Congenital dyserythropoietic anaemias: new acquisitions

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Dyserythropoiesis is defined as a condition of abnormal erythropoiesis in which there are both morphological and functional disorders, with the predominant phenomena being erythroblast abnormalities and ineffective erythropoiesis, respectively. Dyserythropoietic anaemias can be divided into primary and secondary forms and both inherited and acquired forms occur. The congenital dyserythropoietic anaemias (CDA) have been classified into three types (CDA I, II and III) in relation to their pattern of inheritance and bone marrow morphology. There is also a huge group of congenital forms that cannot be included with any of the three canonical types and remain difficult to diagnose (CDA IV-VII)[1].

CDA II is the most widely spread form of dyserythropoietic anaemia and has an autosomal recessive mode of inheritance. A recent epidemiological study established that there are more than 300 cases in Europe, with the prevalence being markedly higher in Mediterranean regions and in Italy in particular[2]. The anaemia is usually mild, although there are transfusion-dependent cases. The onset is sometimes fairly late, but the condition is usually diagnosed in young patients. International registries of families with CDA II have recently been set up with the dual aim of collecting new, precise information on the natural history and epidemiology of this disease and of obtaining a bank of DNA and RNA in order to enable molecular studies.

The blood count of patients with CDA II shows a normocytic, normochromic anaemia, with mild reticulocytosis (particularly in relation to the level of anaemia). Other findings are anisopoikilocytosis, anisochromia and spherocytes. The clinical picture closely resembles that of spherocytosis, being characterised by jaundice, splenomegaly and anaemia. Biliary tract lithiasis and accumulation of iron (haemochromatosis) can complicate the clinical picture[3,4].

The diagnostic laboratory tests include evaluation of osmotic fragility, which is increased, and the demonstration of increased expression of i antigen. Another characteristic of the disease is a positive Ham test, although this test is difficult to perform[4]. Electron microscopy shows a double membrane, which is particularly visible in erythroblasts[5]. For a long time the definitive diagnosis was based on bone marrow studies, which show a 5- to 10-fold higher number of erythoblasts than in normal bone marrow (erythroid hyperplasia)[3,4]; furthermore, more than 10% of the erythroid precursors have a characteristic binuclearity, with two nuclei of equal size[6]. Biochemical analysis of proteins of the red blood cell membrane revealed a second peculiarity of this disease: reduced glycosylation of band 3 (an anion transporter), which is demonstrated as a fast migrating and narrower band on sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)[7,8]. Western blot analysis offers further diagnostic confirmation by showing that there are proteins characteristic of the endoplasmic reticulum (e.g. GRP78) on the surface of the red blood cells[5]. The half-life of the erythrocytes of patients with CDA II is reduced, a phenomenon long considered to be the consequence of a hypothesised membrane defect. We have shown that the mild haemolysis that can be found in these subjects is due to clustering of the band 3 molecules which bind autoantibodies. The red blood cells coated in this way are removed for the circulation during their transit through the spleen[9].

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This observation suggests that splenectomy could be therapeutically useful in CDA II.

The search for a causative gene was first carried out by linkage analysis of three candidate genes (Gnt-II, MANA and MANAx). Such analyses consistently excluded a role for these three genes as causes of CDA-II[10]. A genome wide search was then conducted in 14 family groups, which conclusively demonstrated linkage between some markers on the long arm of chromosome 20 and the CDA II phenotype. The *CDAN2* gene was, thus, initially localised to position 20q11.2[11]. Subsequent intensive study of the chromosomal region remapped the causative gene to the short arm of chromosome 20 and excluded many other candidate genes[12].

Analysis of the gene expression profiles of erythroid precursors during differentiation, together with gene mapping information, recently led to the identification of the causative gene, *SEC23B*[13]. This information enabled identification of the mutations present in most of the cases recorded so far[13-15]. These mutations are distributed throughout the whole length of the gene and include both missense and nonsense mutations. Despite the allelic heterogeneity, the molecular diagnosis can be focused on the few, most frequent mutations: the R14W, E109K, R497C and I318T substitutions account for more than 50% of all the mutations of the *SEC23B* gene. Furthermore, molecular analysis showed a genotype-phenotype correlation, in that patients with a missense mutation and a nonsense mutation have a more severe phenotype than patients with two missense mutations. However, a clear separation between the two genotypic classes is not feasible, given that there is a certain overlap of the phenotypic manifestations. There are no known cases of homozygosity for nonsense mutations, suggesting that the total absence of SEC23B protein is not compatible with life[15].

The SEC23B protein plays a role in cell trafficking of newly formed proteins from the endoplasmic reticulum to the Golgi apparatus[16,17]. Functional studies carried out on $CD34^+$ cells isolated from human bone marrow and zebrafish clarified some of the clinical and biochemical characteristics of the disease (binuclearity, cell cycle changes)[13].

Ongoing studies of SEC23B knock-out mice and transgenic mice will help to reveal the molecular mechanisms underlying CDA II and aid the search for possible pharmacological treatments. Although much is yet to be learnt, identification of the genetic cause of the disease has made early and neonatal diagnosis possible.

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