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REVIEW ARTICLE

Modeling congenital dyserythropoietic anemia in genetically modified mice

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Abstract

Congenital dyserythropoietic anemias (CDAs) are a group of inherited disorders distinguished by ineffective production of red blood cells and peculiar abnormalities in the precursors from which red blood cells arise. The identification of disease-causing mutations and CDA-associated genes is rapidly improving the accuracy of diagnosis, aided by the growing accessibility of next-generation sequencing. Currently, it is much easier to identify the morphological abnormalities and classify different CDA types; however, a range of suitable, experimentally tractable models is needed in order to understand the pathogenic mechanisms at the molecular level.

This review explains the basic concepts related to CDAs, covers different genetically modified mouse lines that are available for CDA researchers, and highlights the challenges inherent to modeling human disease in another species.

Key words: congenital dyserythropoietic anemia, knockout mouse, erythropoiesis

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Erythropoiesis

Erythropoiesis is a 'finely-crafted' cellular differentiation and maturation program that begins with hematopoietic stem cells (HSCs) and eventually leads to the production of mature red blood cells (RBCs) [1, 2]. In this multi-step process, HSCs differentiate into common myeloid progenitors that give rise to either a granulocyte-monocyte progenitor (GMP) or a megakaryocytic-erythroid progenitor (MEP). MEPs can differentiate into megakaryocytes or continue the erythropoietic path by becoming burst-forming unit-erythroid progenitors (BFU-E). Further maturation of these committed erythroid precursor cells (EPCs) into mature RBCs is called 'terminal erythropoiesis'. RBC production is regulated by several cytokines, post-translational modifications of histones, translational factors, cofactors, and miRNAs at different stages of differentiation. Extrinsic cytokines, erythropoietin (EPO) and the stem cell factor (SCF), are known to play important roles in the early stages of erythroid differentiation.

Acquisition of EPO receptors (EPOR) at the BFU-E stage is vital for cell survival and progression through the colony-forming unit-erythroid stage (CFU-E) [3, 4]. Erythroid maturation transpires within erythroblastic islands located in the bone marrow of adults. The fetal liver functions as the main site of erythropoiesis during gestation. In both cases, the process is very similar at the cellular level, and is referred to as 'definitive erythropoiesis'. At a very early stage of embryonic development, a different form of so-called 'primitive erythropoiesis' takes place directly in the bloodstream, without the formation of erythroblastic islands [5].

This review is mainly focused on definitive erythropoiesis, unless indicated otherwise. Each erythroblastic island consists of nearly 30 erythroblasts at different maturation stages, surrounding a central macrophage. The central macrophage interacts with erythroblasts, provides instructions for their proliferation and differentiation, and

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supplies them with iron [6]. EPCs undergo morphological changes from proerythroblasts to basophilic (baso-E), polychromatic (poly-E), and orthochromatic erythroblasts (ortho-E). This phase is marked by increased hemoglobin production, cell size reduction, and nuclear condensation, resulting in nuclear extrusion known as 'enucleation' [7]. Enucleated erythroblasts, called reticulocytes, are released into the bloodstream, where the final maturation stage takes place. Reticulocytes lose the Golgi apparatus, ribosomes, endoplasmic reticulum (ER), and mitochondria. In some mammals, including humans, the reticulocyte loses an additional 20–30% of the cell surface to attain a biconcave shape [8, 9].

The lifecycle of human erythrocytes is c.110–120 days, and about 1% of cells are recognized as senescent and removed from the circulation daily. Therefore, the bone marrow should produce the required number of erythrocytes and release them into the circulation on a daily basis. In cases where the production, differentiation, or maturation of erythrocytes is impaired, this results in an abnormal number of RBCs. If this impairment is permanent, a state of chronic anemia develops. Investigation of genetic factors that lead to various forms of anemia aids the diagnosis and treatment of these disorders, while simultaneously expanding the knowledge of the mechanisms that control erythropoiesis.

This review is focused on congenital dyserythropoietic anemias (CDAs) that are caused by mutations in genes involved in terminal erythropoiesis. The scarcity of samples from CDA patients hampers functional studies. Differentiation of patient-derived induced pluripotent stem cells into erythroid precursors can partially overcome this limitation. However, erythropoiesis is influenced by specific niches of the bone marrow, fetal liver, and spleen. *In vivo* models that can be experimentally manipulated are needed to explain how CDA-associated proteins function, which of their roles are intrinsic to erythropoietic precursors, and which aspects of the disease depend on genetic background, age or erythropoietic stress. To aid progress in this field, we summarize the efforts and challenges of modeling CDA in genetically modified mice.

Congenital dyserythropoietic anemias

CDAs are a diverse group of rare genetic disorders characterized by inefficient erythropoiesis with distinct morphological features in the late erythroblasts that result in cytopenia. CDAs primarily affect the differentiation-proliferation process of terminal erythropoiesis. Characteristic morphological anomalies in the late erythroblasts, accompanied by the presence of binucleated or multinucleated erythroid cells, were one of the original diagnostic features of CDAs [10, 11].

However, these features are not uniquely linked to CDAs, and can also be seen in patients with erythropoietic stress, premature birth, and iron deficiency. Thus, diagnosing CDAs poses a challenge. Some of the symptoms manifested in patients with CDAs are jaundice, splenomegaly, anemia, reduced hemoglobin concentration, and suboptimal reticulocyte response for the degree of anemia [12]. CDAs are a heterogeneous group of hypoproliferative anemias that can be classified into five categories based on specific histological findings and genetics: CDA 1, 2, 3, transcription-factor-related CDAs, and CDA variants [13, 14]. These divisions are useful in clinical practice and reflect differences in the pathogenic mechanisms, although cross-talk between molecular pathways and gene products from separate CDA classes does occur (Figure 1A).

CDA type 1

CDA 1 is an autosomal recessive disorder associated with severe or moderate anemia, which is mostly macrocytic. CDA 1 is also characterized by relative reticulocytopenia and congenital disabilities, which include chest deformity, short stature, and skeletal abnormalities of distal limbs [15]. At the late erythroblast stage, 2.4–10% of the cells are bi-nucleated and the nuclei are often at different stages of maturation [16, 17]. Thin internuclear chromatin bridges are present in between the nuclei. Adjacent erythroblasts may be connected by cytoplasmic bridges and internuclear chromatin, which is observed in c.79% of those affected [18]. The most striking morphological feature of CDA 1 is the appearance of the nucleus under the electron microscope. This is described as "spongy" or "Swiss-cheese" due to the structure of heterochromatin, which is denser than normal. Nuclear-membrane-lined cytoplasmic intrusions may also be present.

Two genes associated with CDA 1 have been identified so far: *CDAN1* and *CDIN1* (C15orf41) [19, 20]. Bi-allelic mutations account for almost 90% of cases [19]. The coincidence of homozygous and compound heterozygous null mutations has not been observed, suggesting that the loss of either is lethal. Chromatin reassembly, DNA replication, and DNA repair are postulated to be the primary functions of codanin-1 [16]. It is also possible that some aspect of intracellular transport of proteins is disrupted by codanin-1 mutations, as the intermediate erythroblasts of CDA 1 show the aberrant distribution of heterochromatin protein-1 (HP-1a) in the Golgi apparatus. In intermediate erythroblasts, codanin-1 is partly colocalized with SEC23B, the protein mutation responsible for CDA 2, indicating a defect in the intracellular transport pathway in both CDA 1 and CDA 2 [21]. In non-erythroid cells, transcription factor E2F1, the main regulator of G1/S transition, directly initiates codanin-1 transcription and increases its levels during the S phase [22]. Furthermore, a cell-cycle regulated codanin-1 binds the anti-silencing factor-1 (ASF1), which acts on the assembly and disassembly of the nucleosome.

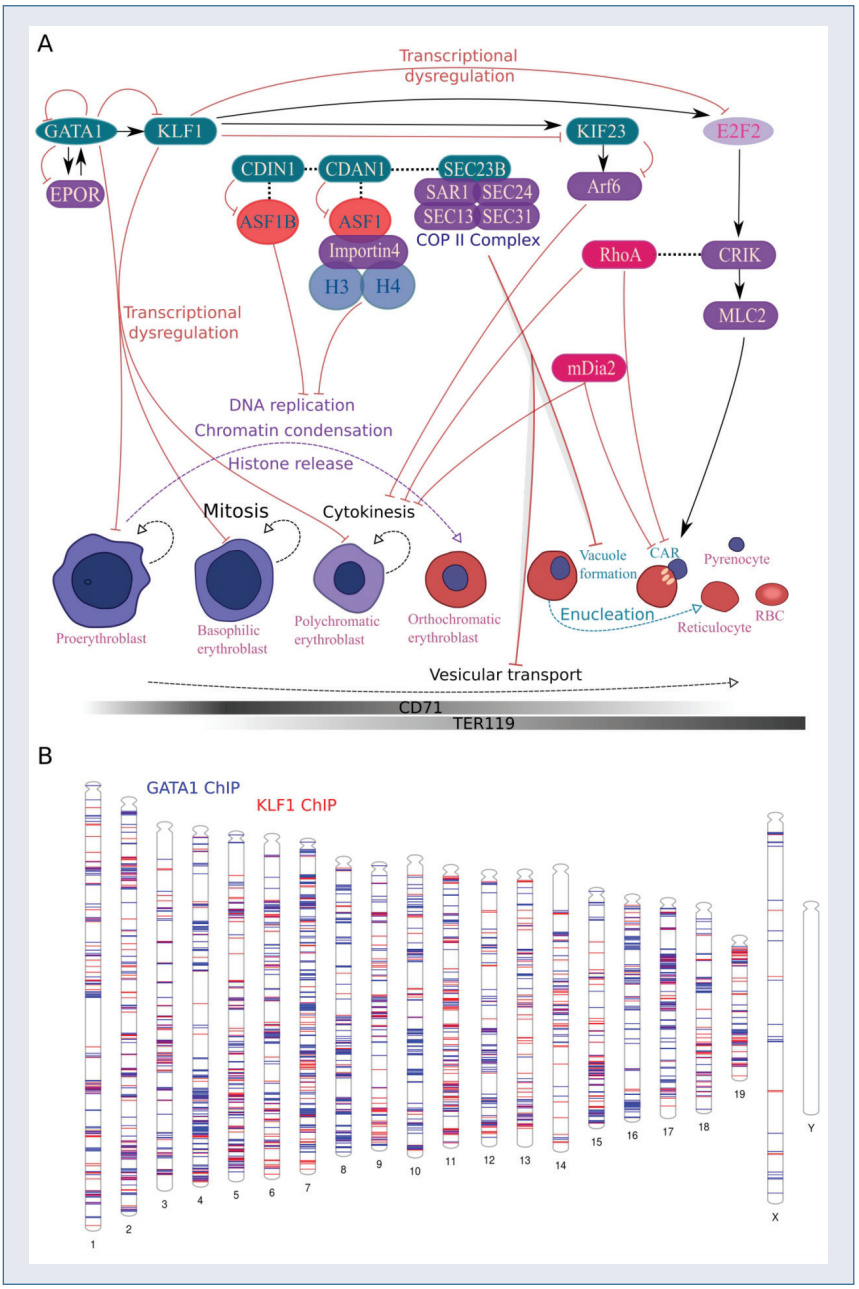

Figure 1. Proteins involved in pathogenesis of congenital dyserythropoietic anemia (CDA) participate in terminal erythropoiesis: **A.** Multiple cellular processes are required for maturation from rapidly dividing proerythroblasts to mature red blood cells (RBCs), including activation of erythropoietic lineage gene expression program, multiple rounds of DNA synthesis, and mitotic division followed by cytokinesis that occurs up to basophilic erythroblast stage, ongoing vesicular transport of membrane proteins, including transferrin receptor CD71 and marker of mature erythrocytes (TER119). Progressive chromatin condensation is accompanied by release of histones from nucleus, nuclear polarization, formation of large vacuoles between nucleus and reticulocyte, and enucleation. Known CDA proteins (cyan blue), functioning as transcription factors (GATA1 and KLF1), interact with histone chaperones (CDAN1, CDIN1), engage COPII complex (SEC23B), and participate in cytokinesis (KIF23). Other proteins that are inactivated in mouse models which mimic CDA III are represented in pink. Solid lines with full arrowheads pointing from upstream to downstream molecules signify functional dependence; dashes indicate known biochemical interactions, dashes with empty arrowheads depict key cellular events, and red lines with blunt stop-ends represent pathways and processes potentially disrupted by CDA-causing mutations. Diagram is simplified and organized around topics discussed herein; **B.** CDA causing mutations in GATA1 and KLF1 must cause pleiotropic effects as these factors bind multiple genomic locations in erythropoietic precursors. GATA1 ChIP-seq peaks confirmed by ChIP-Chip (blue) and KLF1 ChIP-seq peaks in E14.5 fetal liver erythroblasts (red), based on two example studies visualized in PhenoGram [53[Supp GATA1peaks.xls], 54[Table S3], 55]. Actual impact of these mutations needs to be assessed individually, as only some binding sites correspond to genomic regions that regulate transcription, and some mutations may lead to ectopic binding

Codanin-1 is a negative regulator of ASF1 and a part of the ASF1-H3-H4-importin-4 cytosolic complex [23]. In the nucleus, codanin-1 dissociation from the complex allows ASF1 to bind other histone chaperones and helps transport histones for chromatin assembly [16]. CDIN1 is localized in either the cytosol or the nucleus, suggesting a dual function of the protein. Both CDAN1 and CDIN1 are enriched in the nucleoli of erythropoietic cells [24], and nucleolar abnormalities are a recognized feature of CDA 1.

Interestingly, CDIN1 is homologous to restriction endonucleases and a high throughput protein interaction survey has indicated that it interacts with ASF1B [20, 25], adding to the hypothesis that DNA replication and chromatin assembly are hampered in CDA 1. *Cdan1* knockout mice die at an early embryonic stage of development (E6.5) [26]. There is no information on *Cdin1* knockout mice in the literature, but The International Mouse Phenotyping Consortium (www.mousephenotype.org) lists the homozygous *Cdin1*$^{em1(IMPC)J}$ allele as lethal at embryonic day 9.5. Only some of the known mutations in these proteins alter erythropoiesis and lead to CDA 1.

One hypothesis is that sub-lethal mutations primarily affect the rapidly dividing and maturing erythropoietic precursors. Multiple cycles of chromatin remodeling are necessary during the subsequent cell divisions, as well as for the final condensation of the nucleus. Nuclear condensation might require the eviction of specific histones from the DNA [9]. Caspase 3 activity-dependent lamin B cleavage and chromatin condensation induce the release of major histones through a nuclear opening [27]. It is conceivable that CDA 1 erythroblasts fail to evict histones H3 and H4 efficiently enough to prepare for enucleation. Recent analysis of *Asf1b* knockout mice has confirmed the involvement of this gene in erythropoiesis, but the phenotype is relatively mild, with a slight reduction of RBC count and increase of hemoglobin content and mean corpuscular volume (MCV). Persistent embryonic globin expression in adults and enlarged spleen likely result from the activation of compensatory erythropoiesis [28].

CDA type 2

CDA 2 is the most frequent form of CDAs [17], clinically characterized by varying degrees of normocytic anemia, with normal or slightly increased reticulocyte number [10, 29]. CDA 2 patients usually suffer from jaundice and splenomegaly due to the destruction of cell membranes in RBCs, which might result in the release of hemoglobin from disrupted cells. CDA 2 patients show a wide spectrum of clinical symptoms; about 10% of cases are asymptomatic, whereas 20% of them are dependent on blood transfusions [30, 31]. CDA 2 has distinct erythroid hyperplasia, with hypercellularity in the bone marrow just as CDA 1. The most striking morphological feature of CDA 2 is the presence of binucleated cells with both nuclei at the same stage of erythroid maturation. Morphologically, the diagnosis of CDA 2 is definite when at least 10% of erythroblasts are binucleated, with over 2% of the cells having a fragmented nucleus and irregular chromatin distribution in the cytoplasm [17, 32]. A discontinuous double membrane is present in the mature CDA 2 erythroblasts in electron microscopy, which is likely formed by large vesicles or cisterns of the ER that are positioned just below the plasma membrane [16].

SEC23B, the causative gene of CDA 2, encodes the cytoplasmic COPII (coat protein) component, which has a role in the secretory pathway of eukaryotic cells [33]. The COPII complex is responsible for the transport of correctly folded cargo from the ER to the Golgi apparatus after secretory cargo accumulation and membrane deformation [34]. Missense mutations in *SEC23B* are responsible for 52% of CDA 2 cases, nonsense mutations for 20%, and intronic for 13%. The remaining cases are caused by small insertions or deletions. CDA 2 is inherited in an autosomal recessive manner. Nonetheless, only one mutated allele of *SEC23B* has been found in 13% of cases, suggesting that a second unidentified mutation is present in the non-coding, regulatory region of the gene [35]. Occasionally, patients are diagnosed with CDA 2 based on bone marrow and biochemical analysis, but no *SEC23B* mutation is involved, as indicated by linkage exclusion.

This finding suggests that there might be another gene involved in CDA 2 [36]. Clinical symptoms vary depending on the compound heterozygosity of the mutations, such as missense and nonsense mutations [37]. In CDA 2 patients, no homozygous or compound heterozygous null mutations have been found, indicating that the absence of SEC23B is lethal. The expression of *SEC23B* is ubiquitous, but the effects of the known mutations of this gene are limited to the erythroid lineage. This phenotype could be explained by the hypothesis that SEC23B expressed in the hematopoietic system participates in terminal erythropoiesis in humans [33, 38], playing a role in vesicle trafficking by correctly transporting erythroid-specific cargoes from the ER to the Golgi apparatus with the help of the COPII complex [39].

Additionally, impairment of cytokinesis in CDA 2 erythroblasts suggests a possible primary function of SEC23B in the midbody assembly [39]. The presence of two hypomorphic alleles manifests in mild CDA 2 symptoms and is accompanied by increased expression of highly homologous SEC23A that could potentially compensate for the reduced expression of SEC23B [39]. The pathophysiology of CDA 2 is particularly challenging to establish due to the lack of a reliable animal model. *Sec23b*-deficient mice (*Sec23b*$^{gt/gt}$) die shortly after birth, due to pancreas and salivary gland degeneration. Surprisingly, they do not display any obvious signs of anemia [40]. The conditional *Sec23b* knockout specific to erythropoietic cells is viable, yet it does not develop anemia [41]. Moreover, fetal

liver cells (FLCs) isolated from *Sec23b*$^{gt/gt}$ donor mice were equally competent as wild-type FLCs in re-establishing erythropoiesis when transplanted to previously irradiated acceptor mice [41].

This discrepancy between human and mouse phenotypes could be explained by the difference in the temporal expression pattern of SEC23B and SEC23A proteins during erythroblast maturation in the two species [38]. The expression of SEC23B is maintained from the proerythroblast throughout the orthochromatic erythroblast stage in both species. SEC23A protein level drops sharply in transition from polychromatic to early orthochromatic erythroblast in humans, but not in mice.

This notion is supported by a comparative mouse and human mRNA expression study that revealed that human *SEC23A* mRNA level begins to decline at the early basophilic stage, and falls to undetectable levels in late basophilic erythroblasts, while the mouse gene continues to be expressed [42]. An intriguing possibility is that the disparate phenotypes and relative expression level differences of SEC23A and SEC23B that are observed between humans and mice could be a sign of an evolutionary shift in function between these two closely related proteins [41].

It has been recently reported that double conditional (*Sec23a*$^{fl/fl}$*Sec23b*$^{fl/fl}$*Mx1-cre*) knockout mice die *in utero*, presenting symptoms of anemia. This confirms that compensation by *Sec23a* is the reason why *Sec23b* knockouts are not anemic [43].

CDA type 3

CDA 3 has an autosomal dominant inheritance pattern, and is the rarest of the three classical types of CDA [16]. Most CDA 3 patients studied to date have belonged to just two families, one American, the other Swedish [44–46]. The clinical symptoms in CDA 3 patients are usually mild, showing no or moderate anemia, normal or slightly increased MCV, slight relative reticulocytopenia, hemolysis, and jaundice [47]. Typically, splenomegaly does not occur. On the cellular level, erythroid hyperplasia, altered morphology of erythroid cells visible under light microscopy, and the presence of giant multinucleated erythroblasts in the bone marrow are the characteristic features of CDA 3. Electron microscopy reveals the presence of autophagic vacuoles, clefts with heterochromatic, iron-laden mitochondria, and myelin figures in the cytoplasm of the erythroblasts [48]. Additionally, some CDA 3 patients from the Swedish family had disrupted vision, degeneration of the macula, as well as angioid streaks in the retina. Additionally, some patients have developed monoclonal gammopathy and multiple myeloma [45]. *KIF23*, the gene associated with the autosomal dominant inheritance of CDA 3, encodes a kinesin-superfamily molecule, mitotic kinesin-like protein 1 (MKLP1) [49, 50]. MKLP1 is a mitotic protein that interacts with adenosine diphosphate-ribosylation factor 6 (ARF6) and is essential for cytokinesis [49]. The ARF6-MKLP1 complex mediates the interaction between the cell membrane and the microtubule bundle at the cleavage furrow [49]. Reducing expression of ARF6 leads to the formation of binucleated and multinucleated cells, which is a striking morphology of CDA 3 erythroblasts. Transfection of GFP-MKLP1 harboring a CDA 3-specific mutation (P916R) in HeLa cells results in the presence of binucleated cells and the failure of cytokinesis. Wild-type GFP-MKLP1 does not cause this effect [45]. Impaired cytokinesis of erythropoietic precursors could explain the presence of giant multinucleated erythroblasts in the bone marrow of CDA 3 patients [45]. A mouse line created to replicate the exact CDA 3 mutation found in human patients has a P909R substitution in the *Kif23* gene [51]. Neither heterozygous nor homozygous mutant mice showed any symptoms of dyserythropoiesis, suggesting that this mutation is not as deleterious for mouse erythropoietic precursor cells as it is for their human counterparts. Complex alternative splicing of *KIF23/Kif23* that happens in both species, in conjunction with a hypothetical influence of the *KIF23* c.2747C>G, p.P916R mutation on splicing of *KIF23* mRNA in human erythropoietic precursors, has been proposed as a possible explanation for this discrepancy [51].

Transcription factor-related CDAs

Mutations in KLF1 and GATA1, two transcription factors (TFs) involved in erythropoiesis [52], cause CDA IV and X-linked thrombocytopenia associated with dyserythropoietic anemia (XLTDA), respectively. The common feature of TF-related disorders is that the expression of many TF target genes is affected, making the exact pathogenic mechanism difficult to elucidate. The case of KLF1 and GATA1 is no different, as each of them is known to bind several thousand sites in the genome (Figure 1B) [53–55]. Even though only a few hundred of these are associated with experimentally confirmed target genes, the task of evaluating all the potential consequences of disease-causing mutations is daunting.

There have only been eight recorded CDA 4 patients [56–60]. CDA 4 is distinguished by severe hemolytic anemia, normal or slightly increased reticulocyte count, and elevated levels of fetal hemoglobin. Hypercellular bone marrow contains immature erythroid progenitors with binucleated or multinucleated cells. Electron microscopy confirms CDA 4 when an engulfed nuclear membrane, marked heterochromatin, and atypical cytoplasmic inclusions are seen in cells of the erythroid lineage [56, 57]. CDA 4 is inherited in an autosomal dominant manner, and caused by mutations in the erythroid-specific transcription factor 1 gene (*KLF1*). KLF1 is involved in terminal erythropoiesis

and essential for the transition of hemoglobin expression from the fetal to the adult form [58]. Recently, some of the molecular consequences of CDA 4-specific mutation have been deciphered.

Erythroid cells differentiated from induced pluripotent stem cells (IPSCs) derived from a CDA 4 patient presented deregulated expression of genes linked to erythroid identity, involved in membrane transport, iron-utilization, cytokinesis, cell-surface receptors, and cell-cycle regulators [60]. KLF1 mutation E325K alters the sequence of the second zinc-finger, a crucial DNA binding domain. Another CDA 4-causing mutation, G973A results in high fetal hemoglobin expression and a lack of erythroid cell surface markers CD44 and aquaporin 1 (AQP1) in circulating erythrocytes and erythroblasts [56, 57]. However, it is still unclear exactly which KLF1 target genes are responsible for dyserythropoiesis in CDA 4.

One approach to this problem involves analyzing the impact of different genetic mutations or posttranslational modifications on KLF1 activity towards specific targets and correlating the observed changes in transcriptional profiles with the severity of the cellular phenotypes [61–63]. Comparison of homozygous loss-of-function mutations to hypomorphic alleles and haploinsufficiency caused by heterozygous frameshift or nonsense mutations should be performed within a frame of a consistent experimental system *in vitro*, while the most interesting or clinically relevant mutations could be modelled *in vivo*.

The need for new accurate models is evident in light of the documented discrepancies between human disease and the phenotypes of different *Klf1* mutant mouse lines. Complete loss of *Klf1* in mice causes severe anemia that leads to embryonic lethality and a cellular phenotype that recapitulates beta-thalassemia, rather than CDA 4 [64, 65]. In line with this observation, no human patients with a homozygous null *KLF1* mutation were known until a surprising recent report of severe neonatal hemolytic anemia with jaundice and kernicterus, due to compound heterozygous loss-of-function mutations in *KLF1* [66]. Decreased binding affinity of the CDA 4-related KLF1 E325K mutant to the consensus sequence (NCNCNCCCN) in the promoters of several target genes, including HBB, AQP1, and CD44 [67–71]. proves that, in addition to its dominant effect, it is also a partial loss-of-function mutation. Of the existing murine KLF1 mutant lines, the *Nan* strain had the highest potential to mimic CDA 4. *Nan* mice were created by N-ethyl-N-nitrosurea (ENU) mutagenesis [72–74] and carry an amino acid substitution (E339D) that affects the exact same position in the conserved, DNA-binding ZF2 domain as the human E325K mutation [75].

Surprisingly, *Nan* mice present a phenotype that resembles hereditary spherocytosis (HS) more than CDA [74]. It is unclear whether the interspecies differences in KLF1 functions or the opposing biophysical properties of the amino acids introduced by the human E325K and mouse E339D substitutions are responsible for the difference in erythropoietic phenotypes. In support of the latter, homology modeling suggests that *Nan* KLF1 has a higher affinity for the consensus binding motif than wild-type KLF1 [75]. Transcriptomic analysis of erythroid progenitors isolated from *Nan* mice reveals that only 52% of down-regulated, and 18% of upregulated, genes are known KLF1 targets [76, 77].

Prominent *Nan* KLF1 binding to ectopic sites in chromatin immunoprecipitation (ChIP-seq) experiments further highlights the complex consequences of semi-dominant KLF1 mutations [76]. Despite nearly four decades having passed since its appearance on the scene, the *Nan* model continues to yield general insights applicable to both HS and CDA 4.

XLTDA is caused by certain mutations in GATA1, a crucial regulator of erythroid and megakaryocyte differentiation. The exact phenotype differs substantially depending on the mutation. The symptoms include a tendency for bleeding, mild-to-severe anemia, and macro-thrombocytopenia with hypogranulated platelets. Anemia may improve with age. Some patients experience incidents of severe hemorrhage, and require transfusions [78]. Characteristic changes in the bone marrow of XLTDA patients include a decreased number of megakaryocytes that contain cytoplasmic vacuoles but lack platelet membrane demarcation, and dyserythropoiesis that is present in a subset of patients with GATA1 mutations.

The function of GATA1 in erythropoiesis has been extensively studied for several decades, and can be viewed as a model for comprehensive analysis of the role that a single gene plays in a complex biological process. Patients with GATA1 mutations may present with symptoms of beta-thalassemia, Diamond-Blackfan anemia, or CDA. Many of the GATA1 target genes are involved in erythropoiesis. In the context of dyserythropoietic anemia, GATA1 regulates the expression of *SEC23B* [79]. However, the significance of this fact might differ in humans and mice, given the previously outlined interspecies differences in the expression of *SEC23A*. Several mouse models that phenocopy the effects of human GATA1 mutations are available and have been thoroughly reviewed [80]. Complete *GATA1* knockouts die at an early embryonic stage due to severe defects in primitive and definitive fetal erythropoiesis [81]. Only the models best suited to studying GATA1 dependent dyserythropoietic anemia will be briefly discussed here.

Gata1$^{neo\Delta HS}$ is an allele obtained by targeted disruption of upstream regulatory sequence with a floxed neomycin resistance cassette. As a result, GATA1 expression is reduced 4–5-fold. Only 8% of the expected number of males is born, and they have severe anemia. Half of these mice die within 48 hours. Binucleate primitive erythrocytes are present in peripheral blood smears from both E11.5 and

E13.5 embryos. Dyserythropoietic features disappear around 4–5 weeks, suggesting that compensatory mechanisms are able to overcome the reduced expression of GATA1. Colony assays reveal a normal number of erythroid progenitors and a defect in their maturation [82, 83]. *Gata1*$^{\Delta IE}$ mice that lack the erythroid-specific first exon can also be used to study GATA1-dependent CDA. Hemizygous male embryos are severely anemic and die by E12.5, but conditional knockouts obtained using this allele have nucleated erythrocytes present in the peripheral blood [84]. Finally, a transgenic *Gata1*^{V205G} mouse line which expresses a mutated GATA1 that no longer interacts with FOG1 (Friend-of-GATA1) can rescue the complete *Gata1* knockout [85]. Only mice with high transgene expression are viable and suffer from thrombocytopenia and megakaryocyte defects. Surprisingly, the *Gata1* knockouts rescued by crossing to the *Gata1*^{V205G} transgenic mice do not have anemia, even though the V205G mutation targets the very same amino acid that is mutated in patients with severe dyserythropoietic anemia who carry the V205M substitution.

Syndromic forms of CDA

Apart from being a stand-alone condition, CDA is also present in the context of three unrelated genetic disorders. Majeed syndrome is caused by recessive mutations of lipin2 (*LPIN2*). Its manifestations include microcytic dyserythropoietic anemia, inflammatory dermatosis, and osteomyelitis. Erythropoiesis in the bone marrow is increased and 25% of erythroblasts are bi- or trinucleated [86]. LPIN2 is essential for lipid metabolism and affects phosphatidate phosphatase activity. Lpin2 knockout mice are viable and replicate many features of Majeed syndrome, including mild anemia with decreased levels of hemoglobin and reduced RBC volume [87].

A homozygous E138K mutation in COX4I2 causes exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis. *COX4I2* encodes the cytochrome c oxidase (COX) subunit 4 isoform 2, a structural component of the mitochondrial oxidative chain COX complex. The most severe symptoms include dysfunction and degenerative changes of the pancreas, hepatosplenomegaly, episodic jaundice, and life-threatening anemia that requires transfusions. Erythropoietic defects associated with this disorder include the presence of bi- and multinucleated erythroblasts, megaloblastic changes, and erythroid hyperplasia [88]. Surprisingly, *Cox4i2*$^{-/-}$ mice have normal pancreatic function, RBC count, and hematocrit and hemoglobin concentrations. Reduced airway responsiveness, progressive lung dysfunction, and reduced body mass and grip strength in females are the main findings in these mice [89].

There are two possible general classes of explanations for the stark difference between the human disease and the mouse model. The first is a typical interspecies difference in gene function. Since COX4I2 is a facultative subunit of the COX complex, expressed only in some tissues, it is possible that tissue-specific expression of its close paralog, COX4I1, differs between humans and mice. In support of this line of reasoning, *Cox4i2* mRNA expression level in the mouse pancreas is very low [89].

The second possibility is that the E138K mutation leads to more than just a functional loss of COX4I2. Perhaps the presence of the mutated protein prevents the activation of a putative compensatory mechanism that is triggered in *Cox4i2*$^{-/-}$ mice.

Aside from these possible explanations, it should be pointed out that there are only a few known cases of exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis, all of which come from just two families inhabiting two neighboring villages [88]. Is it possible that an undetected mutation, closely linked with the *COX4I2* locus, is responsible for some of the symptoms? The disease-causing mutation maps to an interval on chromosome 20, between markers D20S101 and D20S195. Only 12 of the 117 open reading frames (ORFs) present in this interval have been sequenced. Among others, an interesting candidate is *BCL2L1*, the closest genomic neighbor of *COX4I2* located on the opposite strand. Less than 20 kbp separates the 3' ends of both genes. Several members of the BCL2 family are involved in various aspects of erythropoiesis. *BCL2L1* codes for the BCL-XL (BCL-X) protein that is critically important for erythropoiesis [90–92]. Given the improved availability and the reduced cost of next generation sequencing, it is now feasible to sequence all the remaining genes in this interval to rule out any additional mutations.

Mevalonate kinase deficiency is caused by mutations in the *MVK* gene. Most of the 60+ known mutations cause hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) or mevalonic aciduria (MA), while some patients suffer from a skin condition known as porokeratosis. A compound heterozygous mutation carrier (310M/Y116H), with symptoms intermediate between HIDS and MA, also displays features of CDA [93]. Homozygous *Mvk* knockout mice are not viable, but heterozygous knockouts survive and are a good model of HIDS [94]. Interestingly, some of the *Mvk*$^{+/-}$ mice also display bone marrow hypocellularity, neutropenia and basophilic stippling of circulating RBCs, which indicates that hematological disturbances can be caused by *Mvk* gene dose reduction alone, and not just a specific point mutation.

Conclusions

Systematic progress in the identification of causative genes for the most common CDA types facilitates diagnosis and offers insights into the pathogenic mechanisms; however, the various cellular and molecular mechanisms of dyserythropoiesis are only partially understood.

Table I. Congenital dyserythropoietic anemia types and existing animal models

CDA type	Gene	Mouse model	Erythropoietic phenotype of model
CDA 1	*CDAN1* (AR)	***Cdan1***$^{-/-}$	Embryonic lethal (E6.5)
	CDIN1 (AR)	***Cdin1***$^{-/-}$ [em1(IMPC)J]*	Embryonic lethal (E9.5)
	Unknown	***Asf1b***$^{-/-}$	Mild dyserythropoiesis, persistent embryonic globin, increased fraction of immature EPCs in BM
CDA 2	*SEC23B* (AR)	***Sec23b***$^{-/-}$	Lethal (P0–P1), no anemia
		Sec23b$^{fl/-}$ ***(EpoR-cre***$^{Tg/+}$***)***	Viable, no anemia
	KIF23 (AD)	***Kif23***$^{P909R/+}$	Mice viable, active, no CDA or signs of any disease
		Kif23$^{P909R/\ P909R}$	
CDA 3	Unknown	***mDia2***$^{-/-}$	Lethal (E11.5) anemia, bi- and multinuclear embryonic EBs
		mDia2$^{fl/fl}$ ***(Mx1-cre)***	Defects in BM EBs
		RhoA$^{f/f}$ ***(EpoR-cre***$^{Tg/+}$***)*** ***(Mx1-cre)***	Enlarged primitive RBCs, poikilocytic, often multinucleated, failed definitive erythropoiesis, deletion with *EpoR-cre* is lethal (E16.5)
CDA 4	*KLF1* (AD)	***Klf1***$^{-/-}$	Embryonic lethal, severe anemia
		Nan	Hereditary spherocytosis
XLTDA	*GATA1* (XR)	***Gata1***$^{-/-}$	Lethal (E10.5–11.5), severe anemia
		Gata1^{05}	Embryonic lethal, severe anemia
		Gata1^{V205G} ***(Tg)***	Rescues *Gata1*^{05}, anemia, binucleate primitive RBCs
		Gata1$^{neo\Delta HS}$	Dyserythropoiesis, anemia
?	*VPS4A*	***Vps4a***$^{-/-}$ [tm1b(EUCOMM)Hmgu]*	Embryonic lethal (E18.5)
Majeed syndrome	*LPIN2*	***Lpin2***$^{-/-}$	Mild anemia, lower Hb concentration and MCV
EPIDACH	*COX4I2* (AR)	***Cox4i2***$^{-/-}$	Airway and lung defects, no erythropoietic (or pancreatic) phenotype
Mevalonate kinase deficiency	*MVK* (AR/CH)	***Mvk***$^{-/-}$	Embryonic lethal, heterozygotes survive, mild dyserythropoiesis
		Mvk$^{+/-}$	

*Public consortium allele type in square brackets; AR — autosomal recessive; EPCs — erythroid precursor cells; BM — bone marrow; AD — autosomal dominant; CDA — dyserythropoietic anemia; EBs — erythroblasts; RBCs — red blood cells; XR — X-linked recessive; Tg — transgenic line; Hb — haemoglobin; MCV — mean corpuscular volume; CH — compound heterozygous; EPIDACH — exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis

The complication which arises in studying the mechanism of terminal erythropoiesis in CDAs is the lack of a mouse model. Mouse models which have been generated to date (Table I) die during gestation or shortly after birth. Some of the mouse lines made to replicate specific human mutations do not develop CDA. Nevertheless, they might provide interesting information regarding the interspecies differences in erythropoiesis. Occasionally, a significant disparity between the mouse phenotype and the human disease might warrant re-evaluation of the initial findings, as in the case of *Cox4i2*.

Of the currently available models, the *Gata1*$^{neo\Delta HS}$ and the conditional *RhoA* and *mDia2* (*Diap3*) knockouts are likely to have the greatest impact on CDA research. The

first is close to recapitulating CDA naturally occurring as part of XLTDA, while the other two replicate several features of CDA 3, such as the presence of binucleated and giant multinucleated erythroblasts, due to a defect in cytokinesis [95, 96]. The complete knockouts of each of these genes in mice are lethal.

In the case of *RhoA*$^{fl/fl}$, inactivation with erythroid-specific *EpoR-cre*$^{Tg/+}$ still leads to embryonic lethality (E16.5) but it is late enough to analyze the defects of fetal, definitive erythropoiesis. Inducible inactivation with the *Mx1-cre* allows the study of the function of RhoA in adult erythropoiesis but requires complicated hematopoietic stem cell transplantation experiments into irradiated mice, and leads to defects not only in the erythroid lineage but also in the neutrophils, monocytes, and platelets [97].

Human CDA-causing mutations affecting either the *RHOA* or the *DIAP3* genes have not been identified so far, and therefore the status of the corresponding knockout mouse lines as faithful models of an actual human CDA, or simply as useful tools for replicating some aspects of the disease, is yet to be determined. Despite being instrumental in the above-mentioned studies, the inducible *Mx1-cre* system presents recognized challenges in itself [98]. Perhaps an inducible *EpoR-cre* line could be developed to improve temporal and cellular resolution of phenotypic characterization of the conditional gene knockouts in erythropoiesis.

The symptoms and hematological findings resembling CDA that are seen in certain patients do not fit any of the classical CDA types. Sporadic cases of unknown genetic background also exist. The establishment of the Congenital Dyserythropoietic Anemia Registry [99] is bound to accelerate the identification of novel CDA-associated genes and increase the need for new *in vivo* models. In fact, the first such gene is *VPS4A* [100, 101], a regulator of the ESCRT-III complex. Two probands have heterozygous *de novo* mutations R284W and G203E, and a third carries a homozygous A28V mutation.

Multinuclear erythroblasts in the bone marrow and circulating RBCs displaying transferrin receptor at the surface indicate that defects in cell division and endosomal vesicle trafficking are the main culprits. The gain-of-function or dominant-negative character of the heterozygous mutations is evident from the inheritance mode. It is more difficult to anticipate the degree of functional loss of VPS4A in the proband carrying the homozygous mutation, based on the phenotype and genetics alone. Homozygous knockout mice (*Vps4a*$^{tm1b(EUCOMM)Hmgu}$) die *in utero* (IMPC).

Due to the high throughput nature of these experiments, only four female and two male homozygous mutants have been observed. At E18.5, embryos were unresponsive to tactile stimuli, and were of abnormal size and abnormal body wall morphology. Given the complete penetrance of embryonic lethality of the knockout mice, it is likely that the human CDA-causing A28V mutation leads to only a partial loss of VPS4A function.

Undoubtedly, efforts aimed at developing animal models better suited for CDA research are increasing the understanding of the pathophysiology of this disease, which will eventually lead to new therapeutic strategies.

Authors' contributions

RK and PK wrote the review together.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; uniform requirements for manuscripts submitted to Biomedical journals.

References

1. Palis J. Primitive and definitive erythropoiesis in mammals. Front Physiol. 2014; 5: 3, doi: 10.3389/fphys.2014.00003, indexed in Pubmed: 24478716.
2. Mei Y, Liu Y, Ji P. Understanding terminal erythropoiesis: an update on chromatin condensation, enucleation, and reticulocyte maturation. Blood Rev. 2021; 46: 100740, doi: 10.1016/j.blre.2020.100740, indexed in Pubmed: 32798012.
3. Clarke BJ, Housman D. Characterization of an erythroid precursor cell of high proliferative capacity in normal human peripheral blood. Proc Natl Acad Sci USA. 1977; 74(3): 1105–1109, doi: 10.1073/pnas.74.3.1105, indexed in Pubmed: 265556.
4. Hara H, Ogawa M. Erythropoietic precursors in murine blood. Exp Hematol. 1977; 5(3): 161–165, indexed in Pubmed: 872906.
5. Yamane T. Mouse yolk sac hematopoiesis. Front Cell Dev Biol. 2018; 6: 80, doi: 10.3389/fcell.2018.00080, indexed in Pubmed: 30079337.
6. de Back DZ, Kostova EB, van Kr, et al. Of macrophages and red blood cells; a complex love story. Front Physiol. 2014; 5(9), doi: 10.3389/fphys.2014.00009, indexed in Pubmed: 24523696.
7. Kelley LL, Koury MJ, Bondurant MC, et al. Survival or death of individual proerythroblasts results from differing erythropoietin sensitivities: a mechanism for controlled rates of erythrocyte production. Blood. 1993; 82(8): 2340–2352, doi: 10.1182/blood.v82.8.2340.2340.
8. Waugh RE, McKenney J, Bauserman R, et al. Surface area and volume changes during maturation of reticulocytes in the circulation of the baboon. J Lab Clin Med. 1997; 129(5): 527–535, doi: 10.1016/s0022-2143(97)90007-x.
9. Moras M, Lefevre SD, Ostuni MA. From erythroblasts to mature red blood cells: organelle clearance in mammals. Front Physiol. 2017; 8: 1076, doi: 10.3389/fphys.2017.01076, indexed in Pubmed: 29311991.

10. Crookston JH, Crookston MC, Burnie KL, et al. Hereditary erythroblastic multinuclearity associated with a positive acidified-serum test: a type of congenital dyserythropoietic anaemia. Br J Haematol. 1969; 17(1): 11–26, doi: 10.1111/j.1365-2141.1969.tb05660.x, indexed in Pubmed: 5807784.
11. Heimpel H, Wendt F. Congenital dyserythropoietic anemia with karyorrhexis and multinuclearity of erythroblasts. Helv Med Acta. 1968; 34(2): 103–115, indexed in Pubmed: 5658197.
12. Kato K, Sugitani M, Kawataki M, et al. Congenital dyserythropoietic anemia type 1 with fetal onset of severe anemia. J Pediatr Hematol Oncol. 2001; 23(1): 63–66, doi: 10.1097/00043426-200101000-00016, indexed in Pubmed: 11196275.
13. Wickramasinghe SN, Wood WG. Advances in the understanding of the congenital dyserythropoietic anaemias. Br J Haematol. 2005; 131(4): 431–446, doi: 10.1111/j.1365-2141.2005.05757.x, indexed in Pubmed: 16281933.
14. Iolascon A, Andolfo I, Russo R. Congenital dyserythropoietic anemias. Blood. 2020; 136(11): 1274–1283, doi: 10.1182/blood.2019000948, indexed in Pubmed: 32702750.
15. Tamary H, Dgany O, Proust A, et al. Clinical and molecular variability in congenital dyserythropoietic anaemia type I. Br J Haematol. 2005; 130(4): 628–634, doi: 10.1111/j.1365-2141.2005.05642.x, indexed in Pubmed: 16098079.
16. Gambale A, Iolascon A, Andolfo I, et al. Diagnosis and management of congenital dyserythropoietic anemias. Expert Rev Hematol. 2016; 9(3): 283–296, doi: 10.1586/17474086.2016.1131608, indexed in Pubmed: 26653117.
17. Heimpel H, Kellermann K, Neuschwander N, et al. The morphological diagnosis of congenital dyserythropoietic anemia: results of a quantitative analysis of peripheral blood and bone marrow cells. Haematologica. 2010; 95(6): 1034–1036, doi: 10.3324/haematol.2009.014563, indexed in Pubmed: 20421275.
18. Resnitzky P, Shaft D, Shalev H, et al. Morphological features of congenital dyserythropoietic anemia type I: the role of electron microscopy in diagnosis. Eur J Haematol. 2017; 99(4): 366–371, doi: 10.1111/ejh.12931, indexed in Pubmed: 28755517.
19. Dgany O, Avidan N, Delaunay J, et al. Congenital dyserythropoietic anemia type I is caused by mutations in codanin-1. Am J Hum Genet. 2002; 71(6): 1467–1474, doi: 10.1086/344781, indexed in Pubmed: 12434312.
20. Babbs C, Roberts NA, Sanchez-Pulido L, et al. WGS500 Consortium. Homozygous mutations in a predicted endonuclease are a novel cause of congenital dyserythropoietic anemia type I. Haematologica. 2013; 98(9): 1383–1387, doi: 10.3324/haematol.2013.089490, indexed in Pubmed: 23716552.
21. Renella R, Hall G, Ferguson D, Wood W. Congenital dyserythropoietic anemias. Blood and bone marrow pathology. Elsevier, Philadelpia 2011: 235–244.
22. Noy-Lotan S, Dgany O, Lahmi R, et al. Codanin-1, the protein encoded by the gene mutated in congenital dyserythropoietic anemia type I (CDAN1), is cell cycle-regulated. Haematologica. 2009; 94(5): 629–637, doi: 10.3324/haematol.2008.003327, indexed in Pubmed: 19336738.
23. Ask K, Jasencakova Z, Menard P, et al. Codanin-1, mutated in the anaemic disease CDAI, regulates Asf1 function in S-phase histone supply. EMBO J. 2012; 31(8): 2013–2023, doi: 10.1038/emboj.2012.55, indexed in Pubmed: 22407294.
24. Scott C, Downes DJ, Brown JM, et al. Recapitulation of erythropoiesis in congenital dyserythropoietic anaemia type I (CDA-I) identifies defects in differentiation and nucleolar abnormalities. Haematologica. 2021; 106(11): 2960–2970, doi: 10.3324/haematol.2020.260158, indexed in Pubmed: 33121234.
25. Ewing RM, Chu P, Elisma F, et al. Large-scale mapping of human protein-protein interactions by mass spectrometry. Mol Syst Biol. 2007; 3: 89, doi: 10.1038/msb4100134, indexed in Pubmed: 17353931.
26. Renella R, Roberts N, Sharpe J, et al. A transgenic mouse model for congenital dyserythropoietic anemia type I. Blood. 2008; 112(11): 3455–3455, doi: 10.1182/blood.v112.11.3455.3455.
27. Gnanapragasam MN, Bieker JJ. Orchestration of late events in erythropoiesis by KLF1/EKLF. Curr Opin Hematol. 2017; 24(3): 183–190, doi: 10.1097/MOH.0000000000000327, indexed in Pubmed: 28157724.
28. Papadopoulos P, Kafasi A, De Cuyper IM, et al. Mild dyserythropoiesis and β-like globin gene expression imbalance due to the loss of histone chaperone ASF1B. Hum Genomics. 2020; 14(1): 39, doi: 10.1186/s40246-020-00283-3, indexed in Pubmed: 33066815.
29. Heimpel H, Anselstetter V, Chrobak L, et al. Congenital dyserythropoietic anemia type II: epidemiology, clinical appearance, and prognosis based on long-term observation. Blood. 2003; 102(13): 4576–4581, doi: 10.1182/blood-2003-02-0613, indexed in Pubmed: 12933587.
30. Russo R, Gambale A, Langella C, et al. Retrospective cohort study of 205 cases with congenital dyserythropoietic anemia type II: definition of clinical and molecular spectrum and identification of new diagnostic scores. Am J Hematol. 2014; 89(10): E169–E175, doi: 10.1002/ajh.23800, indexed in Pubmed: 25044164.
31. Bianchi P, Schwarz K, Högel J, et al. Analysis of a cohort of 101 CDAII patients: description of 24 new molecular variants and genotype--phenotype correlations. Br J Haematol. 2016; 175(4): 696–704, doi: 10.1111/bjh.14271, indexed in Pubmed: 27471141.
32. Tandon B, Peterson L, Norwood S, et al. Congenital dyserythropoietic anemia type II (CDA II) diagnosed in an adult patient. J Hematopathol. 2010; 3(4): 149–153, doi: 10.1007/s12308-010-0073-5.
33. Schwarz K, Iolascon A, Verissimo F, et al. Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II. Nat Genet. 2009; 41(8): 936–940, doi: 10.1038/ng.405, indexed in Pubmed: 19561605.
34. De Matteis MA, Luini A. Mendelian disorders of membrane trafficking. N Engl J Med. 2011; 365(10): 927–938, doi: 10.1056/NEJMra0910494, indexed in Pubmed: 21899453.
35. Iolascon A, Esposito MR, Russo R. Clinical aspects and pathogenesis of congenital dyserythropoietic anemias: from morphology to molecular approach. Haematologica. 2012; 97(12): 1786––1794, doi: 10.3324/haematol.2012.072207, indexed in Pubmed: 23065504.
36. Iolascon A, De Mattia D, Perrotta S, et al. Genetic heterogeneity of congenital dyserythropoietic anemia type II. Blood. 1998; 92(7): 2593–2594, indexed in Pubmed: 9746803.
37. Iolascon A, Russo R, Esposito MR, et al. Molecular analysis of 42 patients with congenital dyserythropoietic anemia type II: new mutations in the SEC23B gene and a search for a genotype-phenotype relationship. Haematologica. 2010; 95(5): 708–715, doi: 10.3324/haematol.2009.014985, indexed in Pubmed: 20015893.
38. Satchwell TJ, Pellegrin S, Bianchi P, et al. Characteristic phenotypes associated with congenital dyserythropoietic anemia (type II) manifest at different stages of erythropoiesis. Haematologica. 2013; 98(11): 1788–1796, doi: 10.3324/haematol.2013.085522, indexed in Pubmed: 23935019.

39. Russo R, Langella C, Esposito MR, et al. Hypomorphic mutations of SEC23B gene account for mild phenotypes of congenital dyserythropoietic anemia type II. Blood Cells Mol Dis. 2013; 51(1): 17–21, doi: 10.1016/j.bcmd.2013.02.003, indexed in Pubmed: 23453696.
40. Tao J, Zhu M, Wang He, et al. SEC23B is required for the maintenance of murine professional secretory tissues. Proc Natl Acad Sci USA. 2012; 109(29): E2001–E2009, doi: 10.1073/pnas.1209207109, indexed in Pubmed: 22745161.
41. Khoriaty R, Vasievich M, Jones M, et al. Disparate phenotypes of SEC23B deficiency in humans and mice. Blood. 2013; 122(21): 312–312, doi: 10.1182/blood.v122.21.312.312.
42. An X, Schulz VP, Li J, et al. Global transcriptome analyses of human and murine terminal erythroid differentiation. Blood. 2014; 123(22): 3466–3477, doi: 10.1182/blood-2014-01-548305, indexed in Pubmed: 24637361.
43. King R, Friedman A, Lin Z, et al. Functional overlap between the SEC23 paralogs suggests a novel treatment paradigm for congenital dyserythropoietic anemia type II. Blood. 2019; 134(Suppl_1): 2221–2221, doi: 10.1182/blood-2019-129669.
44. Sandström H, Wahlin A. Congenital dyserythropoietic anemia type III. Hematologica. 2000; 85(7): 753–757, indexed in Pubmed: 10897128.
45. Liljeholm M, Irvine AF, Vikberg AL, et al. Congenital dyserythropoietic anemia type III (CDA III) is caused by a mutation in kinesin family member, KIF23. Blood. 2013; 121(23): 4791–4799, doi: 10.1182/blood-2012-10-461392, indexed in Pubmed: 23570799.
46. Iolascon A, Heimpel H, Wahlin A, et al. Congenital dyserythropoietic anemias: molecular insights and diagnostic approach. Blood. 2013; 122(13): 2162–2166, doi: 10.1182/blood-2013-05-468223, indexed in Pubmed: 23940284.
47. Wickramasinghe SN, Parry TE, Williams C, et al. A new case of congenital dyserythropoietic anaemia, type III: studies of the cell cycle distribution and ultrastructure of erythroblasts and of nucleic acid synthesis in marrow cells. J Clin Pathol. 1982; 35(10): 1103–1109, doi: 10.1136/jcp.35.10.1103, indexed in Pubmed: 6182166.
48. Björkstén B, Holmgren G, Roos G, et al. Congenital dyserythropoietic anaemia type III: an electron microscopic study. Br J Haematol. 1978; 38(1): 37–42, doi: 10.1111/j.1365-2141.1978.tb07106.x, indexed in Pubmed: 638061.
49. Makyio H, Ohgi M, Takei T, et al. Structural basis for Arf6-MKLP1 complex formation on the Flemming body responsible for cytokinesis. EMBO J. 2012; 31(11): 2590–2603, doi: 10.1038/emboj.2012.89, indexed in Pubmed: 22522702.
50. Matuliene J, Kuriyama R. Kinesin-like protein CHO1 is required for the formation of midbody matrix and the completion of cytokinesis in mammalian cells. Mol Biol Cell. 2002; 13(6): 1832–1845, doi: 10.1091/mbc.01-10-0504, indexed in Pubmed: 12058052.
51. Vikberg AL, Malla S, Golovleva I. Differential tissue specific expression of Kif23 alternative transcripts in mice with the human mutation causing congenital dyserythropoietic anemia type III. Blood Cells Mol Dis. 2020; 85: 102483, doi: 10.1016/j.bcmd.2020.102483, indexed in Pubmed: 32818800.
52. Barbarani G, Fugazza C, Strouboulis J, et al. The pleiotropic effects of GATA1 and KLF1 in physiological erythropoiesis and in dyserythropoietic disorders. Front Physiol. 2019; 10: 91, doi: 10.3389/fphys.2019.00091, indexed in Pubmed: 30809156.
53. Cheng Y, Wu W, Kumar SA, et al. Erythroid GATA1 function revealed by genome-wide analysis of transcription factor occupancy, histone modifications, and mRNA expression. Genome Res. 2009; 19(12): 2172–2184, doi: 10.1101/gr.098921.109, indexed in Pubmed: 19887574.
54. Tallack MR, Magor GW, Dartigues B, et al. Novel roles for KLF1 in erythropoiesis revealed by mRNA-seq. Genome Res. 2012; 22(12): 2385–2398, doi: 10.1101/gr.135707.111, indexed in Pubmed: 22835905.
55. Wolfe D, Dudek S, Ritchie MD, et al. Visualizing genomic information across chromosomes with PhenoGram. BioData Min. 2013; 6(1): 18, doi: 10.1186/1756-0381-6-18, indexed in Pubmed: 24131735.
56. Arnaud L, Saison C, Helias V, et al. A dominant mutation in the gene encoding the erythroid transcription factor KLF1 causes a congenital dyserythropoietic anemia. Am J Hum Genet. 2010; 87(5): 721–727, doi: 10.1016/j.ajhg.2010.10.010, indexed in Pubmed: 21055716.
57. Jaffray JA, Mitchell WB, Gnanapragasam MN, et al. Erythroid transcription factor EKLF/KLF1 mutation causing congenital dyserythropoietic anemia type IV in a patient of Taiwanese origin: review of all reported cases and development of a clinical diagnostic paradigm. Blood Cells Mol Dis. 2013; 51(2): 71–75, doi: 10.1016/j.bcmd.2013.02.006, indexed in Pubmed: 23522491.
58. Ortolano R, Forouhar M, Warwick A, et al. A Case of Congenital Dyserythropoeitic Anemia Type IV Caused by E325K Mutation in Erythroid Transcription Factor KLF1. J Pediatr Hematol Oncol. 2018; 40(6): e389–e391, doi: 10.1097/MPH.0000000000001042, indexed in Pubmed: 29200155.
59. Ravindranath Y, Johnson RM, Goyette G, et al. KLF1 E325K-associated congenital dyserythropoietic anemia type IV: insights into the variable clinical severity. J Pediatr Hematol Oncol. 2018; 40(6): e405–e409, doi: 10.1097/MPH.0000000000001056, indexed in Pubmed: 29300242.
60. Kohara H, Utsugisawa T, Sakamoto C, et al. KLF1 mutation E325K induces cell cycle arrest in erythroid cells differentiated from congenital dyserythropoietic anemia patient-specific induced pluripotent stem cells. Exp Hematol. 2019; 73: 25–37.e8, doi: 10.1016/j.exphem.2019.03.001, indexed in Pubmed: 30876823.
61. Siatecka M, Bieker JJ. The multifunctional role of EKLF/KLF1 during erythropoiesis. Blood. 2011; 118(8): 2044–2054, doi: 10.1182/blood-2011-03-331371, indexed in Pubmed: 21613252.
62. Borg J, Patrinos GP, Felice AE, et al. Erythroid phenotypes associated with KLF1 mutations. Haematologica. 2011; 96(5): 635–638, doi: 10.3324/haematol.2011.043265, indexed in Pubmed: 21531944.
63. Perkins A, Xu X, Higgs DR, et al. KLF1 Consensus Workgroup. Krüppeling erythropoiesis: an unexpected broad spectrum of human red blood cell disorders due to KLF1 variants. Blood. 2016; 127: 1856–1862.
64. Nuez B, Michalovich D, Bygrave A, et al. Defective haematopoiesis in fetal liver resulting from inactivation of the EKLF gene. Nature. 1995; 375(6529): 316–318, doi: 10.1038/375316a0, indexed in Pubmed: 7753194.
65. Perkins AC, Sharpe AH, Orkin SH. Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. Nature. 1995; 375(6529): 318–322, doi: 10.1038/375318a0, indexed in Pubmed: 7753195.
66. Magor GW, Tallack MR, Gillinder KR, et al. KLF1-null neonates display hydrops fetalis and a deranged erythroid transcriptome. Blood. 2015; 125(15): 2405–2417, doi: 10.1182/blood-2014-08-590968, indexed in Pubmed: 25724378.
67. Feng WC, Southwood CM, Bieker JJ. Analyses of beta-thalassemia mutant DNA interactions with erythroid Krüppel-like factor (EKLF), an erythroid cell-specific transcription factor. J Biol Chem. 1994; 269(2): 1493–1500, indexed in Pubmed: 8288615.

68. Crossley M, Whitelaw E, Perkins A, et al. Isolation and characterization of the cDNA encoding BKLF/TEF-2, a major CACCC-box-binding protein in erythroid cells and selected other cells. Mol Cell Biol. 1996; 16(4): 1695–1705, doi: 10.1128/MCB.16.4.1695, indexed in Pubmed: 8657145.
69. Tallack MR, Whitington T, Yuen WS, et al. A global role for KLF1 in erythropoiesis revealed by ChIP-seq in primary erythroid cells. Genome Res. 2010; 20(8): 1052–1063, doi: 10.1101/gr.106575.110, indexed in Pubmed: 20508144.
70. Siatecka M, Sahr KE, Andersen SG, et al. Severe anemia in the Nan mutant mouse caused by sequence-selective disruption of erythroid Kruppel-like factor. Proc Natl Acad Sci USA. 2010; 107(34): 15151–15156, doi: 10.1073/pnas.1004996107, indexed in Pubmed: 20696915.
71. Singleton BK, Lau W, Fairweather VSS, et al. Mutations in the second zinc finger of human EKLF reduce promoter affinity but give rise to benign and disease phenotypes. Blood. 2011; 118(11): 3137–3145, doi: 10.1182/blood-2011-04-349985, indexed in Pubmed: 21778342.
72. Lyon M, Glenister P, Loutit J, et al. Dominant hemolytic anemia. Mouse News Lett. 1983; 68: 68.
73. Lyon M, Glenister P. Position of neonatal anaemia (Nan) on chromosome 8. Mouse News Lett. 1986; 74: 95.
74. White RA, Sokolovsky IV, Britt MI, et al. Hematologic characterization and chromosomal localization of the novel dominantly inherited mouse hemolytic anemia, neonatal anemia (Nan). Blood Cells Mol Dis. 2009; 43(2): 141–148, doi: 10.1016/j.bcmd.2009.03.009, indexed in Pubmed: 19409822.
75. Heruth DP, Hawkins T, Logsdon DP, et al. Mutation in erythroid specific transcription factor KLF1 causes hereditary spherocytosis in the Nan hemolytic anemia mouse model. Genomics. 2010; 96(5): 303–307, doi: 10.1016/j.ygeno.2010.07.009, indexed in Pubmed: 20691777.
76. Nébor D, Graber JH, Ciciotte SL, et al. Mutant KLF1 in adult anemic Nan mice leads to profound transcriptome changes and disordered erythropoiesis. Sci Rep. 2018; 8(1): 12793, doi: 10.1038/s41598-018-30839-2, indexed in Pubmed: 30143664.
77. Cantú I, van de Werken HJG, Gillemans N, et al. The mouse KLF1 Nan variant impairs nuclear condensation and erythroid maturation. PLoS One. 2019; 14(3): e0208659, doi: 10.1371/journal.pone.0208659, indexed in Pubmed: 30921348.
78. Shimizu R, Yamamoto M. GATA-related hematologic disorders. Exp Hematol. 2016; 44(8): 696–705, doi: 10.1016/j.exphem.2016.05.010, indexed in Pubmed: 27235756.
79. Russo R, Andolfo I, Gambale A, et al. GATA1 erythroid-specific regulation of SEC23B expression and its implication in the pathogenesis of congenital dyserythropoietic anemia type II. Haematologica. 2017; 102(9): e371–e374, doi: 10.3324/haematol.2016.162966, indexed in Pubmed: 28550189.
80. Ling T, Crispino JD. GATA1 mutations in red cell disorders. IUBMB Life. 2020; 72: 106–118, doi: 10.1002/iub.2177.
81. Fujiwara Y, Browne CP, Cunniff K, et al. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proceedings of the National Academy of Sciences 1996; 12355–8. Proc Natl Acad Sci USA. 1996; 93(22): 12355–12358, doi: 10.1073/pnas.93.22.12355, indexed in Pubmed: 8901585.
82. Shivdasani RA, Fujiwara Y, McDevitt MA, et al. A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. The EMBO Journal. 1997; 16(13): 3965–3973, doi: 10.1093/emboj/16.13.3965.
83. McDevitt MA, Shivdasani RA, Fujiwara Y, et al. A "knockdown" mutation created by cis-element gene targeting reveals the dependence of erythroid cell maturation on the level of transcription factor GATA-1. Proc Natl Acad Sci USA. 1997; 94(13): 6781–6785, doi: 10.1073/pnas.94.13.6781, indexed in Pubmed: 9192642.
84. Kobayashi E, Shimizu R, Kikuchi Y, et al. Loss of the Gata1 gene IE exon leads to variant transcript expression and the production of a GATA1 protein lacking the N-terminal domain. J Biol Chem. 2010; 285(1): 773–783, doi: 10.1074/jbc.M109.030726, indexed in Pubmed: 19854837.
85. Shimizu R, Ohneda K, Engel JD, et al. Transgenic rescue of GATA-1--deficient mice with GATA-1 lacking a FOG-1 association site phenocopies patients with X-linked thrombocytopenia. Blood. 2004; 103(7): 2560–2567, doi: 10.1182/blood-2003-07-2514, indexed in Pubmed: 14656885.
86. Majeed HA, Kalaawi M, Mohanty D, et al. Congenital dyserythropoietic anemia and chronic recurrent multifocal osteomyelitis in three related children and the association with Sweet syndrome in two siblings. J Pediatrics. 1989; 115(5 Pt 1): 730–734, doi: 10.1016/s0022--3476(89)80650-x, indexed in Pubmed: 2809904.
87. Dwyer JR, Donkor J, Zhang P, et al. Mouse lipin-1 and lipin-2 cooperate to maintain glycerolipid homeostasis in liver and aging cerebellum. Proc Natl Acad Sci USA. 2012; 109(37): E2486–E2495, doi: 10.1073/pnas.1205221109, indexed in Pubmed: 22908270.
88. Shteyer E, Saada A, Shaag A, et al. Exocrine pancreatic insufficiency, dyserythropoeitic anemia, and calvarial hyperostosis are caused by a mutation in the COX4I2 gene. Am J Hum Genet. 2009; 84(3): 412–417, doi: 10.1016/j.ajhg.2009.02.006, indexed in Pubmed: 19268275.
89. Hüttemann M, Lee I, Gao X, et al. Cytochrome c oxidase subunit 4 isoform 2-knockout mice show reduced enzyme activity, airway hyporeactivity, and lung pathology. FASEB J. 2012; 26(9): 3916–3930, doi: 10.1096/fj.11-203273, indexed in Pubmed: 22730437.
90. Motoyama N, Wang F, Roth KA, et al. Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science. 1995; 267(5203): 1506–1510, doi: 10.1126/science.7878471, indexed in Pubmed: 7878471.
91. Wagner KU, Claudio E, Rucker EB, et al. Conditional deletion of the Bcl--x gene from erythroid cells results in hemolytic anemia and profound splenomegaly. Development. 2000; 127(22): 4949–4958, indexed in Pubmed: 11044408.
92. Afreen S, Bohler S, Müller A, et al. BCL-XL expression is essential for human erythropoiesis and engraftment of hematopoietic stem cells. Cell Death Dis. 2020; 11(1): 8, doi: 10.1038/s41419-019-2203-z, indexed in Pubmed: 31907357.
93. Samkari A, Borzutzky A, Fermo E, et al. A novel missense mutation in MVK associated with MK deficiency and dyserythropoietic anemia. Pediatrics. 2010; 125(4): e964–e968, doi: 10.1542/peds.2009-1774, indexed in Pubmed: 20194276.
94. Hager EJ, Tse HM, Piganelli JD, et al. Deletion of a single mevalonate kinase (Mvk) allele yields a murine model of hyper-IgD syndrome. J Inherit Metab Dis. 2007; 30(6): 888–895, doi: 10.1007/s10545-007-0776-7, indexed in Pubmed: 18008182.
95. Konstantinidis DG, Giger KM, Risinger M, et al. Cytokinesis failure in RhoA-deficient mouse erythroblasts involves actomyosin and midbody dysregulation and triggers p53 activation. Blood. 2015; 126(12): 1473–1482, doi: 10.1182/blood-2014-12-616169, indexed in Pubmed: 26228485.
96. Mei Y, Zhao B, Yang J, et al. Ineffective erythropoiesis caused by binucleated late-stage erythroblasts in mDia2 hematopoietic specific knockout mice. Haematologica. 2016; 101(1): e1–e5, doi: 10.3324/haematol.2015.134221, indexed in Pubmed: 26471482.

97. Zhou X, Florian MC, Arumugam P, et al. RhoA GTPase controls cytokinesis and programmed necrosis of hematopoietic progenitors. J Exp Med. 2013; 210(11): 2371–2385, doi: 10.1084/jem.20122348, indexed in Pubmed: 24101377.
98. Velasco-Hernandez T, Säwén P, Bryder D, et al. Potential pitfalls of the Mx1-Cre system: implications for experimental modeling of normal and malignant hematopoiesis. Stem Cell Reports. 2016; 7(1): 11–18, doi: 10.1016/j.stemcr.2016.06.002, indexed in Pubmed: 27373927.
99. Niss O, Lorsbach RB, Christakopoulos GE, et al. The First Registry for Patients with Congenital Dyserythropoietic Anemia in North America: design and preliminary results. Blood. 2017; 130: 2210, doi: 10.1182/blood.V130.Suppl_1.2210.2210.
100. Seu KG, Trump LR, Emberesh S, et al. VPS4A mutations in humans cause syndromic congenital dyserythropoietic anemia due to cytokinesis and trafficking defects. Am J Hum Genet. 2020; 107(6): 1149–1156, doi: 10.1016/j.ajhg.2020.10.013, indexed in Pubmed: 33186543.
101. Niss O, Lorsbach RB, Berger M, et al. CDAR consortium. Congenital dyserythropoietic anemia type I: first report from the Congenital Dyserythropoietic Anemia Registry of North America (CDAR). Blood Cells Mol Dis. 2021; 87: 102534, doi: 10.1016/j.bcmd.2020.102534, indexed in Pubmed: 33401150.