

# \* CHAPTER 7

## Congenital dyserythropoietic anaemias

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## 1. Definition and classification

The congenital dyserythropoietic anaemias (CDAs, ICD-10 D64.4) comprise a group of rare hereditary disorders that are characterised by ineffective erythropoiesis as the predominant mechanism of anaemia and by distinct morphological abnormalities of erythroblasts in the bone marrow. The term was first used by Crookston et al. (1) (for cases later classified as CDA II) and by Wendt and Heimpel (2) (for cases later classified as CDA I), but a few reports of similar cases were published previously (3-5). The working classification initially proposed by Heimpel and Wendt (6), including as type III the families with autosomal dominant inheritance described by others (7, 8), is still used in clinical practice. There are, however, families that fulfil the general definition of the CDAs, but do not conform to any of the three classical types (9, 5, 10) (Table 1).

In the majority of CDAs, inheritance is autosomal recessive, and due to the small number of offspring in most European families, single cases in one family are the rule rather than the exception. Together with the rarity of the disorder and the need to obtain bone marrow specimens for diagnosis, this explains why correct diagnosis is often delayed (Figure 1), particularly in mild cases, even when anaemia and/or hyperbilirubinaemia have been evident for many years.

In general, the diagnosis of the CDAs requires the presence of all of the four following criteria:

1. Evidence of congenital anaemia/jaundice or a positive family history
2. Evidence of ineffective erythropoiesis
3. Typical morphological appearance of bone marrow erythroblasts

**Table 1: Characteristic features of different types of congenital dyserythropoietic anaemia**

CDA type	I	II	III familial	III sporadic	Variants
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal dominant	Variable	Autosomal recessive
Cases reported	> 300	~150	3 families	< 20	~70
Morphology	Abnormal chromatin structure, chromatin bridges	Multinuclearity of mature erythroblasts	Giant multinucleated erythroblasts	Giant multinucleated erythroblasts	CDA I-like CDA II-like Others
Gene	<i>CDAN1</i>	unknown	unknown	unknown	unknown
Chromosome	15q (15.1.3)	20	15q (21-25)		
Associated dysmorphism	Skeleton, others	Variable, rare	B-Cells Retina	Variable	CNS Others

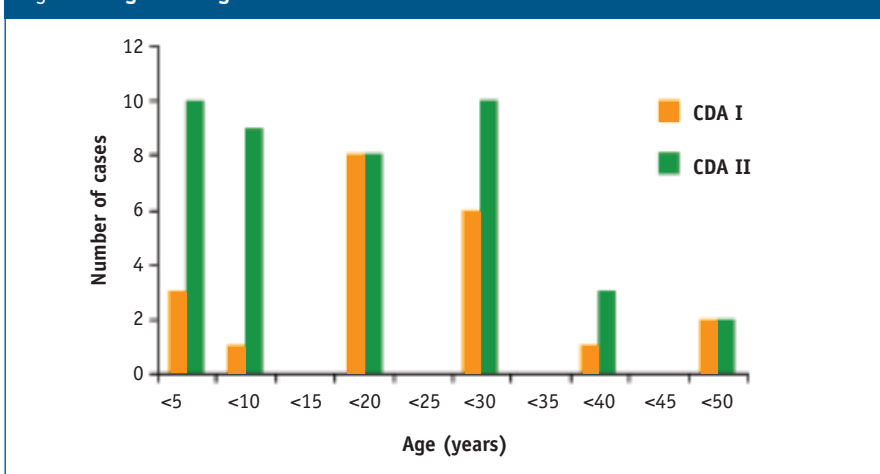
4. Exclusion of congenital anaemias that fulfil criteria 1 and 2, but which are classified according to the specific underlying defect, such as the thalassaemia syndromes, certain types of haemoglobinopathies or hereditary sideroblastic anaemias.

Proof of the first criterion may be difficult in adult patients for whom sufficient previous laboratory data is unavailable. In such cases, acquired types of anaemia involving ineffective erythropoiesis, such as megaloblastic anaemia due to vitamin deficiencies or myelodysplastic syndromes, must be ruled out. In the latter conditions as well as in acute myeloid leukaemia FAB-M6, morphologic abnormalities may mimic CDA of any type.

Ineffective erythropoiesis was identified as the main mechanism for the anaemia by erythroferrokinetic studies (11, 12). In addition, red cell lifespan may be moderately shortened, particularly in CDA type II (13, 14). Today, these techniques are no longer used outside of special studies. Ineffective erythropoiesis is suspected if there are symptoms and signs of increased haemoglobin turnover, such as mild jaundice due to indirect hyperbilirubinaemia and low or absent haptoglobin, but reticulocytosis does not correspond to the degree of anaemia. The bone marrow is always hypercellular, due to an exclusive and pronounced increase of erythroblasts, with erythropoietic/granulopoietic ratios of 4 to 10 (normal reference values 0.3–1.0). A more recently introduced test that reflects the expansion of the erythropoietic tissue is the elevated serum concentration of the soluble transferrin receptor, if iron deficiency is excluded (15).

Characteristic morphological aberrations of the erythroblasts are still the

Figure 1: Age at diagnosis in CDA I and II



cornerstone of the diagnosis, and if they are present in the majority of cells in an anaemia which is definitely congenital they can be regarded as specific for the diagnosis. They are also the first step in determination of CDA type. Recognition is much easier in smears of aspirated bone marrow than in histology specimens, and morphological analysis of both peripheral blood and appropriate bone marrow smears is required for diagnosis of any case of CDA. In addition to panoptic staining, the specimen should be stained for non-haem iron to exclude congenital sideroblastic anaemia with CDA-like morphological aberrations in a minority of cells, and also to estimate tissue iron stores. The number of sideroblasts may be increased in patients with increased iron stores, but ringed sideroblasts are present only in exceptional cases (16).

CDAs are very rare disorders. Because even haematologists only occasionally see such patients, specialised sources of information are needed. A European network (<http://www.enerca.org>) provides information on specialist centres and gives access to new publications for both physicians and patients. A variety of microscopic views can be seen at [http://bildatlas.onkodin.de/bildatlas/content/e1352/e1775/e1872/e2500/e2501/index\\_ger.html](http://bildatlas.onkodin.de/bildatlas/content/e1352/e1775/e1872/e2500/e2501/index_ger.html).

All types of CDA share a high incidence of splenomegaly, cholelithiasis and iron overload (5, 17, 18). As in other forms of anaemia with ineffective erythropoiesis, this is due to up-regulation of iron absorption (19), mediated by hepcidin. Extramedullary haematopoiesis presenting as paravertebral masses may be observed in all types of CDA.

## 2. CDA I (MIM 224120)

### 2.1 Epidemiology and clinical presentation

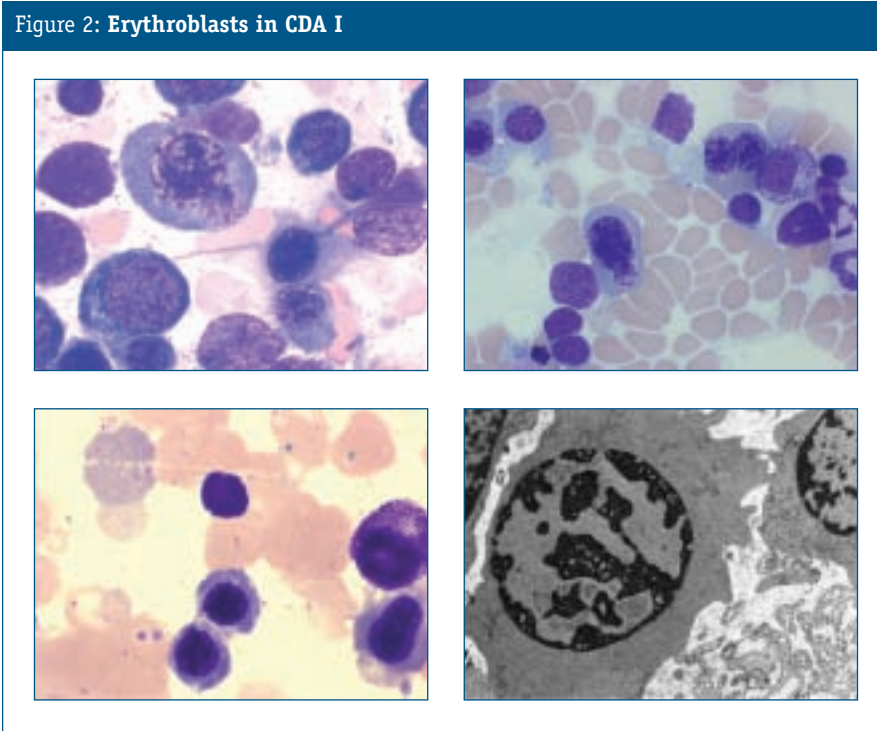
CDA I is less frequent than CDA II, with 89 cases from 82 families collated in the German registry of CDAs and/or identified from published case reports together with 70 additional cases in a large Bedouin tribe described by Tamary et al. from Israel (20, 21). At least for Europe, this probably reflects true differences of prevalence, since there is no evidence that the ascertainment rate of cases of the two types is different. Most families have been detected among Western Europeans and Arabs, but single cases have also been reported from the USA, India, Japan, Australia, New Zealand, Polynesia and China. The degree of anaemia is variable not only between families, but for unexplained reasons (modifier genes) may also be different among siblings (22). Most patients have life-long anaemia with haemoglobin concentration between 7 and 11 g/mL. Occasionally, there are severe cases requiring transfusion *in-utero* (23) or immediately after birth and regular blood transfusions during childhood and adolescence (24, 25). At the other extreme there

are patients with only borderline low haemoglobin but distinct macrocytosis. The anaemia is usually macrocytic with MCV between 100 and 120 fl, but may be normocytic in childhood (22).

As in any case of chronic unclassified anaemia, careful inspection of the peripheral blood smear is the first step in verifying the suspected diagnosis. There is distinct anisocytosis and poikilocytosis. Large poikilocytes and elliptocytes are reminiscent of changes seen in megaloblastic anaemias, which is the most frequent erroneous diagnosis made before the correct diagnosis is recognised. Basophilic stippled cells are always present, and Cabot rings may be seen in some cases, even before splenectomy. Except for the occasional presence of late erythroblasts, the distribution and shape of nucleated cells in the peripheral blood are normal.

Light microscopy of bone marrow erythroblasts in bone marrow smears is the next step toward confirmation of the diagnosis. Detailed illustrated descriptions can be found in previous publications, (5, 26). 30 to 60% of early and late polychromatic erythroblasts show characteristic and bizarre abnormalities of nuclear shape and size and of chromatin structure, but proerythroblasts and immature basophilic erythroblasts usually appear normal. In less severely affected cells, chromatin strands are coarser than normal and interrupted by irregularly shaped translucent areas. In more severely affected erythroblasts the chromatin structure is lost and the nucleus contains weakly stained material that is not sharply delineated from the surrounding cytoplasm. There are large polyploid cells, and a minority of cells show bi- or multinuclearity. In contrast to CDA II, the nuclei of binucleated cells are of different size and shape. A hallmark of CDA I is the presence of incompletely divided cells with thin chromatin bridges between the pairs of erythroblasts, which may also be seen between two nuclei in a single cell (Figure 2). With the exception of a few cases of erythroleukaemia, such bridges are virtually specific for CDA I, but since they may be present in less than 3% of cells, at least 500 consecutive cells should be examined when searching for this abnormality. The cytoplasm of many cells shows Howell-Jolly bodies and/or intensive and irregular basophilic stippling. These changes are sometimes called megaloblastic, but in contrast to megaloblastic anaemia the characteristic loose, fine chromatin structure of erythroblast nuclei is lacking, as are giant granulopoietic cells and hyperlobulation of megakaryocytes. Experts who have seen cases of CDA I before are easily able to make the diagnosis by light microscopy, but electron microscopy shows particularly specific alterations (26-28). They are absent in very early erythroblasts but become distinct as maturation progresses. The heterochromatin is denser than normal and forms sharply delineated clumps with small translucent vacuoles, giving rise to the metaphor of "Swiss cheese appearance" (29), and cytoplasm may penetrate through widened pores of the nuclear envelope (Figure 2).

Figure 2: Erythroblasts in CDA I



As in the other types of CDA, signs of increased haemoglobin turnover such as indirect hyperbilirubinaemia and low or absent plasma haptoglobin are always present. Most patients are not clinically jaundiced, and distinct jaundice with increased concentrations of direct serum bilirubin in adults should raise suspicion of secondary complications such as liver cirrhosis or gallstones. Reticulocyte counts are normal or only moderately increased, with maximum values of 5% or  $150 \times 10^9/L$  (22, 28, 30, 31).

In the majority of patients, the spleen is palpable at the time of diagnosis, and all patients develop splenomegaly in adolescence or adulthood (22). Morphologic body abnormalities are observed in about 20% of affected children and may be the presenting features for the referral of children (25, 32). These patients show skeletal malformations, particularly syndactyly in hands or feet, absence of nails, or supernumerary toes (33-35), and dyskeratosis such as skin pigmentation or neurological deficits (Figure 3) (5). There is indirect evidence that these malformations are caused by a mutation of a single morphogenetic gene rather than by foetal damage

Figure 3: Syndactyly of toes in a case of CDA I (courtesy of J. Goede, Zurich)



(36). Short stature may be the result of pituitary failure due to unrecognised secondary haemochromatosis (37).

## 2.2 Pathophysiology

The ineffective erythropoiesis corresponds to abnormalities of the cell cycle distribution. Most mononucleated polychromatic cells – those stages that show the most distinct morphological abnormalities – have DNA contents between 2c and 4c, but many become arrested during their progress through the S-Phase as shown by the absence of H<sup>3</sup>-thymidine uptake *in vitro*. Exceptionally large mononuclear as well as binucleated cells or those connected by chromatin bridges reach polyploid DNA values up to 8c (29, 38, 39). Erythroblasts showing chromatin abnormalities by EM also show an arrest of protein synthesis, and intramedullary necrobiosis of interphase erythroblasts is more likely than apoptosis. The relationship between mutations in the *CDAN1* gene (the gene responsible for CDA I) and ineffective erythropoiesis is not yet understood. Studies are in progress to define the role of the codanin-1 protein in normal erythropoiesis, but it is known that codanin-1 is localised to nuclear heterochromatin in interphase cells and is expressed in the S-Phase (40).

## 2.3 Genetics

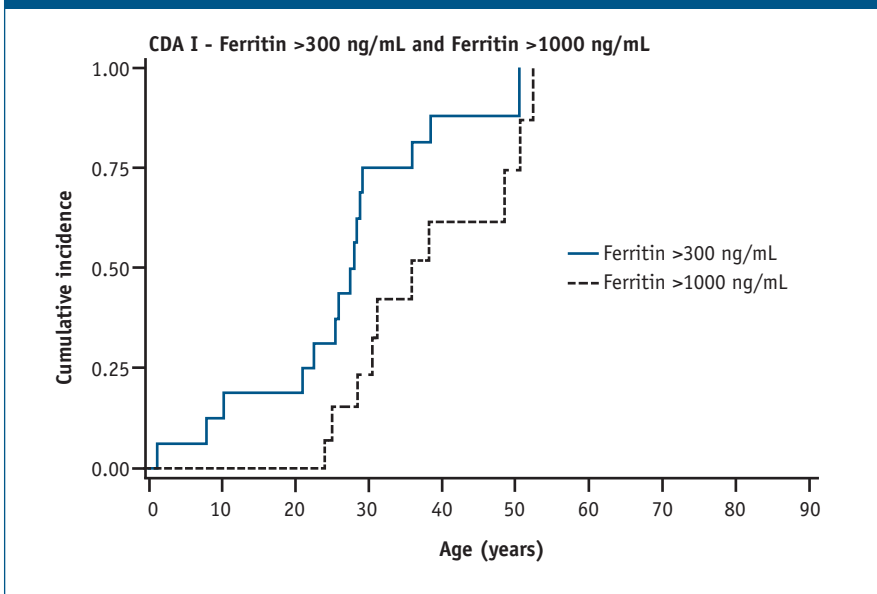
The gene responsible for CDA I (*CDAN1* gene) was mapped to the long arm of chromosome 15 between 15q15.1q15.3 by homozygosity mapping in four Bedouin families with a high degree of consanguinity (20) and could be assigned to a 0.5 cM interval (41). Similar results were reported in six patients from Europe and the Near East (42). The *CDAN1* gene was cloned and found to contain 28 exons spanning

15 kb and encoding a protein named codanin-1. A founder mutation Arg1042Trp was identified in all Bedouin patients. From studies in unrelated patients of European, Bedouin, North-American and Asian origin, altogether 36 different point mutations, distributed over 13 exons have been detected (22, 43-45). The majority of mutations (70%) are located on the 3' half of the gene. In another series of 51 CDA I cases, in 15 patients [29%, originating in Israel (5), Germany (6), and England (4)], only one mutation was identified, although splice site mutations were not excluded. In 6 of the 51 case with the definite phenotype of CDA I, no mutation was found (H. Tamary personal communication), suggesting either a promoter defect or a mutation in another gene (44). As is often the cases in orphan disorders, molecular study was the clue to understanding the role of a number of proteins in erythropoiesis, including the codanin in CDA I that is localised to the nuclear heterochromatin and upregulated during S phase by E2F1, the main regulator of G1/S transition of the cell cycle (40, 43).

## 2.4 Complications

The incidence of gallstones is increased (22, 31). Almost all patients accumulate iron with a steady increase of ferritin values throughout life (17) (Figure 4) (31),

Figure 4: Serum ferritin concentrations in CDA I as related to age



independently of the presence of any HFE gene mutations (46). In severe cases, iron overload becomes apparent in childhood (37, 47). Patients with organ damage and death from secondary haemochromatosis were observed before ferritin levels were systematically monitored and iron depletion therapy introduced (22).

### 3. CDA II (MIM 224120)

#### 3.1 Epidemiology and clinical presentation

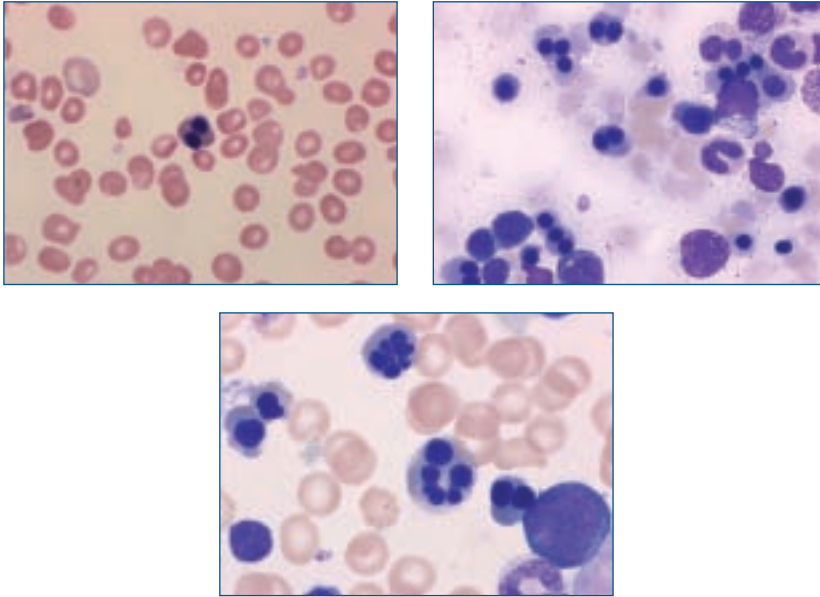
CDA II is the less uncommon form of CDA. The geographic distribution of affected patients suggests a higher frequency of the gene in Italy and in the Mediterranean countries as compared to central and northern Europe. At present it is difficult to assess whether this is due to a clustered distribution (48) or to greater diagnostic awareness.

The International Registry on CDA II (49) includes epidemiology, clinical findings and molecular studies. Through April 2008, 76 Italian and 46 non-Italian patients from 60 and 36 families, respectively, were registered (A. Iolascon, unpublished). To these patients we must add 49 patients from the German CDA registry (14), as well as more than 50 further cases published as case reports (3).

The regional distribution of the Italian patients demonstrated clustering in Southern Italy, and this phenomenon suggests a founder effect. However, molecular studies by means of microsatellites, localised where the gene was mapped, failed to demonstrate the existence of a common haplotype (48).

The main clinical findings are normocytic anaemia, jaundice and variable splenomegaly (14, 49). These features are also present in hereditary spherocytosis (HS) and it is possible to confuse these two conditions, especially because osmotic fragility tests give similar results in both. The most useful pointer to diagnosis of CDA II is an inadequate reticulocyte count for the degree of anaemia. The red cell distribution widths for cell volume (RDW, anisocytosis) and for haemoglobin concentration (HDW, anisochromia) are also helpful in distinguishing HS from CDA II. Characteristically the RDW is increased in CDA II and the HDW is increased in HS, resulting in an RDW/HDW ratio that is significantly greater in CDA than HS (50).

CDA II is associated with a well-defined morphological phenotype: bi- or multinucleated late precursors (6, 49) (Figure 5) and flat vesicles of variable length visualised by electron microscopy (5, 51). Peripheral blood smears show distinct aniso-poikilocytosis with basophilic stippled red cells and a few (occasionally binucleated) mature erythroblasts. The red cells express an antigen that binds to a natural cold-reacting Ig-M antibody present in the serum of 40% to 60% of healthy individuals. Antibody binding can be demonstrated by agglutination or by lysis when

**Figure 5: Peripheral erythrocytes and bone marrow erythroblasts in CDA II**

the serum is acidified to pH 6.7 (52). Therefore, the acronym HEMPAS (haemolytic anaemia with a positive acidified serum test) is commonly used as a synonym for CDA II. Red cells of adult patients with CDA II retain a very high agglutinability by anti-i sera due to increased expression of the i antigen although their I density is comparable to normal adults (52). Enhancement of the i-antigen is not specific, since it occurs in different forms of erythropoietic hyperplasia such as thalassaemia major or haemolytic anaemia. However, since agglutination titers in CDA II are invariably extremely high, a normal score excludes the diagnosis.

Membrane abnormalities can be detected by highly specific biochemical tests. Band 3 (anion exchange protein 1) and band 4.5 (glucose transporter 1) show a narrower band and faster migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (53). Equally specific is the detection of minor proteins derived from endoplasmic reticulum by Western blotting (51) and the decreased binding of tomato lectin (54).

Anaemia is usually noted in infancy or childhood; the degree varies widely, from mild to severe. Median haemoglobin level in a large group (49) was 9.9 g/dL, with

a range from 6.0 to 12.7. In the majority in adult patients untransfused levels of 8 to 11 g/dL are observed (14).

Few patients require regular transfusions, and as in CDA I transfusion may only be needed in infancy. In a number of specific reported cases transfusion dependence was generated by the interaction with another red blood cell defect. Three of these subjects were heterozygotes for CDA II and beta-thalassaemia (Iolascon, unpublished data) or a G-6PDH variant (55).

### 3.2 Pathophysiology

The life-long anaemia results from the combination of ineffective erythropoiesis and moderate peripheral haemolysis.

The principal biochemical feature is the hypoglycosylation of certain proteins, including transferrin and band 3. It appears that in CDA II a genetic factor blocks the glycosylation of glycoprotein acceptors and shifts polylactosamines to lipid acceptors. The reduced glycosylation is associated with abnormal function of band 3 in CDA II patients. Analysis of red cells from CDA II patients demonstrated a narrower- than-usual band 3 with slightly faster migration on SDS-PAGE, while analysis of anion transport (inhibition of sulphate flux by H<sub>2</sub>-DIDS) showed decreased activity of the anion transport for the band 3 molecule. Furthermore the CDA II erythrocytes were found to contain higher amounts of aggregate band 3 than control erythrocytes (56). Aggregated band 3 has been reported to bind naturally occurring antibodies, possibly mediating the phagocytic removal of red blood cells. These results suggested that the haemolysis found in CDA II patients may be ascribed to clusterings of band 3, leading to IgG binding and phagocytosis, rather than to secondary modification of the erythrocytic cytoskeletal structure.

### 3.3 Genetics

The results of structural analysis of CDA II band 3 carbohydrates suggested disruption of the biosynthesis involving the N-acetylglucosaminyltransferase II (GnT-II) and alpha-mannosidase II (MANII) steps. However, linkage analysis in patients from Southern Italy excluded these candidate genes (57), and a genome-wide search yielded conclusive evidence for linkage of CDA II to microsatellite markers on the long arm of chromosome 20 (20q11.2). A maximum two-point lod score of 5.4 at  $q=0.00$  with the marker D20S863 was obtained (58). Mapping of the gene shows that there is genetic heterogeneity in this condition.

### 3.4 Complications

Gallstone formation, apparently related to the increased haemoglobin turnover, is

the most prevalent complication (14, 49). A significant correlation has been observed between the UGT1A (TA)<sub>7</sub>/(TA)<sub>7</sub> genotype, i.e., Gilbert's syndrome, and the increased rate of gallstones in CDA II patients. The effects of Gilbert's syndrome are clearly visible when CDA II patients from the same families but with different UGT1A genotypes are compared (59).

Secondary haemochromatosis is the most important long-term complication. As in CDA I, iron overload is not dependent on (albeit enhanced by) transfusions. It may be alleviated by ongoing iron loss, such as menstrual bleeding or pregnancies. Haemochromatosis can lead to organ damage if not recognised and properly treated (14, 19, 60).

#### 4. CDA III (MIM 105600)

CDA III was first described in 1962 under the name of Hereditary Benign Erythroreticulosis (8) or "Västerbotten anomaly" in members of a large family living in Northern Sweden, and designated as type III after types I and II were classified (6). At present, the fifth generation of this family is being investigated, and most data on CDA III have been described by the investigators from Umea, Sweden (61). Clinical presentation is similar to that of type I and II patients, but anaemia is never severe and transfusions are not required. In contrast to other types, there is no clinically relevant iron overload. In addition to ineffective erythropoiesis, there is intravascular haemolysis, as demonstrated by haemosiderinuria and absence of serum haptoglobin (62). The most marked anomaly in the bone marrow is the presence of giant multinucleated erythroblasts resembling the giant erythroblasts seen in the early phase of the erythroid aplasia initiated by parvovirus B19. Of great interest are additional features, such as abnormalities of the retina with angioid streaks and macular degeneration, and a high incidence of monoclonal gammopathy with or without multiple myeloma. The responsible gene has been mapped to a locus close to the *CDAN1* gene on a 4.5 cM interval between 15q21 and 15q25 (63). The genetic changes associated with the haemopoietic and ocular abnormalities are unknown. There are two more families with similar haemopoietic changes and dominant inheritance living in North and South America, but only a few details are known, and it is not clear whether they share the same genetic basis.

Non-familial CDA III is the rarest type of CDA, with fewer than 20 well-documented cases. They are probably due to other genetic lesions (61). We have observed CDA III-like giant erythroblasts in multiple myeloma (unpublished), raising doubts as to whether reported patients in whom CDA III was detected on examination for malignant lymphoma (64, 65) had true congenital anaemia.

## 5. CDA variants

Before and after the core group of CDAs was recognised, familial and sporadic cases of congenital anaemia were reported that fulfilled the four general criteria of the CDAs but could not be attributed to one of the three groups (5, 9, 66). These variants form an extremely heterogenous group, and failure to attribute such cases to either to one of the three types or to any other defined congenital anaemia may result from incomplete diagnostic workup. However, in many reported patients other known disorders were carefully excluded by extensive and repeated examination, including serological, biochemical and morphological analysis. As in CDA I and II, the mode of inheritance is generally, though not always, autosomal recessive but nothing is known about the genes that may be involved. A preliminary classification based on a proposal by Wickramasinghe (5) and cases in the German CDA Registry defines the following groups:

- a. *CDA Type IV* (67, 68) is described as having typical morphological features of CDA II but with a negative acidified serum test. Some reports were later reclassified when retesting with more sera gave positive results. However, other authors (68, 69) have observed families whose acid serum tests are consistently negative, and in none of them were any of the other tests for recognising the membrane abnormality found to be positive. Patients with CDA type IV have a severe clinical course and require regular transfusions. Hydrops foetalis has been reported in five cases (70, 71).
- b. *CDA with prominent erythroblastosis after splenectomy* (72-75): Clinical features and morphology resemble CDA II, but acidified serum tests are consistently negative. Up to  $50 \times 10^9$  mature erythroblasts/L have been seen for many years following splenectomy.
- c. *CDA with intraerythroblastic inclusions* as described by Wickramasinghe (5).
- d. *Congenital ineffective erythropoiesis and erythroid hyperplasia and absence of erythroid dysplasia*: Such cases have been published under terms such as shunt hyperbilirubinaemia or idiopathic dyserythropoietic jaundice (5). One such patient who was enrolled in the German CDA Registry was followed over 20 years and developed iron overload.
- e. *CDA with thrombocytopenia* (76). One patient had distinct extramedullary haematopoiesis in liver and spleen, and although the authors used the term "CDA" to describe him, this case could also be grouped within the chronic congenital bone marrow failure syndromes. Recently a GATA I mutation (G208R) was detected in this family (C. Kratz, unpublished, as previously described in congenital thrombocytopenia (77, 78).

## 6. Therapy of CDAs

The approach to treatment depends on age, type of CDA, severity of expression and

comorbidity. Most patients with CDA have only mild or moderate anaemia. Transfusions contribute to iron overload, and this risk has to be individually weighed against the failure to thrive in infants and children with severe anaemia, and against the risk of damage to the mother and the foetus in pregnancy. About 50% and 10% of neonates with CDA I (21) and CDA II (49), respectively, need at least one transfusion, and some remain transfusion-dependent during childhood. In most but not all adolescents and adults, the need for transfusions is limited to aplastic crises, pregnancy, periods of severe infections or major operations. Seven cases of transfusion dependence were reported to the International Registry of CDA II (unpublished). Two were characterised by coinheritance with heterozygous beta-thalassaemia. One patient with CDA II and beta-thalassaemia failed to benefit from splenectomy, whereas this procedure had a favourable effect in his brother, who lacked the beta-thalassaemia trait. This suggests that interaction between these two conditions was responsible for a more severe clinical picture, and it may be that excess of globin alpha chains with precipitation within CDA II erythroid cells increases ineffective erythropoiesis. Because of the pronounced erythroid hyperplasia, supportive supplementation with Vitamin B12 and folic acid is frequently given, but without any evidence of efficacy. There is also no evidence of benefit for erythropoietin formulations (79) (own unpublished observations). Iron supplementation should be strictly avoided due to the tendency for iron overload, but unfortunately is often given before the correct diagnosis is made.

Two treatments are effective in improving the chronic anaemia: interferon (IFN)- $\alpha$  and splenectomy.

### 6.1 Interferon- $\alpha$

IFN- $\alpha$  is effective in CDA type I, but there is no evidence of efficacy in other types of CDA. Restoration of erythropoiesis was first observed as an unexpected result in a female with CDA I treated with IFN- $\alpha$ -2a for post-transfusional chronic viral hepatitis (80), and iron uptake returned to normal after continuation of treatment for nine years (81). Reported effective doses in a total of 18 patients ranged from 4 to 9 million IU/week in adults and 7.8 to 12.5 million IU/m<sup>2</sup>/week in children (82), given thrice weekly or on alternate days. The same effects are achieved by pegylated interferon (Peg-IFN)- $\alpha$ -2b 30  $\mu$ g to 50  $\mu$ g in one weekly injection (35). There is no evidence of different activity dependent on gender or the use of interferon- $\alpha$ -2a or  $\alpha$ -2b. Normal haemoglobin concentration was achieved in all treated patients. Erythrokinetic studies demonstrated a striking reduction of the ineffective erythropoiesis, and electron-microscopic studies showed a reduction in nuclear structural abnormalities (81, 83). When IFN therapy was stopped, haemoglobin levels returned to previous

values. A dose below 4 to 9 million IU of IFN or 50 µg Peg-IFN per week is probably sufficient for maintenance in adults. The pathophysiological basis of the beneficial effect of IFN in CDA I is not understood. One study on cell lines treated with IFN- $\alpha$  established which genes were up- or down-regulated by this drug but no clear explanation was found for its mode of action (84).

## 6.2 Splenectomy

Splenectomy leads to a moderate but sustained increase in haemoglobin concentration and decrease of serum bilirubin levels in CDA II (14, 49). Red cell survival normalises (13, 85), demonstrating that, as in hereditary spherocytosis, abnormal CDA II erythrocytes may survive normally in an asplenic individual. Splenectomy does not prevent further iron loading, even in patients whose haemoglobin concentrations become nearly normal (14). This may be explained by the observation that iron loading is more closely correlated to the expansion of the erythroid marrow than to the anaemia itself, which in CDA II is determined by ineffective erythropoiesis as well as shortened red cell survival.

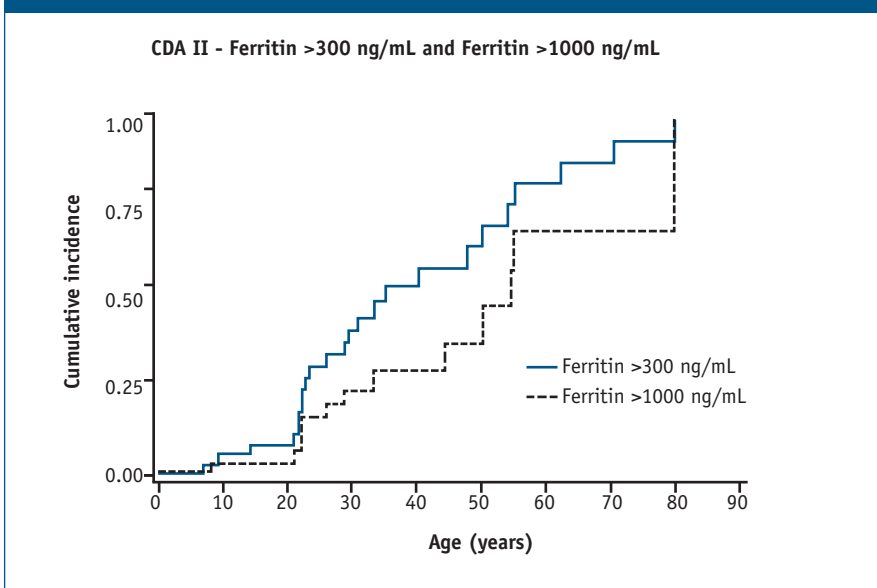
The main benefit of splenectomy is abrogation of transfusion requirements and increase of the haemoglobin concentration in severe cases. In other patients, it is advisable to follow the same guidelines as for splenectomy in mild cases of hereditary spherocytosis (86). Splenectomy is not recommended in CDA I (22), and individual decisions have to be made in CDA variants with transfusion dependency and an enlarged spleen.

## 6.3 Cholecystectomy

Cholecystectomy is often indicated in patients with all types of CDA, and decision making should follow the normal practice for cholelithiasis (87). Morbidity and mortality following cholecystectomy are expected to be lower in the pediatric age group.

## 6.4 Management of iron overload

The main problem encountered by patients after the first years of life is that of iron overload, which is not related to transfusion requirement. It has been known since early observations of CDA that patients with CDA I or II (as well as those with variant types) are at risk of iron overload in the same way patients with other chronic states of ineffective erythropoiesis. This has been confirmed by many case reports (3). Iron accumulates steadily throughout life, with kinetics similar to those in patients with untreated hereditary haemochromatosis (Figures 4 and 6). There is, however, distinct variability among individuals, which is not explained by HFE gene polymorphism (49). Even in patients with mild or moderate anaemia, ferritin levels

**Figure 6: Serum ferritin concentration in CDA II as related to age**

should be checked at least annually, because iron overload may approach risk levels at any age. Adequate treatment of patients with CDA with regular phlebotomy and/or deferoxamine leads to normal ferritin concentrations, indicating reversal of iron overload. However, since data correlating serum ferritin levels to tissue iron in CDA are scarce, prospective studies using noninvasive techniques for liver iron determination are required; at present, management of iron overload should follow the practice for thalassaemia (88).

### 6.5 Allogeneic haematopoietic stem cell transplant (HSCT)

Allogeneic HSCT from an HLA-identical sibling can abolish transfusion dependency in patients with erythroid disorders and so prevent progression of tissue damage related to iron overload. The result should be both longer life expectancy and better quality of life. Successful allogeneic BMT has been reported in five transfusion-dependent children with very severe CDA (71, 75, 89, 90) and in one adult with CDA II and beta-thalassaemia trait (91).

### 6.6 Genetic counselling

CDAs are usually mild, and genetic counselling is only needed in severe cases, which

are the least understood and may depend on the presence of coexistent abnormalities. Counselling has to be based on the pattern of inheritance, since there are no evidence-based methods for early antenatal diagnosis.

## 7. Summary and conclusions

The CDAs are a heterogeneous group of hereditary disorders, both at clinical and genetic levels. Mapping and cloning have shown that these conditions have different molecular mechanisms that induce disturbances of cell maturation and cell division during erythropoiesis. In general the features of dyserythropoiesis, in terms of ineffective erythropoiesis, should be demonstrated by a number of different criteria: bone marrow evaluation by light microscopy, ultrastructural features, assessment of red cell production and destruction, and studies of iron metabolism. Certainly the main assessment method, and one which is easy to perform, is light microscopy. Biochemical (such as in CDA II) or molecular methods (such as in CDA I) are required for exact classification. Effective modalities of therapy include general measures, such as iron depletion, and specific measures such as IFN- $\alpha$  in CDA I and splenectomy in CDA II. In the future, microarray and proteomic studies may prove useful in defining further genes involved in these conditions and possibly new drugs will become available.

## References

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## Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/iron-handbook2009answers.htm>

### 1. Which one of the following criteria is not compatible with the diagnosis of CDA:

- a) Evidence of congenital anaemia/jaundice or a positive family history .....
- b) Evidence of ineffective erythropoiesis .....
- c) Typical morphological appearance of bone marrow erythroblasts .....
- d) MCV reduced to less than 70 fl .....

### 2. Which one of the following findings does not suggest the diagnosis of CDA type I:

- a) Peripheral blood: anisocytosis and poikilocytosis .....
- b) Peripheral blood: basophilic stippled cells .....
- c) Electron microscopy: presence of double membrane in mature erythroblasts .....
- d) Electron microscopy: chromatin bridges between erythroblasts .....

### 3. Which is the causative gene for CDA I?

- a) Codanin .....
- b) BCRA1 .....
- c) wt1 .....
- d) Spectrin alpha .....

### 4. Which of the following tests is not useful for the diagnosis of CDA II?

- a) SDS-PAGE of red cell membrane proteins .....
- b) Western-blot for RE proteins .....
- c) Increased anti-i agglutinability .....
- d) Low iron saturation of serum transferrin .....

### 5. Please identify the common complication(s) of CDA II:

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- a) Iron overload, gallstones .....
- b) Splenomegaly and renal failure .....
- c) Thrombocytopenia and splenomegaly .....
- d) Symptomatic osteoporosis .....