Erythropoietic failure syndromes in paediatrics

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Schematic representation of normal hematopoiesis and erythroid development

Pluripotent hematopoietic stem cells

- LT-HSCs
- ST-HSCs

Multipotent progenitors

- CLP
- MEP
- CMP
- GMP

Committed progenitors

- ProMegB
- Megakaryocyte

Erythropoiesis

- BFU-E
- CFU-E
- ProEB

Mature blood cells

- T and B lymphocytes, NK cells
- Lymphoid DCs
- Mast cells, eosinophils, neutrophils, monocytes, macrophages, myeloid DCs

Defects of oligoelements

- FA; DKC
- DBA; SDS
- CDA

PTLs
Flow diagram for differential diagnosis of BMF

C CBC: (hemolytic) anemia with inadequate reticulocyte count

BMF syndromes

Isolated ineffective erythropoiesis: BM examination

Erythroid hyperplasia

- Reduction to absent trilineage hematopoiesis (FA, AA, PNH, MDS)
- Reduced proliferation and survival of erythroid progenitors (DBA)

- CDA I: incompletely divided cells; thin chromatin bridges between nuclei of pairs of erythroblasts
  - EM: “swiss cheese appearance” of the erythroblasts heterochromatin
  - Molecular diagnosis: CDAN1 sequencing

- CDA II: binucleate and rarely multinucleate late polychromatic erythroblasts
  - EM: double plasma membrane of the erythroblasts
  - Biochemical diagnosis: hypoglycosylation of band 3
  - WB: ER proteins on red blood cells plasma membrane
  - Molecular diagnosis: SEC23B sequencing

- CDA III: giant multinucleated erythroblasts

- Dyserythropoietic morphology variants

- Ineffective erythropoiesis; autosomal dominant inheritance
  - KLF1 sequencing

- Ineffective megakaryopoiesis and erythropoiesis
  - GATA-1 sequencing

A. Iolascon et al, Haematologica 2012
DIAMOND-BLACKFAN ANEMIA (DBA)

- Rare congenital hypoplastic anemia (7/million live births).
- Median age at diagnosis= 3 months
- Intrinsic defect of the erythroid progenitors with differentiation blockade.
  - A regenerative usually macrocytic anemia
  - Erythroblastopenia (absence or < 5% of erythroid precursors)
  - Persistence of the fetal erythropoiesis (increased HbF, persistence of the Agi instead AgI)
  - Elevation of the eADA in 90% of non transfused DBA patients
- Heterogeneous disease in
  - Clinical expression: various malformations in 40% of DBA cases.
  - Therapeutic response: 60 to 80% of the DBA patients respond to steroid therapy.
  - And genetics: heterozygous mutation identified in a RP gene (14 RP genes identified).

DBA is the first "ribosomopathy"
RPS19 mutation map

-83 mutations in 127 DBA families;
-Heterozygous mutation;
-Spread along the entire gene sequence

Prof. U Ramenghi (personal comm)
PATIENTS WITH MUTATION 106/162 (65.4%)  

Deletions 19%

Prof. U Ramenghi (personal comm)
GENOTYPE / PHENOTYPE CORRELATION

RPL5

RPL5

e

RPL11

STEROIDS

RPS26

RPL35A

Prof. U Ramenghi (personal comm)
UPDATE 2012 - therapy -

Prof. U Ramenghi (personal comm)
Congenital dyserythropoietic anemias (CDAs)

CDAs comprise a group of rare hereditary disorders that are characterized by ineffective erythropoiesis as the predominant mechanism of anemia and by distinct morphological abnormalities of erythroblasts in the bone marrow.
Diagnosis of the CDAs requires the presence of all of four criteria:

1. Evidence of congenital anemia/jaundice or of heredity
2. Evidence of ineffective erythropoiesis
3. Typical morphological appearance of bone marrow erythroblasts
4. Exclusion of congenital anemias that fulfill criteria 1 and 2, but were classified according to the underlying defect, such as the thalassemia syndromes, certain types of pathological hemoglobin or hereditary sideroblastic anemias.

H. Heimpel & A. Iolascon, 2005
### Differential diagnosis of CDAs

**Table 1.** Characteristic features of different types of congenital dyserythropoietic anemia

<table>
<thead>
<tr>
<th>CDA type</th>
<th>I</th>
<th>II</th>
<th>III familial</th>
<th>III sporadic</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>Autosomal-recessive</td>
<td>Autosomal-recessive</td>
<td>Dominant</td>
<td>Variable</td>
<td>Autosomal-dominant or X-linked or recessive</td>
</tr>
<tr>
<td>Cases reported</td>
<td>&gt; 300</td>
<td>&gt; 450</td>
<td>2 families</td>
<td>&lt; 20</td>
<td>~ 70</td>
</tr>
<tr>
<td>Bone marrow morphology (light microscopy)</td>
<td>Abnormal chromatin structure, chromatin bridges</td>
<td>Bi-nuclearity Multinuclearity of mature erythroblasts</td>
<td>Giant multinucleated erythroblasts</td>
<td>Giant multinucleated erythroblasts</td>
<td>CDA I-like CDA II-like others</td>
</tr>
<tr>
<td>BM EM findings</td>
<td>&quot;Spongy&quot; heterochromatin, invagination of cytoplasm into the nucleus</td>
<td>Peripheral cysternae beneath the plasma membrane</td>
<td>Clefts in heterochromatin, auto-phagic vacuoles, intranuclear cisternae</td>
<td>various</td>
<td>various</td>
</tr>
<tr>
<td>Mutated Gene</td>
<td>CDAN1 C15ORF41</td>
<td>SEC23B</td>
<td>KIF23</td>
<td>Unknown</td>
<td>KLF1 GATA-1 unknown</td>
</tr>
<tr>
<td>Associated dysmorphology/organ involvement</td>
<td>Skeleton</td>
<td>Variable, rare</td>
<td>Monoclonal gammopathy, myeloma, angioid streaks</td>
<td>Variable</td>
<td>CNS others</td>
</tr>
</tbody>
</table>

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**Iolascon A et al, Blood 2013**
Age of diagnosis of CDAs

![Chart showing the age of diagnosis of CDAs with categories ranging from <5 to <50 years, and two bars representing CDA I and CDA II.]
Clinical findings of CDAs

- Anemia
- Jaundice
- Splenomegaly
- Gallstones
- Iron overload
Hematologic parameters in CDA type I and II

- **Haemoglobin** (g/dL):
  - CDAI (n=40): 10.0 ± 1.0
  - CDAII (n=112): 10.0 ± 1.0

- **Reticulocytes absolute count** (10^6/L):
  - CDAI (n=28): 8000 ± 1200
  - CDAII (n=89): 14000 ± 2000

- **MCV (fl)**:
  - CDAI (n=34): 80 ± 2
  - CDAII (n=56): 80 ± 2

*p = 0.004, p = 8*10^{-16}*

Iolascon A et al, Haematologica 2012
INDIRECT BILIRUBIN LEVELS AND INCIDENCE OF GALLSTONE FORMATION IN CDAN2 PATIENTS DIVIDED BY THEIR UGT1A GENOTYPE

<table>
<thead>
<tr>
<th>UGT1A1</th>
<th>Ind.Bil</th>
<th>Gallstones</th>
<th>No Gallstones</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TA)6/(TA)6 or</td>
<td>1,9 ± 1,4</td>
<td>24%</td>
<td>76%</td>
</tr>
<tr>
<td>(TA)6/(TA)7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(TA)7/(TA)7</td>
<td>7,4 ± 3,2</td>
<td>87.5%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>
Iron Overload in CDAs: relevance of this complication. Pathogenesis in CDA-I and CDA-II.

Classification and distinguishing features of CDAs

**TYPE I**

Clinical features: anemia with neonatal appearance; jaundice, splenomegaly; rare syndactyly;

Common complication: hemochromatosis

Bone marrow morphology
- MO: megaloblastoid erythroid hyperplasia; nuclear bridges
- ME: spongy-appearing nuclei and invagination of the cytoplasm in the nucleus.

Inheritance: Autosomal recessive
Locus: 15q15.1-15.3 (Codanin-1)
MUTATIONS IN CDAN1 GENE

c.156 C>G
p. F52L

c.1078 T>C
p. F360L

c.1367+1 G>A

c.1117_19 delGGTT
p. V373del

c.1789_1791 delAGG
p. E597del

c.2015 C>T
p. P672L

c.3389 C>T
p. P1130L

c.2173 C>T
p. R725W

c.2140 C>T
p. R714W

c.2092 G>A
p. E698K

c.2069 T>C
p. V690A

c.2062 C>T
p. R688W

c.1796 A>G
p. N599S

c.2248 G>T
p. G750C

c.2044 C>T
p. R682X

c.1860+5 G>A

c.2393 C>T
p. D791N

c.2539 C>T
p. Q847X

c.2650 A>G
p. T884A

c.2605 G>A
p. V869M

c.2602 T>A
p. F868I

c.3194 G>A
p. R1065Q

c.3128 A>T
p. D1043V

c.3124 C>T
p. R1042W

c.3107 C>A
p. S1036F

c.2868+2 insCCG

c.3145 insT
p. S1049Fs25X

c.2992 C>T
p. R996X

c.3547 C>T
p. Q1183X

c.3024 insTT
p. G1008Fs23X

c.3558 del-10 to +31
New insights on Codanin-1

- Codanin is a target of E2F1 transcription factor and is cell-cycle regulated (Noy-Lotan S, 2009)
- It binds Asf1a a chaperone involved in chromatin structure dynamics (Tamary H, 2010)

- HP1a in abnormally localized in Golgi apparatus of CDA-I erythroblasts

- Codanin-1 colocalizes with Sec23b (Renella R. Blood 2011)
CDA III clinical findings

- Intravascular hemolysis
- Anemia mild to moderate
- Retinal angioid streaks
- Monoclonal gammopathy of undetermined significance (MGUS)
- Myeloma
- RBC transfusions are rarely needed

Sporadic cases:
- Severe hyperplasia
- Skeletal disorders
- Mental retardation
- Hepatosplenomegaly

Bone marrow smear shows dyserythropoiesis with large multinucleated erythroblasts (gigantoblasts)
CDA III genetics

By linkage analysis CDA III locus was mapped to an 11 cm interval on chromosome 15 (15q21-q25)

Lind et al, Hum Mol Genet 1995

KIF23 localizes in the midbody
KIF23 (MLKP1) localization (green) relative to DNA (blue) in Hela cells
The cumulated prevalence of CDA I and CDA II showed the **highest value in Italy** (2.49/million).

CDA II is more frequent than CDA I, with an overall ratio of approximately 3.0.

*Number of cases collected in a 42-yr period*
1967 - Description and classification (H. Heimpel and F. Wendt)

1982 - Band 3 hypoglycosylation

1990 - Identification of enzyme defects

1996 - Identification of double red blood cell membranes

1997 - Mapping of the CDAN2 gene in 20q11.2

1998 - Reduced expression of band 3

2001 - Description of the natural history of CDA II

2009 - Identification of the causative gene of CDA II
SEC23B is a component of COPII complex

The 3 types of coats: clathrin, COPI and COPII.

CCV mediates Golgi-Vacuole traffic;

COPI mediates retrograde traffic from Golgi to ER;

COPII mediate ER-Golgi transport

Russo R et al, Am J Hematol 2012
Group A: patients with two missense mutations (N=27)

Group B: patients with one nonsense + one missense mutation (N=11)

Correlation between the mutations and various biological parameters showed that addition of one missense mutation and one nonsense mutation tended to produce a more severe presentation than the association of two missense mutations; or rather, there is a trend for patients carrying two missense mutations to be more mildly affected.
The chart showed the analysis performed on 10 mutations shared between two cohorts. The allelic frequency has been assessed on 62 Italian cases and 45 not Italian cases (NI)
Dissemination of the E109K mutation in the Mediterranean area
Human phenotypes associated with GATA1 mutations

Legend

AD: activation domain
NF: N-ter zinc finger domain
CF: C-ter zinc finger domain

XLTDA: dyserythropoietic anaemia and thrombocytopenia, X-linked
XLT: thrombocytopenia, X-linked
XLTT: thrombocytopenia with beta-thalassemia, X-linked
CEP: congenital erythropoietic porphyria
IE-DBA: IVS2-1 G>C(ds)

Mutations which hinder GATA-1:FOG-1 interaction
Mutations which hinder GATA-1:DNA interaction
Clinical phenotypes due to KLF1 mutations

**Legend:**
- MUTATIONS ASSOCIATED TO “LV PHENOTYPE”
- MUTATIONS ASSOCIATED TO CONGENITAL DYSERYTHROPOIETIC ANEMIA
- MUTATIONS ASSOCIATED TO HPFH
- MUTATIONS ASSOCIATED TO HEREDITARY SPHEROCYTOSIS IN MODEL MOUSE
SEC23B is required for the maintenance of murine professional secretory tissues

Tao J. et al. PNAS 2012
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