Ring sideroblasts and sideroblastic anemias

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The sideroblastic anemias are a heterogeneous group of inherited and acquired disorders characterized by anemia of varying severity and the presence of ring sideroblasts in the bone marrow.[1]

Ring sideroblasts

Ring sideroblasts are erythroblasts with iron-loaded mitochondria visualized by Prussian blue staining (Perls' reaction) as a perinuclear ring of blue granules (Figures 1D and 2C). The International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS) recommended that ring sideroblasts be defined as erythroblasts in which there are a minimum of five siderotic granules covering at least one third of the circumference of the nucleus.[2] Ring sideroblasts are found exclusively in pathological conditions, and should not be confused with ferritin sideroblasts, which are present in normal bone marrow. These latter are normal erythroblasts that, after Prussian blue staining, show a few blue granules scattered in the cytoplasm, representing endosomes filled with excess iron not utilized for heme synthesis (siderosomes). While the iron of ferritin sideroblasts is stored in cytosolic ferritin, whose subunits are encoded by the *FTH1* and *FTL* genes, the iron of ring sideroblasts is stored in mitochondrial ferritin, encoded by the *FTMT* gene.[3] Indeed, mitochondrial ferritin is specifically detected in ring sideroblasts, as illustrated in Figure 2D.

Classification of sideroblastic anemias

The sideroblastic anemias include both hereditary and acquired conditions, and the main disorders are reported in Table 1. Representative peripheral blood and bone marrow smears from a patient with X-linked sideroblastic anemia (XLSA) and a patient with refractory anemia with ring sideroblasts (RARS) are shown in Figures 1 and 2, respectively.

X-linked versus *autosomal recessive congenital sideroblastic anemias*

XLSA is caused by germline mutations in the erythroid-specific ALA synthase gene (*ALAS2*). Males with XLSA may present in the first two decades of life with symptoms of anemia or later with manifestations of anemia and/or those of parenchymal iron overload.[4] However, the phenotypic expression of XLSA is highly variable,[5] and occasional patients, both males and females, may present late in life.[6,7] Distinctive laboratory features are microcytic anemia with hypochromic red cells, increased red cell distribution width and evidence of parenchymal iron overload: for a conclusive diagnosis of XLSA, however, identification of the *ALAS2* mutation is required. The management of XLSA involves not only treatment of anemia, but also prevention and treatment of iron overload, family studies to identify additional at-risk individuals, and genetic counseling.[1] Most patients with XLSA are responsive, to some extent, to pyridoxine, and subjects with

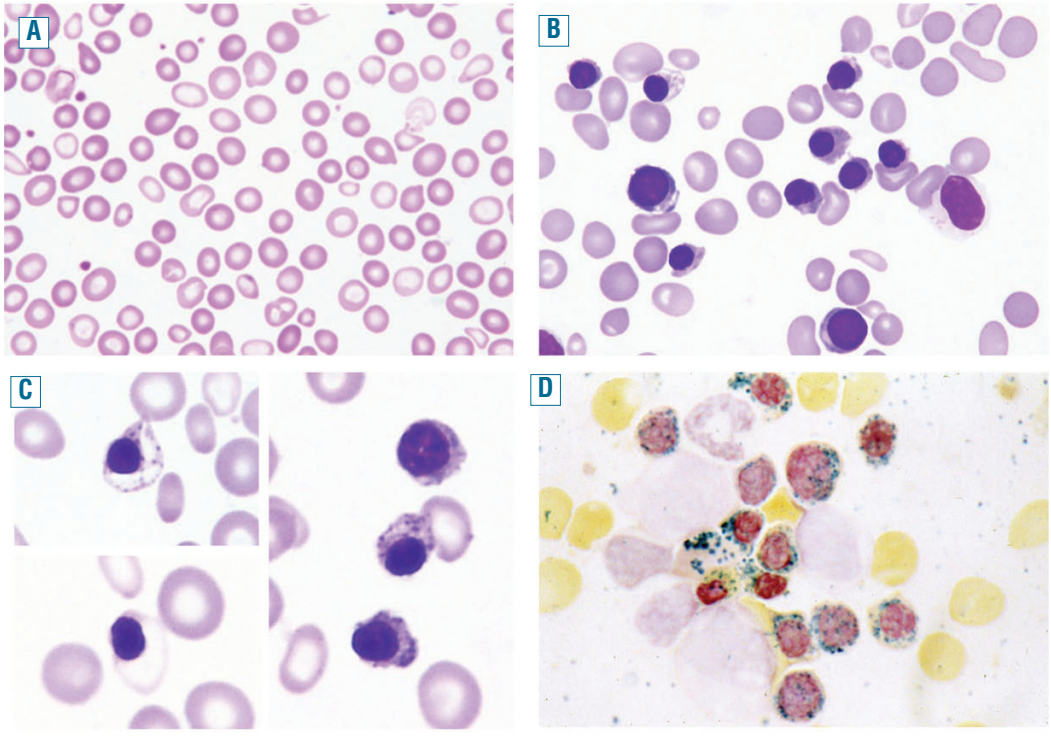

Figure 1. Representative peripheral blood and bone marrow smears from a patient with X-linked sideroblastic anemia. (A) Peripheral blood smear showing many hypochromic and microcytic cells. May-Grünwald-Giemsa (MGG), x1,000. (B) Bone marrow smear showing erythroid hyperplasia: erythroblasts are small with abnormal condensation of nuclear chromatin and ragged cytoplasm with ill-defined edges. MGG, x1,000. (C) Bone marrow smear showing erythroblasts with defective hemoglobinization (left) and erythroblasts containing multiple Pappenheimer bodies (right). MGG, x1,250. (D) Bone marrow smear. Perls' stain shows that most erythroid precursors are ring sideroblasts with at least five positive granules disposed in a ring surrounding a third or more of the circumference of the nucleus. x1,250.

iron overload can safely undergo mild phlebotomy programs under pyridoxine supplementation.

Patients affected with other inherited forms of sideroblastic anemia are not responsive to pyridoxine, and the molecular basis of these autosomal recessive disorders has been clarified only recently. Camaschella *et al.*[8] studied a middle-aged anemic patient with ring sideroblasts and iron overload whose anemia was partially reversed by iron chelation therapy. The phenotype of this patient resembled that of the *shiraz* zebrafish, a mutant resulting from a large deletion encompassing the *GLRX5* gene.[9] In fact, sequencing of *GLRX5* showed that the patient had a homozygous mutation of this gene. GLRX5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts.[10]

Two years ago, Guernsey *et al.*[11] studied three Canadian families, each with one child affected by congenital sideroblastic anemia. The data available were inconsistent with an X-linked recessive inheritance, while the families derived from a local subpopulation isolate, consistent with a possible genetic founder effect. A single nucleotide polymorphism-based genome-wide scan performed in individuals belonging to these families led to the identification of *SLC25A38* as the mutant gene responsible for this type of autosomal recessive pyridoxine-refractory sideroblastic anemia.[11] Studies on additional subjects with familial or sporadic congenital sideroblastic anemia without *ALAS2* mutations showed multiple additional biallelic *SLC25A38*

Table 1. Classification of congenital and acquired sideroblastic anemias.

Condition	Molecular basis	Clinical features
Non-syndromic congenital sideroblastic anemias		
X-linked sideroblastic anemia (XLSA) (MIM ID # 300751)	Germline mutation in the erythroid-specific ALA synthase gene (*ALAS2*, chromosome Xp11.21)	Hemizygous males have hypochromic microcytic anemia due to ineffective erythropoiesis and secondary iron overload (iron-loading anemia). Heterozygous females may have minor red cell abnormalities (in particular, increased red cell distribution width). Most patients are responsive to pyridoxine.
Autosomal recessive pyridoxine-refractory sideroblastic anemia (MIM ID #205950)	Germline mutations in the *SLC25A38* gene (chromosome 3p22.1)	Homozygous males and females have severe microcytic anemia that almost inevitably becomes transfusion-dependent. Heterozygous individuals have no hematologic phenotype. Conservative therapy includes regular red cell transfusion and iron chelation. Allogeneic stem cell transplantation represents the only curative treatment at present.
	Homozygous mutation in the *GLRX5* gene (chromosome 14q32)	The reported case regards a middle-aged male affected with pyridoxine-refractory sideroblastic anemia and iron overload.
Hereditary syndromic conditions		
X-linked sideroblastic anemia and spinocerebellar ataxia (XLSA/A, MIM ID #301310)	Germline mutation in the *ABCB7* gene (chromosome Xq13.1-q13.3)	Hemizygous males have moderate hypochromic microcytic anemia and tend to develop non-progressive ataxia and incoordination early in life.
Myopathy, lactic acidosis and sideroblastic anemia (MLASA1, MIM ID #600462)	Homozygous germline mutation in the *PUS1* gene (chromosome 12q24.33)	Myopathy, lactic acidosis, and anemia (progressive exercise intolerance during childhood).
Myopathy, lactic acidosis and sideroblastic anemia (MLASA2, MIM ID #613561)	Homozygous germline mutation in the *YARS2* gene (chromosome 12p.11.21)	Myopathy, lactic acidosis, and anemia (progressive exercise intolerance during childhood).
Thiamine-responsive megaloblastic anemia (TRMA, MIM ID #249270)	Germline mutations in the *SLC19A2* gene encoding a thiamine transporter protein (chromosome 1q23.3)	Thiamine-responsive macrocytic anemia, diabetes mellitus, and sensorineural deafness.
Pearson marrow-pancreas syndrome (MIM ID #557000)	Mitochondrial DNA deletion	Refractory sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreatic dysfunction.
Myeloid neoplasms with refractory sideroblastic anemia		
Refractory anemia with ring sideroblasts (RARS)	Abnormal expression of genes of heme synthesis and mitochondrial iron metabolism (overexpression of *ALAS2* and reduced expression of *ABCB7*). Overexpression of *FTMT*	Myelodysplastic syndrome with isolated erythroid dysplasia and benign clinical course. A variant with worse prognosis is represented by the condition defined as refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS).
Refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T)	Abnormal gene expression as in RARS plus somatic mutations of *JAK2* and/or *MPL* in about two-thirds of patients	Myelodysplastic/myeloproliferative neoplasm with mild anemia and thrombocytosis (platelets ≥ $450x10^9$/L), resembling essential thrombocythemia.
Acquired sideroblastic anemias		
Ethanol-induced and drug-induced sideroblastic anemia	–	–

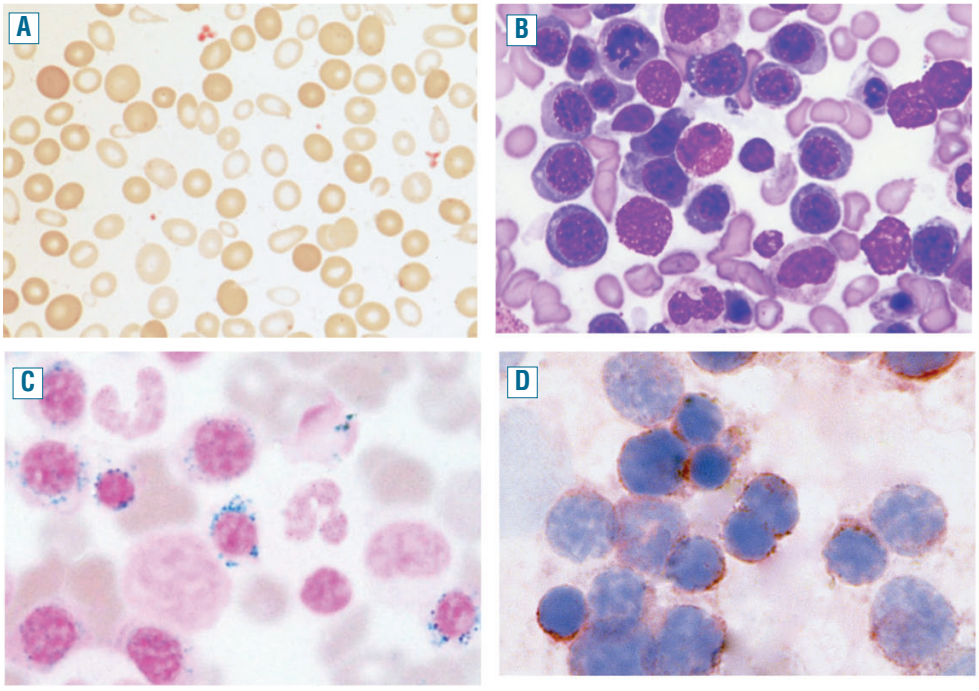

Figure 2. Representative peripheral blood and bone marrow smears from a patient with refractory anemia with ring sideroblasts. (A). Peripheral blood smear showing dimorphic red cells with a population of macrocytes and a population of hypochromic microcytes. MGG, x1,000. (B) Bone marrow smear showing a marked erythroid hyperplasia with megaloblastoid features. The rare granulocytic cells look normal. Upper right, a late erythroblast with defective hemoglobinization; lower right, an early erythroblast with vacuolated cytoplasm and a late erythroblast with Pappenheimer bodies. MGG, x1,000. (C) Bone marrow smear stained by Perls' reaction showing several ring sideroblasts. MGG x1,250. (D) Bone marrow smear. Mitochondrial ferritin is detected in granules surrounding the nucleus. Immunoalkaline phosphatase reaction, MGG x1250.

mutations. *SLC25A38* encodes the erythroid specific mitochondrial carrier protein, which is important for the biosynthesis of heme in eukaryotes.

Following the identification of mutant *SLC25A38* as a novel cause of inherited sideroblastic anemia, Bergmann *et al.*[12] systematically analyzed a cohort of 60 previously unreported patients with congenital sideroblastic anemia, looking for *ALAS2*, *SLC25A38*, *PUS1*, *GLRX5*, and *ABCB7* mutations. Twelve probands had biallelic mutations in *SLC25A38*, while 7 had *ALAS2* mutations and one had a novel homozygous null *PUS1* mutation.

In this issue of the journal, Kannengiesser *et al.*[13] report on a study of 24 patients with congenital sideroblastic anemia who did not have *ALAS2* mutations. Eleven patients of several different ancestral origins carried *SLC25A38* mutations: 9 patients were homozygous and 2 were compound heterozygotes. All patients required blood transfusions that inevitably became regular within the first few years of life. Two patients underwent allogeneic stem cell transplantation with complete correction of anemia. Since the clinical course of congenital sideroblastic anemia associated with *SLC25A38* mutations is very similar to that of thalassemia major, conservative therapy includes regular red cell transfusion and iron chelation. However, as in thalassemia major, allogeneic stem cell transplantation represents the only curative therapy at present, and should, therefore, be considered for young patients with this congenital sideroblastic anemia.

Refractory anemia with ring sideroblasts

What are the implications of recent advances in our understanding of the molecular basis of congenital sideroblastic anemia for the acquired forms, i.e. refractory anemia with ring sideroblasts (RARS) and its variant with marked thrombocytosis (RARS-T)?

RARS is a myelodysplastic syndrome characterized by isolated anemia, erythroid dysplasia only, less than 5% blasts, and 15% or more ring sideroblasts in the bone marrow.[14] The natural history of RARS is characterized by an initial phase of erythroid hyperplasia and ineffective erythropoiesis, which is usually stable for many years but in a proportion of patients may be followed by a phase of marrow failure, with or without the later emergence of leukemic blasts.[15,16] Since the vast majority of patients with this syndrome have no cytogenetic abnormalities, the clonal nature of RARS has been questioned. However, a few studies of X-chromosome inactivation patterns performed in female patients have suggested that RARS derives from the clonal proliferation of a multipotent hematopoietic stem cell with the potential for myeloid and lymphoid differentiation.[17]

Unfortunately none of the candidate genes, i.e. genes mutated in the different types of congenital sideroblastic anemia, has been found to be mutated in RARS. Of note, $CD34^+$ cells from patients with RARS have a particular gene expression profile characterized by upregulation of mitochondria-related genes and, in particular, genes involved in heme synthesis (e.g., *ALAS2*),[18] and reduced expression of *ABCB7*, a gene encoding a protein involved in the transport of iron/sulfur clusters from mitochondria to the cytoplasm.[19] In addition, RARS is characterized by over-expression of mitochondrial ferritin (Figure 2D), encoded by the *FTMT* gene.[3,20-22]

RARS-T is a myelodysplastic/myeloproliferative neoplasm characterized by anemia with ring sideroblasts and marked thrombocytosis.[23] Our recent studies suggest that RARS-T is indeed a myeloid neoplasm with both myelodysplastic and myeloproliferative features at the molecular and clinical levels, and that it may develop from RARS through the acquisition of somatic mutations of *JAK2*, *MPL*, or other as-yet-unknown genes.[24]

Thus, the available evidence suggests that the clonal

hematopoiesis of RARS and RARS-T is associated with abnormal expression of several genes of heme synthesis and mitochondrial iron processing. Identifying the somatic mutation(s) that can be responsible for these abnormalities represents the current challenge in this field.

Conclusions

The two most common forms of congenital sideroblastic anemia, i.e. the X-linked form due to an *ALAS2* mutation and the autosomal recessive form due to *SLC25A38* mutations, have similar hematologic pictures but completely different clinical courses. Overall, XLSA is a benign disorder that generally responds to pyridoxine with substantial amelioration of anemia; prevention and treatment of iron overload is also important and can be generally achieved through phlebotomy. By contrast, the congenital autosomal recessive congenital sideroblastic anemia due to *SLC25A38* mutations is a severe disease, not responsive to pyridoxine and with a clinical course very similar to that of thalassemia major: allogeneic stem cell transplantation should, therefore, be considered in young patients with this disease.

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