Close to unraveling the secrets of congenital dyserythropoietic anemia types I and II

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The congenital dyserythropoietic anemias (CDA) are a heterogeneous group of rare genetic disorders characterized by anemia, the occurrence of multinuclear erythroid precursors in the bone marrow, ineffective erythropoiesis and iron overload. Among the vast number of CDA that have been reported, which are differentiated mainly by the morphological appearance of the erythroid precursors, CDA I and CDA II, both recessively inherited disorders, appear to be two rather homogeneous groups. Long-term clinical follow-ups, therapeutic assessments and in depth studies into the molecular genetics of these disorders have been performed. One of the genes mutated in CDA I, *CDAN1* (15q15.1-15.3), codes for codanin-1. In this issue of Haematologica, Noy-Lotan *et al.* describe that codanin-1 is a cell cycle-dependent nuclear protein and that its synthesis is promoted by transcription factor E2F.[1] The other gene involved in CDA I has yet to be eludicated. CDA II is characterized by multiple glycosylation abnormalities affecting red cell components, but also non-erythrocytic proteins. It has been viewed as a congenital disorder of glycosylation. However, the absence of mutations in all genes governing glycan metabolism that have been investigated so far and, in particular, those lying in the region where the responsible gene has been mapped (20q11.2), suggests that this view should be dismissed. A third type of CDA, CDA III, for which the causative gene has been localized (15q22-25), remains poorly understood and will not be dealt with in this perspective article. The other forms of CDA costitute a motley set about which little progress can be hoped for unless the causative gene is discovered.

Congenital dyserythropoietic anemia type I at present

Eighty familial or individual cases of CDA I were recorded after merging the German, Italian, French, Spanish, Polish and British registries, which include patients from most European countries, eliminating the redundant entries. (A. Matuschek *et al.*, personal communication, 2008). CDA I is thus two to three times less frequent than its counterpart, CDA II. CDA I has also been observed among North Africans, Saudi Arabians, Japanese, Indians, Chinese, and Polynesians. A genetic isolate was found among Bedouin tribes living in the Negev.[2] There is, however, a strong recruitment bias depending on the countries. Most probably, CDA I, like many rare genetic disorders, occurs in all ethnic groups with a relatively comparable frequency.

CDA I is an autosomal recessively inherited disorder. It becomes manifest in infancy, childhood, or adolescence. The most severe forms may appear *in utero*. It is associated with hemolytic anemia, which is usually moderate (hemoglobin of about 9.0 g/L), a normal or slightly elevated reticulocyte count, and macrocytosis. Jaundice, splenomegaly, hepatomegaly, and gallstones should be looked for. Jaundice remains mild, but may be aggravated by the A[TA]$_7$TAA polymorphism in the promoter of the uridine diphosphate glucuronosyltransferase gene.[3]

CDA I is often associated with a variety of dysmorphologies and other abnormalities: syndactyly in hands and/or feet, absence of distal phalanges and nails and, sometimes, of other phalanges in some fingers and toes, additional metatarsal bones, an additional phalanx in the left middle finger, duplication of the distal phalanx of the big toes, hypoplastic nails, flexed finger tips, short stature, brownish skin pigmentation, patches of skin depigmentation, flattened bodies of vertebrae, hypoplastic right third rib, bilateral conduction and sensory deafness and impairment of speech.[4] Almond-shaped blue eyes, hypertelorism, micrognathism, and partial duplication of L3 vertebra have also been recorded. Blood smears often exhibit marked poikilocytosis, associated with a reduction in the membrane protein 4.1R. This secondary phenomenon is yet to be accounted for. Although not constant, it represents a valuable diagnostic clue. Bone marrow studies show intense erythroid hyperplasia, with an increase in binucleate polychromatic erythroblasts and the presence of chromatin bridges linking two nuclei. Electron microscopy reveals the spongy appearance of early and late polychromatic erythroblasts. Nuclei have electrolucent areas within electron-dense heterochromatin. The nuclear envelope is lined with cytoplasmic intrusions sometimes carrying cytoplasmic organelles.[5] Growth differentiation factor 15 (GDF-15) appeared to be increased in 17 patients, but a reduction of serum hepcidin failed to reach statistical significance.[6]

Several complications are possible. Very severe forms may be manifested by hydrops fetalis. Iron overload is the main concern during the evolution of the disorder. It has not been demonstrated so far that the *HFE* gene mutation H63D has an adverse effect.[3] Pulmonary hypertension[7] and retinal angioid streaks may also occur.[8] The latter disorder had long been thought to belong to another CDA, CDA III.

Transfusions should be avoided because of the risk of iron overload. Iron chelation should be performed when ferritinemia exceeds 500-1000 ng/mL. Provided that the anemia is well compensated, phlebotomy may be resorted to under careful control. The effect of splenectomy has not been established unambiguously.[9] Gallstones can be treated by cholecystectomy. Interferon-α is efficient in as far as it raises the hemoglobin concentration and normalizes liver iron overload.[10] Transplantation has been tried occasionally.[11]

One causative gene, *CDAN1* (minus strand), has been mapped to 15q15.1-15.3 between markers D15S779 and D15S778,[12] and identified,[13] based on the Negev Bedouin isolate. *CDAN1* spans 15 kbp and has 28 exons. It is ubiq-

uitously expressed. The encoded protein is codanin-1, containing 1227 amino-acids. At present, *CDAN1* mutations have been found in 88% of CDA I patients. In a number of patients, however, only one *CDAN1* allele carried a mutation and, in a few of them, none of them bore any change.[14] In monozygotic twins, cDNA analysis failed to uncover any mutation (Delaunay *et al.*, unpublished data). Microsatellite analysis in the 15q15.1- 15.3 region, performed in English patients, suggested that the gene responsible lies outside this region.[15] In a Pakistani kindred with CDA I, chromosome 15 abnormalities were excluded.[16] The existence of another gene responsible for CDA I appears, therefore, nearly certain.

The Bedouin mutation (A1042T) has been found in a Caucasian patient whose parents were very probably consanguineous.[8] This mutation, which is prone to happen in a recurrent way since codon 1042 contains a CpG dinucleotide (a *hot spot*), stemmed, in all likelihood, from an event independent of the founding mutation among the Bedouins. Another mutation, N598S in the homozygous state, was found in a very unusual situation. Three siblings, not known to be born from consanguineous parents, combined, in a very misleading fashion, CDA I, deafness and teratoasthenospermia.[17] Telomeric to the *CDAN1* gene, a 70 kbp deletion in the homozygous state removed a tandem repeat comprised of two clusters of four genes. Among them, in the centromeric cluster, i.e., in the one closest to the *CDAN1* gene, lie the *STRC* and the *CATSPER2* genes coding for inner ear stereocilin and a calcium channel in sperm cell precursors, respectively. The genes of the telomeric cluster are pseudogenes. Taken together, this remarkable association had initially suggested, wrongly, that the *CDAN1* gene lay within the deletion.

Using synchronized HeLa cells codanin-1 was found to be localized to the heterochromatin in interphase cells. During the cell cycle, a high level of codanin-1 was noted during the S phase. During mitosis, codanin-1 became phosphorylated and this coincided with its exclusion from condensed chromosomes.

The proximal promoter region of the *CDAN1* gene contains five putative binding sites for E2F, a member of a transcription factor family involved in cell cycling. This region was found to be the target of E2F.[1]

Congenital dyserythropoietic anemia type II at present

CDA II is the most common form of CDA. Examination of the main European Registries of this type of CDA suggests a higher frequency of *CDAN2* in Italy and in Mediterranean countries than in central and northern Europe. So far it has been difficult to assess whether this is due to a clustered distribution or to hematologists' attention.

Up to April 2008, these registries (Italian, French, German) had collected at least 341 cases coming from all European countries. (A. Matuschek *et al.*, unpublished data, 2008). The regional distribution of the Italian patients demonstrated clustering in southern Italy, suggesting a founder effect. However, molecular studies by means of microsatellites, localized to where the gene was mapped,[18] failed to demonstrate the existence of a common haplotype.[19] These data are compatible with the condition generally being due to sporadic or familial mutations, and that in the case of recurrent mutation a founder effect could be supposed or hot-spot mutation codons could exist. Moreover, it is not possible to exclude that the *CDAN2* locus is outside this published region (20q11.2), but close to it; this phenomenon is not so rare in a gene located near the centromere.

The main clinical findings of CDA II are normocytic anemia, jaundice and variable splenomegaly, which are also present in hereditary spherocytosis.[20] Indeed, these two conditions can be confused because osmotic fragility tests give similar results in both. An inadequate number of reticulocytes for the hemoglobin level in CDA II is the most relevant observation for the differential diagnosis. It is noteworthy that in a European investigation, 62 CDA II patients were splenectomized and that in 12 of these the diagnosis prior to the operation was hereditary spherocytosis. (A. Iolascon, unpublished data, 2008).

The vast majority of CDA II patients seem to have a variable degree of anemia, but usually mild. Approximately 10% or less require frequent transfusions or are dependent on them; it has not been possible to establish yet whether these cases are due to more severe mutations in CDA II protein structure, or whether they are not due to the *CDAN2* gene but to other gene(s) causing the same phenotype, or to the interaction with other intra-erythrocytic defects, such as thalassemia.

CDA II is associated with a well-defined cellular and ultrastructural phenotype: bi- or multinucleated late erythroid precursors, karyorrhexis, and the presence of pseudo-Gaucher cells. These findings could suggest a defect in the final part of mitosis (cytokinesis) and in vesicle trafficking. Moreover flat vesicles of variable length have been visualized by electron microscopy.[21] This finding is associated with the presence of several reticulum endoplasmic proteins on the cell surface (calreticulin, glucose-regulated protein 78 and protein disulfide isomerase). This is a hallmark of CDA II and could be used for diagnosis. From a pathogenic point of view this feature could suggest that the defect is localized soon after reticulum endoplasmic production of proteins and that normal processing does not occur, causing the abnormal presence of reticulum endoplasmic proteins on the cell surface. Membrane abnormalities can also detected by highly specific biochemical tests. Band 3 (anion exchange protein-1) and band 4.5 (glucose transporter-1) show thinner and faster migration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This is due to the main biochemical feature of this hereditary disease: defective glycosylation. This is present not only in erythroid precursors but also in liver cells; in fact transferrin is also hypoglycosylated. Truncation of N-glycans is a consistent finding in CDA II erythrocytes indicating the diagnostic value of tomato lectin studies. However, structural data of erythrocyte N-glycans indicate that CDA II is not a distinct glycosylation disorder but is caused by a defect disturbing Golgi processing in erythroblasts.[22] Thus, glycoconjugate abnormalities in CDA erythrocytes do not cause the disease, but rather the disease brings about the abnormalities. Nevertheless, they are useful for diagnosis, especially of CDA type II,

and detection of *CDAN2* carrier status in heterozygotes in whom a clear dosage effect is observed.[22]

Analysis of the hypoglycosylated band 3 (inhibition of sulfate flux by H_2-DIDS) showed decreased activity of the anion transporter and a greater aggregation capacity in CDA II erythrocytes. Aggregated band 3 was reported to bind naturally occurring antibodies, possibly mediating the phagocytic removal of red blood cells. These results suggest that the mild hemolysis found in CDA II patients may be ascribed to clustering of band 3, leading to IgG binding and phagocytosis, rather than to secondary modifications of the structure of the erythrocyte cytoskeleton. These data are consistent with the possible beneficial role of splenectomy in CDA II patients, even when there are some concerns regarding the possible increase of iron overload in other tissues.[23] Interestingly, band 3 zebrafish mutant *ret* showed high numbers of binucleated erythroblasts, the presence of *double membranes*, and a reduction in post-translational glycosylation of anion exchanger-1. This candidate gene in humans was excluded by linkage analysis.[24]

The results of structural analysis of CDA II band 3 carbohydrates suggested disruption of the biosynthesis around the N-acetylglucosaminyltransferase II and alpha-mannosidase II steps. Linkage analysis in patients from southern Italy excluded candidate genes. A genome-wide search obtained conclusive evidence for linkage of CDA II to microsatellite markers on the long arm of chromosome 20 (20q11.2). A maximum two-point lod score of 5.4 at q=0.00 with the marker D20S863 was obtained. Mapping of the gene allows for the demonstration of genetic heterogeneity in this condition and does not confirm the existence of a founder effect.

Seven candidate genes, which map to 20q11.2, were analyzed in a large series of CDA II patients but none turned out to be the causative gene.[25] So the search is still open for the gene that could explain all the findings of this condition. There are descriptions of a few subjects with a mutation in the *GATA1* gene which results in an amino acid substitution (V205M) within the highly conserved portion of the GATA1 N-terminal finger domain, leading to dyserythropoietic anemia and macrothrombocytopenia.[26] It seems that in these cases mutations in the *GATA1* gene induce a reduction or the total absence of *CDAN2* gene expression.

One complication of dyserythropoiesis is iron overload, because iron absorption increases secondary to expanded erythropoiesis. Serum ferritin, a reliable marker of iron overload, increases in the majority of cases with age, with kinetics comparable to those in untreated hereditary hemochromatosis or thalassemia intermedia. Severe organ damage (mainly liver) was seen in the majority of cases not treated by iron depletion. Upregulation of iron is only partially prevented by splenectomy in CDA II.[20] Several studies support the notion that high levels of GDF-15 contribute to iron loading, as was recently demonstrated by Tamary *et al.* in CDA I patients.[6] They proposed that apoptosis associated with ineffective erythropoiesis in humans provides the molecular trigger for the pathologic increases of GDF-15 in CDA I patients. Their results were recently confirmed by Muckenthaler M *et al.* (personal communication, 2008) who showed that GDF-15 levels are significantly increased in the serum of CDA patients (with the highest values being detected in CDA I patients), in addition to the elevated ferritin, reticulocyte and bilirubin concentrations and diminished transferrin and hemoglobin values found in such patients. GDF-15 levels significantly correlate with reticulocyte counts and bilirubin levels (Muckenthaler M *et al.* personal communication, 2008). It is not yet known whether the iron metabolism abnormality in CDA-II is a defect due to dyserythropoiesis with an increased request for iron from bone marrow to duodenal cells or whether post-translational modifications of some proteins playing an important role in this metabolism could be causative.

Future directions

At this juncture, many lines of research become apparent.

With regards to CDA I: (i) it is urgent to understand the role of codanin-1 better; (ii) at what stages and on which cells codanin-1 mutants act to produce the dysmorphologies need to be determined; (iii) the other gene, which most probably causes CDA I with an identical phenotype, needs to be identified, but this is a challenge because no large families are currently available for linkage analysis; (iv) the acquired dysregulation of iron metabolism in CDA I which has only started being investigated requires greater exploration in view of the new factors recently discovered; and (v) the mechanism of action of interferon-α in the treatment of CDA I ought to be elucidated.

With regards to CDA II: (i) although CDA II is a probably not a direct disorder of glycosylation, one may surmise that it stems from some abnormality involving glycosylation in an indirect way. The mitosis alteration would then appear in a further indirect manner. These aspects need further study; (ii) the acquired dysregulation of iron metabolim needs to be explored, as has been underscored for CDA I.

CDA I and CDA II are destined to appear increasingly different from one another as their molecular mechanisms are better understood. One day, they may even be separated into distinct nosological frames, with only their bone marrow appearance remaining in common.

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Dr. Iolascon's studies discussed in this article have been supported by the Italian Ministero dell'Università e della Ricerca (PRIN- MIUR 2006) and project PS 35-126/IND, "Grants Convenzione CEINGE-Regione Campania-Ass. Sanità" and by Telethon (Italy).

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Clinical relevance of extra-hematologic comorbidity in the management of patients with myelodysplastic syndrome

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Malignancies have an increasing incidence with age. At present, 60% of cancers and two-thirds of cancer deaths occur over the age of 65 years in developed countries. This proportion is expected to increase markedly in the next decades as a consequence of the ageing of the population.[1]

One aspect of ageing is an increased prevalence of comorbidity. In a typical geriatric population, subjects aged 65 years and older suffer on average from three or more concomitant diseases. Similarly, older patients with malignancy present a high prevalence of comorbidity.[2] As a result, hematologists and oncologists will increasingly treat patients who have concomitant diseases.

Comorbidity is recognized to have an unfavorable effect on life expectancy of patients with cancer, as well as to influence clinical decision making. There is a negative clinical effect *per se* in terms of competing risk of death with primary disease. Furthermore, presence of comorbidity significantly affects therapeutic strategies,