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Diagnosis and management of congenital dyserythropoietic anemias

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\textbf{ABSTRACT}

Congenital dyserythropoietic anemias (CDAs) are inherited disorders hallmarked by chronic hyporegenerative anemia, relative reticulocytopenia, hemolytic component and iron overload. They represent a subtype of the inherited bone marrow failure syndromes, characterized by impaired differentiation and proliferation of the erythroid lineage. Three classical types were defined by marrow morphology, even if the most recent classification recognized six different genetic types. The pathomechanisms of CDAs are different, but all seem to involve the regulation of DNA replication and cell division. CDAs are often misdiagnosed, since either morphological abnormalities or clinical features can be commonly identified in other clinically-related anemias. However, differential diagnosis is essential for guiding both follow up and management of the patients.

\textbf{ARTICLE HISTORY}

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\textbf{KEYWORDS}

Inherited anemia; ineffective erythropoiesis; molecular genetics; differential diagnosis; follow up; patient management

\textbf{Introduction}

The term dyserythropoiesis refers to a condition of abnormal erythropoiesis affecting the differentiation and proliferation pathways of the erythroid lineage with a consequent defective production of red blood cells (RBCs).\cite{1} Dyserythropoietic anemias can be divided into primary and secondary forms, and both inherited and acquired types can occur. Among these different conditions, the congenital dyserythropoietic anemias (CDAs) are hereditary diseases that embrace a highly heterogeneous set of rare or very rare anemias that result from various kinds of abnormalities during late stages of erythropoiesis. They are counted as subtypes of inherited bone marrow failure syndromes (IBMFS), characterized by morphological abnormalities of erythroblasts in the bone marrow (BM) and ineffective erythropoiesis as predominant mechanism of anemia, accompanied by a hemolytic component.\cite{2}

Dyserythropoiesis appears to be a morphological feature common to several conditions, and this could account for the difficulties in diagnosis of CDAs. However, the specific morphological alterations of the erythroid precursors justify the heterogeneity of these disorders. Indeed, the three classical types of CDAs (types I, II and III) are defined on the basis of BM morphology.\cite{2} Inclusions of additional CDAs, the so-called CDA variants, despite remarkable morphological studies, gradually led to overlapping entities and imposed a limitation on classification. In spite of these difficulties, morphological classification is still widely used in clinical practice. Nevertheless, the identification of the causative genes of the most common forms among CDAs in the last two decades represented an evident advantage for reclassifying these disorders, as well as in understanding their pathogenesis. Moreover, uncovering the molecular basis of CDAs helped to unravel novel aspects of the molecular biology of erythropoiesis. From the genetic standpoint, six different types of CDAs are included in the Online Mendelian Inheritance in Man (OMIM) compendium of human genes and genetic phenotypes so far (Table 1). However, this list is bound to extend. Indeed, as described later in this review, the discovery of new causative genes is a continuous evolving process thanks to the development of high-throughput technologies, such as next-generation sequencing (NGS). The identification of genetic variations underlying hereditary disorders marked the opening of a new era for genetic and clinical research. Indeed, beyond, obtaining definitive diagnosis and planning patient management, knowledge of the genetic basis of these disorders is crucial in estimating their prevalence and geographical distribution. According to the estimation by Heimpel and colleagues in 2010, the prevalence of CDAs varies widely among European regions, with minimal values of 0.04 cases/million in North Europe and the highest
value in Mediterranean countries, particularly in Italy (2.49/million). This is mainly true for CDA II, which is more frequent than CDA I with an overall ratio of approximately 3.0.[3] The studies on molecular epidemiology of CDA I and II highlighted the elevated allelic heterogeneity of both conditions as most of the causative variations are inherited as private mutations.[4] However, recurrent mutations have been found in both CDA I and CDA II patients. In particular, the R1042W mutation in the CDA I causative gene CDAN1 is a founder mutation in the Bedouin population,[5] whereas the amino acid substitutions E109K and R14W in the CDA II causative gene SEC23B show the highest frequency in Mediterranean area (Morocco, Israel and Italy), where a founder effect for both variants has been described.[6] However, molecular geocode of CDA II pathogenic alleles suggested the presence of multiple founder effects in different geographical areas of the world.[7]

Some comprehensive overviews of CDAs have been already published, mostly describing the clinical features and the molecular genetics of these disorders. [2,4] This review will deal with new insights on the diagnosis and the follow-up of CDA patients. We will also discuss the role of the new technologies in the assessment of diagnosis and prognosis, as well as in the research area.

### Differential diagnosis of CDAs and related hereditary anemias

CDAs can be suspected in the presence of anemia and hemolytic signs, accompanied with reticulocytosis inadequate to the degree of anemia. In particular, the following criteria are required: (1) evidence of anemia, jaundice, splenomegaly; (2) evidence of ineffective erythropoiesis; (3) occurrence of typical morphological features of erythroblasts at BM examination; and (4) exclusion of other congenital anemias that fulfill criteria 1 and 2, such as thalassemia syndromes and other IBMFS.[8] However, differential diagnosis between CDAs and clinically related anemias is often difficult.

Thalassemias and hemoglobinopathies are the first conditions to be excluded. The presence of microcytic anemia, pathological hemoglobin (Hb) electrophoresis and positive familial anamnesis can direct the diagnosis toward these disorders. In particular, the differential diagnosis with thalassemia is important in suspected cases of either CDA IV or CDA variants-GATA1 related as described later in this review. Among IBMFS, Diamond–Blackfan anemia (DBA) and Fanconi anemia (FA) are disorders that most frequently undergo differential diagnosis with CDAs. Unlike the CDAs, FA generally presents reduction to

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<table>
<thead>
<tr>
<th>Disease symbol</th>
<th>Phenotype</th>
<th>MIM number</th>
<th>Gene location</th>
<th>Inheritance</th>
<th>No. cases&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Optical microscopy</th>
<th>Electron microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDA Ia</td>
<td>Congenital dyserythropoietic anemia type Ia</td>
<td>224120 CDAN1 15q15.2</td>
<td>AR</td>
<td>&lt;100</td>
<td>Binucleate erythroblasts (3–7%); thin chromatin bridges between nuclei of erythroblasts</td>
<td>'Swiss cheese appearance' of the erythroblasts heterochromatin</td>
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<tr>
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<td>Congenital dyserythropoietic anemia type Ib</td>
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<td>Binucleate (10–30%); rare multinucleate erythroblasts</td>
<td>Double plasma membrane of the erythroblasts</td>
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<td>Congenital dyserythropoietic anemia type II</td>
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<td>AR</td>
<td>&gt;200</td>
<td>Giant multinucleate (up to 12 nuclei) erythroblasts</td>
<td>Clefts within heterochromatin, autophagic vacuoles, iron-laden mitochondria, myelin figures in the cytoplasm Inagination of nuclear membrane, intranuclear precipitated and nuclear blebbing</td>
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</tr>
<tr>
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<td>Congenital dyserythropoietic anemia type III</td>
<td>105600 KIF23 15q21</td>
<td>AD</td>
<td>&lt;20</td>
<td>Tri- and multinucleate erythroblasts</td>
<td>Reduced numbers of platelet alpha granules and dysplastic features in megakaryocytes and platelets</td>
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</tr>
<tr>
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<td>Congenital dyserythropoietic anemia type IV</td>
<td>613673 KLF1 19p13.2</td>
<td>AD</td>
<td>&lt;10</td>
<td>Erythroblasts: megaloblastic features, nuclear irregularities, bi- and multinucleation Megakaryocytes: small, dysplastic with signs of incomplete maturation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XLTDA</td>
<td>Thrombocytopenia X-linked with or without dyserythropoietic anemia</td>
<td>300367 GATA1 Xp11.23</td>
<td>XLR</td>
<td>&lt;10</td>
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<sup>a</sup> Number of cases with positive molecular analysis.

AD: autosomal dominant; AR: autosomal recessive; CDAs: congenital dyserythropoietic anemias; OMIM: Online Mendelian Inheritance in Man; XLR: X-linked recessive.

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### Table 1. Classification of CDAs by OMIM database.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Phenotype</th>
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absent trilinear hematopoiesis, acute myelogenous leukemia or solid tumors; moreover, it can also present developmental abnormalities more frequently compared to CDAs, particularly CDA I. The positivity to the diepoxybutane (DEB) test is a very sensitive and specific tool for guiding FA diagnosis.\cite{9} Similarly to CDAs, DBA presents as isolated inherited red cell production failure. However, conversely to CDAs, DBA BM exhibits reduced proliferation and survival of erythroid progenitors. Moreover, growth retardation, congenital malformations and increased HbF levels are more frequent features of DBA compared to CDAs. Increased activity of erythrocyte adenosine deaminase is a good II level test for establishing the diagnosis of DBA (sensitivity 84%, specificity 95%, positive and negative predictive values 91%) (Figure 1).\cite{10}

CDAs can be also misdiagnosed with hereditary hemolytic anemias. For example, CDA II shares several clinical findings with hemolytic anemias due to red cell membrane defects, such as hereditary spherocytosis (HS).\cite{11} Of note, CDA II patients are often erroneously diagnosed as HS, and consequently, they undergo unnecessary splenectomy. The lack of substantial improvement after intervention leads to a re-examination of the case, allowing the correct diagnosis of CDA II. The most useful pointer to correctly establish the diagnosis of CDA II is the inadequate reticulocyte count for the degree of anemia. Indeed, the marrow stress is higher in CDA II compared to HS for the same Hb level as attested by the increased sTfR levels observed in CDA II patients.\cite{7} Other parameters could be also used for distinguishing both conditions. For example, the RBC distribution width (RDW) is characteristically increased in CDA II, while the Hb distribution width (HDW) is increased in HS, resulting in an RDW/HDW ratio that is significantly greater in CDA II than in HS.\cite{8} Recently, a new clinical index, named BM responsiveness index, has been developed to discriminate a hemolytic anemia from ineffective erythropoiesis one. This index resulted to be a high sensitive parameter (90.4%) to achieve a clinical diagnosis of CDA II (Figure 1).\cite{7}

In addition, other RBCs membrane defects as dehydrated and overhydrated hereditary stomatocytosis (HST) should be evaluated in the differential diagnosis of CDA I. Both CDA I and HST present macrocytosis associated with hemolytic signs. Moreover, it has been described as a novel variant of HST due to a de novo band 3 mutation, transmitted in a dominant fashion, characterized by conversion of band 3 from an anion exchanger to a cation transporter, associated with a dyserythropoietic phenotype.\cite{12}

It is important to underline that marrow abnormalities can be found in several common conditions. Indeed, dyserythropoiesis may be frequently observed in anemias due to iron, folate, vitamin B6 and vitamin E deficiencies. Moreover, conditions of relative hypoxia, as erythropoiesis during the neonatal period, congenital cardiopathies and chronic bronchopathies, can lead to secondary dyserythropoiesis.

![Figure 1. Flow diagram for the differential diagnosis of congenital dyserythropoietic anemias (CDAs) and related hereditary anemias. The flow diagram shows the main steps (light blue) for guiding the clinical suspicion toward the diagnosis of a CDA. DBA: Diamond–Blackfan anemia; FA: Fanconi anemia; IBMF: inherited bone marrow failure syndromes; RBC: red blood cell; XLTDA: X-linked thrombocytopenia with or without dyserythropoietic anemia.](https://example.com)
Differential diagnosis among CDAs

CDA type I

Most of the CDA I patients exhibit lifelong macrocytic anemia with variable values of Hb and mean cell volume (MCV) ranging between 100 and 120 fL; however, it can be normocytic in childhood.[1315] Relative reticulocytopenia is typically observed accompanied to increased Hb turnover as attested by indirect hyperbilirubinemia, high lactate dehydrogenase (LDH) value and low or absent plasma haptoglobin.[14] All patients develop splenomegaly in adolescence or adulthood,[4,13] while about 20% of cases show congenital anomalies, particularly syndactyly in hands or feet, absence of nails or supernumerary toes,[16] pigeon chest deformity[17] and short stature.[18] In some cases, the skull radiograph shows deformity due to the marked expansion of the BM space in the flat bones. Retinal angioid streaks and macular abnormalities were also reported in two cases with deterioration of visual acuity at age 25 and 57 years.[5,19]

Occasionally, symptoms are present in prenatal age, with hydrops fetalis and anemia requiring in utero transfusions.[20] Moreover, 27% of infants born to affected mothers are small for gestational age.[21] At birth, CDA I patients can also present hepatomegaly and jaundice,[15,16] and can often require at least one blood transfusion. Recently, persistent pulmonary hypertension has been described in CDA I newborns, with or without pigeon chest deformity.[17,22]

Peripheral blood smear of CDA I patients shows anisocytosis and poikilocytosis with tear drop cells, large irregularly shaped macrocytes that may suggest megaloblastic disease; mature erythroblasts can also be found. BM smear shows hypercellularity and erythroid hyperplasia (E:C ratio of four and eight times the normal).[23] Approximately 30–60% of polychromatic erythroblasts show abnormalities of nuclear and chromatin structure. Indeed, the morphological pathognomonic feature of CDA I is the presence of thin chromatin bridges between the nuclei pairs of erythroblasts. A minority of erythroblasts shows bi- or multinuclearity, but in contrast to CDA II, the nuclei of binucleated cells are of different size and shape. At electron microscopy (EM), heterochromatin is denser than normal and forms demarcated clumps with small translucent vacuoles, giving rise to the metaphor of ‘Swiss cheese appearance’.[23]

CDA I is inherited in an autosomal-recessive manner. The first causative gene in which pathogenic variants were identified has been CDAN1 (chr15q15.2) [24] that encodes a ubiquitously expressed protein, codanin-1 (Table 1). More than 100 patients (CDA Ia) and 30 unique disease-causing mutations have been described so far.[2,24] Codanin-1 protein localizes in the nucleus, preferentially in the nuclear heterochromatin, and is transcriptionally regulated by E2F1, resulting in a rise in its levels during S phase. Although the function of codanin-1 during the S phase is still unknown, the occurrence of spongy heterochromatin in CDA I marrow could suggest a role of this protein in heterochromatin organization during DNA replication.[25] An additional pathogenic mechanism involves codanin-1 as a part of the cytosolic Asf1-H3-H4-importin-4 complex, which is implicated in nucleosome assembly and disassembly (Figure 2).[26] Cdan1 knockout mice die in utero before the onset of erythropoiesis, suggesting a critical role of codanin-1 in developmental processes beyond erythropoiesis.[27] Of note, no homozygous patients for null mutations have been described so far. Homozygous or compound heterozygous in CDAN1 gene cover approximately 50% of CDA I patients, while in 30% of cases only a single mutant allele can be identified.[28]

Recently, the second causative gene of CDA I has been identified. In particular, two different mutations in C15orf41 gene (chr 15q14) were found in three unrelated Pakistani families, classified as affected by CDA Ib (Table 1).[29] C15orf41 is predicted to encode a divalent metal ion-dependent restriction endonuclease, with a yet unknown function. In cultured erythroblasts, C15orf41 produces a spliced transcript encoding a protein with homology to the Holliday junction resolvases (Figure 2). However, it has been shown that C15orf41 interacts with Asf1b, supporting the hypothesis that the primary defect in CDA Ib is in DNA replication and chromatin assembly.[29] CDA Ib patients show clinical features similar to CDA Ia ones. Anyhow, a difference in Hb level and in MCV value can discriminate between CDA Ia and Ib patients (Figure 3). Nevertheless, more patients should be identified to validate this observation.

CDA type II

The main clinical finding to diagnose CDA II is the presence of normocytic anemia of variable degree, with normal or only slightly increased reticulocyte count, but not adequate to the degree of anemia (ineffective erythropoiesis); it is often accompanied with jaundice and splenomegaly due to the hemolytic component.[2,4] As described in a recent survey on 205 patients, CDA II generally presents mild anemia (mean Hb 9.6 ± 0.2 g/dL), but a wide spectrum of clinical presentations can occur, from asymptomatic...
to severe (Hb range 3.6–16.4 g/dL). Indeed, approximately 10% of cases result symptomless, whereas 20% of patients undergo a regimen of transfusion dependence.[7]

The term ‘congenital’ could sound inappropriate in some cases since this disorder is often diagnosed during adulthood. Of note, the mean age of onset symptoms is approximately 3–4 years, but six patients were reported to have a prenatal onset. However, the age of diagnosis is delayed, 22.2 ± 1.7 years.[7] This could be explained either by the occurrence of mild symptoms or by the misdiagnosis of CDA II with HS, as already stated.

The morphological evaluation of peripheral blood smear highlights the presence of anisopoikilocytosis (ovalocytes, microspherocytes, tear drop cells) with basophilic stippling of cells and few occasionally mature erythroblasts. Similarly to CDA I, the BM is hypercellular with distinct erythroid hyperplasia and subsequent increased E:G ratio. The most specific finding of CDA II marrow is the presence of more than 10%
mature binucleated erythroblasts with equal size of two nuclei.[23]

Upon EM examination, mature erythroblasts show discontinuous double membrane, which is due to the presence of vesicles loaded with proteins of endoplasmic reticulum (ER) that appear to be running beneath the plasma membrane.[23,30]

Analysis of RBC membrane proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis reveals a narrower band size and faster migration of band 3 in most of the CDA II patients (95%).[7] Thus, this biochemical feature represents a specific diagnostic hallmark of the disease. Moreover, the hypoglycosylation of band 3 has been associated to the occurrence of the hemolytic component observed in CDA II patients by means of increased clusterization of this protein on RBC surface, which in turn leads to IgG binding and phagocytosis of RBCs.[31]

Both morphological features of the BM and biochemical alterations of RBC membrane proteins can be explained by the mutations in the causative gene SEC23B (chr 20p11.23) (Table 1). CDA II is an autosomal-recessive disease that belongs to the group of cytoplasmic coat protein (COP) II-related human genetic disorders.[32] SEC23B gene encodes the homonymous member of COPII complex, which is involved in the secretory pathway of eukaryotic cells. This multisubunit complex mediates anterograde transport of correctly folded cargo from the ER toward the Golgi apparatus. [33,34] Most of the cases (86%) show biallelic mutations in the SEC23B gene, although a subset of patients with an incomplete pattern of inheritance (14.0%) has been identified.[7] More than 80 different mutations in SEC23B have been described so far, even if recurrent variants have been also described.[6,7,35] An association between clinical expression and molecular heterogeneity was observed; in particular, we were able to define at least three different phenotypic groups SEC23B-related. However, it remains a broad margin of overlap between the various groups of patients.[7,36]

Moreover, most severe cases can be explained by the co-inheritance of an additional intra-erythrocytic defect (such as β- or α-thalassemia); for example, in six patients with transfusion-dependent (TD) anemia, we identified a mutation in HBB or HBA gene (lolascon, unpublished data). Despite the elevated number of described cases and identified mutations, the pathomechanism of CDA II is not yet well understood. Although no direct evidence exists, SEC23B could play an active role in assembly or deconstruction of the midbody, where it was identified in a proteomic screen.[37] The presence of SEC23B in this subcellular compartment could account for an explanation of the impaired cytokinesis observed in CDA II erythroblasts (Figure 2). Otherwise, the multinuclear phenotype could be secondary to the aberrant glycosylation of specific proteins required for cell division, leading to defects in this process.[38] However, it remains to unravel how alterations in an ubiquitously gene can result in clinical manifestations restricted to the erythropoietic tissue. The specificity of the CDA II phenotype seems to be due to the tissue-specific expression of SEC23B during erythroid differentiation.[33] Alternatively, it could be explained by the presence of erythroid-specific cargos (such as band 3), which might require high levels and full function of a specific COPII component to be correctly transported.[32]

The pathophysiology study is difficult mainly due to the absence of a reliable animal model. Different models of SEC23B-deficient mice have been generated without reproducing CDA II phenotype. Indeed, SEC23B deficiency results in different phenotypes in humans and mice.[39,40] In particular, the absence of phenotype in SEC23B-deficient mouse seems to be related to the different SEC23B/SEC23A expression ratio in murine and human tissues. Indeed, this ratio is higher in mouse pancreas compared to BM, whereas it is higher in human BM relative to pancreas.[40] Of note, SEC23A and SEC23B are paralogous components of the COPII complex. This observation is in agreement with the compensatory expression of SEC23A that seems to ameliorate the effect of low SEC23B expression alleles in CDA II patients.[38,41] So far, the only reliable in vitro model for CDA II is the SEC23B-silencing in K562 cells, which recapitulates the cytokinesis defect, with a significant increase of percentage of binuclarity and an increased size of nuclei in SEC23B-silenced cells.[33]

CDA type III

CDA III is the rarest form among classical CDAs. Wolff and Von Hofe in 1951 described four patients from an American family (a mother and her three affected children) with giant-size erythrocytes in peripheral blood and giant erythroblasts with up to 12 nuclei in the BM slides. They named this condition ‘familial erythroid multinuclearity’. Later, the largest CDA III family (actually six generations) living in the North Swedish county Västerbotten was described.[42,43] Most of our clinical knowledge about CDA III stems from affected individuals of this family. Patients show absent to moderate anemia with normal or slightly elevated MCV, and normal or faintly low relative number of reticulocytes. Twenty percent of patients received blood transfusions. Common symptoms are weakness, fatigue and headache, and these are more severe during infections,
following trauma, and during pregnancy. Jaundice and biliary symptoms are also reported; some patients required cholecystectomy.[44] However, unlike from other CDAs, none of the described patients show enlarged liver or spleen.

Hemolysis is also present as attested by low or absent haptoglobin and increased LDH. No significant changes in serum iron, transferrin or ferritin concentrations are observed. Iron staining of the urinary sediment shows hemosiderinuria. Serum thymidine kinase is markedly increased in all patients. In some cases, serum electrophoresis analysis showed a M-component, IgG-k type; in particular, one patient with myeloma and four with monoclonal gammopathy were described. Peripheral blood films show macrocytes, poikilocytes and occasional extremely large forms (gigantocytes). At optical microscopy, BM highlights erythroid hyperplasia, with the characteristic giant multinucleate erythroblasts; at EM, clefts within heterochromatin, autophagic vacuoles, iron-laden mitochondria and myelin figures in the cytoplasm were described.[42,43]

In 2013, after localization of the gene locus for CDA III to chromosome 15q21-q25,[45] both aforementioned families were selected for target sequencing: the same mutation, P916R, in KIF23 gene (chr 15q21) was identified as causative of the familial CDA III (Table 1).[44] KIF23 encodes a kinesin-superfamily protein MKLP1 that is a component of central spindle, a subcellular structure required for proper formation of the central spindle and the midbody and thus essential for cytokinesis. MKLP1 mutant affects the function of this protein during cytokinesis, leading to the formation of the large multinucleated erythroblasts found in BM of the patients (Figure 2).

In addition to familial cases, with autosomal-dominant inheritance pattern, some sporadic cases of CDA III were reported. They can exhibit severe anemia, mongoloid facies and mental retardation, malignant T-cell lymphoma, Hodgkin’s disease, development of severe iron overload and cirrhosis, and extramedullary hematopoiesis.[43] Recently, a CDA III sporadic case associated to antiphospholipid syndrome has been also reported.[46] The pattern of inheritance for these cases seems to be autosomal recessive: this can be explained with a variable phenotypic expression of the same genetic alteration or by the occurrence of different genetic abnormalities that lead to a similar phenotype.

**CDA type IV**

To date, four patients with CDA IV have been reported. [47,48] All of them are characterized by the occurrence of normocytic anemia, generally severe, with Hb ranging between 5 and 9.5 g/dL. Conversely to classical CDAs, reticulocyte count is normal or slightly increased with respect to the degree of anemia; it ranges between 16% with Hb 7–9 g/dL to 5–12% with Hb 7.4–9.5 g/dL.[48] Moreover, elevated values of Hbf (>30%) are observed. In two cases, anemia has been found in prenatal age, associated with hydrops fetalis and treated with intrauterine transfusions. Furthermore, elevated LDH and bilirubin, reduced haptoglobin and increased RBC fragility configure this anemia as hemolytic. Similarly to other forms of CDAs, it has been reported a case of hemosiderosis, whose blood transfusion regimen does not justify the iron accumulation.[48]

At physical examination, almost all patients exhibit splenomegaly; in two patients with short stature, increasing transfusion rate improved the growth. Thalassemic faces and female genitalia in a patient with male karyotype have been also described.[47,48]

At peripheral blood smear, anisopoikilocytosis, schistocytes, polychromasia and nucleated RBCs are noted. At BM, light microscopy erythroid hyperplasia, dyserythropoiesis signs, as basophilic stippling of polychromatic erythroblasts and erythrocyte, and internuclear bridging are observed. Finally, EM shows immature erythroid progenitors with atypical cytoplasmic inclusions, enlarged nuclear pores, invagination of nuclear membrane and marked heterochromatin.[47,48]

All four patients exhibit the same autosomal-dominant mutation (E325K), in heterozygous state, in KLF1 gene (chr 19p13.2) (Table 1). KLF1 (previously named ELKF, erythroid Krüppel-like factor) encodes the homonymous protein, which is an essential erythroid-specific transcription factor, member of the Krüppel-like factor family.[49] The aminoacidic sequence of KLF1 comprises three C2H2 zinc finger domains with highly conserved DNA-binding domains, essential for activation of target genes (Figure 2). KLF1 is a well-known transcriptional activator in erythropoiesis, but it also exerts transcriptional repression in megakaryopoiesis. It plays a critical role in regulating the switch between fetal and adult Hb expression and is required in terminal erythroid differentiation for the cell-cycle progression.[50]

There are several target genes under the regulation of KLF1; among these, CD44 and AQP1 show a reduced expression in CDA IV patients.[47,48] The role of transcriptional regulator of different genes and pathways may explain how different mutations in the same gene can result in disparate phenotypes.[49] Beyond CDA IV, in which the heterozygous KLF1-E325K mutation is classified as dominant-negative, there are several additional mutations classified as ‘loss of function’. In particular, KLF1 loss-of-function mutations in heterozygous state
have been associated to in(Lu)blood type and hereditary persistence of fetal hemoglobin; recently, homozygosity for null mutations has been associated to embryonic lethality.\[51\]

\textbf{CDA variants}

A lot of CDA variants have been described so far. They are either isolated CDA-like conditions or syndromic CDAs. The first is the X-linked thrombocytopenia with or without dyserythropoietic anemia, characterized by anemia of variable range, ranging from hydrops fetalis and transfusion-dependency to dyserythropoiesis without anemia, macrothrombocytopenia with hypo-granulated platelets and bleeding tendency. BM features are dyserythropoiesis, megakaryocytes decreased in number with cytoplasmic vacuoles and absence of platelet membrane demarcation. This is an X-linked recessive disease (Table 1), due to mutations in the X chromosomal gene GATA1 gene (chr Xp11.23), encoding for the zinc finger DNA binding protein GATA1. This latter belongs to the GATA family of transcription factors, involved in the regulation of hematopoiesis. In particular, GATA1 plays an essential role in the development and maintenance of both erythroid and megakaryocytic lineages (Figure 2).\[52\] There are different syndromic conditions associated to GATA1 mutations, in which thrombocytopenia can be associated to thalassemia, congenital erythropoietic porphyria or DBA-like disease.\[53\] Moreover, the co-inheritance of GATA1 and other CDA-gene mutations could explain the occurrence of more severe phenotypes.\[54\] GATA1 has two zinc finger domains: the C-terminal is necessary for DNA binding, while the N-terminal mediates interaction with FOG1 (friend of GATA1), a cofactor of GATA1. Of note, the most likely pathogenic mechanism in these disorders involves the GATA1–FOG1 interaction.\[55\]

Recently, X-linked dominant macrocytic dyserythropoietic anemia with elevated serum ferritin level (800 ng/mL) has been described in multiple female individuals within the same family. BM examination in the proband showed trilineage hematopoiesis with dyserythropoiesis, and no excess iron or sideroblasts. This condition is related to the presence of a coding mutation (Y365C) in the X chromosomal gene ALAS2 (chr Xp11.21). The pathomechanism of macrocytosis and dyserythropoiesis in this condition seems to be related to the loss of nonviable erythroid progenitors expressing the mutant allele that leads to a survival advantage for the WT allele-expressing cells, which in turn undergo an erythropoietic ‘effort’ affecting the differentiation and the cell divisions.\[56\]

A CDA has been described as part of the clinical picture of the Majeed syndrome, a rare autosomal-recessive disorder hallmarked by early onset of chronic recurrent multifocal osteomyelitis, inflammatory dermatosis and hypochromic microcytic anemia. In these patients, BM examination shows dyserythropoiesis, erythroid hyperplasia, up to 25% bi- and tri-nucleated erythroblasts. The responsible gene is LPIN2 (chr 18p11.31), encoding lipin-2, an ER-phosphatidate phosphatase, involved in the lipid metabolism.\[57\]

In 2009, a syndromic case of dyserythropoiesis associated with exocrine pancreatic insufficiency and calvarial hyperostosis, due to mutations in COX4I2 gene, was described. Blood smear showed anisopoikilocytosis, basophilic stippling and few normoblasts, while BM highlighted erythroid hyperplasia, megaloblastic changes, and bi- and multinucleated erythroblasts.\[58\]

Finally, a case of CDA and mevalonate kinase deficiency due to recessive mutations in MVK gene has been also reported. Severe anemia, recurrent episodes of fever, rash, cervical adenitis and abdominal pain were the main clinical findings. Moreover, the patient showed mild dysmorphic facial features, including downsloped palpebral fissures, hypertelorism and frontal bossing. BM film presented hypercellular with 6–8% of erythroid precursors exhibiting dyserythropoiesis, including forms of irregular nuclei, nuclear budding, binucleation and karyorrhexis.\[59\]

\textbf{Complications}

As effect of chronic anemia and hemolytic component, several complications are associated to CDAs. Hereinafter are described the most common complications reported in CDA patients.

\textbf{Iron loading}

In all CDAs, except in familial forms of CDA III, altered iron balance is observed. Indeed, secondary hemochromatosis represents the most harmful complication in CDA patients. This finding is mainly due to the increased but ineffective erythropoiesis, as seen in other inherited BM erythroid failures as well as in thalassemia syndromes, but it is surely also linked to both the transfusion regimen and the hemolytic component. This deregulation is mediated by the downregulation of the hepatic hormone hepcidin, which leads to increased iron absorption and systemic iron overload.\[60\] Several erythroid regulators have been proposed as signaling mediators for the transcriptional regulation of hepcidin. Among these, growth differentiation factor 15
(GDF15) and soluble HJV were suggested as suppressor of hepcidin in CDA I and II; indeed, increased levels of both modulator were found in CDA patients.[6163] The most recently described hepcidin regulator is the erythroid cell-derived hormone erythroferrone (ERFE), an erythropoietin-responsive gene whose expression is increased in BM and spleen from β-thalassemia intermedia mice.[64] However, the function of ERFE in humans remains to be investigated.

Approximately 30% of non-TD CDA II patients exhibit a tendency to iron overload (ferritin > 300 ng/mL) while 17% of them show marked hemosiderosis (ferritin > 600 ng/mL), more severe in adulthood than childhood. As expected, iron overload in TD patients is more relevant, depending on the frequency of transfusions. It represents the most harmful complication of the chronic anemia as it can lead to organ damage with subsequent failure of the involved tissues.[7] Moreover, in some cases iron overload can be the first sign of a CDA. In particular, in severe patients we can observe liver damage, up to cirrhosis, heart failure and diabetes mellitus. Furthermore, iron overload in the hypothalamus can induce hypergonadotropic hypogonadism with related subfertility and osteopenia. Severe cases could be related to co-inheritance of modifier mutations as polymorphic variants in the HFE gene.[65]

To prevent clinical complications, it is crucial to recognize early iron overload: easily available biochemical signs are high transferrin saturation and serum ferritin levels. Moreover, low levels of hepcidin and high values of GDF15 may be useful markers of iron overload.[66] In the past, liver biopsy was frequently performed to establish the amount of liver iron accumulation; currently, noninvasive techniques are preferred. SQUID is not common and gives information only about hepatic iron, while MRI-T2* is actually the most used for the evaluation of liver, pancreatic or cardiac iron.[67]

Hydrops fetalis

In severe cases of CDAs, anemia can be present also in prenatal age. In utero Hb low levels can induce hydrops fetalis. In this condition, treatment consists in intrauterine RBC transfusions. This clinical finding is more common in CDA I,[68] but it can be also found in other classical CDAs.[51,69]

Aplastic crisis

Aplastic crisis in CDA patients may occur as a result of viral infections, particularly from parvovirus B19, a member of the *Erythrovirus* genus. In these cases, a first prodromal phase with fever, abdominal pain and vomiting is observed and often underestimated by the patient. Afterward, important tiredness and paleness (even in patients before jaundiced) appear. Biochemical signs are severe anemia, which often needs RBC transfusions, and very low reticulocyte count. The aplastic crisis usually resolves by the appearance of specific antibodies, generally within few weeks, with subsequent progressive improvement of anemic state.

**Gallstones and hyperbilirubinemia**

Jaundice is a very common sign of CDAs. High levels of unconjugated hyperbilirubinemia are due to increased production associated with ineffective erythropoiesis. In most of the CDAs patients, it was already noted in neonatal period: we estimated that almost 62% of CDA II patients have neonatal jaundice.[7] Moreover, in adult CDA II patients the co-inheritance of SEC23B biallelic mutations and UGT1A1 (TA)7/(TA)7 genotype, causative of Gilbert’s syndrome, leads to a further rise of the bilirubin levels with subsequent increased rate of gallstones. This is clearly visible in CDA II siblings with different UGT1A1 genotypes.[70] In the presence of symptomatic gallstones, cholecystectomy is indicated in patients with each type of CDA, following the normal practice for cholelithiasis.[71]

**Hypersplenism**

Hypersplenism is one of the most common signs found in CDAs: about 84% of adults CDA II show splenomegaly,[7] and three out four CDA IV patients described.[47,48] It was never observed exclusively in familial CDA III. Although it is a very common feature, currently spontaneous rupture are not reported. Generally, the size of the spleen is not clinically relevant; only in few patients with most pronounced hypersplenism is it possible to observe thrombocytopenia, but spontaneous bleeding was not described [lolascon, unpublished data; 7].

**Refractory leg ulcers**

Rare CDA patients with refractory leg ulcers were described, but this occurrence is less common when compared to individuals with other congenital anemias, such as sickle cell anemia and thalassemia. Etiology seems to be multifactorial, probably an important role is played by tissue hypoxia due to either anemia or thrombosis, which is more frequent in postsplenectomized patients.[72]
Management of CDA patients

CDAs are a complex group of diseases with several genetic subtypes; within each subgroup, a wide range of phenotypic manifestations can be observed. In particular, in severe cases and in young patients it is important to prevent, detect early and properly treat the complications of these diseases.

Follow-up

For CDA patients with positive family history, the prevention starts in utero, with careful search of hydrops at prenatal ultrasound scanning; in case of fetal anemia diagnosed by Doppler ultrasound, in utero transfusions should be performed.

At birth, CDA patients show jaundice and anemia more frequently compared to the normal population. Moreover, in the early childhood (2–3 years), the BM undergoes greater effort compared to middle childhood. Thus, a frequent evaluation of the complete blood count (CBC) should be achieved (at least every three months); otherwise, the iron balance may be assessed less frequently due to the low probability of iron overload in the youngest patients (Figure 4).

In middle childhood/adolescence, CBC can be performed every six months in the absence of TD. Instead, in TD child/adolescents, CBC and iron balance should be evaluated in relation to the transfusion regimen. This is true also for adult TD patients, in which CBC and iron overload have to be assessed dependently to the degree of anemia (Figure 4). Finally, we should also consider that during childhood/adolescence the infection by parvovirus B19 is more frequent, leading to possible aplastic crises, as mentioned in this review. In non-TD CDA adults with moderate-to-severe anemia (Hb ≤ 9 g/dL), we recommend to perform CBC evaluation more often than ones with mild anemia.

Figure 4. Flow diagram for the management of congenital dyserythropoietic anemia (CDA) patients. The three flow diagrams show the management of CDA patients divided according to (1) the age of the patients (early/middle childhood and adolescence vs. adulthood); (2) the transfusion regimen (transfusion dependent (TD) vs. non-TD patients). CBC: complete blood count; Hb: hemoglobin.
In the absence of sign and symptoms of hemolysis, iron balance should be examined annually by evaluation of ferritin and transferrin saturation. Furthermore, since the second decade, MRI-T2* should be recommended every five years or with ferritin >600 ng/mL in order to evaluate hepatic and cardiac iron loading.

Except for children younger than 2 years, spleen size and gallbladder contents have to be valued every year in the absence of symptoms (Figure 4).

Finally, supplementation of vitamin B12 and folic acid is recommended in all CDA patients to prevent their deficiency, which is common in ineffective erythropoiesis syndromes.

**Therapeutic approaches**

Presently, the standard treatment for severe anemia (Hb < 7 g/dL) in CDA patients is RBC transfusions. In the presence of chronic severe anemia, TD regimen can be carried out with a frequency of transfusions that depends on the degree of anemia.

In selected cases, bone marrow transplantation (BMT) is a possible treatment: so far, BMT has been successfully performed in two CDA I[73,74] and seven CDA II patients.[75,76]

In some TD CDA II patients, splenectomy has abolished or reduced the number of transfusions. However, in non-TD CDA II patients, this intervention does not revert the phenotype, but generally induces only a slight increase of Hb. Conversely, splenectomy in seven CDA I patients does not seem to result in any Hb improvement.[11]

For some CDA I patients, a response to interferon (IFN)-α2a, IFN-α2b or PegIFN-α2b has been documented; to date, only a limited number of individuals have been treated, with a high variability.[77]

Finally, for the treatment of iron overload, regular phlebotomies are often contraindicated due to anemia; instead, iron chelators are preferred. Currently, there are no guidelines for chelation treatment in the CDAs, but guidelines for chelation in thalassemic patients are commonly used.[78]

**Expert commentary**

One of the most debated aspects on the management of CDA patients is the indication for splenectomy. Currently, seven cases of splenectomized CDA I patients have been reported, in all cases with no Hb improvement. No cases of splenectomized patients affected by CDA III or CDA IV have been described. Conversely, splenectomy is often performed in CDA II patients, particularly before achieving a correct diagnosis. Non-TD CDA II patients show a moderate increase in Hb concentration (approximately 1 g/dL) after splenectomy intervention.[7] However, the extent of response is highly variable even within the same family. Currently, the reason for this variability is not yet known. This surgical intervention may reduce or abolish the need for transfusion in TD patients or in those with severe symptoms, thus avoiding recourse to the BMT. Moreover, in these subjects splenectomy leads to a decrease of the iron accumulation, enhanced by the transfusion regimen. Despite splenectomy being indicated in TD patients and in those with very severe anemia, it does not seem to reverse the phenotype and, therefore, it is not indicated in CDA II patients with mild or moderate anemia.

**Five-year view**

The variety of unspecific and overlapping phenotypes observed in CDA patients, even in those sharing the same genetic pathogenesis, often hampers a correct clinical management of affected individuals. In the past decades, scientific community has lavished much effort and achieved great success in identifying genes and their variants that contribute to an array of diseases. While the identification of these genetic variants has enriched our knowledge on the etiological bases of diseases, there continues to be a substantial gap in our understanding of the genetic factors that modify disease severity.[79] Thus, beyond achieving a definitive diagnosis, knowing the genetic basis of these patients can be valuable also for guiding treatment. NGS plays a pivotal role in this context; indeed, this technology is largely used either in disease gene discovery or in clinical use for establishing a genetic diagnosis. The implementation of NGS in clinical practice is already a matter of fact. This will result in increased knowledge of genetic and genomic differences among individuals that gradually will lead to shift the focus from population-based to patient-individualization of the clinical management.[80]

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Key issues

- Congenital dyserythropoietic anemias (CDAs) are rare/very rare hereditary diseases that are counted as subtypes of bone marrow failure syndromes, with morphological erythroid abnormalities and ineffective erythropoiesis as predominant mechanism of anemia.
- CDA Ia and CDA II show the highest prevalence, particularly in Europe where founder mutations have been identified in CDA1 and SEC23B genes.
- Three classical types were defined by marrow morphology; however, six different genetic types were recognized.
- The pathomechanisms of CDAs involve the regulation of DNA replication and cell division.
- CDAs can be misdiagnosed with clinically related hemolytic anemias. This is the case of CDA II, which shares several clinical findings with hereditary spherocytosis.
- Differential diagnosis is essential for addressing both follow-up and management of the patients.
- The most harmful complication of CDAs is iron overload, which is due to the increased but ineffective erythropoiesis, but also enhanced by transfusion regimen.
- Follow-up of CDA patients is essential for avoiding the complications related to the chronic anemia.
- The application of new technologies, as next-generation sequencing, will allow understanding the genetic factors that modify disease severity beyond achieving definitive diagnosis.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest


**First demonstrated that CDA Ia is due to mutations in the CDAN1 gene.**


* This elegant study opens new perspectives in knowledge of CDAI pathogenesis.


**First demonstrated that CDA Ib is due to mutations in the C1SORF41 gene.**


**First demonstrated that CDA II is due to mutations in the SEC23B gene.**


**Demonstrated that SEC23B deficiency results in different phenotypes between mice and humans.**


**First demonstrated that CDA III is due to mutations in the KIF23 gene.**


**Describes the KLF1 as a causative gene of CDA IV.**


**An interesting study first described X-linked dominant macrocytic dyserythropoietic anemia in female individuals with a specific mutation in the ALAS2 gene.**


**First describes the identification of the new erythroid hepcidin regulator.**


