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EuroBloodNet

Congenital dyserythropoietic anemias

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ERN-EuroBloodNet subnetwork:
Red blood cell defects, Bone marrow failure
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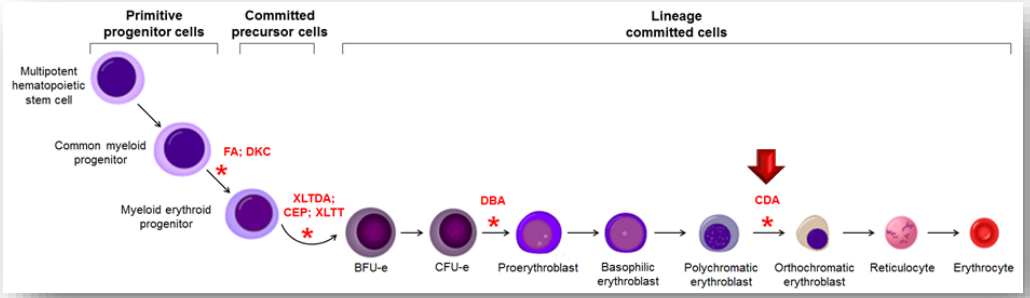
Conflicts of interest

Nothing to disclose

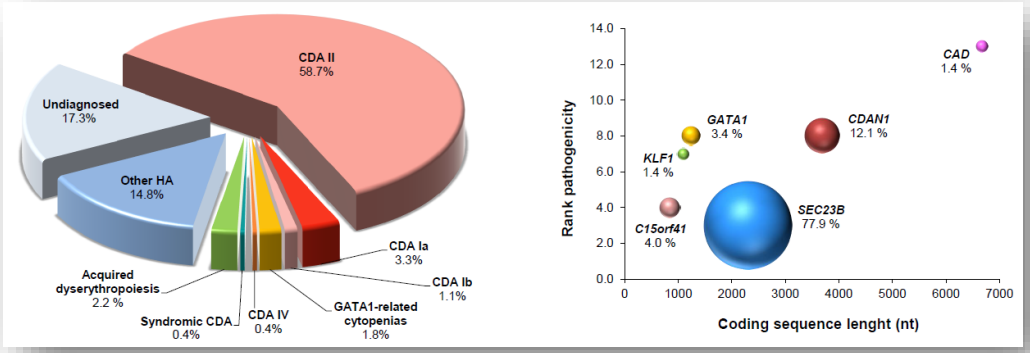
Learning objectives of the webinar

- Description of the clinical, biochemical, and molecular features of CDAs



- NGS-based identification of genetic modifier variants

- Understanding the problem of establishing a correct classification of patients affected by CDA



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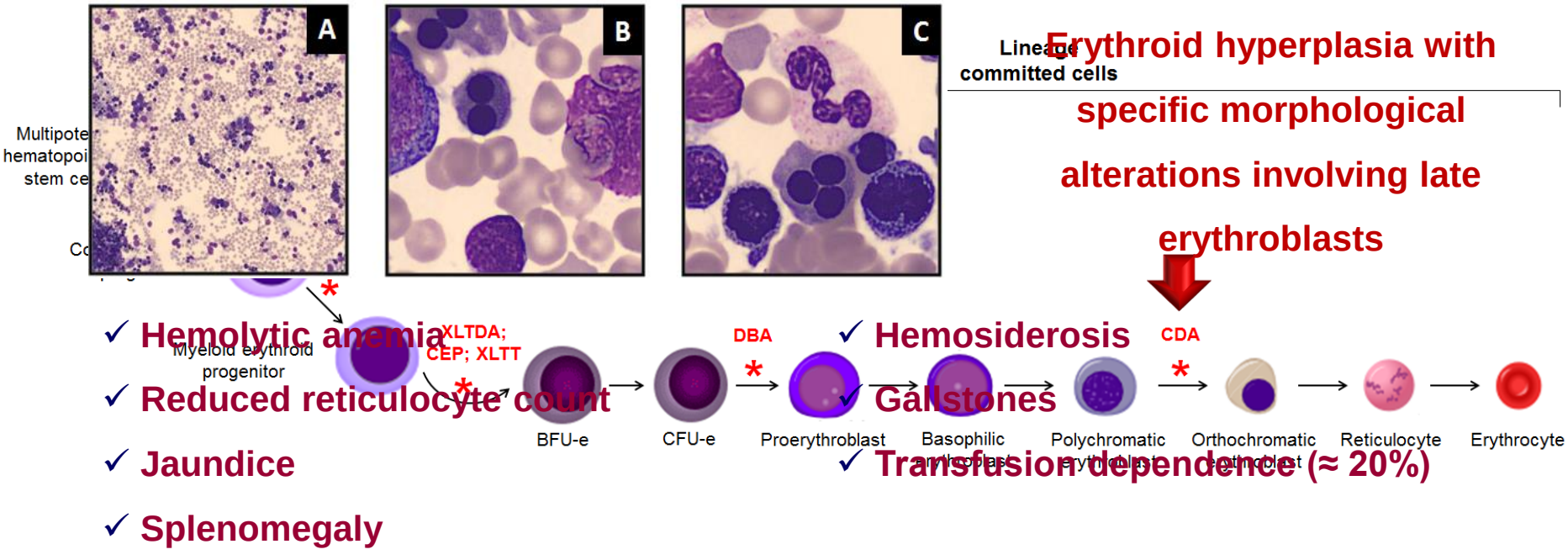
Congenital Dyserythropoietic Anemias

- ✓ **CDAs** are **Mendelian** diseases affecting the normal differentiation-proliferation pathway of the erythroid lineage
- ✓ They belong to a subtype of **bone marrow failure syndromes** characterized by **monolineage** involvement and morphological abnormalities in **erythroid** precursor cells



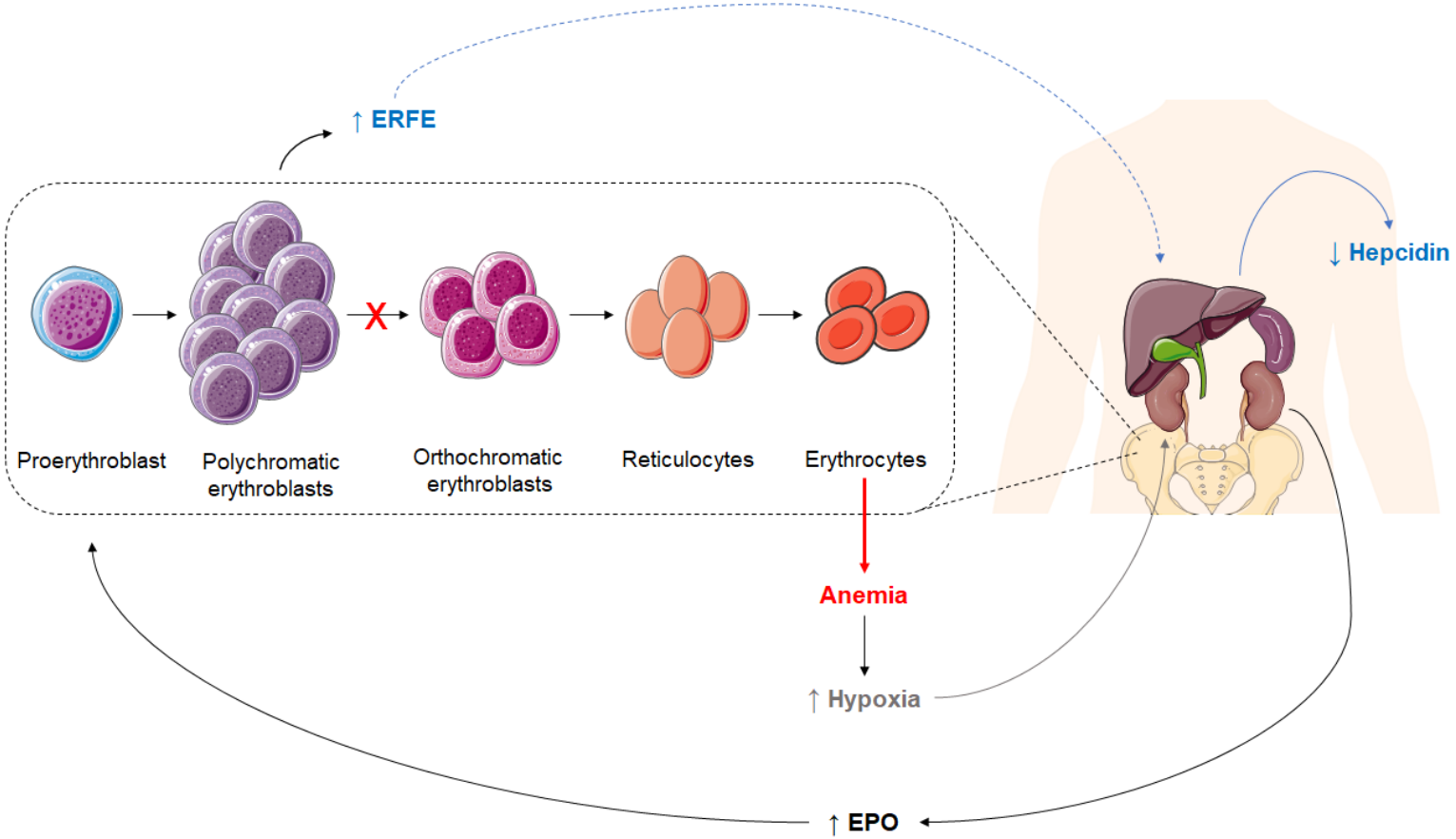
Erythroid hyperplasia with specific morphological alterations involving late erythroblasts

- ✓ **Hemolytic anemia**
- ✓ **Reduced reticulocyte count**
- ✓ **Jaundice**
- ✓ **Splenomegaly**
- ✓ **Hemosiderosis**
- ✓ **Gallstones**
- ✓ **Transfusion dependence (≈ 20%)**

Williams Hematology, Ninth Edition, Chapter 39 by A. Iolascon - McGraw-Hill

Physiopathology of CDAs (at systemic level)

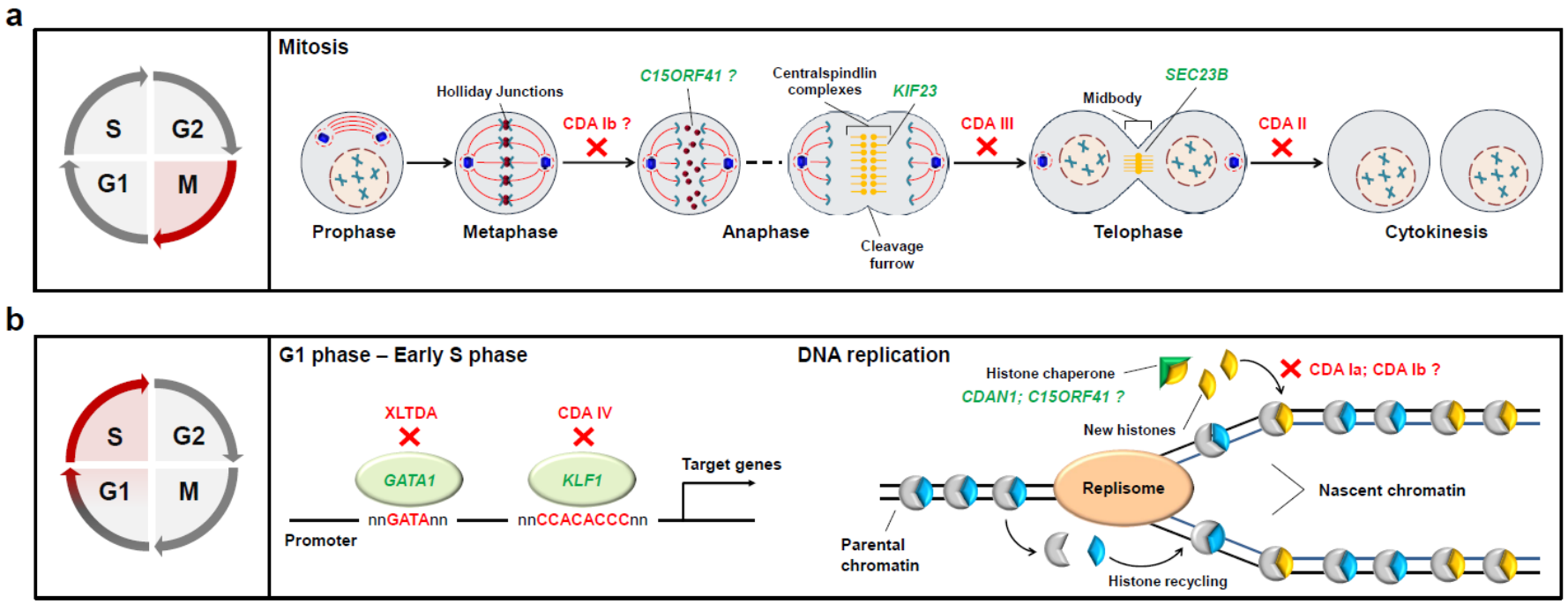
✓Anemia with reduced reticulocyte count



✓ **EPO** is not able to increase the production of RBCs

Iolascon A, Andolfo A, Russo R. Blood 2019 in press

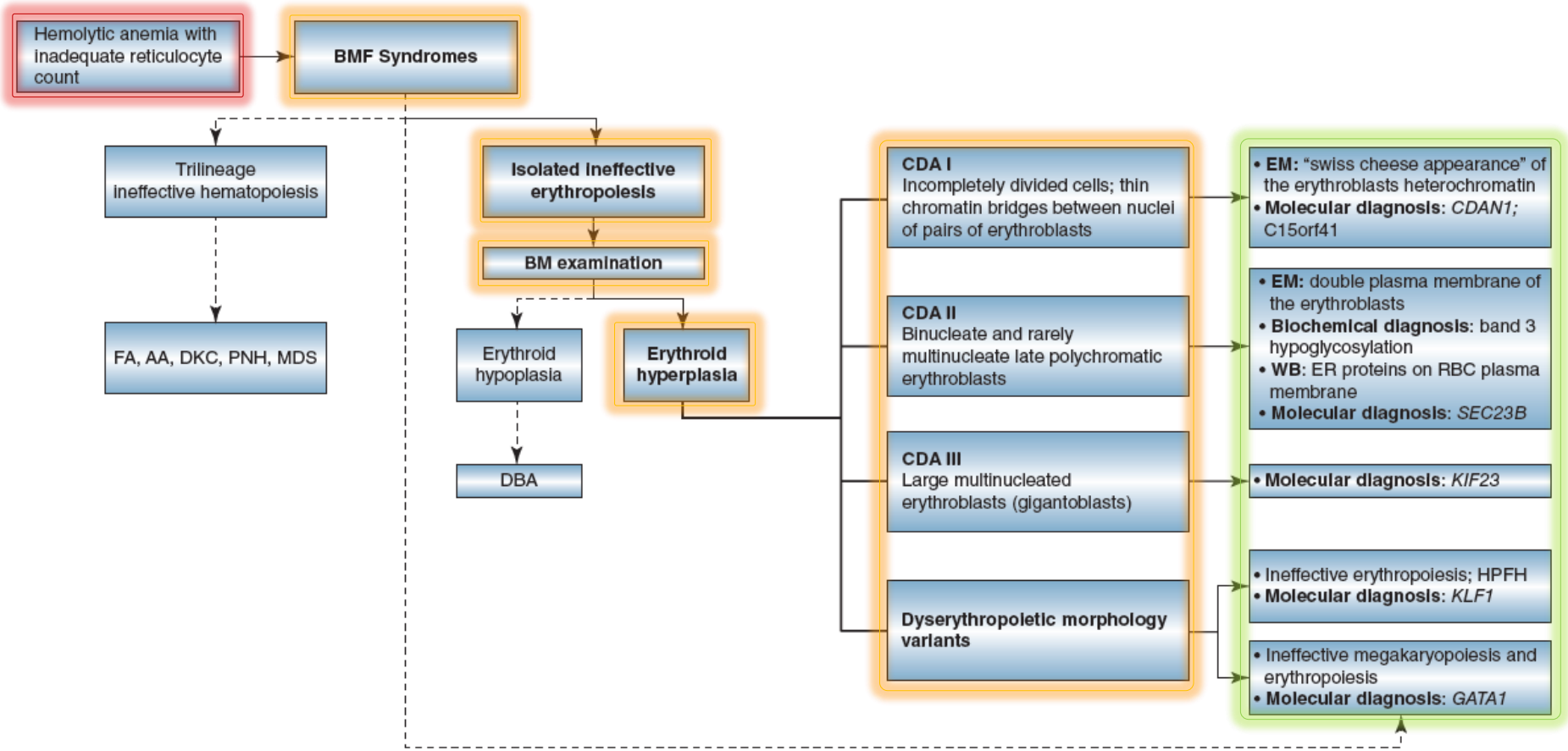
Pathogenic mechanisms of CDAs (at cellular level)



a. Deregulation of mechanisms involved in **cell division**
b. Impairment of mechanisms involved in **DNA synthesis and chromatin assembly**

Gambale A, Iolascon A, Andolfo I, Russo R. Expert Rev Hematol. 2016

Traditional diagnostic workflow for CDAs



Williams Hematology, 9th Edition, Chapter 39 by A. Iolascon - McGraw-Hill

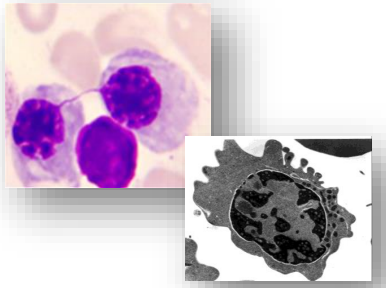
Different subtypes of CDAs (I)

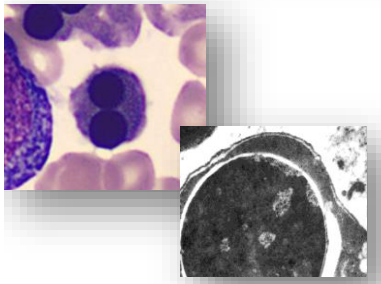
Table 1. Classification of CDAs by OMIM database.

Disease symbol	Phenotype	Phenotype MIM number	Gene location	Inheritance	No. cases[a]	Bone marrow biopsy: Optical microscopy	Bone marrow biopsy: Electron microscopy
CDA Ia	Congenital dyserythropoietic anemia type Ia	224120	CDAN1 *15q15.2*	AR	<100	Binucleate erythroblasts (3–7%); thin chromatin bridges between nuclei of erythroblasts	'Swiss cheese appearance' of the erythroblasts heterochromatin
CDA Ib	Congenital dyserythropoietic anemia type Ib	615631	C15orf41 *15q14*	AR	<10		
CDA II	Congenital dyserythropoietic anemia type II	224100	SEC23B *20p11.23*	AR	>200	Binucleate (10–30%); rare multinucleate erythroblasts	Double plasma membrane of the erythroblasts
CDA III	Congenital dyserythropoietic anemia type III	105600	KIF23 *15q21*	AD	<20	Giant multinucleate (up to 12 nuclei) erythroblasts	Clefts within heterochromatin, autophagic vacuoles, iron-laden mitochondria, myelin figures in the cytoplasm
CDA IV	Congenital dyserythropoietic anemia type IV	613673	KLF1 *19p13.2*	AD	<10	Tri- and multinucleate erythroblasts	Invagination of nuclear membrane, intranuclear precipitated and nuclear blebbing
XLTDA	Thrombocytopenia X-linked with or without dyserythropoietic anemia	300367	GATA1 *Xp11.23*	XLR	<10	*Erythroblasts*: megaloblastic features, nuclear irregularities, bi- and multinucleation *Megakaryocytes*: small, dysplastic with signs of incomplete maturation	Reduced numbers of platelet alpha granules and dysplastic features in megakaryocytes and platelets

[a] Number of cases with positive molecular analysis.

AD: autosomal dominant; AR: autosomal recessive; CDAs: congenital dyserythropoietic anemias; OMIM: Online Mendelian Inheritance in Man; XLR: X-linked recessive.

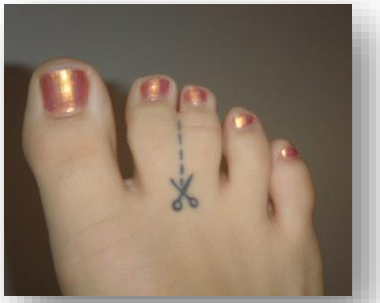




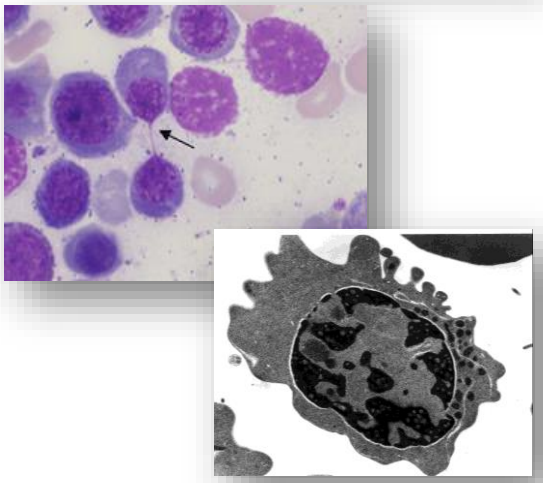
Gambale A, Iolascon A, Andolfo I, Russo R. Expert Rev Hematol. 2016

Main features of CDA type I

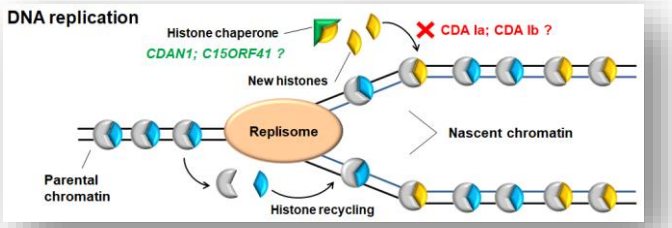
- ✓ **Clinical features:** severe or moderate anemia (generally ***macrocytic***) with neonatal appearance; jaundice; splenomegaly; common complication: hemosiderosis
  → **Morphologic body abnormalities** (10% of patients): skeletal malformations, syndactyly in hands or feet, absence of nails, or supernumerary toes



- ✓ **Morphology** (BM)**:** 2.4-10% of late erythroblasts are bi-nucleate; megaloblastic erythroid hyperplasia; internuclear bridges (1-8% of cells)
  → EM: spongy-appearing nuclei and invagination of the cytoplasm in the nucleus



- ✓ **Inheritance:** autosomal recessive
  Locus: 15q15.2 → ***CDAN1* (CDA Ia)**
  Locus: 15q14 → ***C15orf41* (CDA Ib)**



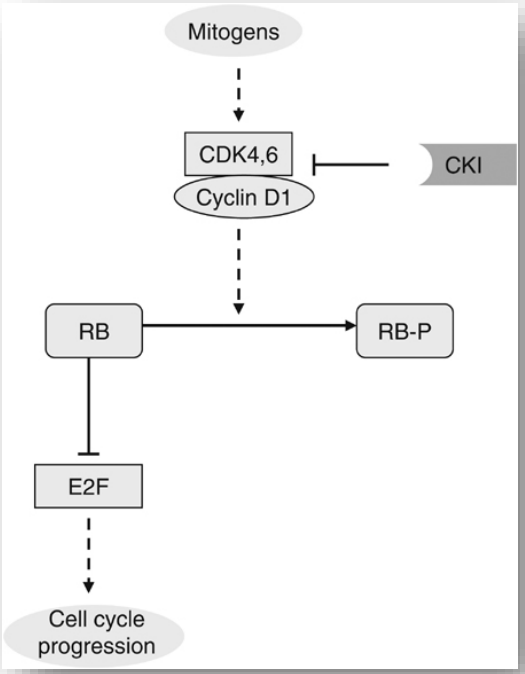
Roy NB, Babbs C. BJH 2019
Iolascon A, Andolfo A, Russo R. Blood 2019 in press

Locus: 15q15.2 – *CDAN1*

> 50 disease variants (2019)

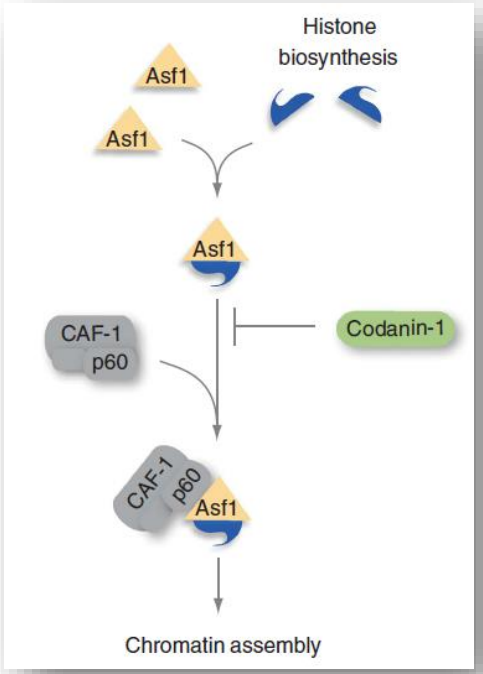
- ✓ **Codanin-1** is a target of E2F1 transcription factor and **is cell-cycle regulated** protein

Noy-Lotan et al, Haematologica 2009



- ✓ **Codanin-1** sequesters **Asf1a** in the cytoplasm, restraining histone deposition and thereby limiting DNA replication

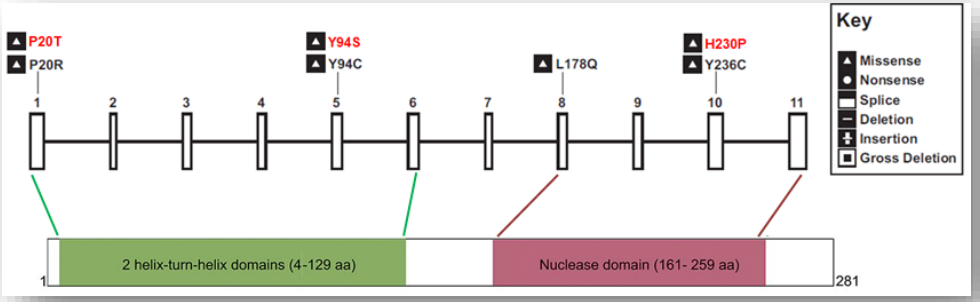
Ask et al, EMBO J 2012



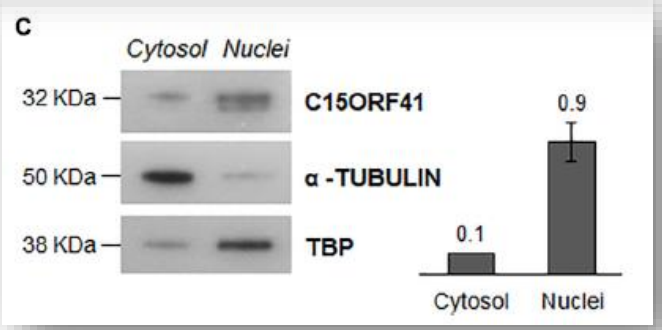
Locus: 15q14 – *C15orf41*

7 disease variants described – 8 unrelated families (2020)

- ✓ Uncharacterized gene
- ✓ Widely transcribed; mainly expressed in B lymphoblasts, **CD34+ cells**, cardiomyocytes, and fetal liver



- ✓ *C15orf41* is **uniformly expressed during erythroid differentiation**
- ✓ *C15orf41* binds **Asf1b**
- ✓ *C15orf41* endogenous protein exhibits mainly **nuclear (*nucleolus*) localization**



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Roy NB, Babbs C. BJH 2019
Russo R, Marra R, et al. Front Physiol. 2019
Olijnik AA, et al. Med Genet. 2020

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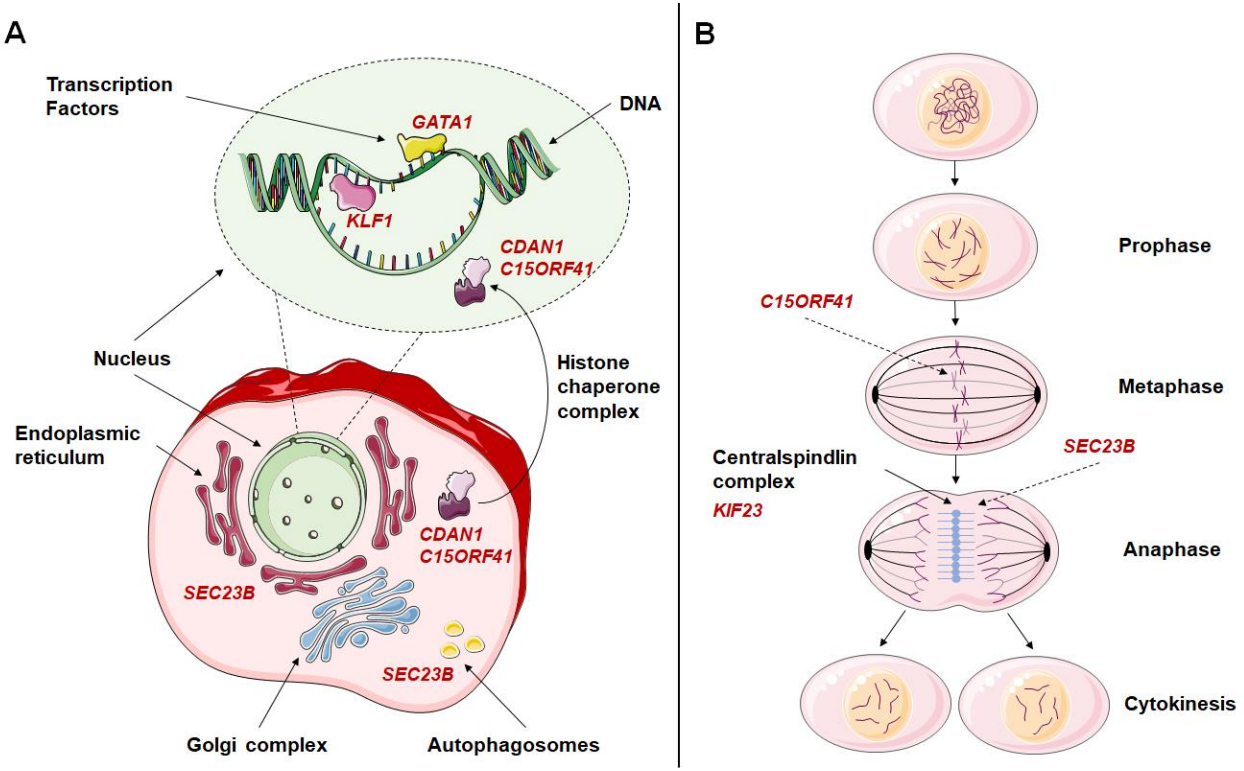
The dual role of C15orf41 protein

- C15orf41 **shuttles** between **nucleus** and **cytosol**

- **C15orf41** forms a tight, near-stoichiometric **complex** with the **C-terminal region of Codanin-1**
- Codanin-1 stabilizes C15orf41 in the cytosol
- C15orf41 has homology to the Holliday junction resolvases → **DNA repair, mitosis**

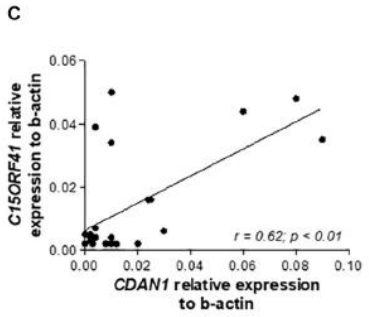


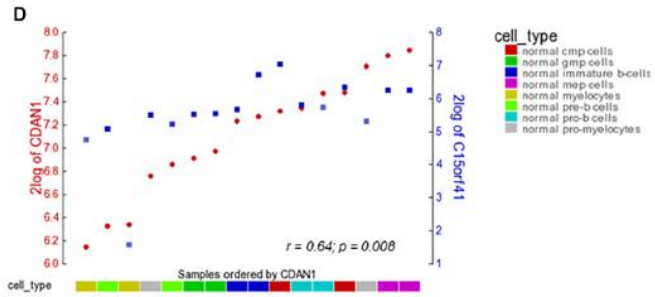
Iolascon A, Andolfo A, Russo R. Blood 2019 in press
Swickley G et al. BMC Mol Cell Biol. 2020
Shroff M et al. Biochem J. 2020

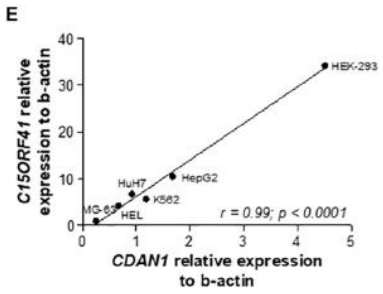
CDAN1 - C15orf41: differential diagnosis

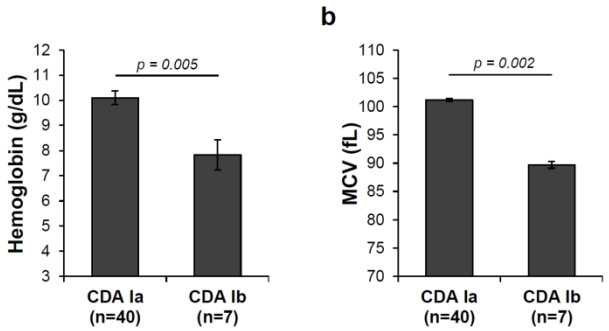
C15orf41 patients show clinical features similar to *CDAN1* patients

- ***C15orf41*** and ***CDAN1*** gene expression is tightly correlated
- Shared mechanism of regulation between the two genes









Hb levels and **MCV values** may differentiate *C15orf41* and *CDAN1* patients

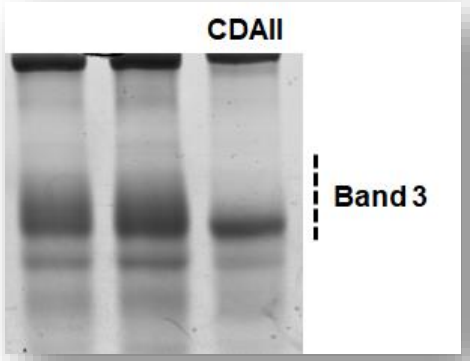
Most of the ***C15orf41*** patients described so far (n=8) are transfusion-dependent

Gambale A, Iolascon A, Andolfo I, Russo R. Expert Rev Hematol. 2016
Russo R, Marra R, et al. Front Physiol. 2019

Main features of CDA II patients

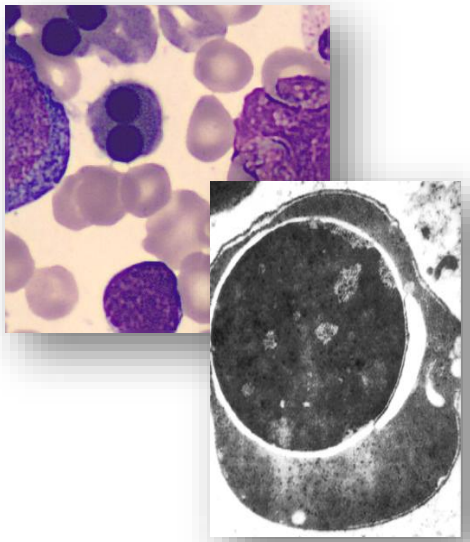
✓ **Clinical features:**

- Average age of onset symptoms: 3.7 ± 0.6 y
- Mean age at diagnosis: 22.2 ± 1.7 y
- Normocytic mild anemia: **Hb 9.6** ± 0.2 g/dL with **MCV 87.3** ± 1.0
- Reticulocyte index: 1.7 ± 0.1
- Mean serum ferritin: 464.8 ± 55.9 ng/mL
- Splenomegaly: 102/122, **83.6% of patients**
- Transfusion dependency: 25/126, **19.8% of patients**



✓ **Biochemical features**:

- Hypoglycosylation of band 3 at SDS-PAGE: **95.1% of patients**



✓ **Morphology**: erythroid hyperplasia
bi-nucleated erythroblasts **> 10%**
→ EM: double-membrane appearance

✓ **Inheritance**: Autosomal recessive
Locus: 20p11.23 → ***SEC23B***

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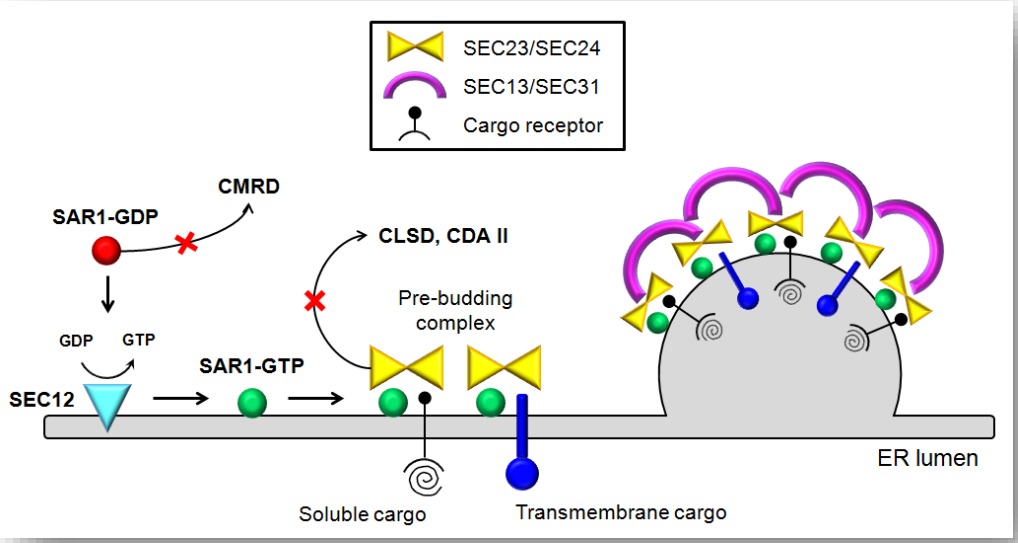
Russo R. et al. Am J Hematol. 2014

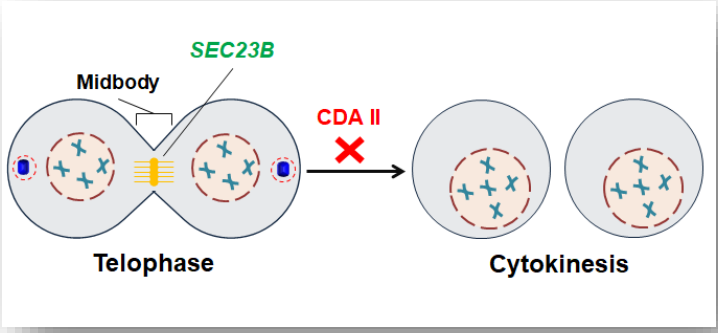
CDA II belongs to COPII-related human genetic disorders



- ✓ CDA II is caused by biallelic pathogenic variants in ***SEC23B*** gene
- ✓ Approximately 100 pathogenic variants
- ✓ *SEC23B* CDAII-mutations are mostly LoF (loss-of-function)

CMRD, chylomicron retention disease; CLSD, cranio-lenticulo sutural dysplasia

- ✓ In a proteomic screening, **SEC23B** has been found expressed in the **midbody**, a subcellular structure fundamental during telophase
- ✓ It is supposed to have a role in the cytokinesis



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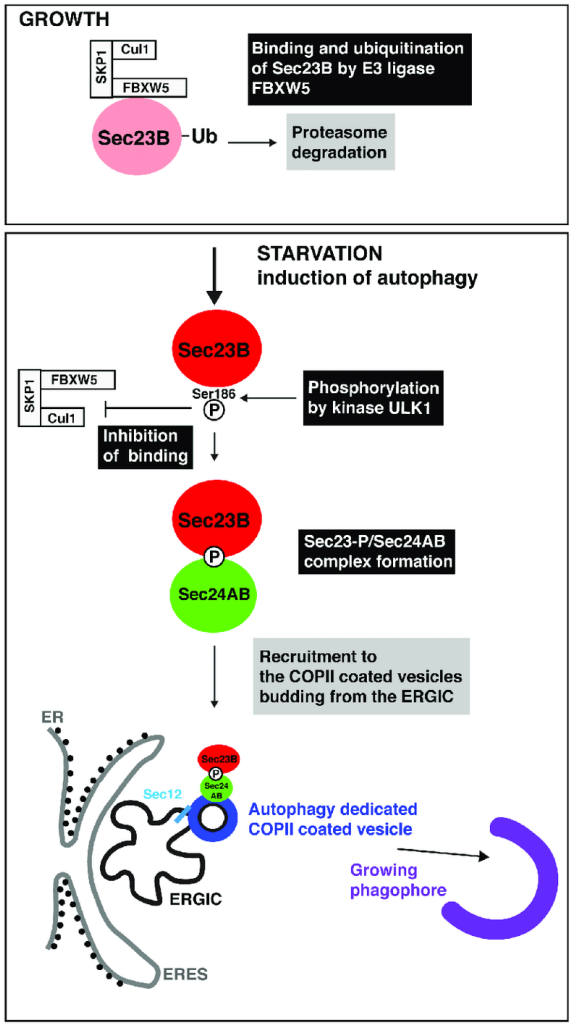
Russo R et al, Am J Hematol 2012
Gambale A, Iolascon A, Andolfo I, Russo R. Expert Rev Hematol. 2016

New pathogenic hypothesis for CDA II

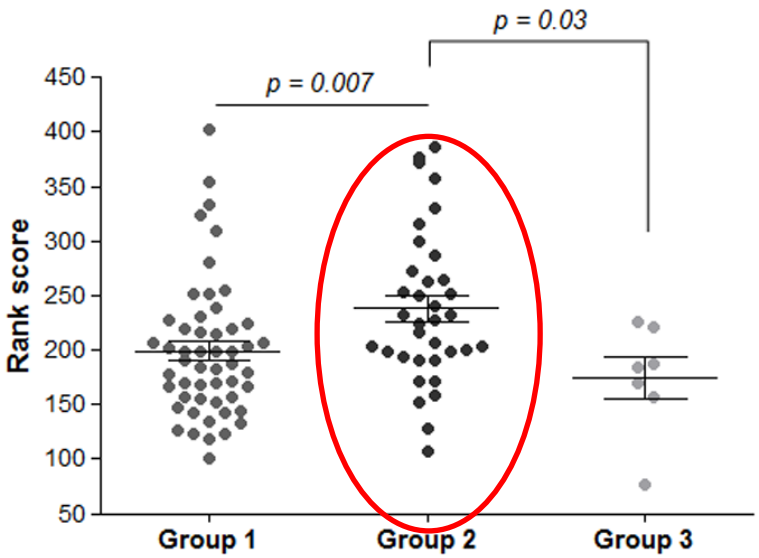


- ✓ A recent study investigated the involvement of **SEC23B** in the **autophagy**
- ✓ The protein **FBXW5** targets **SEC23B** for **proteasomal degradation**
- ✓ In response to starvation, **phosphorylated SEC23B** is not able to interact with FBXW5
- ✓ Phosphorylated and stabilized SEC23B associates with other COPII components, promoting **autophagic flux**

Jeong YT, Elife 2018

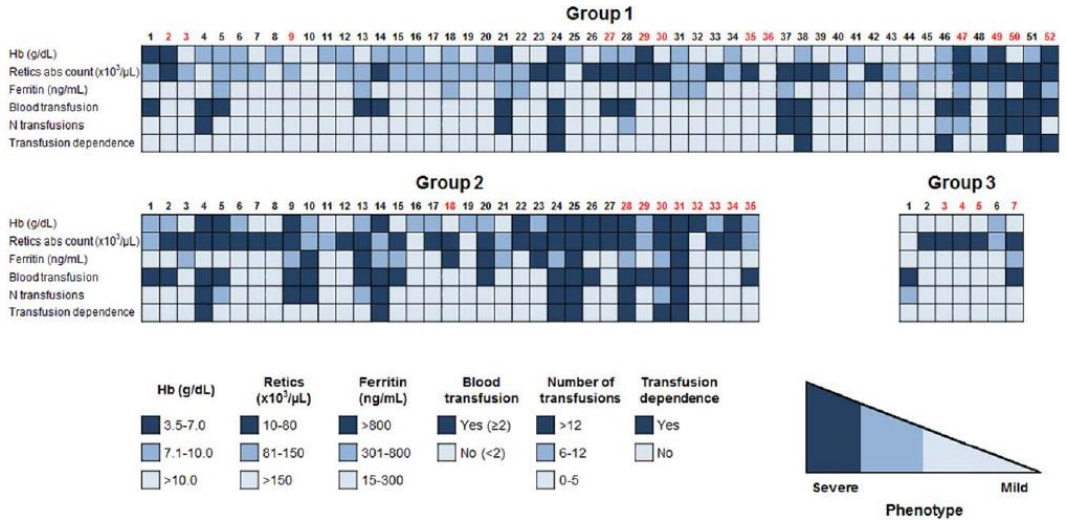
Genotype-phenotype correlation in CDA II patients

94 CDAII SEC23B-biallelic patients



- **Group 1**: two missense alleles
- **Group 2**: a missense allele + a nonsense/hypomorphic allele
- **Group 3**: two hypomorphic alleles

Overlapping features among the different genotype subgroups



Russo R, et al. AJH 2014

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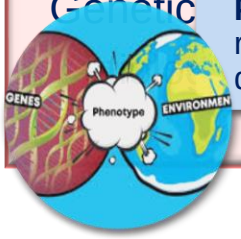
NGS-based approach for the identification of phenotype modifiers

Overlapping features among the different genotype subgroups

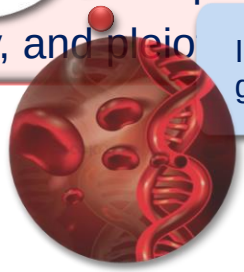


Genetic … e not only in … ariability of Mendelian diseases

Phenotype stratification*: ranking of the patients by a quantitative score

Selection of patients (**phenotype classes**)

Genetic mod… affect penetrance, expressivity, and pleio…



Identification of genetic **modifiers**

***Critical variables:**
- Hb levels
- Abs retics count
- BMRI
- Ferritin levels
- Transfusion need

Andolfo et al. AJH 2019

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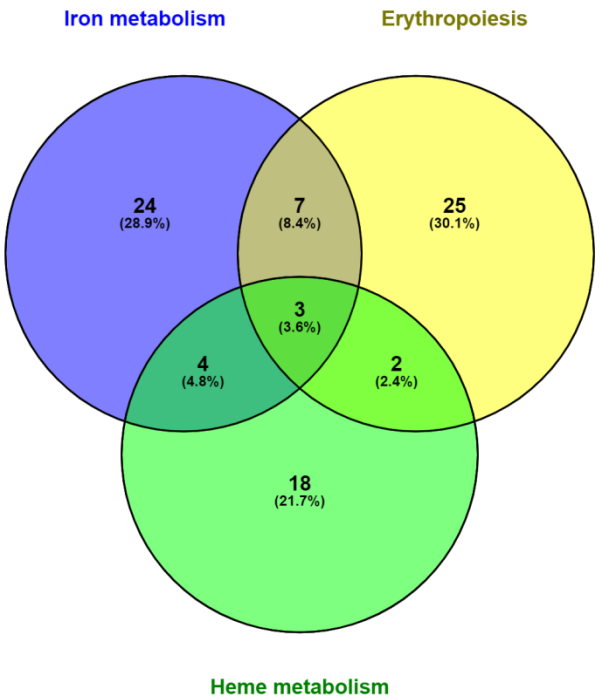
NGS-based approach for the identification of phenotype modifiers

- ✓ Custom gene panel of **81 genes** related to anemias
- ✓ Patients (**Test-set**):
  - High-rank (n =14): severe
  - Low-rank (n=17): mild



- ✓ **44**% of most enriched genes within **high-rank patients** were included in the **iron metabolism** subgroup
- ✓ Recurrent low-frequency variant (rs111241405, MAF=0.03) in ***ERFE*** gene: c.778G>T, p.Ala260Ser (A260S) in high-rank patients

Andolfo et al. AJH 2019

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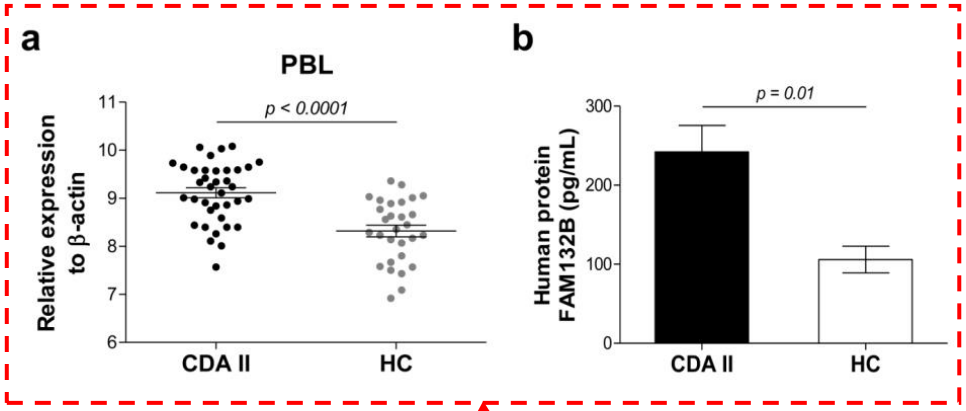
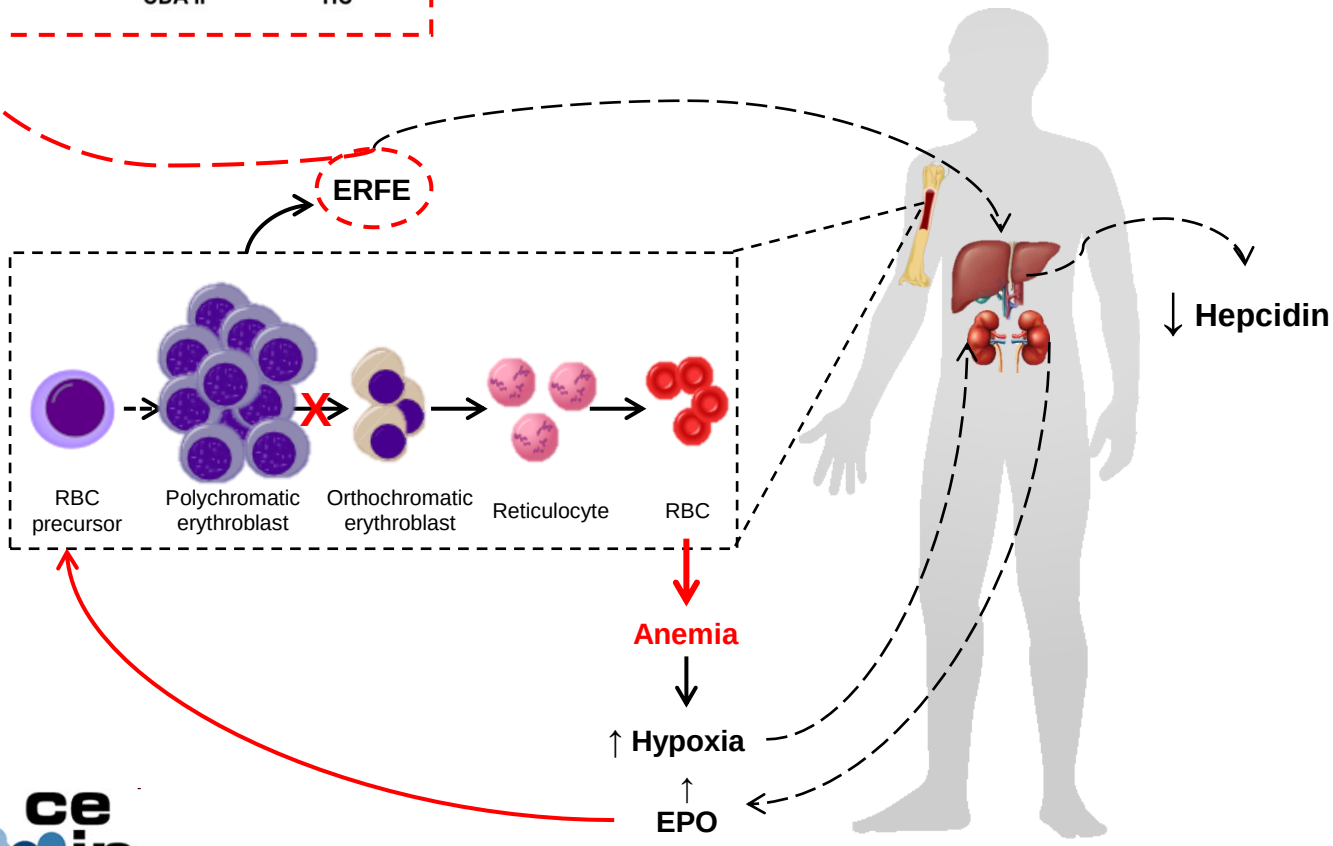
CDAII patients have increased ERFE levels



✓ CDA II is hallmarked by **ineffective erythropoiesis**, **iron overload**, **reduced expression of** hepatic hormone **hepcidin** ...

… and **increased** levels of **ERFE**

Russo et al. Blood 2016



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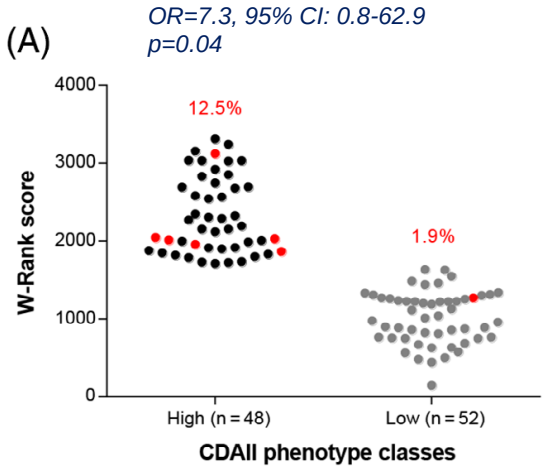
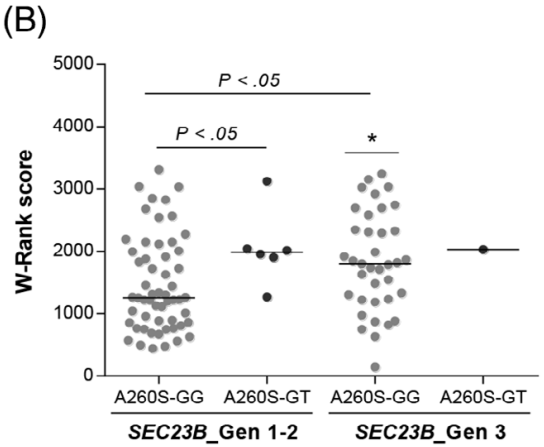
CDAII severe phenotype correlates with ERFE-GT genotype





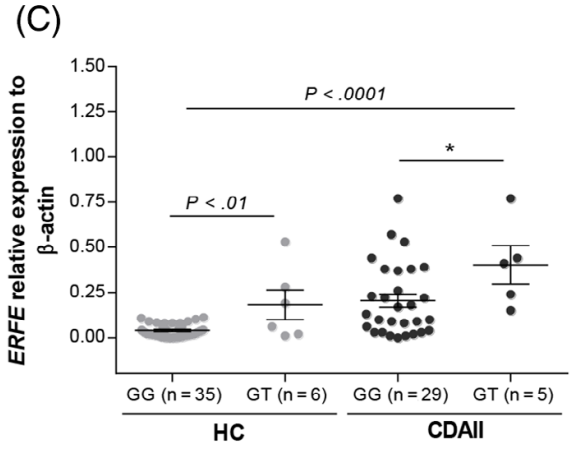
- Test-set: **31** CDAII
- Validation-set: **69** CDAII

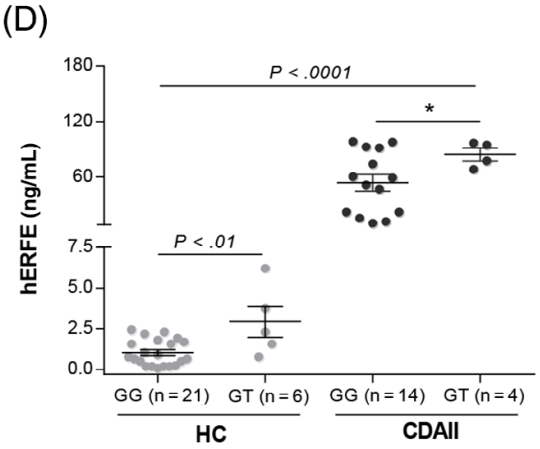
A. Overall CDAII patients: 100

B. Polygenic W-Rank score by combining *SEC23B* genotypes and *ERFE*-rs111241405 alleles

C. Gene expression analysis in PBL

D. Human ERFE protein in plasma samples





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Andolfo et al. AJH 2019

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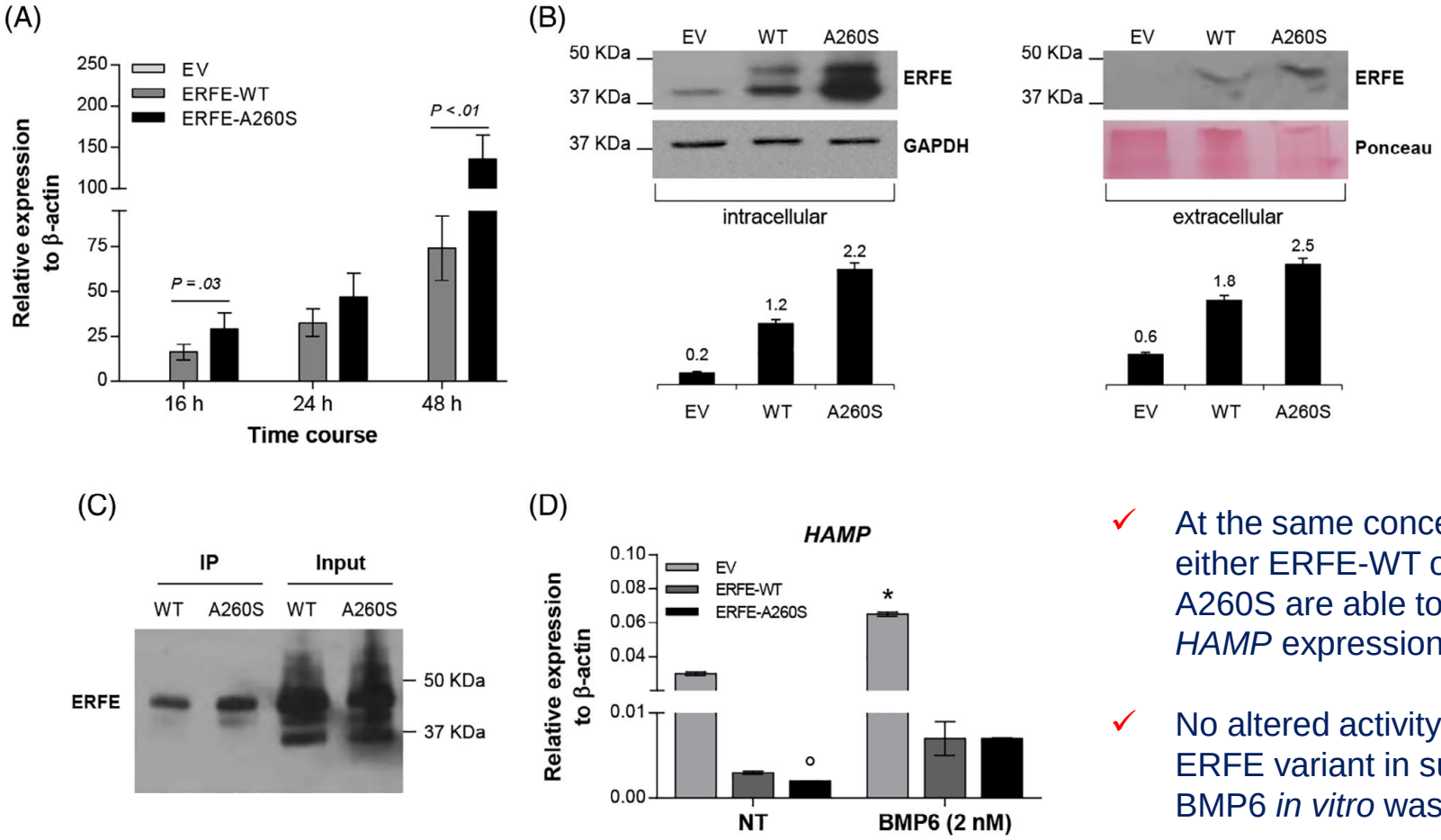
ERFE-A260S variant accounts for increased expression of *ERFE*



- ✓ At the same concentration, either ERFE-WT or ERFE-A260S are able to suppress *HAMP* expression
- ✓ No altered activity of the ERFE variant in suppressing BMP6 *in vitro* was observed

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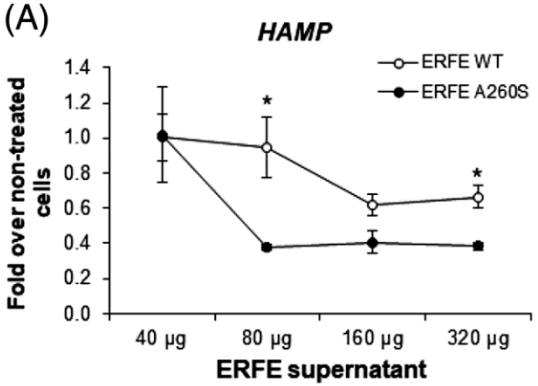
ERFE-A260S alters key effectors of iron metabolism in hepatic cells

- We treated HuH7 cells with purified extracellular media of HEK-293 cells overexpressing either **ERFE-WT** or **ERFE-A260S**
- In cells treated with **mutant ERFE**, we observed:

(A)



A. Down-regulation of *HAMP* gene expression

B. Reduction of the pSMAD1/5/8

C. Down-regulation of the targets genes of the BMP/SMAD pathway

Correlation analysis on ERFE levels

Table 1. *FAM132B* expression and clinical correlations in CDAII patients

	Low *FAM132B* (*n* = 20)	High *FAM132B* (*n* = 17)	p‡
Age (years)	25.3 ± 4.9 (16.0; 19)	12.1 ± 2.5 (10.0; 17)	0.03
Onset symptoms (years)	7.5 ± 2.5 (5.0; 16)	3.2 ± 1.3 (1.3; 16)	0.14
Gender (Female/Male)	9 (45.0)/11 (55.0)	9 (52.9)/8 (47.1)	0.63
Complete blood count			
RBC (10^6/µL)	3.6 ± 0.2 (3.5; 20)	3.2 ± 0.1 (3.3; 17)	0.05
Hb (g/dL)	10.7 ± 0.5 (10.4; 20)	9.2 ± 0.4 (9.5; 17)	0.02
Ht (%)	31.7 ± 1.4 (30.6; 20)	27.5 ± 1.2 (28.0; 17)	0.03
MCV (fL)	89.7 ± 1.8 (90.2; 20)	86.0 ± 2.2 (84.7; 17)	0.20
MCH (pg)	30.6 ± 0.7 (31.0; 18)	28.9 ± 0.9 (27.9; 17)	0.12
MCHC (g/dL)	33.8 ± 0.4 (33.5; 19)	33.3 ± 0.3 (33.1; 16)	0.32
RDW (%)	19.9 ± 2.5 (18.9; 12)	21.8 ± 1.2 (22.0; 15)	0.48
PLT (10^3/µL)	373.0 ± 41.1 (290.0; 17)	459.2 ± 69.2 (390.0; 17)	0.30
Retics abs count (10^3/µL)	67.4 ± 9.2 (59.2; 20)	87.3 ± 17.5 (79.7; 16)	0.30
Retics (%)	2.0 ± 0.3 (1.5; 20)	2.7 ± 0.6 (2.2; 16)	0.25
Reticulocyte Index	1.3 ± 0.2 (1.2; 20)	1.7 ± 0.3 (1.5; 16)	0.38
Iron balance			
Hepcidin/ferritin	0.04 ± 0.01 (0.02; 16)	0.01 ± 0.003 (0.006; 16)	0.01
Hepcidin (nM)	5.8 ± 1.9 (2.7; 17)	1.0 ± 0.3 (0.6; 16)	0.02
Ferritin (ng/mL)	372.1 ± 107.7 (200.0; 19)	168.5 ± 36.0 (99.8; 17)	0.10
Ferritin level/dosage age§	32.9 ± 17.2 (14.9; 18)	26.1 ± 8.6 (12.7; 17)	0.73
Transferrin saturation (%)	67.7 ± 6.8 (62.5; 19)	81.8 ± 7.8 (86.0; 8)	0.23
Serum iron (µg/dL)	157.8 ± 13.6 (159.5; 18)	162.7 ± 20.4 (172.0; 13)	0.84
sTfR (mg/L)	3.7 ± 0.4 (3.7; 12)	5.1 ± 0.5 (5.7; 8)	0.04
Laboratory data and transfusion regimen			
EPO (mIU/mL)	82.5 ± 19.1 (61.9; 14)	154.3 ± 14.5 (170.1; 13)	0.01
GDF15 (pg/mL)	814.9 ± 251.1 (503.5; 13)	781.9 ± 140.6 (804.0; 9)	0.92
Total bilirubin (mg/dL)	3.7 ± 0.8 (2.5; 19)	2.3 ± 0.3 (2.1; 16)	0.15
Unconjugated bilirubin (mg/dL)	3.1 ± 0.8 (2.2; 17)	1.9 ± 0.3 (1.5; 12)	0.22
Transfusion need (Yes/No)	7 (46.7)/8 (53.3)	10 (58.8)/7 (41.2)	0.49

Data are not available for all patients. For quantitative variables data are presented as average ± SE (median; n). For qualitative variables data are presented as n (%)/n (%)
‡ Student t test for quantitative unpaired data; chi square test for categorical data
§ Normalization of ferritin by means of "Ferritin level/dosage age ratio", as described by Iolascon et al, Haematologica 2010; 95(5)

High-ERFE patients:

- Reduced Hb
- Reduced Ht
- Increased EPO
- Increased sTfR

- Reduced Hepcidin
- Reduced Hepcidin/ferritin
- Increased Transferrin saturation

However …

The iron balance data do not differ significantly between the two CDA II sub-groups

Russo R et al. Blood 2016

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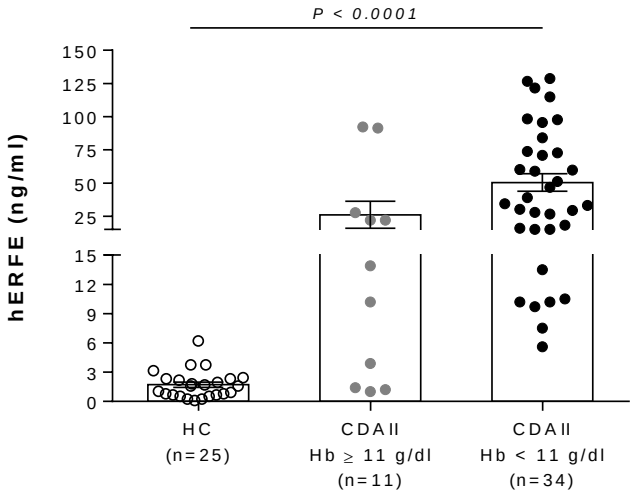
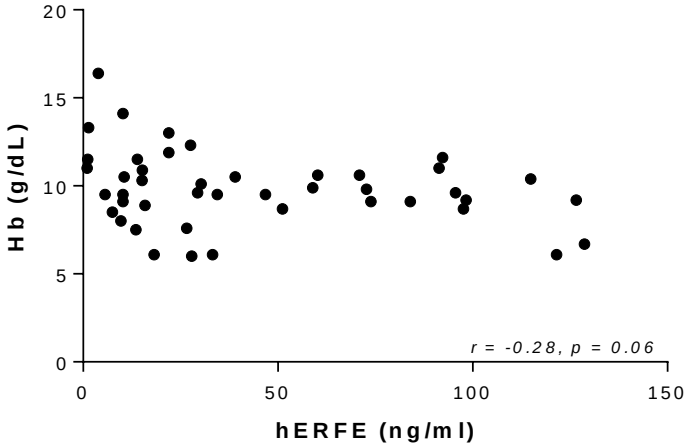
SEC23B loss-of-function impairs iron metabolism in hepatic cells





- **ERFE** levels do not stickily correlate with **Hb** levels in CDA II patients

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Rosato BE. et al. E-Poster EP1579
EHA25 virtual meeting

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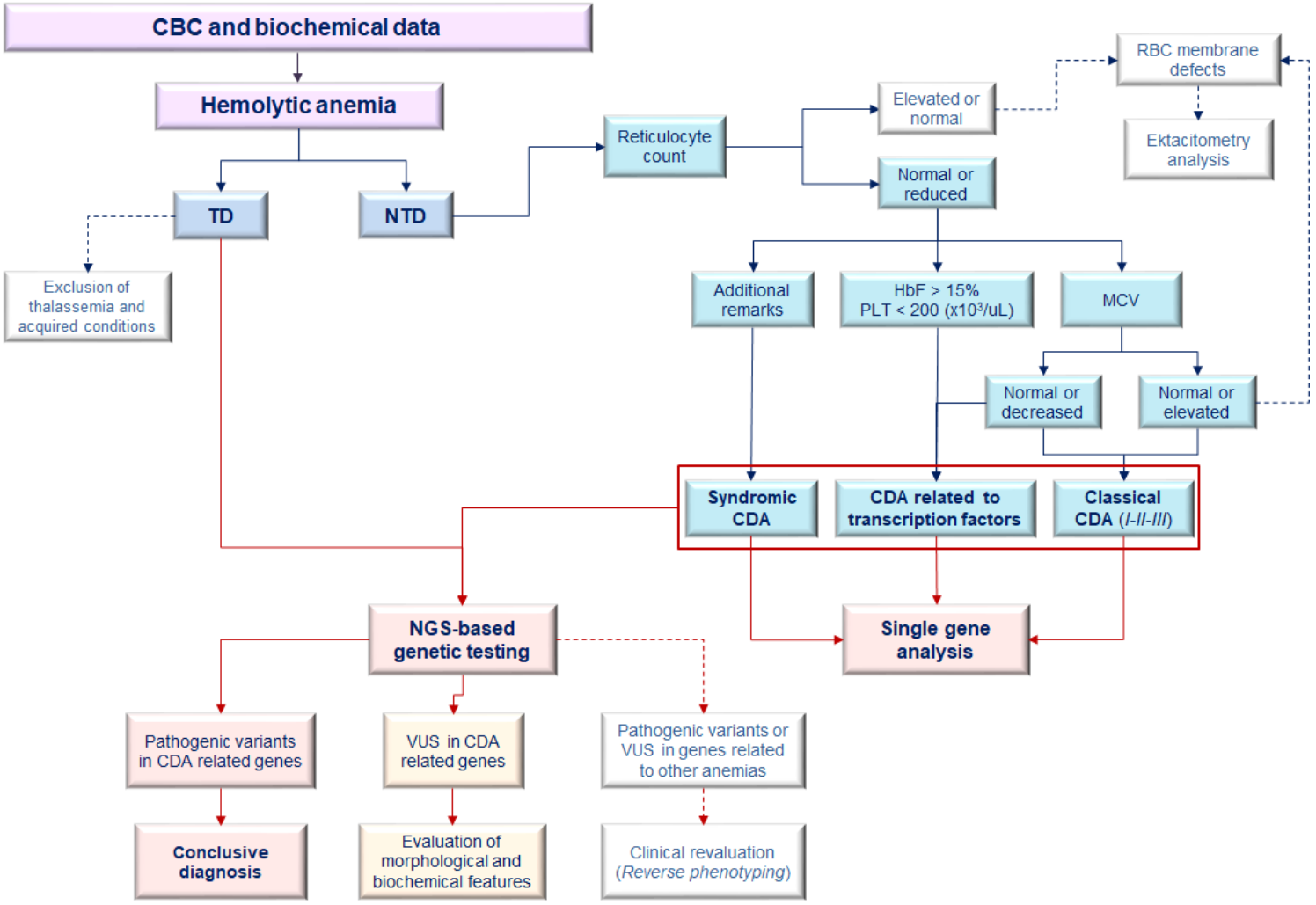
New diagnostic workflow for CDAs

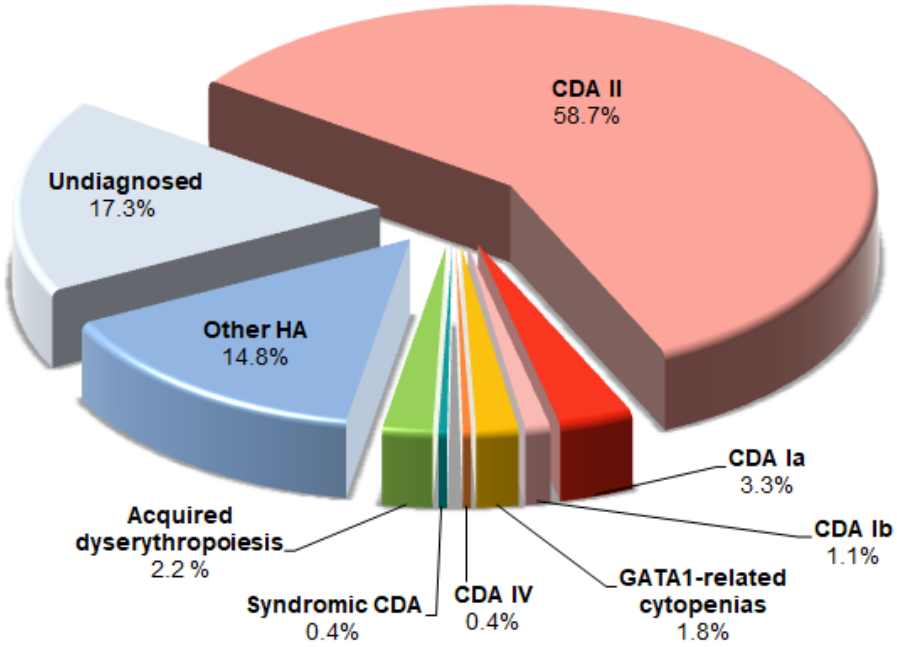


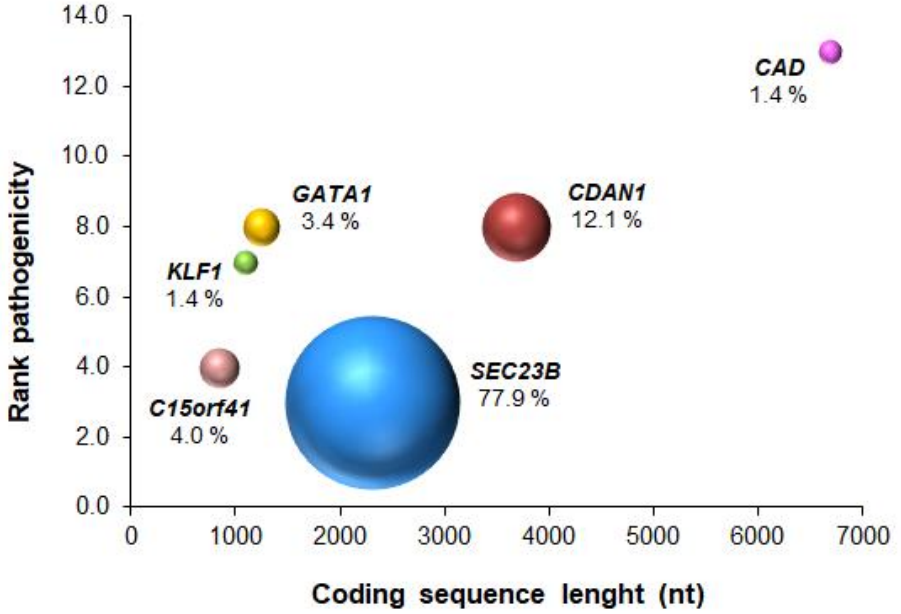
Iolascon A, Andolfo A, Russo R. Blood 2019 in press

Different subtypes of CDAs (II)

CDA II is the most common form among CDAs

SEC23B is the most frequently mutated gene





218 patients clinically suspected to have CDA *(1995 to 2019)*

78 patients genetically diagnosed as CDA *(2008 to 2019)*

More intolerant-to-variation is a gene, the less it is mutated

Iolascon A, Andolfo A, Russo R. Blood 2019 in press

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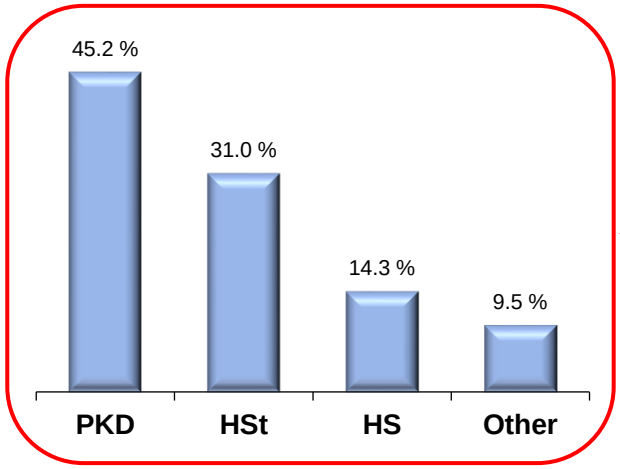
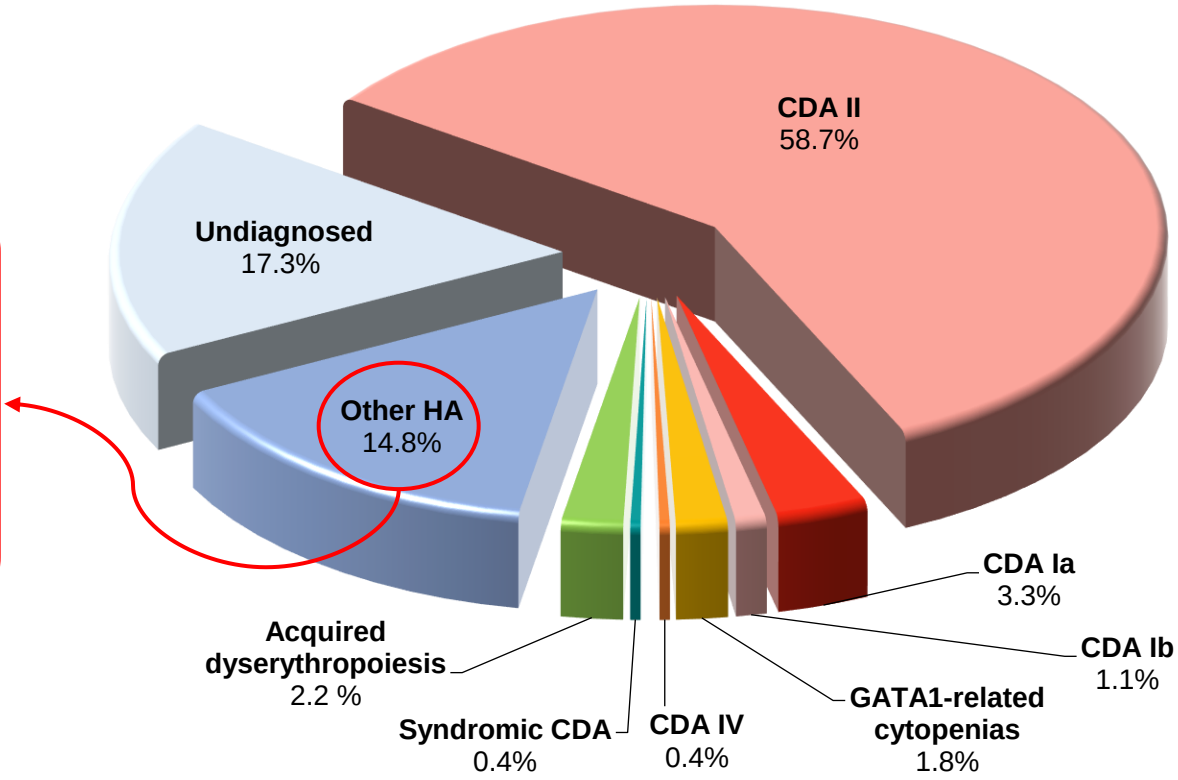
Different subtypes of CDAs (genetic classification)

- ✓ CDA patients enrolled by the Medical Genetics Unit of Naples:

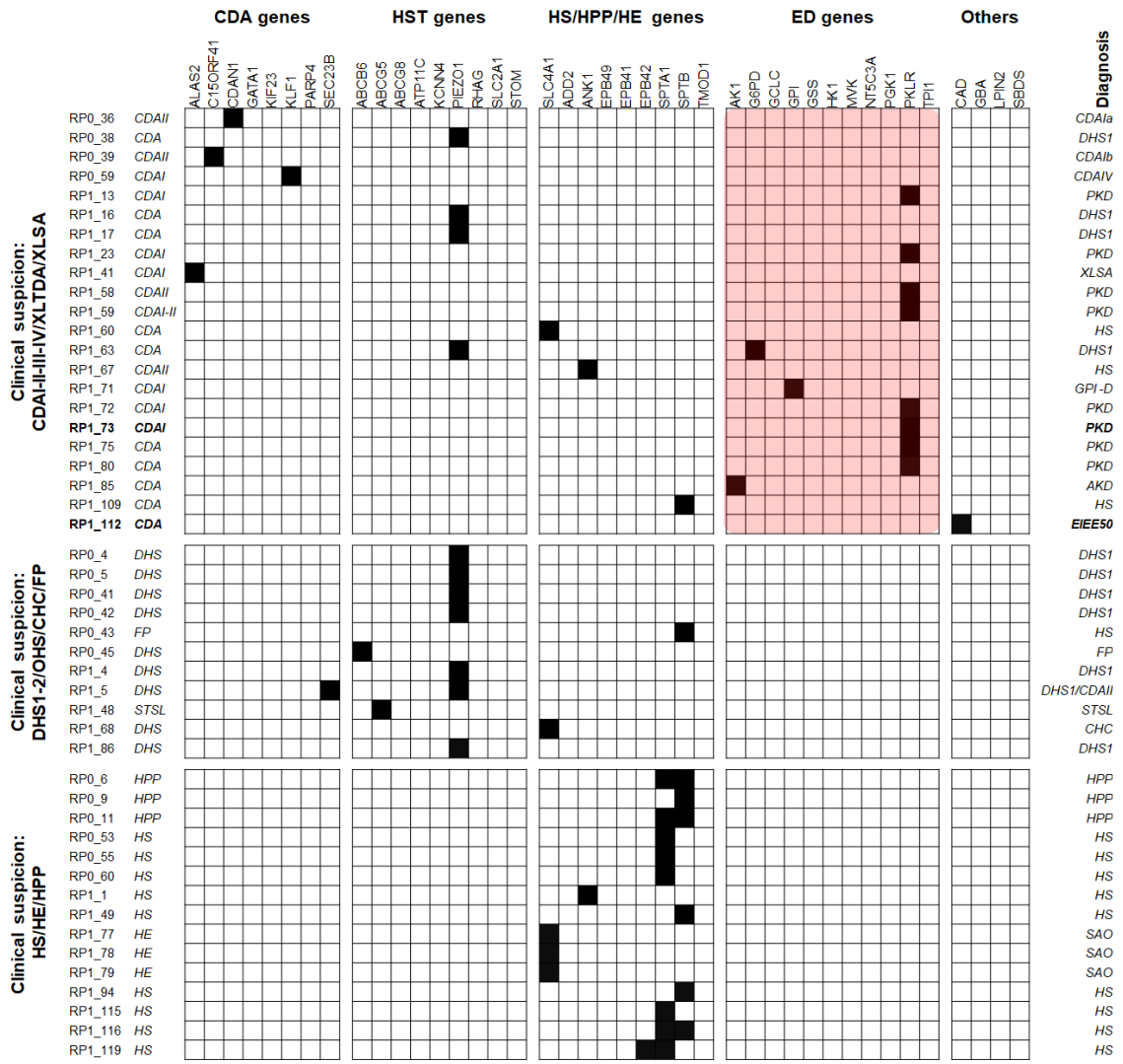
218 patients clinically suspected to have CDA *(1995 to 2019)*





Iolascon A, Andolfo A, Russo R. Blood 2019 in press

NGS-based genetic testing of CDAs



- ✓ The multi-gene approach modified the original diagnosis in **45.8%** of patients (**non-matched phenotype-genotype**)
- ✓ 81.8% of **non-matched patients** were clinically suspected to suffer from **CDA**

Russo R, et al. Am J Hematol. 2018

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Different subtypes of CDAs (genetic classification)

- **36.4%** of **CDA** patients within our cohort exhibited mutations in ***PKLR*** gene → overlapping phenotypes among these disorders

TABLE 3 Clinical features of CDA patients conclusively diagnosed as PK deficiency

	RP1_13	RP1_23	RP1_58	RP1_59	RP1_72	RP1_73	RP1_75	RP1_80
Age (years)	1.4	5.2	2.0	1.7	7	0.8	1.6	14
Onset symptoms (years)	At birth	Neonatal	Neonatal	At birth	4	At birth	At birth	At birth
Gender	Male	Female	Male	Male	Female	Female	Male	Male
Ethnicity	Turkish	Turkish	Colombian	Turkish	Italian	Turkish	Hungarian	Venezuelan
Complete blood count								
RBC (10^6/μL)	2.1	2.9	2.6	2.9	3.2	1.7	1.7	2.7
Hb (g/dL)	6.8	7.7	7.6	7.9	9.6	5.5	6.1	9.5
Ht (%)	18.0	23.4	21.6	23.3	29	15.8	17.5	32
MCV (fL)	104.9	80.6	82.0	81.2	89.6	90.1	103.6	117.8
MCH (pg)	32.5	26.1	29.0	28.1	33	31.4	35.3	35.2
MCHC (g/dL)	-	32.4	35.0	34.4	36.8	34.9	34.3	29.9
RDW (%)	-	13.7	14.0	13.2	-	14.9	16.7	18.2
PLT (10^3/μL)	387.0	287.0	361.0	276.0	295	284	362	1010
Retics %	0.6	0.1	3.2	1.8	7.2	2.0	8.56	18.2
Retics abs count (x10^3/μL)	12.8	3.8	83.5	51.5	23.3	35.2	144.7	215.0
Transfusion rate	8/year	7–8/year	25/year	12/year	-	6/year	10/year	12/year
Bone marrow examination	Erythroid hyperactivity, 10% double nucleated normoblasts (asymmetric nuclei)	Hypercellular with megaloblastic changes in erythroid cells	-	Erythroid hyperactivity, megaloblastic elements (bi- and multi-nucleated with internuclear bridges)	Erythroid hyperactivity with dyserythropoiesis, mostly bi- and multi-nucleated with internuclear bridges	Normoblasts with double nuclei and internuclear bridges	Hypercellular with megaloblastic changes and bi-nucleated normoblasts	Erythroid hyperactivity with dyserythropoiesis
Laboratory data								
Total bilirubin (mg/dL)	1.7	1.9	3.7	6.1	5.6	3.5	2.2	7
Unconjugated bilirubin (mg/dL)	0.5	1.5	3.1	5.4	5	3.1	2.1	6.3
Ferritin (ng/mL)	554	2554	1042	389	132	-	198	238
PKLR molecular analysis								
HGVS (coding[a]; protein; status)	c.1349A>G; p.Asp450Gly; Hom	c.1117-1G>C; Hom	c.1116 + 2T>G; Hom	c.67_68delTA; p.Leu23Cysfs*55c.287C>A; p.Pro96Gln; Comp het	c.1492C>T; p.Arg498Cysc.994G>A;p.Gly332Ser Comp het	c.353A>G; p.Asn118Ser; Hom	c.1594C>T; p.Arg532Trpc.1529G>A; p.Arg510Gln; Comp het	c.1528C>T; p.Arg510Ter; Hom

Hom, homozygous; Comp het, compound heterozygous.
[a]Reference Transcript ID: NM_000298.

- Bone marrow features mostly resembling those of CDA I patients
- TD patients → enzymatic assay is not reliable

Russo R, et al. Am J Hematol. 2018

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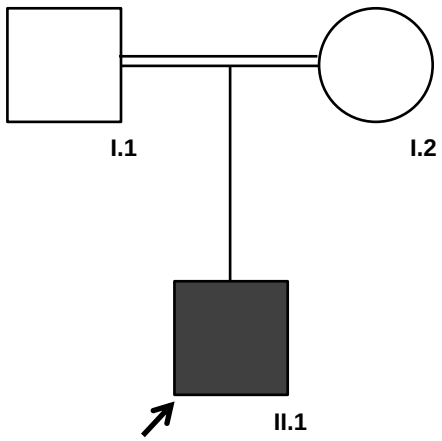
Clinical case



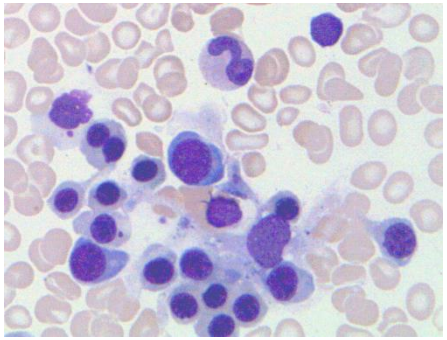
- 7-years-old boy
- Consanguineous parents from Cambodia
- Mild to borderline anemia (**Hb 9.8-10.5 g/dL**)
- Red cell macrocytosis (**MCV 92 fL**)
- Increased RDW (**19-24%**)
- Reduced reticulocyte count (**25000-53000/µL**)
- Peripheral blood smear: **marked anisopoikilocytosis**

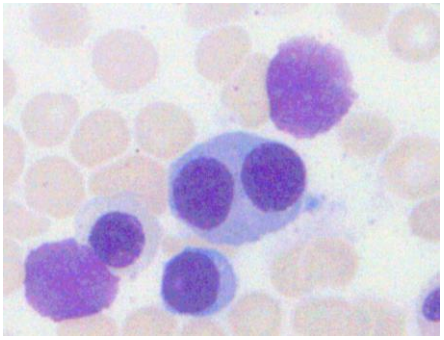
*First referred to the Monash Children's Hospital of Melbourne at **3 years** of age with:*

- **autism**
- **seizures**

Bone marrow:

- hypercellular marrow with moderate dyserythropoiesis
- **binucleated erythroblasts** (5-10%), cytoplasmic bridging
- **no internuclear bridging**,
- karyorrhexis, nuclear-cytoplasmic asynchrony, normal granulo- and megakaryopoiesis.





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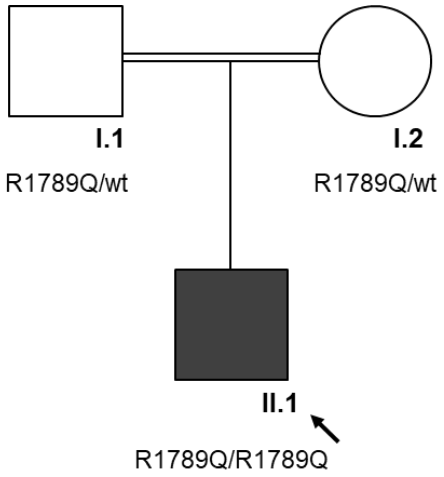
Russo R, et al, AJH 2018

Clinical case: *genetic testing*

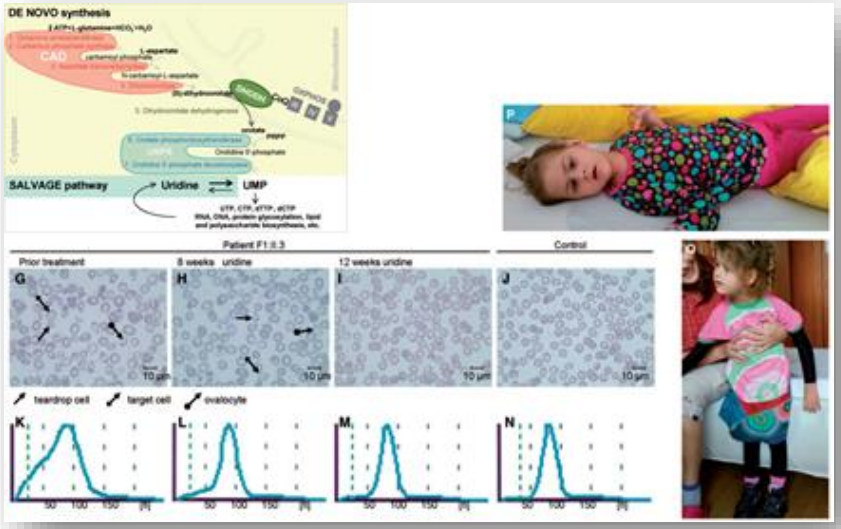
- First-line genetic testing**:** *SEC23B* gene analysis
  - no causative variants were identified

- Second-line genetic testing: ***t-NGS analysis***
  - variant in homozygous state in ***CAD*** gene: **c.5366G>A, p.Arg1789Gln**



✓Epileptic encephalopathy, early infantile, 50 (OMIM #616457): **neurodegenerative disorder** associated to **mild CDA II-like anemia** with **abnormal glycosylation** of the erythrocyte proteins band-3 and RhAG

- ***CAD*** gene (2p23.3), which encodes a trifunctional enzyme that catalyzes the first steps of *de novo* pyrimidine biosynthesis



Ng BG et al. Hum Mol Genet. 2015
Koch J et al. Brain 2017
Russo R et al. AJH 2018

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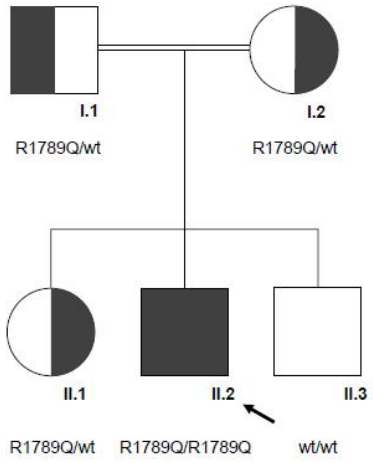
Clinical case: *treatment*

- ✓ A supplementation with oral uridine has been suggested as treatment for CAD-deficient patients
- ✓ Uridine is an FDA-approved drug for treatment of Uridine Monophosphate Synthase (UMPS) deficiency

a



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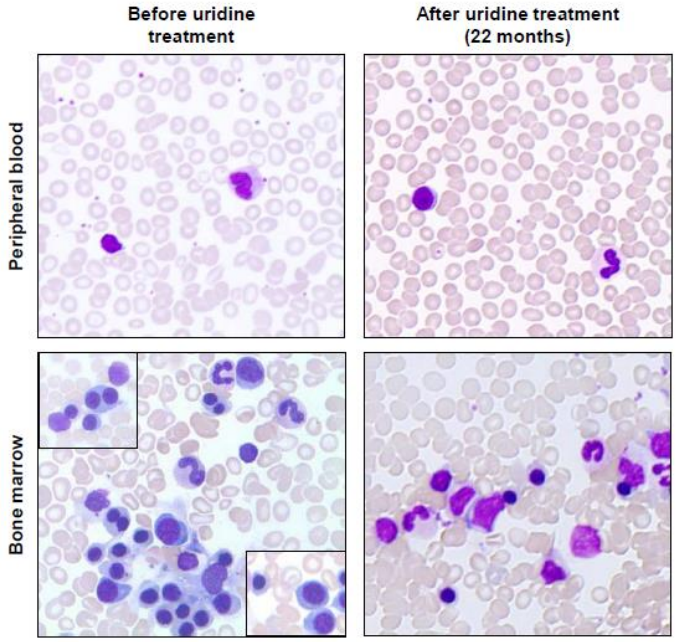

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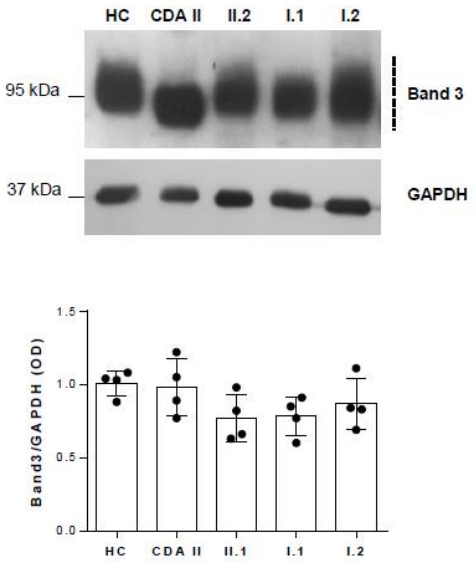

100 mg/kg/day

- increase of Hb levels
- reduction of the HbA2 and HbF levels
- reduction of RDW values

- *the frequency and severity of the seizures were markedly reduced;*
- *the patient has weaned off all antiepileptic drugs, started talking, and increased socialization*

Russo R, et al. AJH 2020 (under revision)

Take-home messages

- CDAs are characterized by high clinical and genetic heterogeneity that result in difficulties in reaching a correct differential diagnosis
- To diagnose these conditions, it is crucial to have **detailed phenotyping** and to perform **correct genetic testing**
- **Genetic testing** is fundamental for the correct **patient management**
- Implementation of NGS-based approach for the identification of **genetic phenotype-modifier variants**

- The most harmful complication of CDA II is **iron overload**. This is mainly related to increased levels of **ERFE** in CDA II patients.
- Nevertheless, a specific involvement of ***SEC23B* LoF** in the regulation of hepcidin expression at hepatic level has been recently demonstrated

Acknowledgments

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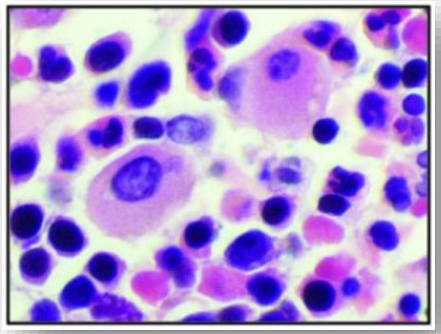
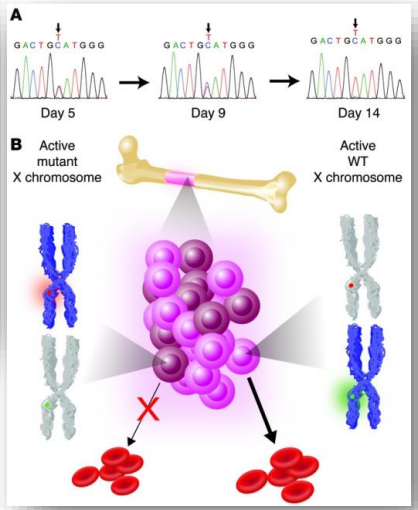
CDA variants

- ✓ **X-linked dominant** macrocytic dyserythropoietic anemia with iron overload in female individuals
- ✓ Heterozygous loss-of function mutations in the ***ALAS2*** gene (Xp11.21)

Sankaran VG et al. J Clin Invest. 2015
Russo R. et al. AJH 2018
Moreno-Carralero MI et al. EJH 2018





Loss of nonviable erythroid progenitors expressing the mutant allele

- ✓ **<u>Single cases/families</u>**:

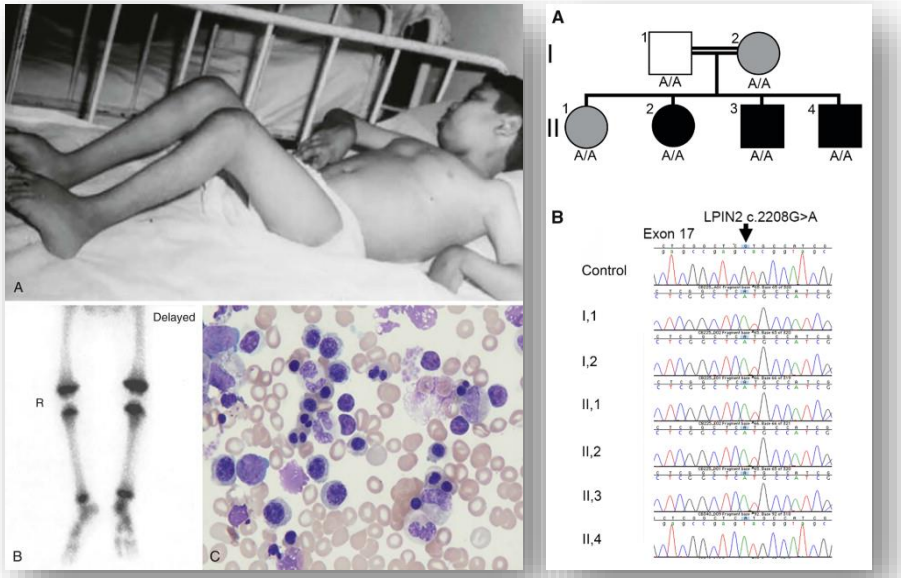
- A case with CDA II-like due to bi-allelic mutations in the ***MVK*** gene
  Samkari A et al. Pediatrics. 2010
- Two siblings with a CDA II-like anemia due to bi-allelic mutations in the ***PARP4*** gene
  Bianchi P et al. Blood. 2015 (ASH)
- A case of CDA-I like anemia due to a *de novo* variant in ***VPS4A*** gene
  Giger K et al. Blood. 2017 (ASH)
- A family with an atypical, dominantly-inherited CDA due to a variant in ***PRDX2*** gene
  Emberesh M et al. Blood. 2018 (ASH)

CDA variants: syndromic conditions

- ✓ Majeed syndrome (OMIM #609628): **chronic recurrent multifocal osteomyelitis**, inflammatory dermatosis, and **hypochromic microcytic anemia with dyserythropoiesis**

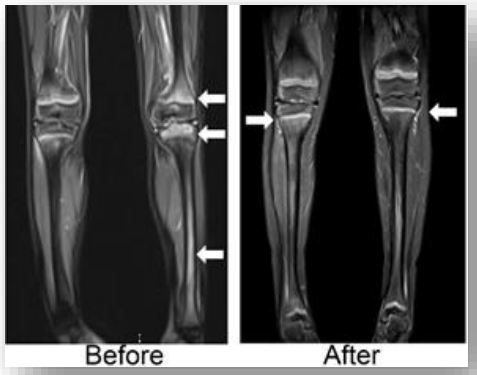
- ***LPIN2*** gene (18p11.31), encoding a phosphatidate phosphatase important in lipid metabolism

Ferguson PJ et al. J Med Genet. 2005
Rao AP et al. J Rheumatol. 2016
Roy NBA et al. Rheumatology 2019



- *Several case reports describe the clinical response to biologic anti-interleukin-1 (IL-1) therapy*

Herlin T, Ann Rheum Dis. 2013
Pinto-Fernández C, Allergol Immunopathol (Madr). 2017
Al Mosawi Z, Arch Rheumatol. 2019
Roy NBA et al. Rheumatology 2019



Models of studying (I)

- ✓ The main hurdle to advancing our understanding of the pathogenic mechanisms underlying CDA is the lack of appropriate models of studying

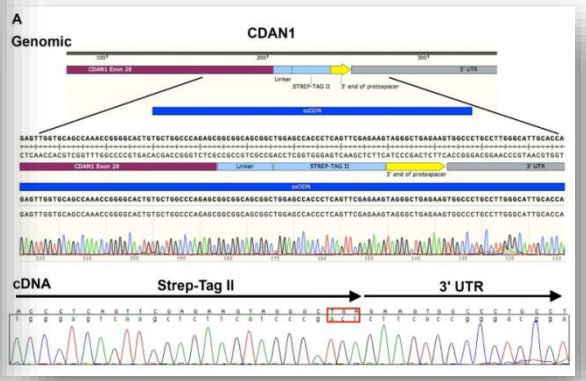
For ***CDAN1*** gene:

- Transgenic mouse model → embryonic lethality

Renella R et al. Blood. 2011

- Engineered Human Umbilical Derived Erythroid Precursors (**HUDEP-2**) cells by CRISPR/CAS9 technology

Moir-Meyer G et al. Methods Protoc. 2018



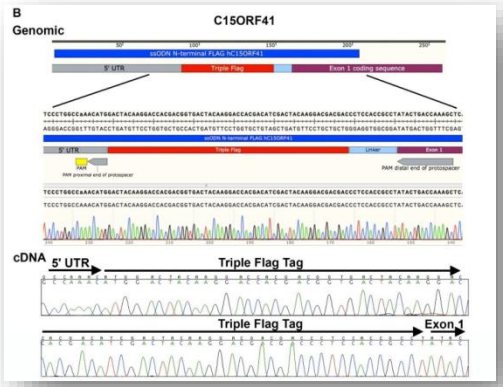
For ***C15ORF41*** gene:

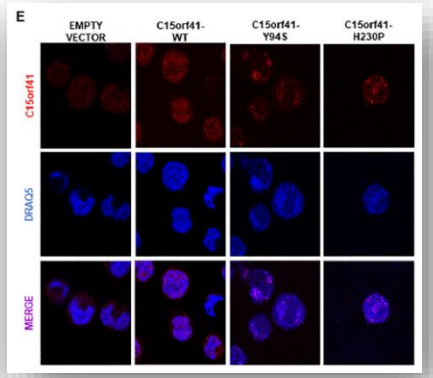
- Engineered **HUDEP-2** cells by CRISPR/CAS9 technology

Moir-Meyer G et al. Methods Protoc. 2018

- **K562 cells** overexpressing the H230P and Y94S mutations

Russo R, Marra R. et al. Front Physiol. 2019





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Models of studying (II)

For ***SEC23B*** gene:

- Knockdown of **zebrafish sec23b** by morpholino → aberrant erythrocyte development

  Schwarz K, Iolascon A. Nat Genet. 2009

- Knock-in **zebrafish** for a hom 53-bp deletion in exon 5 of **sec23b** (sec23b-/-) by CRISPR/Cas9 → lethal within 3 weeks of age

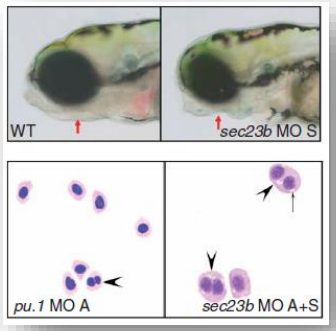
  Khoriaty R et al. Proc Natl Acad Sci U S A. 2018

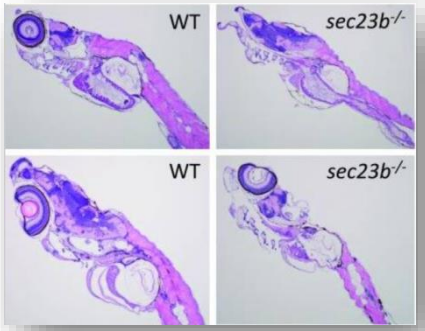
- Knock out **mice** for ***SEC23B*** gene die perinatally, showing huge pancreatic degeneration with no features of CDA II or other signs of anemia

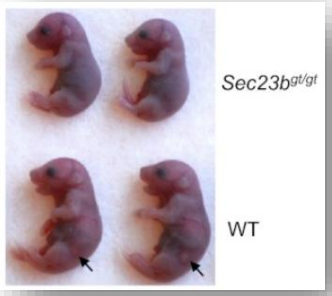
  Tao J et al. Proc Natl Acad Sci U S A. 2012
  Khoriaty R et al. Mol Biol Cell. 2017

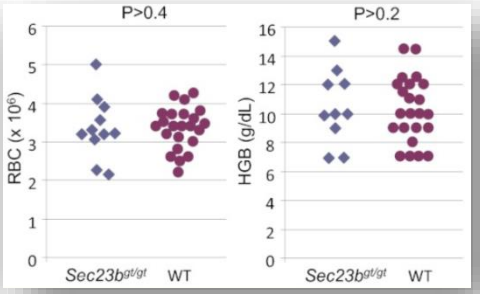
- Interference of ***SEC23B*** expression in **K562** cells by both plasmid transfection and stable lentivirus infection, recapitulates the cytokinesis defects observed in CDA II patients

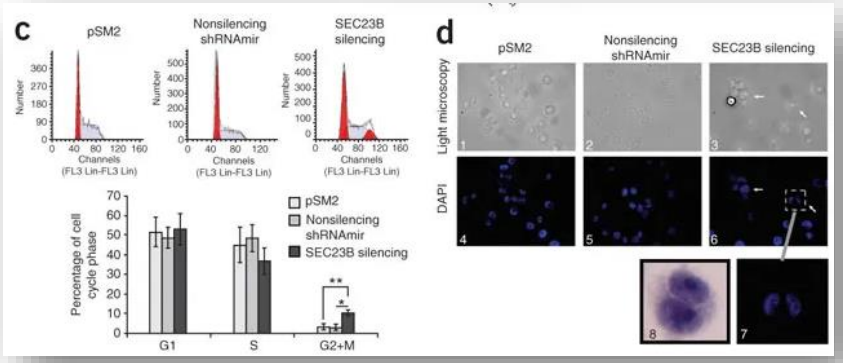
  Schwarz K, Iolascon A. Nat Genet. 2009
  Russo R et al. Blood 2016











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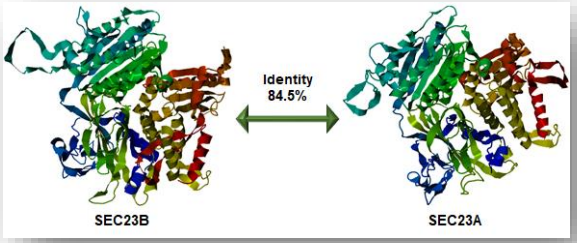
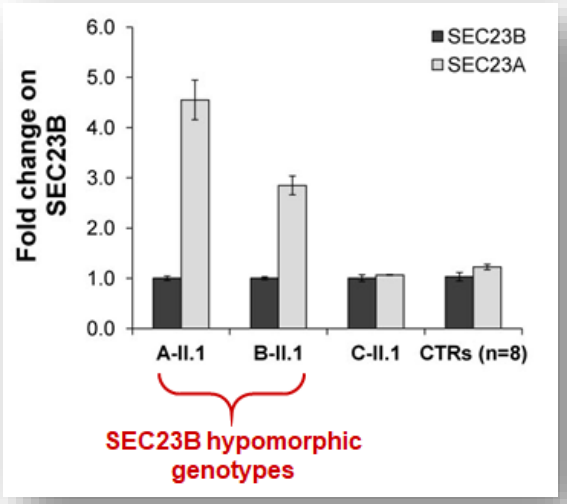
From pathogenic mechanisms to new therapeutic approaches

✓ Both **Sec23** genes carry specific but partially **redundant roles**



- Compensatory mechanism of ***SEC23A*** in *SEC23B* deficient cells
- ***Sec23a*** expression is maintained during **murine** terminal erythroid differentiation
- ***Sec23a***-expressing transgene rescues the lethality resulting from sec23b disruption in zebrafish
- The ***Sec23a*** coding sequence inserted into the endogenous *Sec23b* locus rescues the mortality and the pancreatic phenotype of *SEC23B* deficient mice



In vitro gene therapy on primary human erythroblasts with lentivirus p60-BBF2H7, **a transactivator of SEC23A**, led to the **normalization** of **SEC23 levels** to compensate for the mutated SEC23B in CDA II patients

Satchwell T et al, Haematologica 2013;
Khoriaty R et al, Mol Cell Biol. 2014
Russo R et al, Blood Cells Mol Dis 2013
Khoriaty R et al. Proc Natl Acad Sci U S A. 2018
Pellegrin S et al. BJH 2'019