## A new type of transfusion-dependent congenital dyserythropoietic anemia

**Cases of congenital dyserythropoietic anemia (CDA) that do not conform to any of the three classical types often present diagnostic difficulties and are at risk of developing secondary hemochromatosis. Here, we report a case of a six year old boy with transfusion dependency and gross abnormalities of the erythroblasts.**

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Case 484/01 of the German Registry of Congenital Dyserythropoietic anemias, male, is the only child of healthy, non-consanguineous German parents. Birth weight was 3,750 g, and there are no dysmorphologies. Scleral icterus and pallor was first noticed at the age of two months. Lowest hemoglobin concentration was 5.4 g/dL, with an MCV of 89 fL and a reticulocyte count of 1.4%. No splenomegaly was noted at the most recent examination at the age of six years. Regular red cell transfusions were given at monthly intervals which ensured a target hemoglobin concentration between 7-12 g /dL. Serum ferritin reached 1,850 ng/mL at the age of 3 years. Non-invasive estimation by superconducting quantum interference device biomagnetometry (SQUID) showed a greatly increased liver iron.

Bone marrow aspiration at the age of five months showed increased cellularity, with greatly increased numbers of erythroblasts. Granulocytic cells and megakaryocytes did not show any abnormalities. About 65% of the early and late polychromatic erythroblasts and some more mature basophilic erythroblasts showed distinct dyserythropoietic changes (Figure 1). These include irregular nuclear outlines, formation of nuclear blebs and Howell-Jolly bodies, karyorrhexis, as well as small clear areas and clefts in the nucleus. Five percent of mature cells were bi- or tri-nucleate. Coarse basophilic stippling and large cytoplasmic vacuoles often associated with the nuclear membrane were seen in some cells. Identical aberrations were seen in subsequent marrow aspirates. At the age of six years, storage iron was greatly increased. Ringed sideroblasts were absent.

Electron microscopy showed multiple long stretches of double membranes within the cytoplasm, but neither close to or parallel to the cell membrane as in CDA type II. Parts of these membranes were dilated, forming large cytoplasmic vacuoles. Nuclear abnormalities consisted of multiple long intranuclear clefts, dilation of the space between the two layers of the nuclear membrane, loss of parts of the nuclear membrane, duplication, sometimes repeatedly, of stretches of the nuclear membrane, and irregular nuclear outlines (Figure 2). In some sections, the nuclear membrane was continuous with the cytoplasmic membranes or large cytoplasmic vacuoles.

Serum concentration of vitamin $B_{12}$ was initially decreased (102 ng/L; reference range 200-900 ng/L) but normal on subsequent controls. Repeated treatment with hydroxycobalamin had no effect on reticulocyte count, hemoglobin concentration or transfusion requirements. Serum vitamin $B_{12}$ binding and transcobalamin II serum concentration were normal.

Acidified serum lysis tests were negative, and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was normal. Sequencing of the CDANI-gene[1] failed to show mutations. Hb A2 and HbF were not increased. Iron studies consistently showed data compatible with secondary hemochromatosis which was not sufficiently controlled by deferoxamine therapy. At the age of six, serum ferritin was still increased at 930 ng/mL, transferrin was decreased at 1.3 g/L, transferrin saturation was 100% and the soluble transferrin receptor 4.6 mg/L (reference 0.8-1.8 mg/L). Creatinine, ALT, AST and serum concentrations of albumin, cortisol, TSH and glucose were all within normal limits.

The case described fulfills the general definition criteria of the CDAs,[2] but cannot be attributed to CDA I, II or III, or to any of the other entities described as CDA variants or groups.[3] Its congenital nature is shown by anemia and

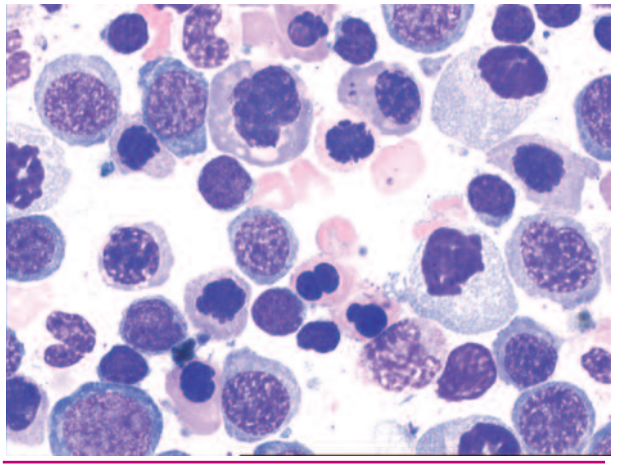

**Figure 1.** Marrow smear showing erythroblasts with irregular nuclear outlines, nuclear blebs, Howell-Jolly bodies or large cytoplasmic vacuoles or combinations of these. In two cells, the vacuoles are clearly associated with the nucleus. There is one binucleate erythroblast.

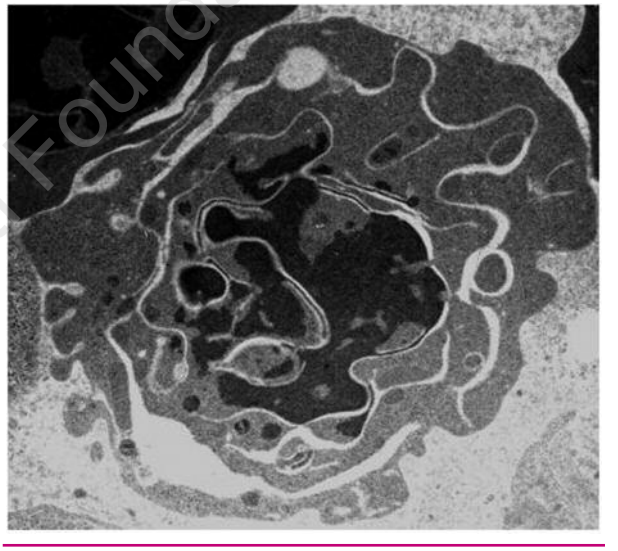

**Figure 2.** Electron micrograph of an erythroblast showing several long cytoplasmic double membranes the space between which is more dilated in two areas than in others. The nuclear outline is markedly irregular, there are several intranuclear clefts, and some regions of heterochromatin are not associated with nuclear membrane.

jaundice in infancy and consistency of the abnormal laboratory data during the observation period of six years. Erythropoiesis is ineffective, as shown by severe anemia requiring regular red cell transfusions with low reticulocyte counts, erythropoietic hyperplasia, and increased serum concentration of the soluble transferrin receptor. The disorder is the result of autosomal inheritance or due to *de novo* mutations, since both parents and their siblings had normal blood counts. Analysis of cases of CDA reported as *variants* did not reveal a similar case, with the exception of a patient described from Spain,[4] who was reported to present similarly extensive morphological aberrations. In contrast to out report, the anemia was less severe and transfusions were only required in the first three months.

In the case reported here, transfusions still had to be given at the age of six. This resulted in iron overload which standard therapy with deferoxamine could not adequately control. Since there is no intervention available to correct the unknown genetic defect leading to ineffective erythropoiesis, matched unrelated hemopoietic stem cell transplantation is under consideration.

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## References

1. Heimpel H, Schwarz K, Ebnöther M, Goede J, Heydrich D, Kamp T, et al. Congenital dyserythropoietic anemia type I (CDA I): Molecular genetics, clinical appearance and prognosis based on long-term observation. Blood 2006; 107: 334-40.
2. Heimpel H, Iolascon A. Congenital dyserythropoietic anemia. In: Beaumont C, Beris Ph, Beuzard Y, Brugnara C, eds. Disorders of homeostasis, erythrocytes, erythropoiesis. Paris: European School of Haematology. 2006. p. 120-42.
3. Wickramasinghe SN. Congenital dyserythropoietic anaemias: clinical features, haematological morphology and new biochemical data. Blood Rev 1998;12:178-200.
4. Woessner S, Trujillo M, Florensa L, Mesa MC, Wickramasinghe SN. Congenital dyserthropoietic anaemia other than type I to III with a peculiar erythroblastic morphology. Eur J Haematol 2003;71:211-4.