To the Editor:

Congenital dyserythropoietic anemia type II (CDA-II, CDAN2, or HEMPAS) (MIM224100) is an autosomal recessive trait and it represents the most frequent form of congenital dyserythropoiesis. It is characterized by normocytic anemia, variable jaundice, and hepatosplenomegaly. Gallbladder disease and secondary hemochromatosis are frequent complications. Bone marrow histology shows binucleated and multinucleated (10% to 40%) erythroblasts with karyorrhexis. Electron microscopy of these cells shows the presence of the so-called double membrane, ie, peripheral cisternae running parallel to and beneath the plasma membrane. The diagnosis could be confirmed by Positive Acidified Serum test (Ham test) and by presence of enhanced agglutination with anti-i antibodies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of red blood cell membrane proteins shows a narrower aspect and a faster migration of band 3 (anion exchange transporter), and Western blot of membrane proteins shows the presence of reticulum-endotelial proteins (calreticulin, glucose regulated protein 78, protein disulphide isomerase).

Recently we have recruited a panel of well-characterized CDA II families and used them to search for CDA II gene by linkage analysis. We first excluded three candidate genes (α-Mannosidase II, α-Mannosidase x, and N-Acetylglucosaminyltransferase II) (MANA, MANAx, Gnt-II), and obtained conclusive evidence for linkage of CDA II to microsatellite markers on the long arm of chromosome 20 (20q11.2). Strong evidence of allelic association with the disease was also detected with marker D20S863. Here we describe two unrelated families in which CDA-II disease was not linked with the CDAN2 locus, demonstrating for the first time the presence of genetic heterogeneity.

The first family comes from a little town on the Ionian sea (southern Italy). The parents could be related because three of the grandparents had the same family name. The propositus was born in 1982; at 3 days of age a severe icterus demanded an exchange transfusion. A severe thrombocytopenia was observed. During the next years anemia (range, 61 to 92 g/L) seldom required transfusions and the platelet count was always low (15 to 50 × 10^9/L). Bone marrow observation and electronic microscopy showed the characteristic feature of CDA-II associated with severe reduction of megakaryocytes, which did not show double membranes. Analysis of SDS-PAGE of red blood cell membrane protein and the Western blot showed the characteristic feature of CDA-II. The second family comes from Lecce province (southern Italy) and it consisted of two affected and one unaffected sibs (Fig 1). The mother and the sons (II-2 and II-3) had β-thalassemic trait. The probands’ (II-1 and II-2) anemia was documented since the newborn period when jaundice required exchange-transfusion. During infancy and childhood the patients received an unknown number of red blood cell transfusions, until splenectomy. Physical examination showed moderate hepatomegaly. After splenectomy the affected brothers had a very mild anemia requiring one transfusion only during an episode of infection. Bone marrow observation showed characteristic pattern of CDA-II as well as SDS-PAGE and Western blotting.

Linkage analysis by means of microsatellite markers localized at long arm of chromosome 20 performed in these families showed that the CDA-II locus was not linked to chromosome 20 (Fig 1). As a matter of fact, there is no segregation of alleles of each marker with the disease. Furthermore, in literature a reduced activity of Gnt-II has been shown in two cases and of MANA in another CDA-II case. Since it was suggested that these alterations could be directly involved in CDA-II, we performed a linkage analysis with these genes. For this purpose, highly informative markers located at the same chromosomal region where MANA-II and Gnt-II genes mapped were used as previously described. Negative results obtained indicated that none of the investigate regions contains the gene involved in determining CDA-II in these families (Fig 1).

The CDA-II anemia is often mild to moderate but may be severe and, rarely, causes fetal distress. Approximately 300 cases are present in the literature, but the prevalence of this disorder is almost certainly higher because it is likely that many asymptomatic cases with little or no anemia are underdiagnosed. The clinical heterogeneity could be caused by genetic heterogeneity or by the association with another red blood cell membrane protein.
Increased Levels of Endothelin-1 in Plasma of Sickle Cell Anemia Patients

To the Editor:

Interactions between circulating blood cells and the vascular endothelium are tightly regulated to maintain the integrity of a functional circulatory system. Endothelial cells, which are bipolar, provide the vascular system with a nonthrombogenic surface on the luminal side and perform a number of specialized metabolic and transport functions while in contact with the subendothelial matrix on the basal side. Injury to the vascular endothelium can expose the thrombogenic subendothelium and upset the delicate balance between blood flow and hemostasis. In sickle cell (SS) disease, injury to the vascular endothelium has been shown to result from increased adherence of SS red blood cells (RBCs) to endothelial cells and by vaso-occlusion of abnormally shaped SS RBCs. Vaso-occlusion by SS RBCs can produce prolonged hypoxia to local areas of the microvasculature resulting in endothelial cell damage. In addition, vaso-occlusion can contribute to the adherence of activated polymorphonuclear neutrophils, which can further damage endothelial cells by release of reactive oxygen metabolites.

One endothelial-cell–derived component extremely sensitive to cell injury is the vasoconstrictor peptide, endothelin-1 (ET-1), which has been found to be increased in the plasma of patients with diabetes, uremia, myocardial infarction, cardiogenic shock, and in patients after hemodialysis. Damage to endothelial cells in SS disease may be another system in which plasma ET-1 is increased. If this were the case, the local vasoconstriction produced by ET-1 could decrease the diameter of some blood vessels and cause slower microvasculature transit times hypothesized to be necessary for cell sickling in vivo. If ET-1 were elevated in SS disease, it would not only be a marker for endothelial cell damage but could also be a factor in exacerbating a vaso-occlusive event.

We measured the plasma levels of immunoreactive ET-1 in patients with SS disease in both steady state and crisis, and in normal age- and race-matched controls (AA) using an enzyme-linked immunosorbent assay (ELISA) method (Amersham Pharmacia Biotech, Arlington Heights, IL). Thirty-seven homozygous SS patients, (13 in crisis and 24 in steady state) from the Bronx Comprehensive Sickle Cell Center and Milford, MA. ET-1 was eluted from the columns with 2 mL 80% acetic acid. Plasma from 1 mL of heparinized blood was acidified with 0.25 mL assay buffer. The median ET-1 plasma level for AA in steady state was 18.79 pg/mL (n = 24).

REFERENCES

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Genetic Heterogeneity of Congenital Dyserythropoietic Anemia Type II

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