Molecular basis of congenital dyserythropoietic anemia type II and genotype-phenotype relationship

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The Online Mendelian Inheritance in Man (OMIM) compendium of human genes and genetic phenotypes includes three types of congenital dyserythropoietic anemia as reported in Table 1. A comprehensive overview of these disorders has been published recently.[1]

Congenital dyserythropoietic anemia type II is the most common of these inherited disorders. Typical morphological abnormalities of this condition are shown in Figure 1: these abnormalities clearly indicate that incomplete cytokinesis is one of the key features of erythroid cells in this condition.

More than 30 years ago, we investigated the pathophysiology of anemia in patients with congenital dyserythropoietic anemia type II in studies of iron kinetics.[2] A wide variation in effectiveness of erythroid activity was observed, and a significant inverse relationship was found between ineffective erythropoiesis and peripheral hemolysis. In 4 patients with prominent peripheral hemolysis, splenectomy was carried out. Marked improvement in their clinical condition and in hemoglobin level resulted, indicating that splenectomy was able to improve anemia in a significant portion of patients.

We later realized that many patients with congenital dyserythropoietic anemia type II developed parenchymal iron overload during the clinical course of their disease. Therefore, we studied the relationship between body iron status, degree of anemia, erythroid expansion, age and sex in 8 patients with congenital dyserythropoietic anemia type II and 2 patients with congenital sideroblastic anemia, who had received no or very few blood transfusions and no medicinal iron during the course of their illness.[3] All patients had increased iron stores. Iron load was mild in 3 women of reproductive age and severe in 2 middle-aged men who had evidence of parenchymal organ dysfunction. Iron loading, as judged by the plasma ferritin concentration, was independent of the degree of anemia while it was closely related to the patient age and the degree of increase in the total erythropoietic activity. We concluded that patients with congenital anemias associated with ineffective erythropoiesis are at high risk of developing hemochromatosis in middle age, and that prophylactic phlebotomy or iron chelation therapy should be considered for such patients.

Congenital anemias due to ineffective erythropoiesis and associated with marked increase in dietary iron absorption and progressive iron loading, are commonly defined as "iron loading anemias". They typically include thalassemia intermedia, congenital dyserythropoietic anemias and congenital sideroblastic anemias. The mechanism by which the erythroid marrow expansion combined with ineffective erythropoiesis (and therefore with excessive apoptosis of immature red cells) induces a positive iron balance[4] has been debated for years. Finch[5] introduced the concept of the "erythroid regulator" of iron balance, defining it at that time solely in physiological terms.

The identification of the ferroportin/hepcidin axis has allowed the effect of erythroid activity on iron balance to be studied and has created the basis for better defining the erythroid regulator(s).[6] In iron-loading anemias, expanded but ineffective erythropoiesis suppresses hepcidin production dysregulating iron homeostasis. Miller and co-workers showed that release of cytokines like growth differentiation factor 15 (GDF15)[7] and twisted gastrulation (TWSG1)[8] during the process of ineffective erythropoiesis inhibits hepcidin production, thus defining a molecular link between ineffective erythropoiesis, suppression of hepcidin production and parenchymal iron loading.[9] Indeed, patients with congenital dyserythropoietic anemia type I were found to express very high levels of serum GDF15, and this contributed to the inappropriate suppression of hepcidin with subsequent secondary overload.[10]

In 2001, Iolascon and co-workers[11] studied the natural history of disease in 98 patients from unrelated families enrolled in the International Registry of congenital dyserythropoietic anemia type II. They found that median age at presentation was five years, and that anemia was present in two thirds and jaundice in half of the cases. Splenectomy improved anemia and reduced jaundice, while it had little impact on iron overload. The authors underscored that this condition was difficult to diagnose and, due to the presence of a hemolytic component, was not infrequently misdiagnosed as hereditary spherocytosis.

Abnormalities of the erythroid cells in congenital dyserythropoietic anemia type II include protein and lipid dysglycosylation and endoplasmic reticulum double-membrane remnants.[12,13] In 2009, Schwarz and co-workers[14] found that that the *SEC23B* gene is mutated in patients with congenital dyserythropoietic anemia type II. SEC23B is an essential component of coat protein complex II (COPII)-coated vesicles that transport secretory proteins from the endoplasmic reticulum (ER) to the Golgi complex. Interestingly, knockdown of zebrafish sec23b also leads to aberrant erythrocyte development,[14] indicating a SEC23B selectivity in erythroid differentiation. *SEC23B* mutations were confirmed by Zanella and co-workers.[15]

In this issue of the journal, Iolascon and co-workers[16] report on a study of 42 patients with congenital dyserythropoietic anemia type II that was aimed at defining a genotype-phenotype relationship. The authors divided patients into two groups: (i) patients with two missense mutations and (ii) patients with one nonsense and one missense mutation. Overall, they found 22 mutations in *SEC23B*. Compound heterozygosity for a missense and a nonsense mutation tended to produce a more severe clinical presentation, a lower reticulocyte count and a higher serum ferritin level

Table 1. OMIM classification of congenital dyserythropoietic anemias.

OMIM n.	Disease name	Clinical features	Molecular basis
#224120	Congenital dyserythropoietic anemia type I	Congenital macrocytic anemia associated with ineffective erythropoiesis, increased iron absorption and parenchymal iron loading. Megaloblastic appearance of erythroblasts on bone marrow smear. Anemia may improve with administration of recombinant human interferon α.	Autosomal recessive disease caused by mutation in the gene encoding codanin-1 (*CDAN1*)
#224100	Congenital dyserythropoietic anemia type II (CDAN2)	Congenital anemia associated with ineffective erythropoiesis, jaundice, progressive splenomegaly, increased iron absorption and parenchymal iron loading. Erythroblast binuclearity or multinuclearity on bone marrow smear; nuclei of equal size and DNA content, suggesting a cytokinesis disturbance. Improvement of anemia and jaundice with splenectomy in a portion of patients.	Autosomal recessive disease caused by mutation in the *SEC23B* gene
%105600	Congenital dyserythropoietic anemia type III (CDAN3)	Congenital anemia associated within effective erythropoiesis. Erythroblast multinuclearity (up to 12 nuclei per cell)	Gene map locus 15q21

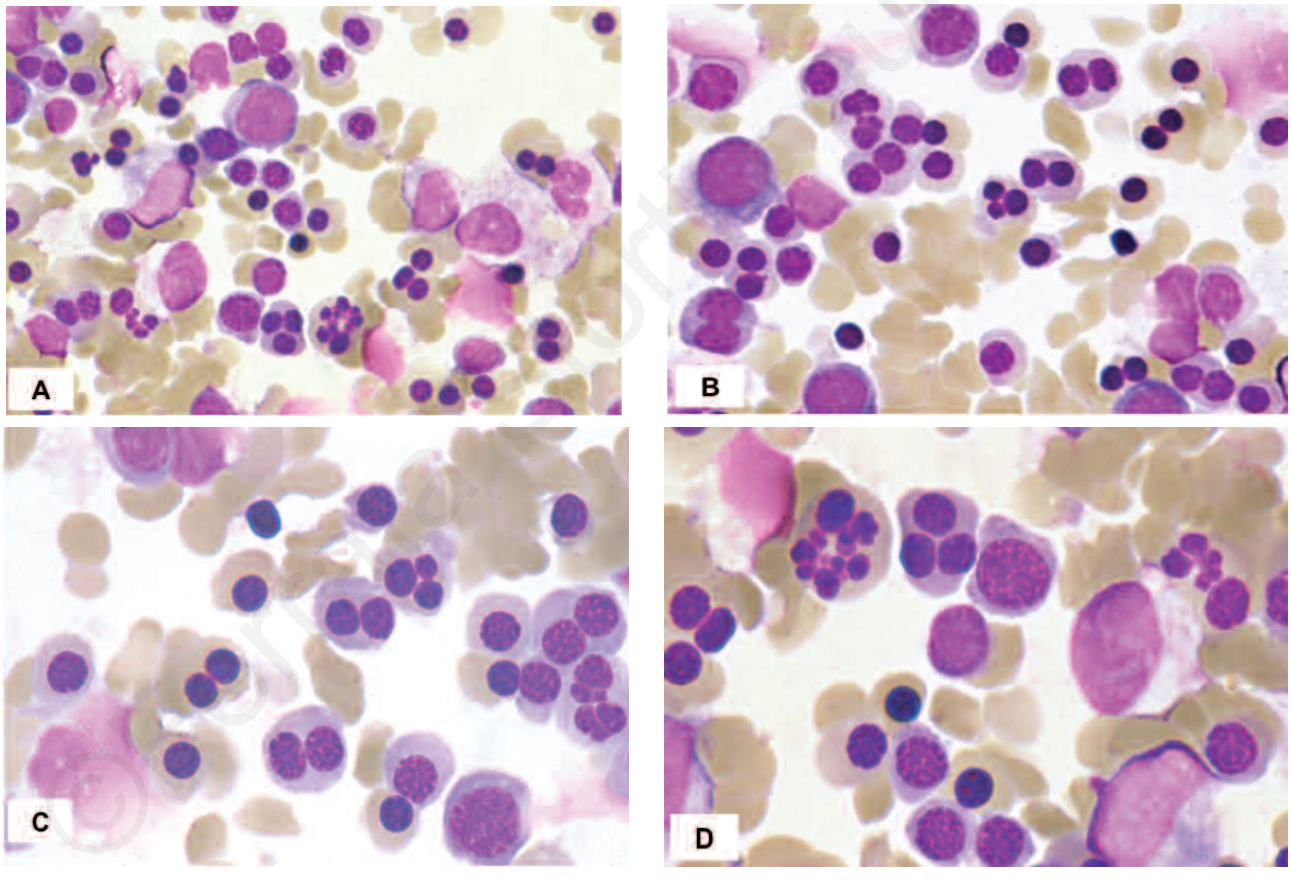

Figure 1. Bone marrow smear from a patient with congenital dyserythropoietic anemia type II. (A and B). Marked erythroid hyperplasia with binuclearity and multinuclearity, especially of late erythroblasts. MGG, x480. (C and D). Binucleate erythroblasts and erythroblasts with a multilobulated nucleus. MGG, x1200.

than homozygosity or compound heterozygosity for two missense mutations. These findings suggest that the association of one missense mutation and one nonsense mutation is significantly more deleterious than the association of two missense mutations. As illustrated in Figure 2, the former combination is more likely to result in predominant ineffective erythropoiesis, while the combination of two missense mutations is more likely to involve both ineffective erythropoiesis and peripheral hemolysis. Homozygosity for two nonsense mutations

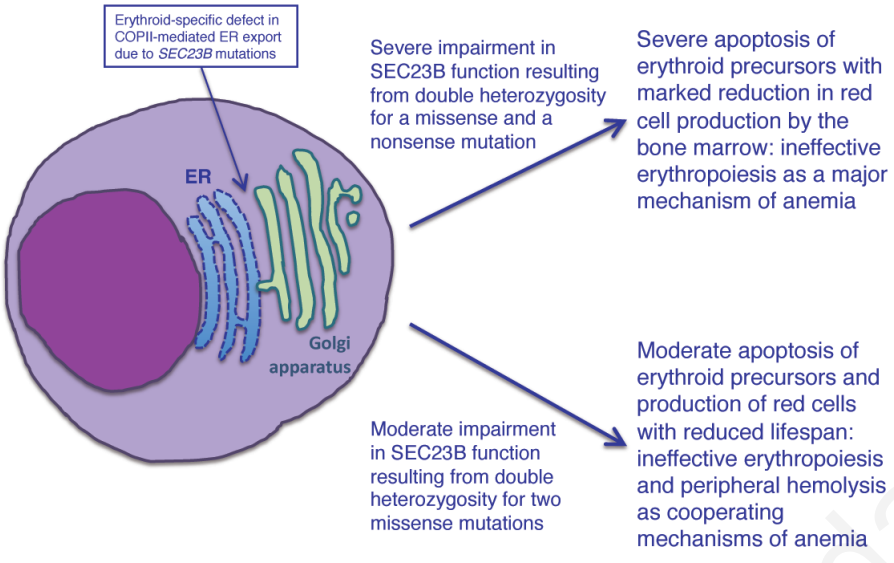

Figure 2. **Patients with congenital dyserythropoietic anemia type II may be homozygous or double heterozygous for *SEC23B* mutations. SEC23B is an essential component of coat protein complex II (COPII)-coated vesicles that transport secretory proteins from the endoplasmic reticulum (ER) to the Golgi complex in erythroid cells. In general, a nonsense mutation results in nonfunctional protein product, while a missense mutation is more commonly associated with a less efficient protein product. Abnormalities in SEC23B activity may affect both erythroblast maturation in the bone marrow and red cell survival in circulation. Findings of the study by Iolascon and co-workers in this issue[16] suggest that the association of one missense mutation and one nonsense mutation is significantly more deleterious than the association of two missense mutations. The former combination is more likely to result in severe apoptosis of immature red cells and therefore in predominant ineffective erythropoiesis as a mechanism of anemia. On the other hand, the combination of two missense mutations is more likely to involve a lower degree of apoptosis of erythroid cells, allowing many more immature red cells to mature and to give rise to circulating red cells. However, their membrane abnormalities resulting from defective SEC23B function during erythroid maturation are responsible for reduced red cell survival and excessive hemolysis. The above considerations may have clinical implications. Patients with predominant ineffective erythropoiesis are less likely to respond to splenectomy and are more likely to develop parenchymal iron loading associated with release of molecules like GDF15 and TWSG1 during the process of massive erythroid apoptosis. In contrast, patients with a significant hemolytic component are more likely to respond to splenectomy with correction of anemia and are less likely to develop parenchymal iron loading associated with suppression of hepcidin production, increased iron absorption and excessive reticuloendothelial iron release.**

was never encountered and might, therefore, be fatal.

This interesting study illustrates the importance of genomic medicine, not only in defining the molecular basis of disease but also in establishing relationships between molecular abnormalities and clinical phenotype that may be relevant to clinical decision making.

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