Diagnòstic Genètic Avaçant de les Malalties del Metabolisme del Ferro
Mayka Sanchez
Systemic Iron homeostasis

Heme  Hemoglobin
Bone marrow ~ 300mg
Red blood cells ~ 1800mg
Reticuloendothelial macrophages ~ 600mg
Liver ~1000mg
Other cells and tissues ~ 400mg
Fe$_{2}^{3+}$ - Tf ~ 3mg

Hepcidin
Iron loss
Duodenum
Ferroportin
1-2mg/day
The importance of Iron balance

Too much… …too little

IRON

DISEASE

Overload

Hereditary Hemochromatosis

Deficiency

Rare Iron-related Anaemias
Hereditary Hemochromatosis

- Iron deposits in tissues.
- Clinics: Cirrosis, Hepatic cancer, Cardiomyopathy, Diabetes, Arthritis, Impotence, Hyperpigmentation, Hypogonadotropic hypogonadism
- Lab findings: High serum Iron, High sFerritin (>1000 ng/ml) + High Transferrin saturation (>50%)

<table>
<thead>
<tr>
<th>Type</th>
<th>Rare disease</th>
<th>Common Mutation</th>
<th>Rare genetic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>HH 1 Classic</td>
<td>HFE (Chr 6p21.3)</td>
<td>TfR2 (Chr7q22)</td>
<td>Hemojuvelin (Chr1p21) Hepcidin (Chr19q13.1) Ferroportin (Chr2q32)</td>
</tr>
<tr>
<td>HH 3</td>
<td>HFE (Chr 6p21.3)</td>
<td>TfR2 (Chr7q22)</td>
<td>Hemojuvelin (Chr1p21) Hepcidin (Chr19q13.1) Ferroportin (Chr2q32)</td>
</tr>
<tr>
<td>JH 2b</td>
<td>HFE (Chr 6p21.3)</td>
<td>TfR2 (Chr7q22)</td>
<td>Hemojuvelin (Chr1p21) Hepcidin (Chr19q13.1) Ferroportin (Chr2q32)</td>
</tr>
<tr>
<td>JH 2a</td>
<td>HFE (Chr 6p21.3)</td>
<td>TfR2 (Chr7q22)</td>
<td>Hemojuvelin (Chr1p21) Hepcidin (Chr19q13.1) Ferroportin (Chr2q32)</td>
</tr>
<tr>
<td>HH 4a/4b</td>
<td>HFE (Chr 6p21.3)</td>
<td>TfR2 (Chr7q22)</td>
<td>Hemojuvelin (Chr1p21) Hepcidin (Chr19q13.1) Ferroportin (Chr2q32)</td>
</tr>
</tbody>
</table>

Onset: adult, juvenil, adult

Heredity: Autosomal recessive, Autosomal dominant
HH type I: C282Y +/- but NOT H63D!

• HH type I is due to mutations in HFE gene
  • C282Y homozygous => high predisposition for HH.
    • In Spain C282Y+/+ genotype frequency: 1/1000
  • Other rare mutations in HFE have been identified
  • In HFE gene there are common variations and polymorphism!

• What is new about with H63D variant/polymorphism?
  H63D variant is a COMMON variant very frequent in SPAIN
  • In Spain H63D +/- genotype frequency: 1/23!!
  • In Spain H63D +/- genotype frequency: 1/3!!

EASL GUIDELINES in HFE-HH:
Definition for diagnosis of HFE-HH: C282Y homozygosity and increased body iron stores with or without clinical symptoms.
Any other HFE genotype must be interpreted with caution.
Homozygosity for H63D is not a sufficient genetic cause of iron overload
C282Y/H63D compound heterozygotes should first be investigated for other causes of hyperferritinemia
Genetic testing of ‘other hemochromatosis genes’ (TFR2, SLC40A1, HAMP, HJV) could be considered in patients with increased iron stores after exclusion of C282Y homozygosity.

Sanchez et al., J Hepatol 2003
EASL HFE Hemochromatosis 2011
Hereditary Hemochromatosis versus Hyperferritinemia

• High sFerritin is also a hallmark of other genetic entities such as:
  • Hereditary hyperferritinemia cataract Syndrome (HHCS)
  • Bening hyperferritinemia

Hyperferritinemia
A problem for the clinical diagnostic

FERRITIN as a dual marker…
Iron levels or inflammation?
Hereditary Hyperferritinemia-Cataracts Syndrome (HHCS)

Clinical Synopsis
Eyes: Binucleated congenital cataracts.
Inheritance: Autosomic dominant

Molecular Mechanism for HHCS
Healthy control
Patient
Hereditary Hyperferritinemia-Cataracts Syndrome

New mutations in HHCS cases

Minor disturbance of IRP binding BUT DISEASE!

Hyperferritinemia project

HIGHFERRITIN Web Server

Algorithms and recommendations for diagnosis and management of hyperferritinemia

HOME  CAUSES  MANAGEMENT  RESOURCES  FOR DOCTORS  COMMENTS  CONTACT

HIGHFERRITIN

The HIGHFERRITIN Web Server is a tool for helping the general practitioners (GP) or medical specialists in the diagnosis and management of patients with high levels of serum ferritin or hyperferritinemia. HIGHFERRITIN Web Server provides suggestions and recommendations about the appropriate medical tests and procedures to be done and the possible treatments to follow.

These recommendations have been agreed by a group of experts and they follow the recommendations of international guidelines in Hereditary Hemochromatosis and other diseases together with the personal experience of these experts.

To use this tool, please go to the diagnostic section or just click here.

http://highferritin.imppc.org
HIGHFERRITIN Web Server

Thanks to:
- Albert Altés (Althaia Foundation)
- Maria José Pérez-Lucena (CAP Canaletes)
- Miquel Bruguera (Hospital Clínic de Barcelona)
- Rosario López (Althaia Foundation)
- Maria Àngles Ruiz-Minguez (Hospital de l'Esperit Sant)
- Miquel Torres (Hospital de l'Esperit Sant)
- Mayka Sánchez (IMPPC)
- Jordi Fèlix-Brugués (CAP Canaletes)
- David Benéitez (Hospital Universitari Vall d'Hebron)
- María Fátima Matute (Hospital Clínico San Carlos)
- Ángel F. Remacha (Hospital de la Santa Creu i Sant Pau)
- Cristina Sanz (Hospital Clínic de Barcelona)

PUBLICATION:
# Hereditary Hemochromatosis versus Hyperferritinemia

<table>
<thead>
<tr>
<th>Gene (protein) defect</th>
<th>HFE</th>
<th>HFE2 (HJV)</th>
<th>HAMP (Hepcidin)</th>
<th>TFR2</th>
<th>SLC40A1 (FPN)</th>
<th>FTL</th>
<th>FTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
<td>AD</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>Adult</td>
<td>&lt;20 years</td>
<td>&lt;20 years</td>
<td>&lt;35 years</td>
<td>Adult</td>
<td>Infancy to Adult</td>
<td>Infancy to Adult</td>
</tr>
<tr>
<td>Liver iron overload</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Other organ damaged</td>
<td>Heart</td>
<td>Heart</td>
<td>Heart</td>
<td>Retina</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High/Normal</td>
<td>Normal/low</td>
<td>Normal/low</td>
</tr>
<tr>
<td>Hepcidin levels</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Ferritin</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Treatment</td>
<td>Phlebotomies</td>
<td>Phlebotomies</td>
<td>Phlebotomies</td>
<td>Phlebotomies</td>
<td>No phlebotomies, cataracts removal</td>
<td>Benign</td>
<td></td>
</tr>
<tr>
<td>Other Symptoms</td>
<td>Cirrhosis, Hepatic cancer, Cardiomyopathy, Diabetes, Arthritis, Impotence, Hyperpigmentation, Hypogonadotropic hypogonadism</td>
<td>Cirrhosis, Cardiomyopathy</td>
<td>Cirrhosis, Cardiomyopathy</td>
<td>Cirrhosis, Hepatic cancer, Cardiomyopathy, Diabetes, Arthritis, Impotence, Hyperpigmentation, Hypogonadotropic hypogonadism</td>
<td>Depend of type 4a or 4b</td>
<td>Benign</td>
<td></td>
</tr>
</tbody>
</table>

C282Y IRE mutations
Causes of Iron Deficiency Anaemia (IDA)

• Blood loss (intestinal gastric bleeding)
• Limited supply diet (iron, folic, vitamins)
• Increased requirements

• Iron Malabsorption

Adquired (Common form):
• Refractory (no explanation): Helicobacter pylori, Celiac Disease, Autoimmune Atrophic Gastritis

Hereditary (Rare form): Microcytic & hypocromic Anaemia
• Non-sideroblastic
• Sideroblastic
# Genetics of non-sideroblastic forms of Iron Deficiency Anaemia

<table>
<thead>
<tr>
<th>Gene Symbol (Protein)</th>
<th>Chr.</th>
<th>Protein Function</th>
<th>Inheritance</th>
<th>Disease Caused by Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMPRSS6 (Matriptase -2)</td>
<td>22</td>
<td>Regulates hepcidin expression</td>
<td>AR</td>
<td>Iron-refractory Iron-deficiency Anemia (IRIDA)</td>
</tr>
<tr>
<td>SLC11A2 (DMT1)</td>
<td>12</td>
<td>Transmembrane iron transporter</td>
<td>AR</td>
<td>Autosomal recessive hypochromic, microcytic anaemia with hepatic iron overload</td>
</tr>
<tr>
<td>TF (Transferrin)</td>
<td>3</td>
<td>Plasma iron binding protein; ligand for TFR1 &amp; TFR2</td>
<td>AR</td>
<td>Atransferrinaemia: Iron deficiency anaemia with tissue iron overload</td>
</tr>
<tr>
<td>CP (Ceruloplasmin)</td>
<td>3</td>
<td>Plasma ferroxidase</td>
<td>AR</td>
<td>Aceruloplasminaemia: Mild iron deficiency anaemia associated with iron accumulation in the liver and brain</td>
</tr>
</tbody>
</table>
Aceruloplasminemia: Deficiency of Ceruloplasmin

- Ceruloplasmin (CP), a copper-containing ferroxidase, cooperates to export iron with ferroportin \(^1\)
- Laboratory and clinical expression of aceruloplasminemia includes low or absence serum ceruloplasmin, low serum copper levels, mild-moderate microcytic anemia with low serum iron and high serum ferritin, diabetes mellitus (iron in pancreas), and late-onset neurological symptoms (iron in brain), including retinal degeneration, ataxia, involuntary movements and dementia\(^1,2\)
- Differential diagnosis\(^1\)
  - Anaemia of chronic diseases (ACD)
  - Wilson’s disease (Cu accumulation, ATP7B)
  - Menkes’s disease (Cu and Cp deficiency, ATP7A)
  - Hypotransferrinaemia or atransferrinaemia
- Treatment: chelation therapy; oral zinc sulfate\(^3\); no benefit from phlebotomy\(^1\)

4 cases of Aceruloplasminemia (from Lithuania, Neurogenetics Unit Univ Collegue London, UK)

- Case 100U
- 75 years old woman
- Lithuania

2 NEW FRAMESHIFT MUTATIONS
NM_000096.3:c.[1783_1787delGATAA(;)2520_2523delAACA];[=]
NP_000087.1:p.(D595Yfs*2;T841Rfs*52);(=)

NEW HOMOZYGOUS SPLICING MUTATION
NM_000096.3:c.[1864+5G>A];[1864+5G>A]

Exon 10

Exon 14

Intron 10

- Case 102U, 66 years old man
- Case 106U, 67 years old man
- Case 107U, 50 years old man
- 1 patient from Pakistan origin

4 cases of Aceruloplasminemia (from Lithuania, Neurogenetics Unit Univ Collegue London, UK)
Hypotransferrinaemia or Atransferrinaemia


- Hereditary atransferrinaemia has been reported in 12 families world-wide.

- Functional deficiency of Transferrin (TF gene Chr 3q21).
Hypotransferrinaemia or Atransferrinaemia

• Onset in early infancy

• Clinically **defective expression associated with mutations in the TF gene and results in:**
  - reduction in delivery of iron to erythroid cells in the bone marrow
  - reduced haemoglobin synthesis

=> **Severe iron deficiency** hypochromic–microcytic **anaemia** resistant iron therapy.
  - Massive but futile iron absorption

=> **Severe iron overload** in parenchymal organs (liver and heart hemosiderosis)

=> Recurrent infections

• **Treatments:**
  - fresh plasma infusions
  - purified human apotransferrin

Participation in clinical Trial: NCT01797055 (Apotransferrin in Atransferrinemia)
SANQUIN BLOOD (The Netherlands). Measurements of hepcidin and NTBI
Atransferrinaemia cases (4 families, 6 affected cases)

Transferrin normal values: 204-360 mg/dl
Bioinformatics and computational analysis of missense mutations

- Bioinformatics functional predictions: Ala418Glu, Arg609Trp, Asp647Val mutations are damaging
- Multiple sequence alignments: Aminoacids Ala418, Arg609, Asp647 are highly conserved through evolution among 17 species
- Structural analysis indicates that these mutations destabilize TF structure and cause protein malfunction and disease.

**PUBLICATION: 2 Indian cases families C and D**
Iron Refractory Iron Deficiency Anemia (IRIDA)

- Myelocytic hypochromic Anemia without response to oral iron treatment, partial response to iv iron
- AR Disease (OMIM #206200). Mutations in TMPRSS6 (MT-2). Negative regulator of hepcidin
- Hepcidin levels inappropriately high for the level of Anemia (ELISA at UDGAEMH)

Manuscript in preparation (20 new mutations, 16 families)
IRIDA (Iron-Refractory, Iron-Deficiency Anaemia)

**Treatment**

- Oral iron administration is ineffective
- Response to parenteral iron administration is partial
- Anaemia becomes less severe in adulthood as a consequence of the greater availability of the limited amount of available iron to erythropoiesis
Iron Refractory Iron Deficiency Anemia (IRIDA)
Mutations in the gene SLC11A2 (DMT1)

- Severe microcytic anaemia with high transferrin saturation
- Severe hypochromia with liver iron overload and normal ferritin levels
- 5 cases described world-wide

France


Czech


Italy


Spain

# Main Biologic and Clinical Differences in Genetic and non-sideroblastic forms of Iron Deficiency Anaemia

Hypochromic microcytic ANAEMIA: LOW MCV, LOW MCHC, LOW Hemoglobin

<table>
<thead>
<tr>
<th></th>
<th>DMT1-deficiency</th>
<th>IRIDA</th>
<th>Atransferrinaemia</th>
<th>Aceruloplasminaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene (protein) defect</td>
<td>SLC11A2 (DMT1)</td>
<td>TMPRSS6 (MT2)</td>
<td>TF</td>
<td>CP</td>
</tr>
<tr>
<td>Inheritance</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>At birth</td>
<td>18–24 mo</td>
<td>Late onset provided some transferrin is present</td>
<td>Late onset with moderate anaemia</td>
</tr>
<tr>
<td>Liver iron overload</td>
<td>Yes</td>
<td>No (or yes due to transfusion treatment)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Brain damage</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Serum iron</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Ringed sideroblasts</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hepcidin levels</td>
<td>Low</td>
<td>High for low iron values</td>
<td>Low</td>
<td>Not yet measured</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Low or normal</td>
<td>Normal</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Cases (families)</td>
<td>5 (5)</td>
<td>41(24)</td>
<td>12 (14)</td>
<td>32 (6, Japan)</td>
</tr>
<tr>
<td>Inheritance</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td>Very low levels TF</td>
<td>Very low levels CP</td>
</tr>
</tbody>
</table>
Congenital Sideroblastic Anemia (CSA)

Classification
• Hereditary forms (RARES)
  • Syndromic or Non-syndromic
  • Microcytic, Normocytic or Macrocytic (80>MCV>100)
    ▪ X-linked
    ▪ Autosomic Recessive
    ▪ Autosomic Dominant
    ▪ Mitochondrial DNA

• Acquired forms (Common)
  • Idiopathic: RARS Refractory anemia with ringed sideroblasts (acquired stem cell disorders)
  • Secondary: tumors, alcoholism, Pb or Zn intoxication
Microcytic Congenital Sideoblastic Anemias (CSA)

Pathophysiology
• Disturbances of mitochondrial proteins regulating haem synthesis or Fe/S cluster synthesis

• Ringed sideroblasts form when iron accumulated inside the mitochondria that circle the normoerythroblast nucleus

• Perceived by body as increased need for iron
  - Increased iron absorption results in iron overload
### Genetics of Congenital Sideroblastic Anaemias CSA (Microcytic)

<table>
<thead>
<tr>
<th>Gene Symbol (Protein)</th>
<th>Chr.</th>
<th>Protein Function</th>
<th>Inheritance</th>
<th>Disease Caused by Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAS2</td>
<td>X</td>
<td>Heme biosynthetic pathway mitochondrial enzyme</td>
<td>X-linked</td>
<td>CSA linked to chromosome X (XLSA)</td>
</tr>
<tr>
<td>SLC25A38</td>
<td>3</td>
<td>Erytroid-specific transporter (Glycine?/ALA?)</td>
<td>AR</td>
<td>Autosomal recessive nonsyndromic CSA</td>
</tr>
<tr>
<td>ABCB7</td>
<td>X</td>
<td>Fe/S cluster transporter</td>
<td>X-linked</td>
<td>CSA associated with spinocerebral ataxia (XLA/A)</td>
</tr>
<tr>
<td>GLRX5</td>
<td>14</td>
<td>Fe/S cluster assembly pathway</td>
<td>AR</td>
<td>Sideroblastic anemia with hepatic iron overload</td>
</tr>
<tr>
<td>STEAP3</td>
<td>2</td>
<td>Ferrireductase, acquisition of iron by erythroblasts</td>
<td>AR</td>
<td>Congenital hypochromic anaemia associated with STEAP3 mutations</td>
</tr>
</tbody>
</table>
Microcytic CSA: dysfunctional mitochondrial iron metabolism
AR non-syndromic CSA: gen SLC25A38

- Non-syndromic severe type of autosomal recessive sideroblastic anemia
  - Microcytic & hypochromic sideroblastic anemia (low MCV, low MCHC)
  - Refractory to treatment with pyridoxine and folic acid
  - Increase transferrin saturation and ferritin levels before significant amount of blood received by transfusion
- Early onset
- Iron overload problems
- Patients are transfusion dependent specially first few years of life
- Few patients underwent bone marrow transplantation
  - Genetic diagnostic allows SLC25A38 genotyping of potential bone marrow donors

- Second type more frequent of CONGENITAL SIDEROBLASTIC ANEMIA after XLSA (ALAS2 gene)

NEW MISSENSE MUTATION
NM_017875.2:c.[152T>C];[=];
NP_060345.2:p.(Leu 51Pro);(=)

NEW NONSENSE MUTATION
NM_017875.2:c.[559C>T];[=];
NP_060345.2:p.(R187*);(=)

• Girl 4 years old from Peru
• Severe Anaemia, onset: 1 month
• Transfusion dependent
• Hb:3.5g/dl (1 month)

• 50% BM ring sideroblasts
  (22/05/2013)

ANEMIA WITH RING SIDEROBLASTS

UTILITY OF GENETIC DIAGNOSTIC: It is not going to respond to pyridoxine!
A novel syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay (SIFD)
Sideroblastic Anemia, Immunodeficiency, Fevers and Developmental Delay (SIFD)

- Anemia
  - Severe (Hb 7.1g/dL; range 4.8-8.3). Markedly microcytic (MCV 62 fL; range 53.6-73.2)
  - Hypochromasia, Microcytosis, Schistocytosis, Basophilic stippling, Frequent nucleated erythrocytes
  - No hemoglobinopathies, iron deficiency, RBC mb defects, erythropoietic or porphyrias
  - BM examination reveals ringed sideroblasts >45-50% (1 not performed)
  - Erythroid hyperplasia and dyserythropoiesis, with deficient cytoplasmic hemoglobinization
  - Most children hyperferritinemia (due inflammation and secondary iron overload)
- Recurrent high fever syndrome requiring multiple hospitalizations (11), elevated inflammatory markers, Vomiting, Diarrhea.
- Immunodeficiency
  - Recurrent inflammatory episodes, B-cell lymphopenia and hypogammaglobulinemia (11),
  - No good response to intravenous replacement with immunoglobulins (Ig)
- Developmental delay variable but alarming:
  - Generalized and truncal hypotonia, often severe, progressive and associated with gross motor developmental delay
  - Comprehension and communication were impaired in many
- Other symptoms were present in some of the patients: sensorineural hearing impairment (5), Recurrent seizures (5), Neurological/neuromuscular abnormalities, Nephrocalcinosis/renal tubular dysfunction (3), Aminoaciduria (6), Cardiomyopathy (2) and cardiac failure (contribution to death in 5 cases), Pigmentary retinitis (4), hepatosplenomegaly (4), brittle hair (3), chronic ichthyotic skin changes (1)
# Main Biologic and Clinical Differences in Genetic and Congenital Microcytic Sideroblastic Anaemia

<table>
<thead>
<tr>
<th></th>
<th>XLSA</th>
<th>Non-syndromic SA</th>
<th>XLSA/A</th>
<th>GLRX5-deficiency</th>
<th>SIFD</th>
<th>STEAP3-deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene (protein) defect</td>
<td>ALAS2</td>
<td>SLC25A38</td>
<td>ABCB7</td>
<td>GLRX5</td>
<td>?</td>
<td>STEAP3</td>
</tr>
<tr>
<td>Inheritance</td>
<td>X-linked</td>
<td>AR</td>
<td>X-linked</td>
<td>AR</td>
<td>AR</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>Variable</td>
<td>Variable</td>
<td>Neonatal/infancy</td>
<td>Usually midlife</td>
<td>Neonatal/infancy</td>
<td>Infant/Adolescent</td>
</tr>
<tr>
<td>Liver iron overload</td>
<td>Yes</td>
<td>Yes</td>
<td>Variable</td>
<td>Yes</td>
<td>Yes</td>
<td>Secondary to blood transfusions</td>
</tr>
<tr>
<td>Brain damage</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Serum iron</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
<td>High</td>
<td>Normal (High after few transfusions)</td>
<td>High</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>Variable</td>
<td>High</td>
<td>Normal</td>
<td>High</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Ringed sideroblasts</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Hepcidin levels</td>
<td>Not yet measured</td>
<td>Normal/high</td>
<td>Not yet measured</td>
<td>Not yet measured</td>
<td>Not yet measured</td>
<td>Normal/high</td>
</tr>
<tr>
<td>Ferritin</td>
<td>High</td>
<td>Normal/high</td>
<td>Variable</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Cases (families)</td>
<td>&gt;70</td>
<td>29 (26)</td>
<td>10 (3)</td>
<td>2 (2)</td>
<td>12 (10)</td>
<td>3(1)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Pyridoxine</td>
<td>BM transplantation?</td>
<td>Chelation!</td>
<td>Transfusion + Iv Ig. Iron chelation, BM transplantation (1)</td>
<td>Transfusion + chelation</td>
<td></td>
</tr>
<tr>
<td>Symptoms</td>
<td>Anaemia</td>
<td>Anaemia</td>
<td>Anaemia and ataxia</td>
<td>Anaemia</td>
<td>Severe anaemia, B-lymphopenia or panhypogammaglobulinemia, Fevers, developmental delay</td>
<td>Anaemia, growth retardation, hepatosplenomegaly</td>
</tr>
</tbody>
</table>
Congenital dyserythropoietic anemia (CDA)

- Rare Anaemias with ineffective erythropoiesis (iron overload) and distinct morphological abnormalities of erythroblasts in the Bone Marrow

**General characteristics:**

- Fatigue
- Pallor
- Scleral icterus
- Jaundice
- Splenomegaly
- Abnormal fingernails
- Macrocytic/Normocytic RBC
- Anaemia
- Ineffective erythropoiesis
- Increased iron absorption
- Abnormal expression of membrane antigens
# Congenital dyserythropoietic anemia (CDA)

##_classification

<table>
<thead>
<tr>
<th>CDA TYPE</th>
<th>PROTEIN</th>
<th>GENE</th>
<th>CHROMOSOME LOCATION</th>
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</thead>
<tbody>
<tr>
<td>CDA I</td>
<td>Codanin-1</td>
<td>CDAN1</td>
<td>15q15.1-15.3</td>
</tr>
<tr>
<td></td>
<td>Endonuclease</td>
<td>C15orf41</td>
<td>15q14</td>
</tr>
<tr>
<td>CDA II</td>
<td>SEC23B</td>
<td>SEC23B</td>
<td>20p11.23-20p12.1</td>
</tr>
<tr>
<td>CDA III</td>
<td>KIF23</td>
<td>KIF23</td>
<td>15q22</td>
</tr>
<tr>
<td>CDA IV</td>
<td>KLF1</td>
<td>KLF1</td>
<td>19p13.13-p13.12</td>
</tr>
<tr>
<td>CDA with thrombocytopenia</td>
<td>GATA1</td>
<td>GATA1</td>
<td>Xp11.23</td>
</tr>
</tbody>
</table>
Main Biologic and Clinical Differences in Congenital Dyserythropoietic Anaemias (CDAs)

Intravascular hemolysis low haptoglobin, high LDH. High bilirubin.
Inappropriately low reticulocyte count for the degree of anemia compared with other hemolytic anemias (normal or slight increased absolute reticulocytes).
Jaundice, erythroid hyperplasia, splenomegaly and or hepatomegaly. Iron overload

<table>
<thead>
<tr>
<th></th>
<th>CDA I</th>
<th>CDA II (HEMPAS)</th>
<th>CDA III</th>
<th>CDA IV</th>
<th>CDA with thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene (protein) defect</td>
<td>CDAN1, C15ORF14</td>
<td>SEC23B</td>
<td>KIF23</td>
<td>KLF1</td>
<td>GATA1</td>
</tr>
<tr>
<td>Inheritance</td>
<td>AR</td>
<td>AR</td>
<td>AD</td>
<td>AD</td>
<td>X-linked</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>Infancy-Adult</td>
<td>Infancy-Adult</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Liver iron overload</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>High</td>
<td>High</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hepcidin levels</td>
<td>Low</td>
<td>Low</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ferritin</td>
<td>High</td>
<td>High</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bone marrow (BM)</td>
<td>E/G ratio 3-8 (N:0.2-1)</td>
<td>BM: Bi, Tri-nucleated erythroblasts.</td>
<td>BM: large number 10-35% binucleated erythroblasts.</td>
<td>BM: Multinucleated (up to 12 nuclei) erythroblasts</td>
<td>BM: Multinucleated erythroblasts</td>
</tr>
<tr>
<td>Hyperplasia of erythroblasts lineage</td>
<td>Electron microscopy (EM) BM: spongy heterochromatin Swiss cheese appearance.</td>
<td>EM:Double membrane</td>
<td>Megakaryocytes display also ultrastructural abnormalities</td>
<td>Iron filled mitochondria</td>
<td></td>
</tr>
<tr>
<td>Peripheral blood (PB)</td>
<td>Macrocytosis (normo in childhood)</td>
<td>Normocytosis</td>
<td>Low reticulocytes</td>
<td>Absence exp CD44 and AQP1 in RBC</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1/3 Congenital malformations (limbs, heart, kidney, hip dysplasia, nail deformities), scoliosis</td>
<td>Positive test Ham (acidified-serum test)</td>
<td>Gallstones</td>
<td>High levels of fetal and embryonic hemoglobin</td>
<td></td>
</tr>
<tr>
<td>Cholelithiasis. Gallstones</td>
<td>Retinal angioid streaks</td>
<td>Gallstones</td>
<td>Biliary problems</td>
<td>Hydrops fetalis, dysmorphic features (1 case)</td>
<td></td>
</tr>
<tr>
<td>Retinal angioid streaks</td>
<td></td>
<td>Gallstones</td>
<td>High serum thymidine kinase</td>
<td>Thrombocytopenia with b-Thalassemia</td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>150</td>
<td>370</td>
<td>43 cases in 2 fam (Sweden 39, American 4, Argentinien (n=?)</td>
<td>4 + 1 no publicado (AI)</td>
<td></td>
</tr>
<tr>
<td>Exclude</td>
<td>Hemolitic anaemias</td>
<td>Hereditary spherocytosis (HS)</td>
<td>Macrocytic anaemias</td>
<td>KLF1 mut also in HPFH+/+ZnPP</td>
<td></td>
</tr>
<tr>
<td>Gilbert syndrome</td>
<td>Macrocytic anaemias (B12, folic acid defic)</td>
<td>Hemolytic anaemias</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CDA: Congenital dyserythropoietic anemias

CDAI = gene CDAN1

CDAII = gene SEC23B

Microscopy:
Chromatin bridges
Electron microscopy:
Heterochromatin “swiss cheese appearance”

Microscopy: bi or multi-nucleated erythroid precursors.
Electron microscopy: double mb inside cytoplasmic membrane
The future is here

Manual sequencing
1 gene at a time
Sanger Sequencing

Gene PANELS
Many genes at one time
MiSeq NGS
Take home message!

- Rare non-HFE Hemochromatosis can be caused by mutations in TFR2, FPN, HJV or HAMP (and other genes?). Easy treatment: pheblotomies.
- Hyperferrritinemias with cataract. Mutations in the FTL IRE. NO pheblotomy please!!
- Recent advances in iron metabolism led to the recognition of new entities of iron deficiency anaemia
- New technology (NGS gene panels, exome, genome) is going to change the Clinical Genetics in the close future
- These genetic forms of iron deficiency anaemia should be recognized by haematologists, as they are refractory to classical oral or intravenous iron administration
Agradecimientos

• Advanced Genetic Diagnostic Unit for Rare Iron Metabolism Disorders
  • Dr. Erica Morán
  • Jessica Aranda
• Grupo Ibérico de Ferropatología (GIF)
• Iron Metabolism Community (EIC, IBIS)

Research Projects and Contracts
Ramón y Cajal Research Contract
Technician Contrat (Carlos III)
Plan Nacional (SAF) – 2013-2015
European Project E-rare –2009-2012
Proyectos de Investigación Fundamental no Orientada - SAF 2012.
European Project e: e-ENERCA –2013-2016
Postdoctoral Fellowship FEBS 2012-2015
<table>
<thead>
<tr>
<th>CATEGORIES</th>
<th>GENE</th>
<th>DISEASE</th>
<th>PRICE (euros)</th>
<th>OMIM</th>
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</thead>
<tbody>
<tr>
<td>IRON OVERLOAD</td>
<td>Classical Hemochromatosis</td>
<td>HFE</td>
<td>HH1</td>
<td>200</td>
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<tr>
<td></td>
<td>Non-HFE Hemochromatosis</td>
<td>HAMP</td>
<td>HH2 (juvenile form)</td>
<td>150</td>
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<tr>
<td></td>
<td></td>
<td>HFE2</td>
<td>HH2 (juvenile form)</td>
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<tr>
<td></td>
<td></td>
<td>TFR2</td>
<td>HH3</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLC40A1</td>
<td>HH4</td>
<td>240</td>
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<tr>
<td>HYPERFERRITINEMIA</td>
<td>Hyperferritinemia</td>
<td>FTL</td>
<td>Hereditary hyperferretnemia with cataracts</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FTL</td>
<td>Benign Hyperferretnemia</td>
<td>150</td>
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<tr>
<td></td>
<td></td>
<td>FTH</td>
<td>Hyperferretnemia with iron overload</td>
<td>120</td>
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<tr>
<td>RARE IRON RELATED ANEMIAS</td>
<td>Non-sideroblastic anemia</td>
<td>CP</td>
<td>Aceruloplasminemia</td>
<td>440</td>
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<tr>
<td></td>
<td></td>
<td>TF</td>
<td>Hypotransferrinemia</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLC11A2</td>
<td>Familial microcytic hypochromic anemia with hepatic iron overload</td>
<td>320</td>
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<tr>
<td></td>
<td></td>
<td>TMPRSS6</td>
<td>IRIDA, Iron-refractory iron deficient anemia</td>
<td>360</td>
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<td></td>
<td>Congenital dyserythropoietic anemia</td>
<td>CDAN1</td>
<td>CDA type I</td>
<td>460</td>
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<td></td>
<td></td>
<td>SEC23B</td>
<td>CDA type II</td>
<td>400</td>
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<td></td>
<td></td>
<td>KIF23</td>
<td>CDA type III</td>
<td>480</td>
</tr>
<tr>
<td></td>
<td>Sideroblastic anemia</td>
<td>ALAS2</td>
<td>XLSA</td>
<td>240</td>
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<td></td>
<td></td>
<td>STEAP3</td>
<td>Sideroblastic anemia associated to STEAP3</td>
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<td></td>
<td></td>
<td>ABCB7</td>
<td>XLSA with ataxia</td>
<td>300</td>
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<td></td>
<td></td>
<td>GLRX5</td>
<td>SA with hepatic iron overload</td>
<td>150</td>
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<td></td>
<td></td>
<td>SLC25A38</td>
<td>Non syndromic autosomal recessive CSA</td>
<td>200</td>
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<td></td>
<td>Acquired anaemia</td>
<td>SF3B1</td>
<td>Myelodysplastic Syndrome Ring Sideroblasts</td>
<td>150</td>
</tr>
</tbody>
</table>

Dr. Mayka Sánchez  
Dr. Erica Morán  
udgaemh@imppc.org  
Tlf: 935543064  
http://www.imppc.org/resources-and-services/index.html
Unidad de Diagnóstico Genético Avanzado de Enfermedades del Metabolismo del Hierro (UDGAEMH)

Jefe: 
• Dra. Mayka Sánchez
Técnicos: 
• Dr. Erica Morán  
• Jessica Aranda

Servicios
- Hepcidina sérica/plasmática (ELISA)
- Diagnóstico Genético de enfermedades raras del metabolismo del hierro

http://www.imppc.org/resources-and-services/index.html

udgaemh@imppc.org

Acreditación de Calidad:
EMQN EQA en HH-HFE
Designada como unidad EXPERTA por ORPHANET
Acreditación asistencial Generalitat Catalunya
Hyperferritinemia project

HIGHFERRITIN Web Server

Algorithms and recommendations for diagnosis and management of hyperferritinemia

HIGHFERRITIN

The HIGHFERRITIN Web Server is a tool for helping the general practitioners (GP) or medical specialists in the diagnosis and management of patients with high levels of serum ferritin or hyperferritinemia. HIGHFERRITIN Web Server provides suggestions and recommendations about the appropriate medical tests and procedures to be done and the possible treatments to follow.

These recommendations have been agreed by a group of experts and they follow the recommendations of international guidelines in Hereditary Hemochromatosis and other diseases together with the personal experience of these experts.

To use this tool, please go to the diagnostic section or just click here.

http://highferritin.impppc.org
HIGHFERRITININ Web Server

Thanks to:
Albert Altés (Althaia Foundation)
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Rosario López (Althaia Foundation)
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Miquel Torres (Hospital de l'Esperit Sant)
Mayka Sánchez (IMPPC)
Jordi Félez-Brugués (CAP Canaletes)
David Benéitez (Hospital Universitari Vall d'Hebron)
María Fátima Matute (Hospital Clínico San Carlos)
Ángel F. Remacha (Hospital de la Santa Creu i Sant Pau)
Cristina Sanz (Hospital Clínica de Barcelona)

PUBLICATION:
A novel syndrome of congenital sideroblastic anemia, B cell immunodeficiency, periodic fevers and developmental delay (SIFD)
**Sideroblastic Anemia, Immunodeficiency, Fevers and Developmental Delay (SIFD)**

- **Anemia**
  - **Severe** (Hb 7.1g/dL; range 4.8-8.3). Markedly *microcytic* (MCV 62 fL; range 53.6-73.2)
  - Hypochromasia, Microcytosis, Schistocytosis, Basophilic stippling, Frequent nucleated erythrocytes
  - No hemoglobinopathies, iron deficiency, RBC mb defects, enzynopathies or porphyrias
  - BM examination reveals *ringed sideroblasts* >45-50% (1 not performed)
  - Erythroid *hyperplasia* and dyserythropoiesis, with deficient cytoplasmic hemoglobinization
  - Most children *hyperferritinemia* (due inflammation and secondary iron overload)

- **Recurrent high fever syndrome** requiring multiple hospitalizations (11), elevated inflammatory markers, Vomiting, Diarrhea.

- **Immunodeficiency**
  - Recurrent inflammatory episodes, B-cell lymphopenia and hypogammaglobulinemia (11),
  - No good response to intravenous replacement with immunoglobulins (Ig)

- **Developmental delay** variable but alarming:
  - Generalized and truncal hypotonia, often severe, progressive and associated with gross motor developmental delay
  - Comprehension and communication were impaired in many

- Other symptoms were present in some of the patients: *sensorineural hearing impairment* (5), Recurrent seizures (5), Neurological/neuromuscular abnormalities, Nephrocalcinosis/renal tubular dysfunction (3), *Aminoaciduria* (6), *Cardiomyopathy* (2) and *cardiac failure* (contribution to death in 5 cases), *Pigmentary retinitis* (4), hepatosplenomegaly (4), brittle hair (3), chronic ichthyotic skin changes (1)
Clinical presentation in SIFD

• 12 cases from 10 families (Europe and North America, multiple ethnicities)

• Healthy parents (Consanguineous parentage in 3 families)

• (8) in the neonatal period or within 3 months of live, (3) before 7 months and (1) at 18 months

• Initial presentation involved:
  - Sideroblastic, severe, and markedly microcytic anemia
  - B-lymphopenia and/or panhypogammaglobulinemia
  - Febrile illness with inflammatory markers
  - Poor feeding,
  - Gastrointestinal upset
  - (2) severe pallor at birth (before illness)
  - (1) clinical vomiting with metabolic acidosis, without prominent fevers
### Clinical interventions and outcome

At the time of publication 7/12 children died (range 16m-14y)

<table>
<thead>
<tr>
<th>Case #</th>
<th>INTERVENTIONS</th>
<th>OUTCOME</th>
<th>Terminal Event/Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regular Transfusions</td>
<td>IVlg Replacement</td>
<td>BONE MARROW TRANSPLANT</td>
</tr>
<tr>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>No (Awaited)</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Yes*</td>
<td>Yes*</td>
<td>Yes (aged 9 months)</td>
</tr>
<tr>
<td>12</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*IMPPC Institute of Predictive and Personalized Medicine of Cancer*
Patient’s recruitment (2010-2013)

- **13 Hyperferritinemias with cataracts** => Seq. IRE FTL: 7 known Mutations, 2 new.
  - **2 NEW MUTATIONS** Publication in *OJRD* Luscieti et al., 2013. IF: 5.074.

- **3 Hyperferritinemias without cataracts** => Seq. FTL / FTH, No mutations.
  - **30 Hemochromatosis** => Seq. genes HFE, TFR2, HAMP, HJV, SLC40A1.
    - 1 case C282Y HFE.
    - 4 cases TFR2, molecular studies ongoing, **3 NEW MUTATIONS**. Publication in preparation
    - 3 cases SLC40A1 (FPN), **2 NEW MUTATIONS**

- **23 non-sideroblastic Anemias** => Seq. genes TF, CP, TMPRSS6, DMT1.
  - 4 families TF (5 **NEW MUTATIONS**). Publication in *BJH* Athiyarath R et al., 2013 IF: 4.941 + Publication in preparation
  - 5 families IRIDA 5 **NEW MUTATIONS**. Publication in preparation
  - 4 families CP (3 **NEW MUTATIONS**, del 4 ntd, del 5 ntas, splicing in10)
  - 1 new entity case NGS5 ongoing.

- **17 CDAI/II** => Seq. genes SEC23B, CDAN1, KLF1
  - SEC23B: 4 **NEW MUTATIONS** (2 missense, 1 splicing, 1 insertion), 2 known mutations
  - CDAN1: 1 **NEW** missense MUTATION

- **16 Congenital Sideroblastic Anemias (CSA)** => Seq. genes ALAS2, SLC25A38, ABCB7, GLRX5, STEAP3.
  - 2 ALAS2 (known mutation R452C, P520L).

- **4 Myelodysplastic Syndrome (MDS-RARS)** => Seq. gene SF3B1.
  - 4 SF3B1 (known mutation K700E)

Total Cases 106, 125 affected patients, 200 studied persons (affected and non-affected)
Treatments for Aceruloplasminemia

• Treatment with iron chelating agents (i.e., desferrioxamine, deferasirox) can be considered for symptomatic aceruloplasminemic individuals. Iron chelating treatments can decrease serum ferritin concentration as well as brain and liver iron stores, and can prevent progression of the neurologic signs/symptoms (Miyajima et al. Ann Neurol. 1997; Skidmore et al. J Neurol Neurosurg Psychiatry. 2008).

• Repetitive intravenous administration of fresh-frozen human plasma containing ceruloplasmin has been also reported to improve neurologic signs/symptoms (Yonekawa et al, Eur Neurol. 1999).

• Vitamin E (antioxidant) may be used along with a chelator or oral administration of zinc to prevent tissue damage, particularly to the liver and pancreas (Kuhn et al. Brain Dev. 2007).

• No benefit from phlebotomy (DiRaimond D, et al. Intern Emerg Med. 2008)