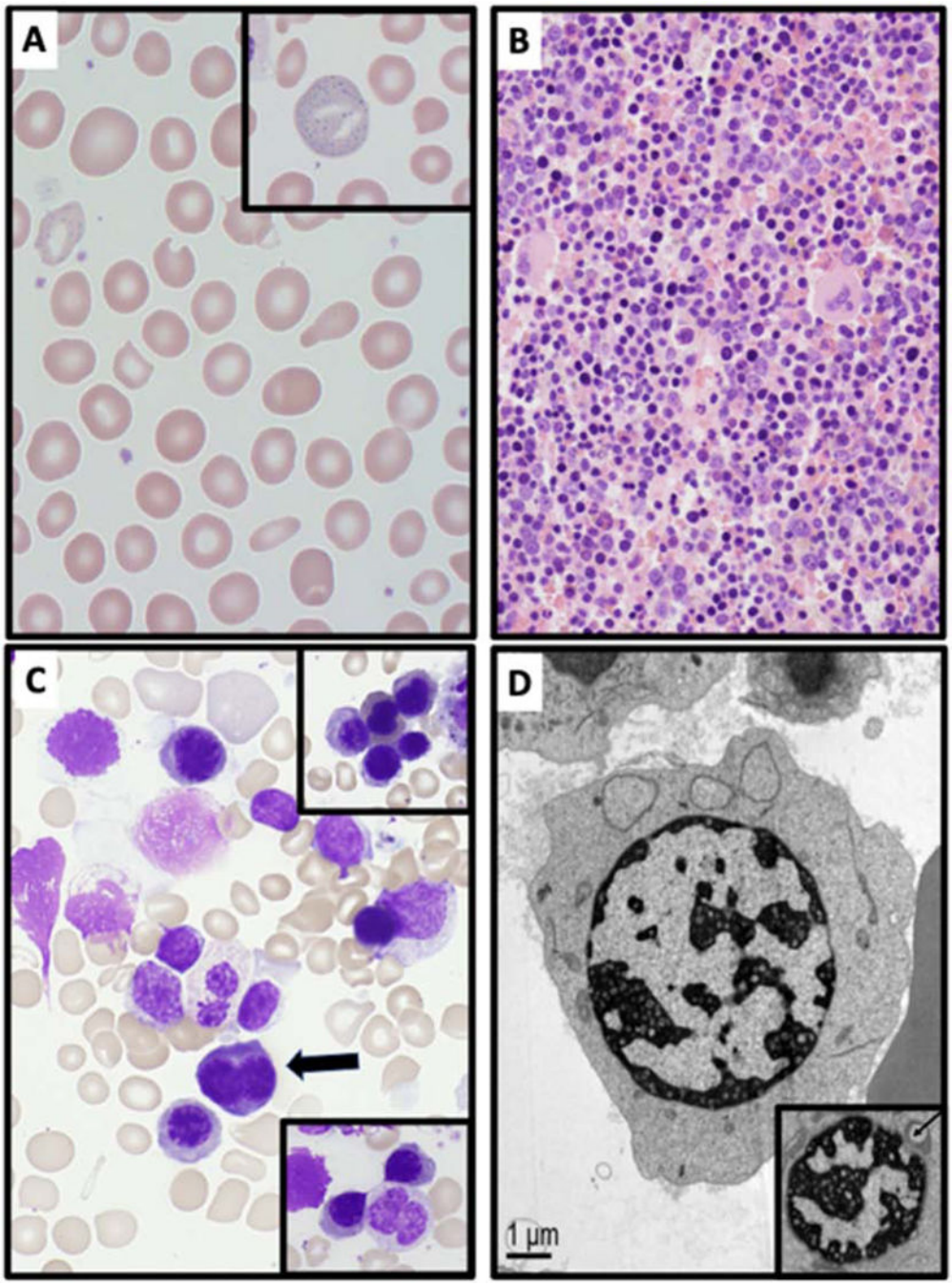

Figure 2. Peripheral blood and bone marrow abnormalities in CDA-I.

(A) Peripheral blood smear of CDA-I patient showing marked anisopoikilocytosis, RBC fragmentation and basophilic stippling (inset). (B) Bone marrow biopsy (H&E, 400x) showing erythroid hyperplasia in a patient with CDA-I. (C) Bone marrow aspirate (100x) demonstrating binucleated erythroblast (arrow). The lower inset shows erythroid cells with a chromatin bridge, and the upper inset showing nuclear lobation. (D) Electron microscopy image of erythroblasts in CDA-I showing the characteristic “spongy” or “swiss cheese”

appearance of nuclear heterochromatin and widened pore membrane dilations (arrow in inset).

Table 1.

Characteristics of participants with CDA-I due to *CDAN1* mutations

Pt #	*CDAN1* mutations (compound heterozygous)	Age, Sex, & Ethnic Background	Laboratory findings				Age at first transfusion	Age at last transfusion	Other treatments	Non-hematologic findings
			Hb	ARC	MCV	Ferritin/LIC				
1	c.3025dupG (p.E1009Gfs*10) and c.3389C>T (p.P1130L)	8 y.o., M, Hispanic-American	9.4	74	88	F 410	Neonate	4 m.o.	None	Macrocephaly and dolichocephaly, one café au lait spot, midline hyperpigmented macule
2	c.2015C>T(p.P672L) and c.2173C>T(p.R725W)	5 y.o., F, Japanese and European-American	10.3	91	89	F 809	Neonate	4 m.o.	None	curved toenails, one café au lait spot
3	c.1003C>T (p.R335W) and c.2174G>A (p.R725Q)	2 y.o., F, European-American	8.2–10.7	11–45	88–95	F 1189→361 LIC 9.3→3.26	Neonate	13 m.o.	Deferasirox (1–2 y.o)	Transient pulmonary hypertension (as neonate), hepatomegaly
4	c.156C>G (p.F52L) and c.3024_3025insTT (p.E1009Lfs*24)	7 y.o., M, European-American	7.8–10.1	50–88	89–91	F 397	Neonate	11 m.o.	IFNα_{2b} (1–3 y.o)	Hepatomegaly, transient pulmonary hypertension (as neonate), thalamic infarction
5	c.2072dupT (p.T692Hfs*13) and c.2093A>T (p.E698V)	5 y.o., F, European-American	7–8	96	87	F 1162 LIC 8.2	Intrauterine	Ongoing at 5 y.o	Deferasirox, (EPO-no response)	web between 4th and 5th toe
6	c.156C>G (p.F52L) and c.2015C>T (p.P672L)	20 y.o., F, European-American and Ashkenazi Jewish	10.1–12.4	34.9–120.4	90.9–93.4	F 2200→680 LIC 6 cardiac T2* 27–39 (normal > 20 ms)	Neonate	19 y.o.	Deferoxamine, Deferasirox, IFNα_{2b}	Transient pulmonary hypertension (as neonate), digital clubbing, joint laxity, osteoarthritis
7	c.2868+1G>A and c.3389C>T (p.P1130L)	30 y.o., M, African-American	7.4	365	93	F 2012 LIC 33.5 cardiac T2* 17.8 (normal > 20 ms)	Neonate	11 y.o. before splenectomy, now again on chronic transfusions since 27 y.o.	Deferasirox, Hydroxyurea for extramedullary erythropoiesis	Short stature, scoliosis, pulmonary hypertension post splenectomy, paraspinal extramedullary erythropoiesis causing kyphosis and obstructive lung disease

Abbreviations and units: Hb: hemoglobin concentration (g/dL); ARC: absolute reticulocyte count (K/μL); MCV: mean corpuscular volume (fL); F: serum ferritin (ng/mL); LIC: liver iron content (mg/g dry liver weight); IFNα_2: interferon-alpha; EPO: erythropoietin.