HIGHLIGHTS IN EMATOLOGIA

“Parliamo di ferro”

Treviso 17 Novembre 2017

Emocromatosi ereditaria

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UNIMORE

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Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell’Adulto

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA

Azienda Ospedaliero - Universitaria di Modena

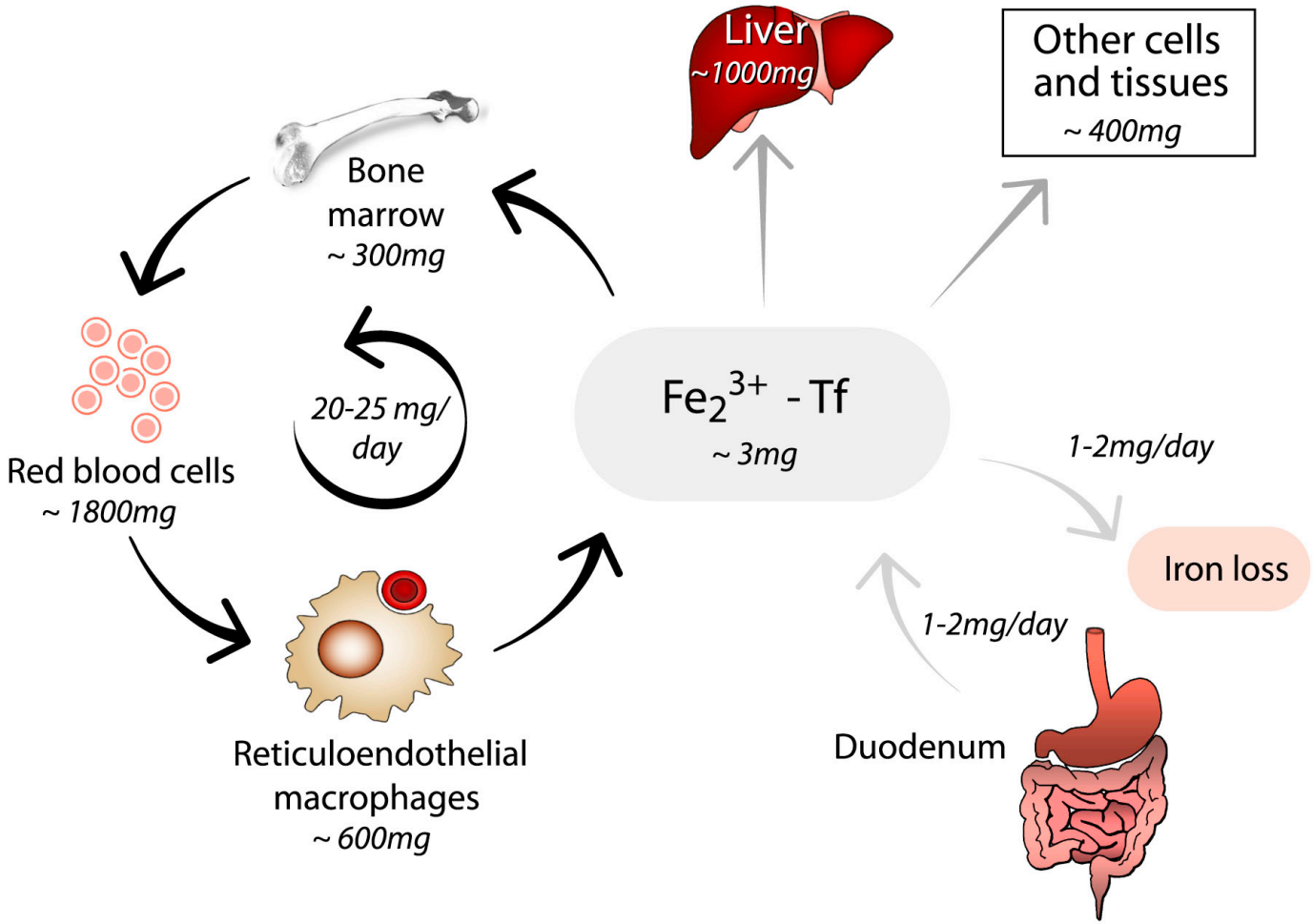
Ai sensi dell'art. 3.3 del Regolamento applicativo dell'Accordo Stato-Regioni 05.11.2009,

dichiaro che negli ultimi due anni non ho avuto rapporti, anche di finanziamento, con soggetti portatori di interessi commerciali in campo sanitario

Slides content

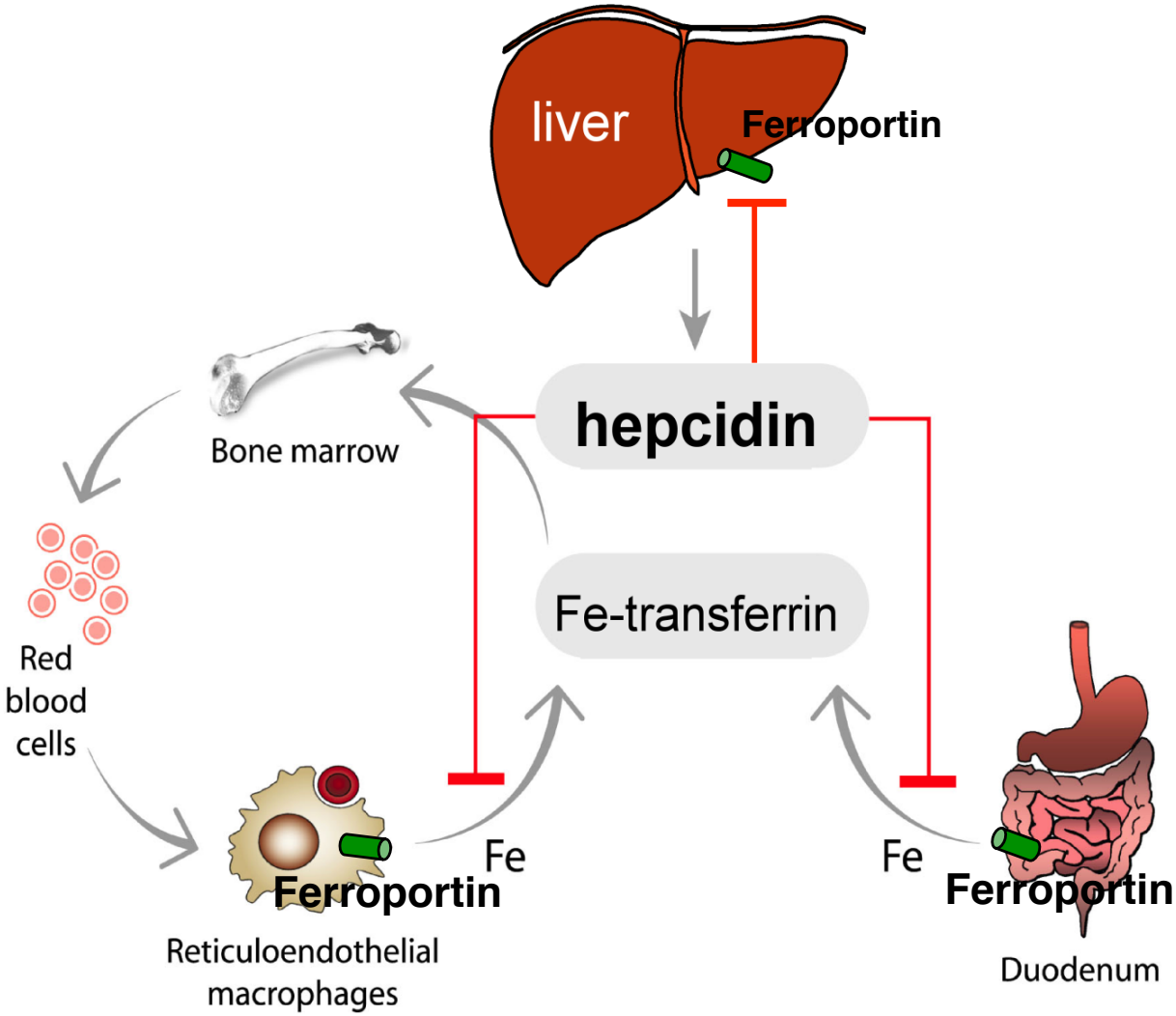
- Systemic iron homeostasis
- Hereditary hemochromatosis disease
- Therapeutic strategies

Systemic Iron Homeostasis



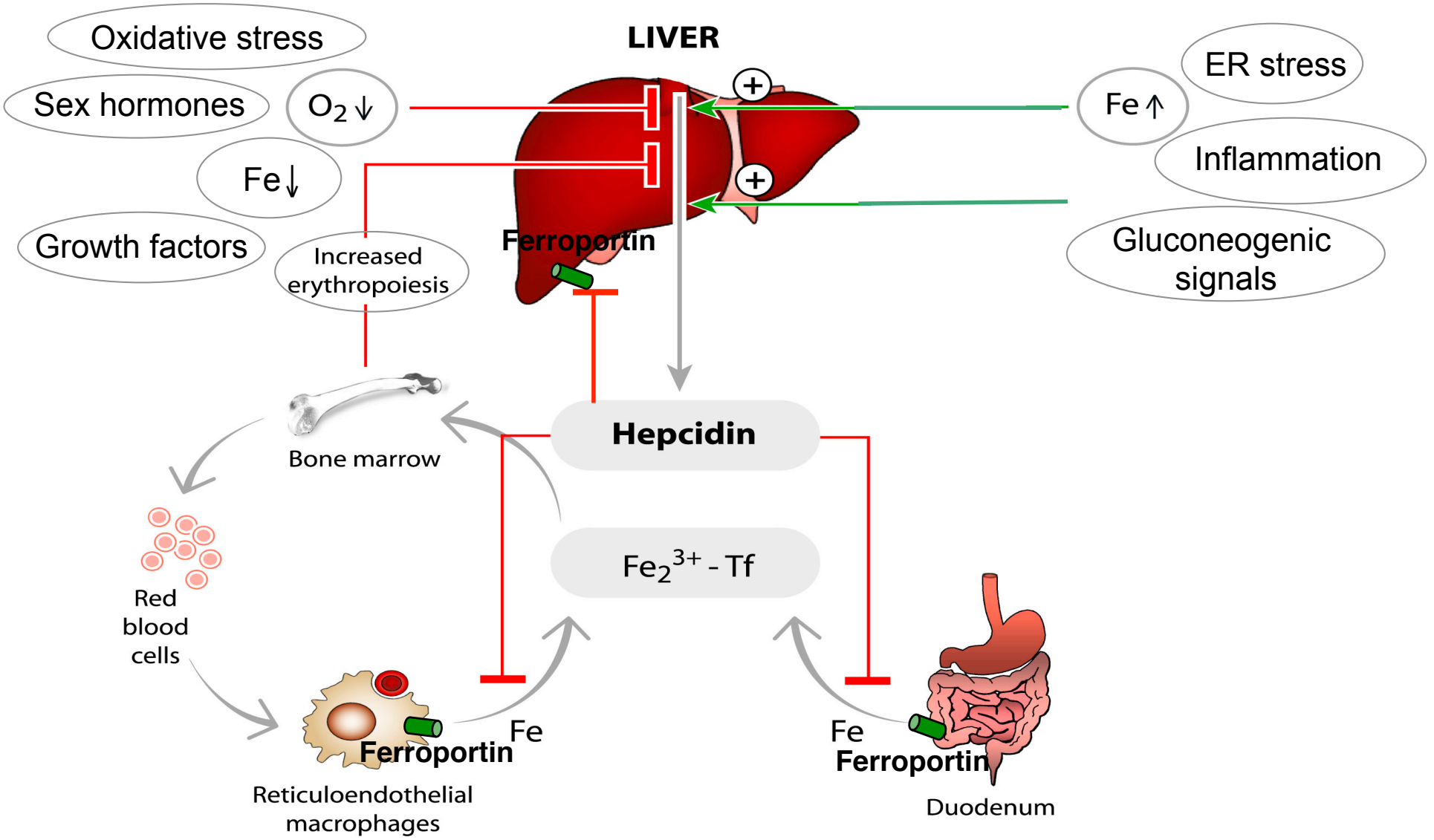
adapted from Hentze et al. Cell 2004

Hepcidin regulates body iron levels



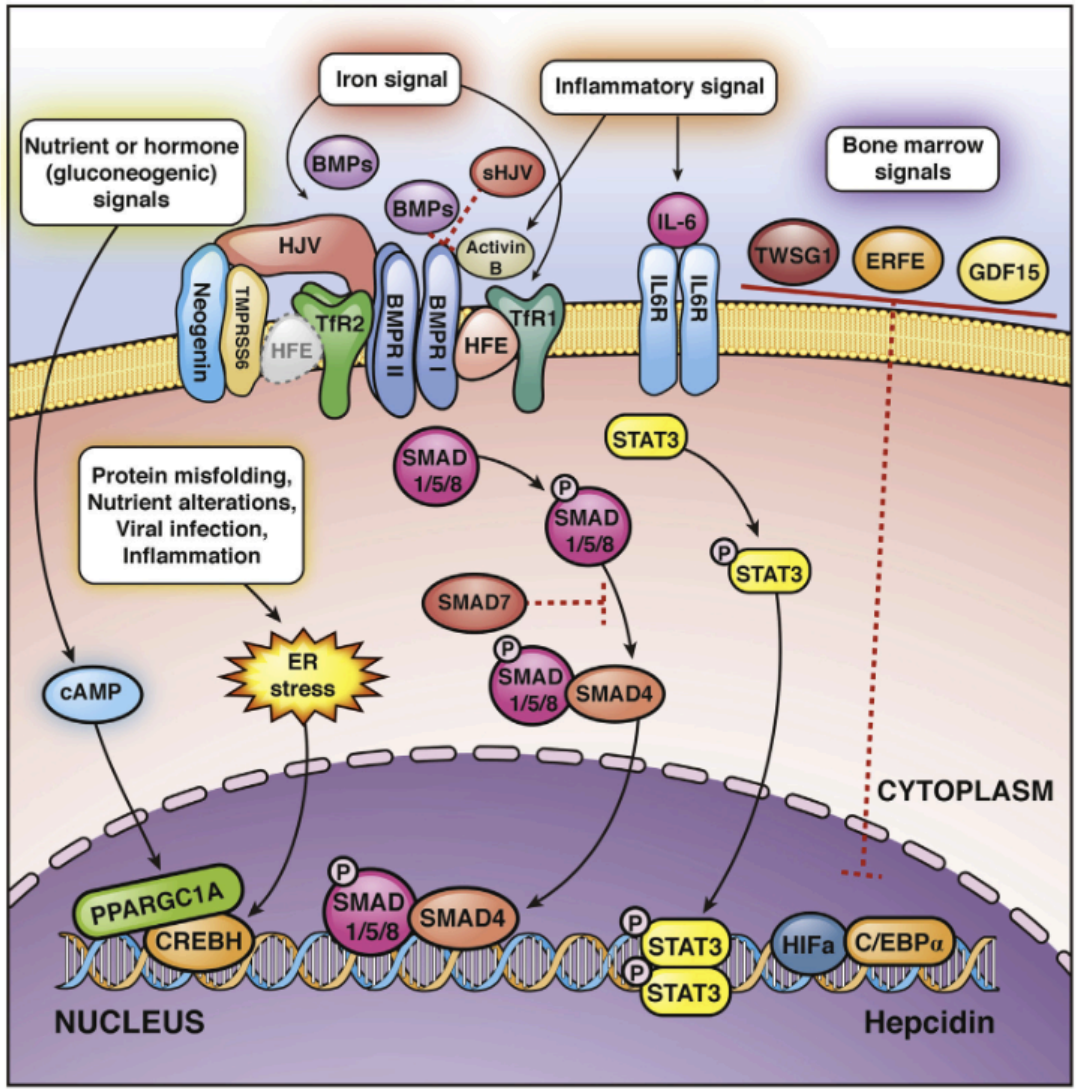
Krause et al. FEBS Lett 2000, Park et al. J Biol Chem 2001, Pigeon et al. J Biol Chem 2001, Fung et al. Hematologica 2013, Xiao et al. AAPS J 2010

A number of stimuli modulate hepcidin expression to influence systemic iron balance



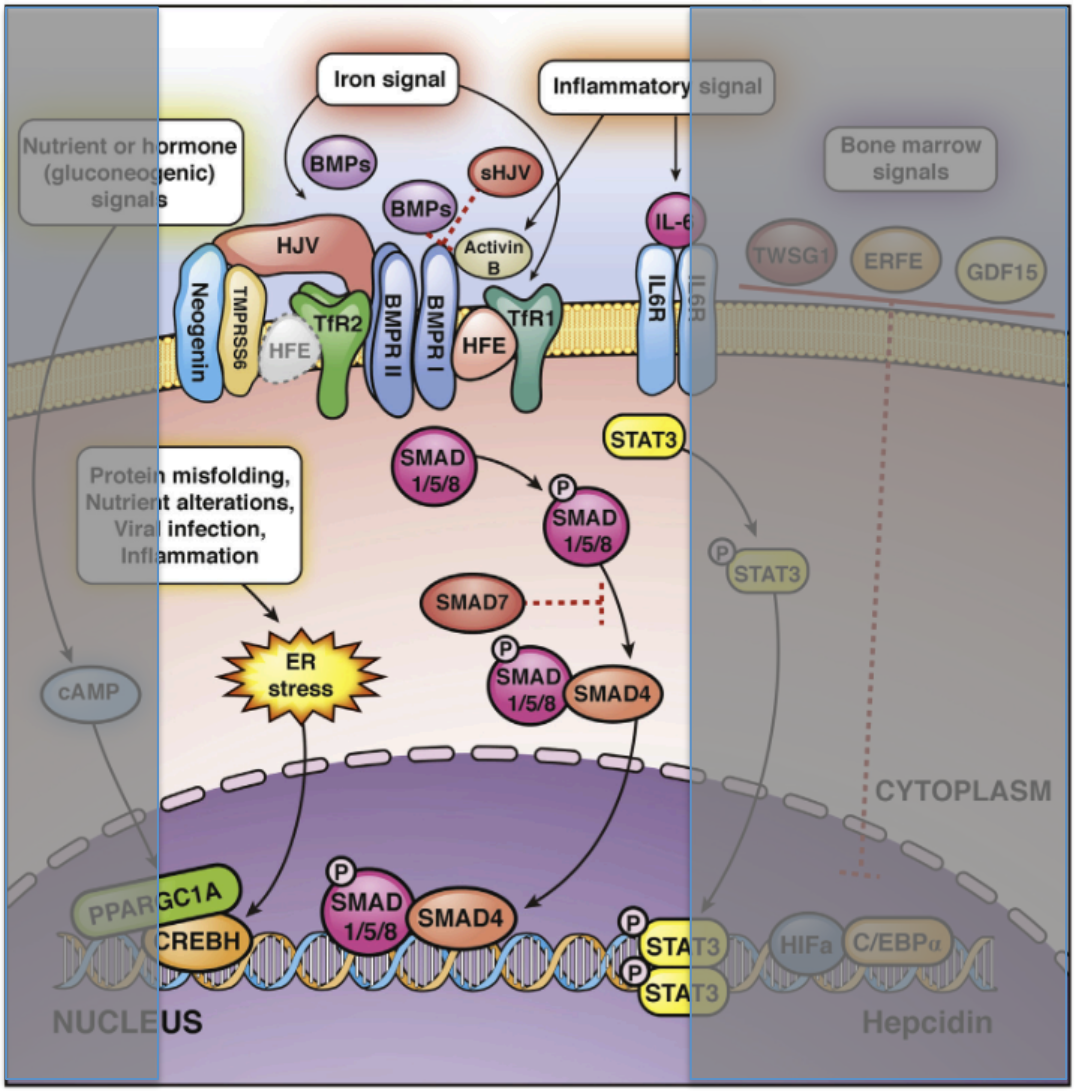
Reviewed in Sangkhae et al. Adv Nutr 2017

Signaling pathways in hepcidin transcription



Reviewed in Pietrangelo. Gastroenterology 2015

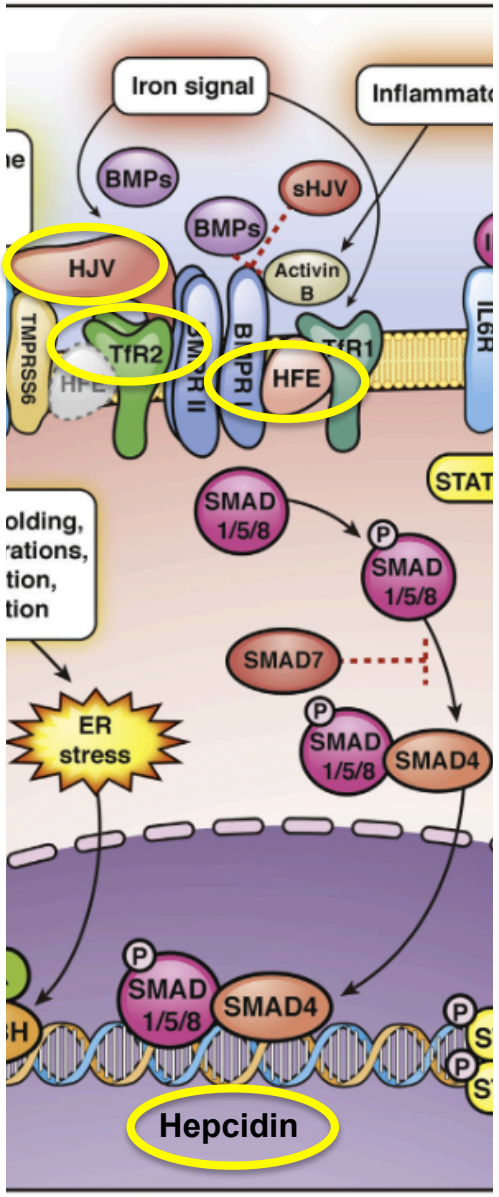
Signaling pathways in hepcidin transcription



Babitt et al. 2001. JBC 2005; Babitt et al. JCI 2007, and Nat Genet 2006
Reviewed in: Core et al. Front Pharmacol 2014; Pietrangelo. Gastroenterology 2015

Hereditary Hemochromatosis

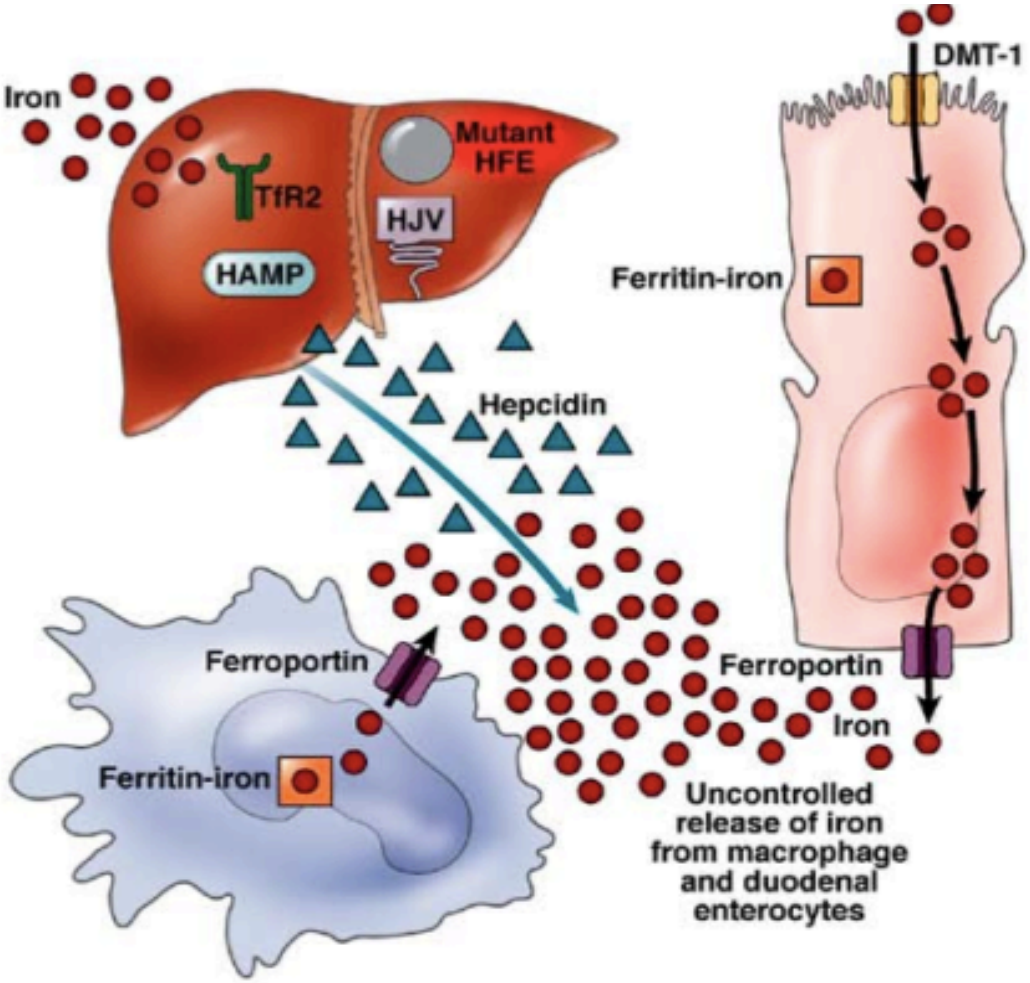
- Caused by mutations that:
  - affect proteins involved in the production of hepcidin in response to iron:
    - **HFE**
    - **Transferrin-Receptor 2 (TfR2)**
    - **Hemojuvelin (HJV)**
  - affect **hepcidin (HAMP)** *per se*
  - make its receptor **Ferroportin** resistant to hepcidin



Reviewed in Pietrangelo. Gastroenterology 2010 and 2015

Hepcidin deficiency:
a unifying pathogenetic mechanisms for HH

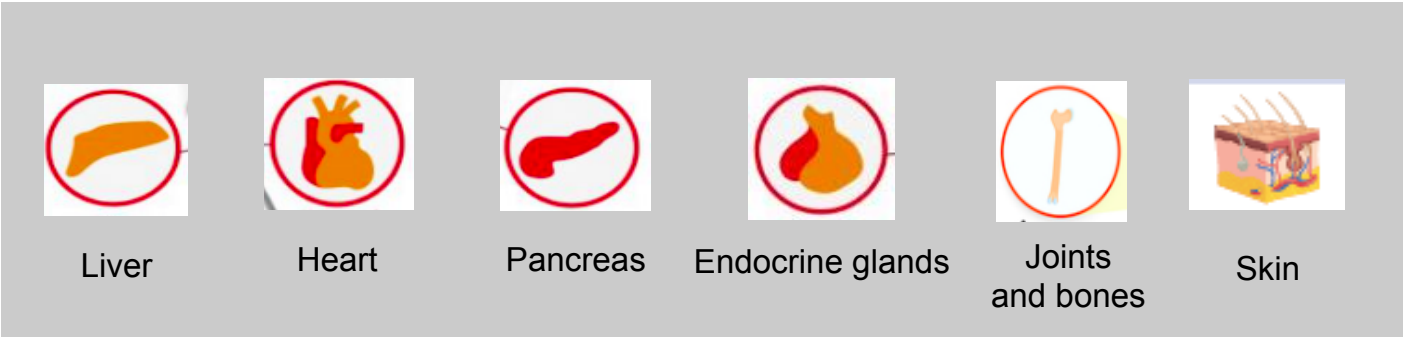
- **Endocrine disease due to the genetic loss of hepcidin (synthesis or function)**



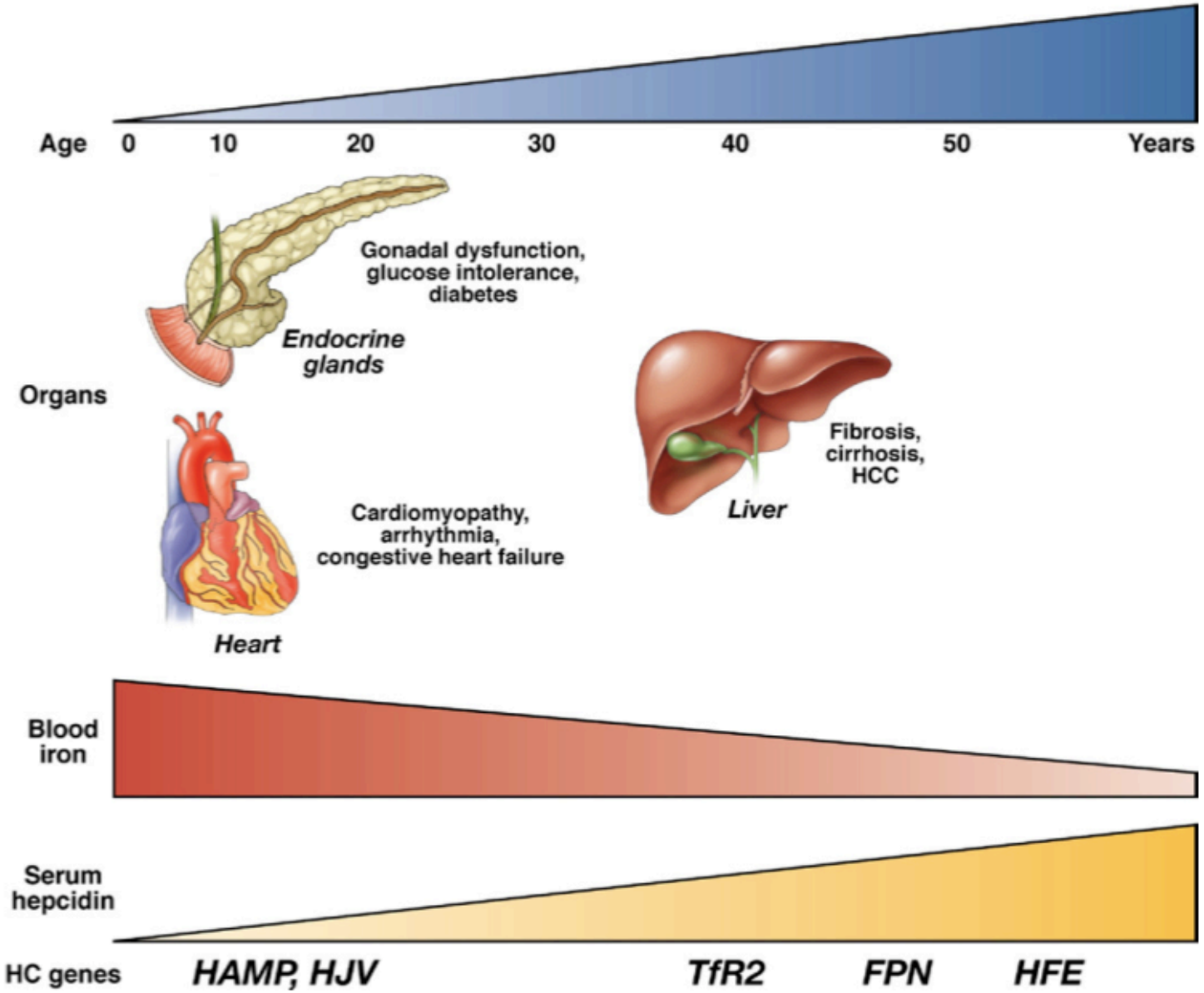
Reviewed in Pietrangelo. Gastroenterology 2010 and 2015

Hemochromatosis

- Unchecked transfer of **iron into the bloodstream**
- ↑ transferrin and non-transferrin bound iron
- **Iron loading and iron toxicity** of tissues and organs
- Target organs: **liver, hearth, endocrine glands, joints**



5 different genes for a similar clinical syndrome



From Pietrangelo. Gastrenterology 2010

HFE-hemochromatosis

- **The most common inherited disease in Caucasians**
- **AR**
- Highly prevalent: ~**1/250**
- **80% cases**: substitution of tyrosin for cystein at position 282 (**C282Y**)
- **Founder effect**: Celtic or Viking ancestor 2000 years ago
- C282Y allelic frequency ~6% (12,5% Ireland-0% Southern Europe)

Reviewed in: EASL CPG on HFE Hemochromatosis. J Hepatol. 2010; Pietrangelo. Gastroenterology 2010 and 2015; Zoller et al. Dig Dis 2016, Wallace et al. Genet Med 2016

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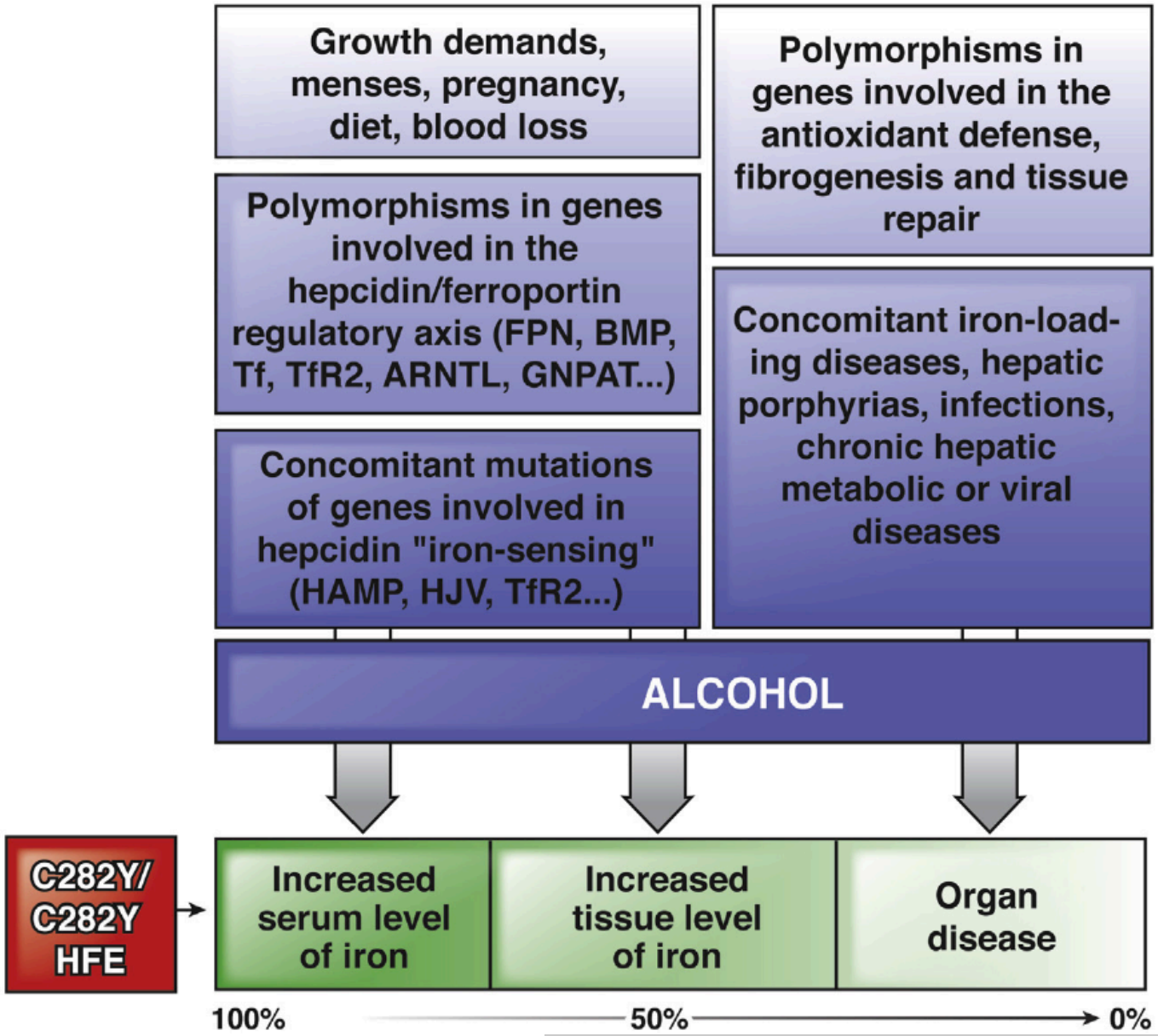
- H63D: allelic frequency ~14%
- **C282Y/H63D** in 5,3% of HH patients (cofactors)
- **C282Y/H63D or H63D/H63D** may present with abnormal iron parameters or mild liver iron deposits (cofactors)

Reviewed in: EASL CPG on HFE Hemochromatosis. J Hepatol. 2010; Pietrangelo. Gastroenterology 2010 and 2015; Zoller et al. Dig Dis 2016, Wallace et al. Genet Med 2016

Incomplete and low disease penetrance

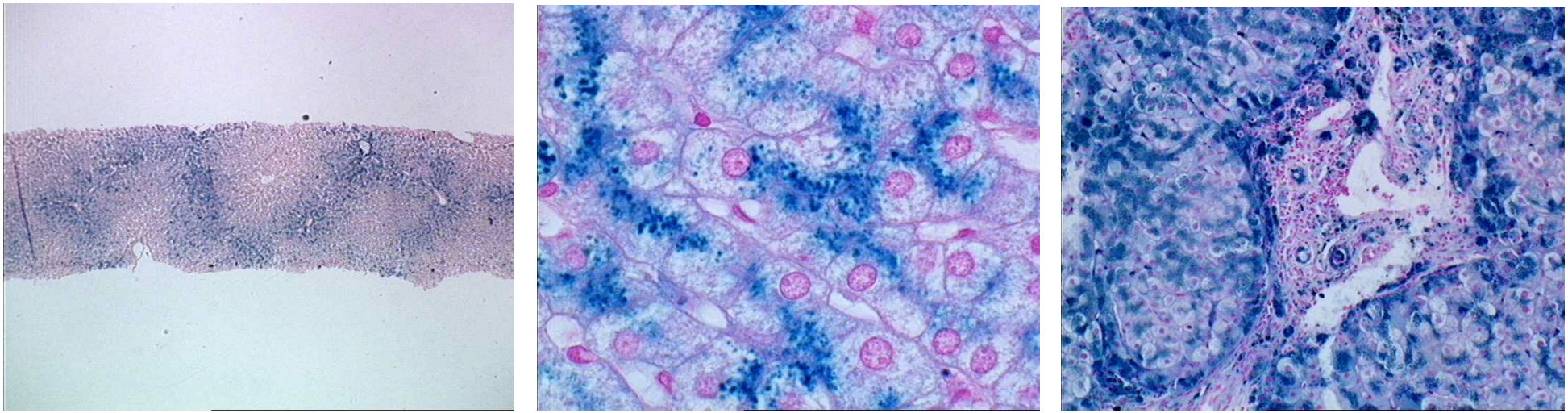


Reviewed in Pietrangelo. Gastroenterology 2015

Liver in HFE-Hemochromatosis

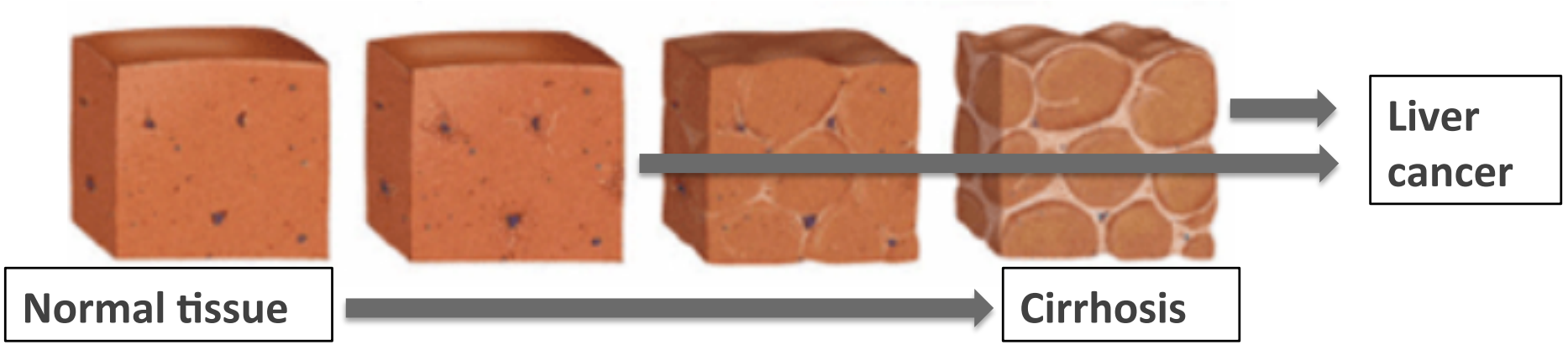
Typical **parenchymal iron overload:**

- **decreasing gradient from periportal to centrolobular areas**
- **biliary pole location** of iron within hepatocytes
- with the time, sideronecrosis leads to **distribution of iron towards KCs**



Perls' stain, from SLD 2011 Deugnier and Turlin

Liver disease progression



Progression to Fibrosis and Cirrhosis:

- ***LIC*** > 60 umol/g: stellate cell activation
- ***LIC*** > 250-300 umol/g: organelle damage/cell death/fibrosis>cirrhosis
- ***LIC*** > 350 umol/g: extrahepatic/cardiac deposition
- ***Duration*** of iron exposure is crucial

Basset et al. Hepatol 1986; Ramm et al. Hepatol 1997, Iancu et al. J Hepatol 1997; Adams et al. Am J Gastroenterol2001; Powell LW et al. Arch Intern Med 2006; Olynyk et al. Am J Gastro 2005; Reviewed in Deugnier et al. SLD 2011

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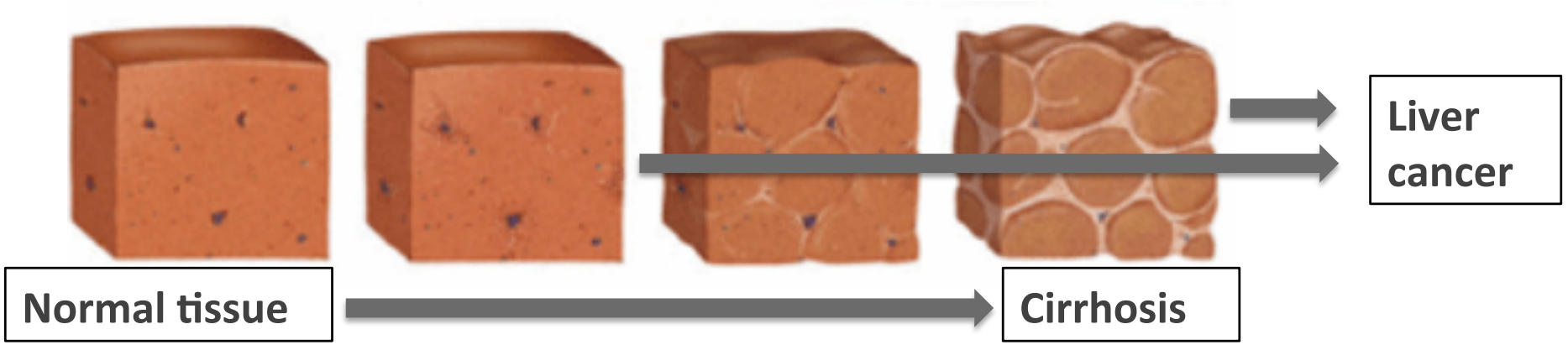


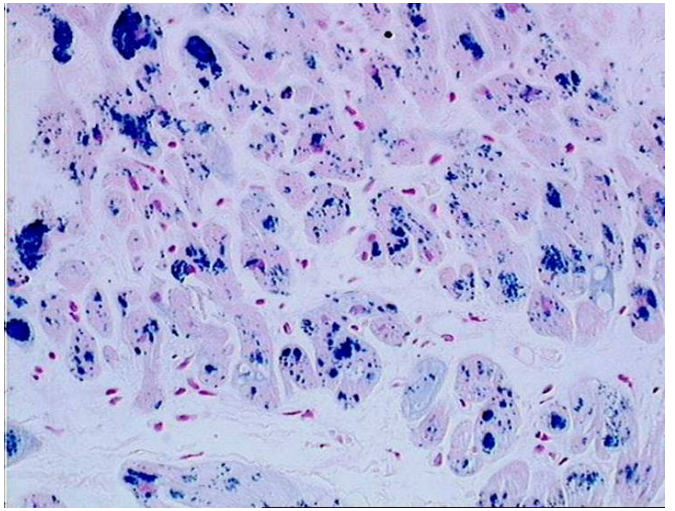
Liver cancer:

- RR for liver cancer ~100
- **HCC** (2/3) and **colangiocarcinoma** (1/3)
- male, > 50y, co/carcinogenic factors
- may develop in non cirrhotic liver and in treated patients

Reviewed in Deugnier et al. SLD 2011

Iron mediated cardiomyopathy

Perls' stain,
SLD 2011 Deugnier and Turlin



- **Iron deposition** and **fibrosis**
- Iron heterogenously distributed
- Not linear relationship to LIC or ferritin

- **Contractile disfunction**:
  - ✓ early abnormal diastolic function >> restrictive cardiomyopathy
  - ✓ impaired systolic LV function >> dilated cardiomyopathy
  - ✓ hearth failure
- **Electrical disturbances**:
  - ✓ slow heart rate/bradyarrhythmias, heart block, AF

Reviewed in Allen at al. NEJM 2008; Murphy et al. J Card Fail 2010; Zhabyeyev et al. Can J Cardiol 2017;

Endocrinopathy

- **Pituitary gland**: **hypogonadotropic hypogonadism**, hypothyroidism, adrencortical insufficiency

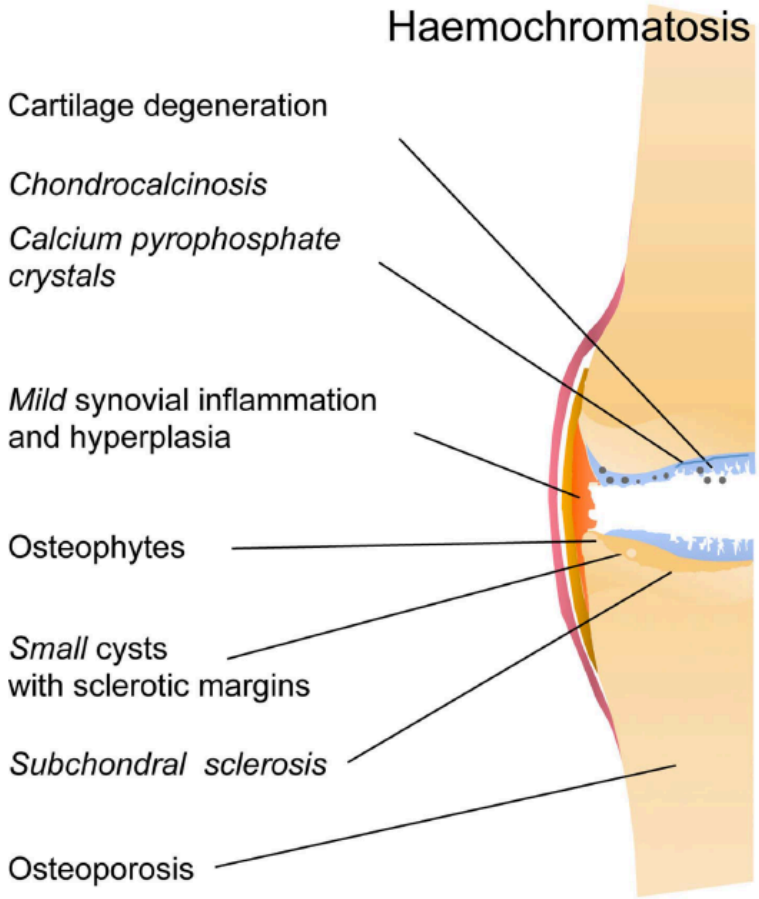
- **Pancreas**: **DM** *(Type1>Type2)*
  - ✓ multifactorial pathogenesis (beta cells oxidant stress, loss of insulin secretion, superimposed insulin resistance..)
  - ✓ <u>significant decrease due to early diagnosis</u>

- Gonads, thyroid, parathyroids

Reviewed in EASL CPG on HFE Hemochromatosis. J Hepatol. 2010; Pietrangelo. Gastroenterology 2015; Wallace et al. Genet Med 2016

Joints and bones

- 2/3 HH patients **joint symptoms**
- 1/3 HH revealed by articolar pain
- II and III MCP, hips, knees, ankles,...
- **Condrocalcinosis,** osteoarthritis
- Osteoporosis
  - even in absence of hypogonadism, liver disease, alcohol
- High number of prosthetic replacement joints



van Vulpen et al. J Clin Pathol 2015

Reviewed in Jeney V.Front Pharmacol 2017; van Vulpen et al. J Clin Pathol 2015

Non specific symptoms

- Unrelated to ferritin levels:
  - ✓ Fatigue, weakness, lethargy, apathy, weight loss
  - ✓ Progressive skin hyperpigmentation

Non-HFE Hemochromatosis

- After identification of HFE in 1996: **not all patients with an HH hemochromatosis-phenotype carried pathogenic mutations in the HFE gene:**
  - ✓ C282Y >90% in the UK and Brittany
  - ✓ C282Y 64% in Italy and 30% and Greece

Reviewed in: Pietrangelo et al. SLD 2011; Piperno. Expert Opin Med Diagn 2013; Bardou-Jacquet. Clin Res hepatol Gastroenterol 2014; Pietrangelo. Gastroenterology 2015; Zoller et al. Dig Dis 2016; Wallace et al. Genet Med 2016

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- New iron genes and related diseases have been recognized

  ➔**"Non-HFE hemochromatosis":**

  – rare or very rare

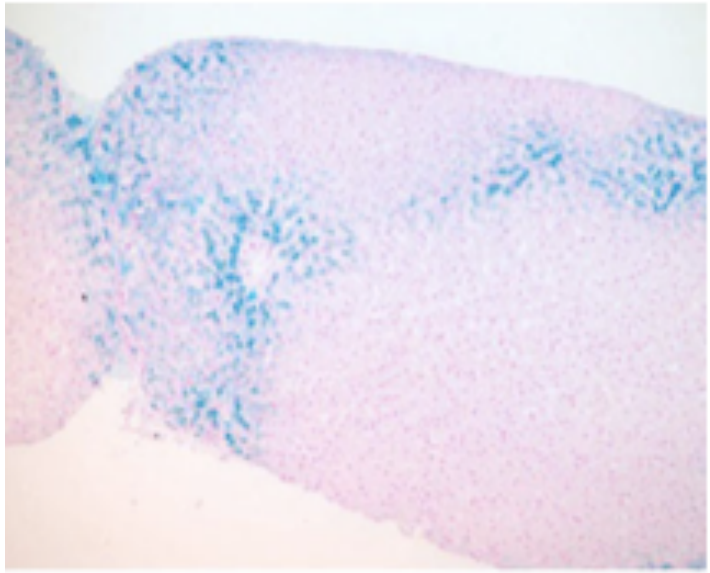
  – not restricted to northern European descent

  – often private mutations

Reviewed in: Pietrangelo et al. SLD 2011; Piperno. Expert Opin Med Diagn 2013; Bardou-Jacquet. Clin Res hepatol Gastroenterol 2014; Pietrangelo. Gastroenterology 2015; Zoller et al. Dig Dis 2016; Wallace et al. Genet Med 2016

Non-HFE Hemochromatosis

- **TfR2-hemochromatosis**
  - AR
  - **similar phenotype to the HFE-form**
  - **earlier age** and/or with **more severe phenotype**



Camaschella et al. Nat Gen 2000; Roetto et al. Nat Gen 2003; Papanikolau et al. Nat Gen 2004
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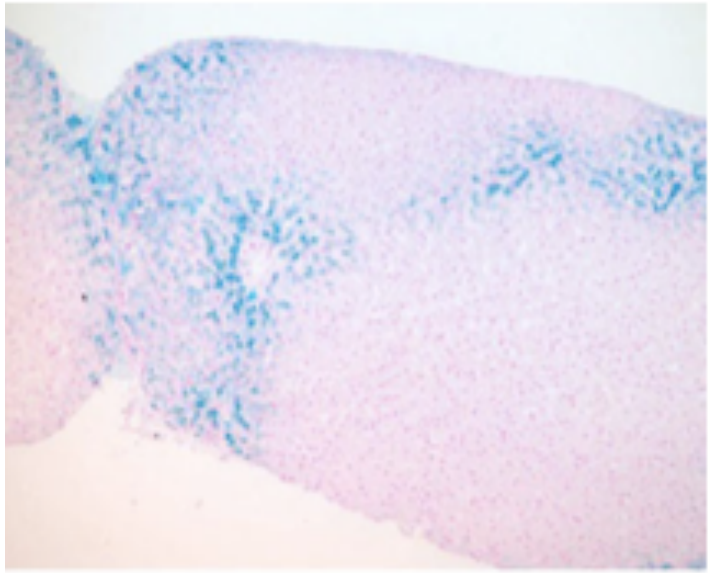
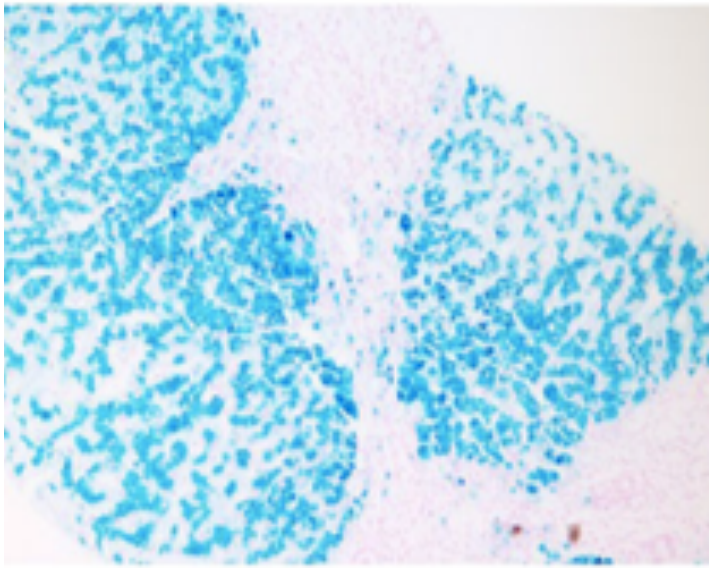


- **Juvenile-hemochormatosis (HAMP and HJV-related)**
  - AR
  - **early onset** (II-III decade)
  - **more severe** iron loading
  - **cardiac and endocrine system** involvement dominate the picture



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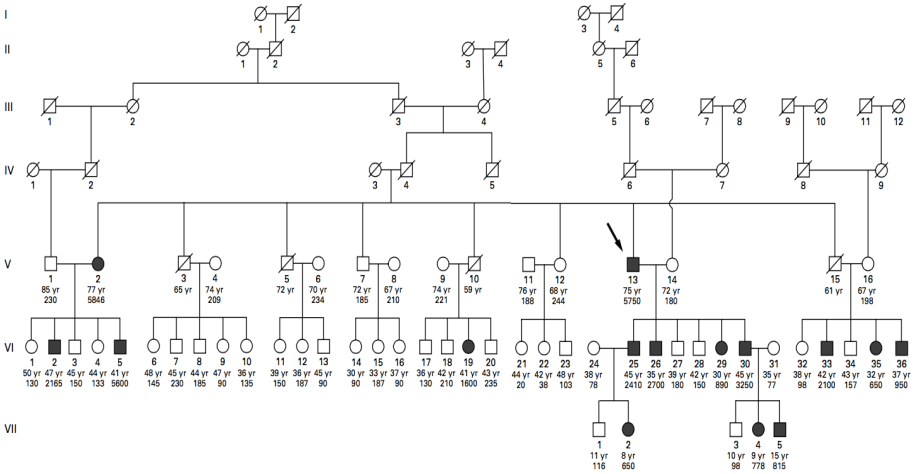
Ferroportin-related iron overload syndromes

In 1999 and in 2001

- **two different iron-overload syndromes**
- associated to mutations of **Ferroportin** gene
- **autosomal dominant** inheritance



Pietrangelo et a. NEJM 1999; Montosi et al. JCI 2001

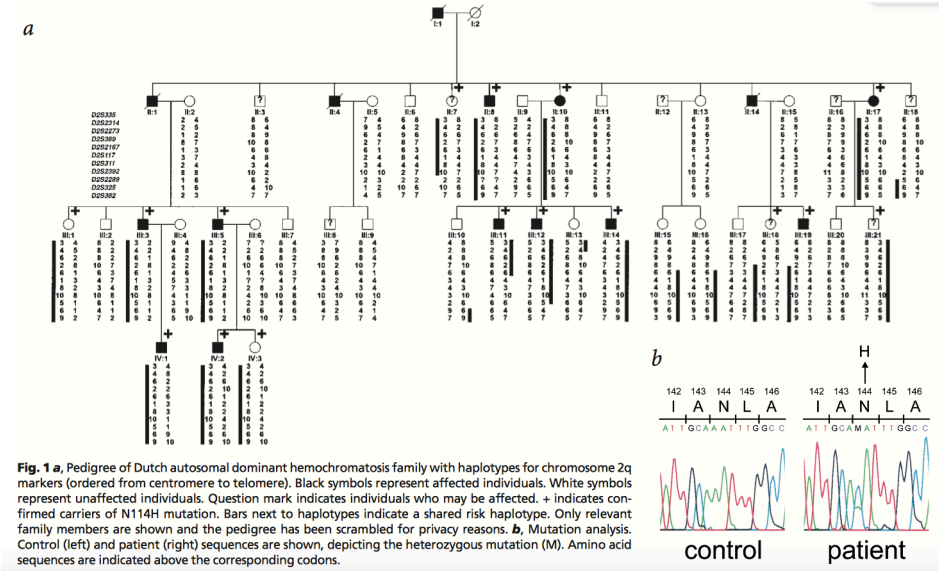


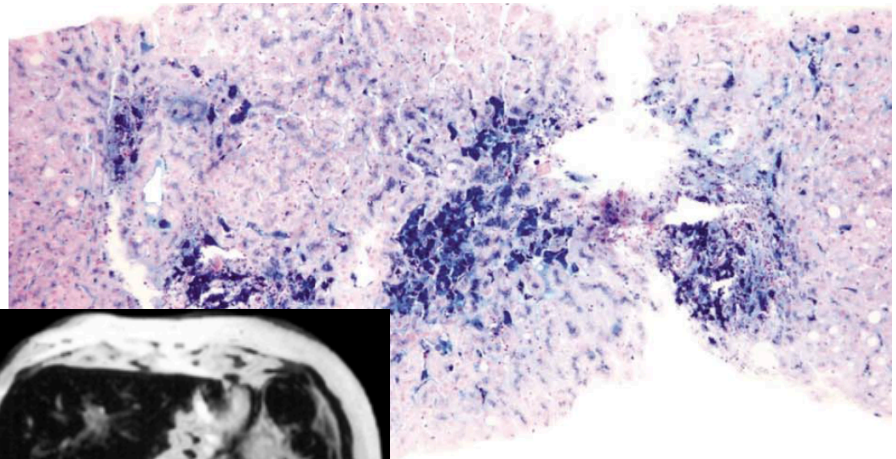
Fig. 1 ***a***, Pedigree of Dutch autosomal dominant hemochromatosis family with haplotypes for chromosome 2q markers (ordered from centromere to telomere). Black symbols represent affected individuals. White symbols represent unaffected individuals. Question mark indicates individuals who may be affected. + indicates confirmed carriers of N114H mutation. Bars next to haplotypes indicate a shared risk haplotype. Only relevant family members are shown and the pedigree has been scrambled for privacy reasons. ***b***, Mutation analysis. Control (left) and patient (right) sequences are shown, depicting the heterozygous mutation (M). Amino acid sequences are indicated above the corresponding codons.

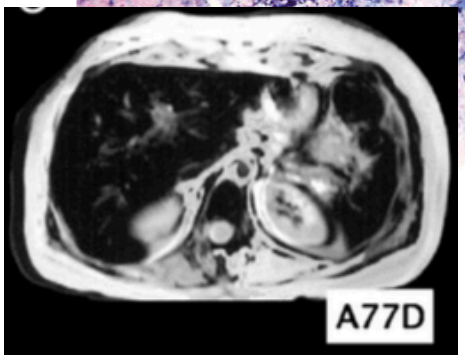
Njajou et al, Nat Gen 2001.

Ferroportin Disease *versus* FPN-hemochromatosis

Loss of Function mutations:
reduced ferroportin activity mainly in tissue macrophages

- Elevated ferritin with normal/low serum iron
- Kupffer cell iron loading pattern
- Spleen and BM iron loading
- Marginal iron restricted erythropoiesis
- Clinically mild phenotype





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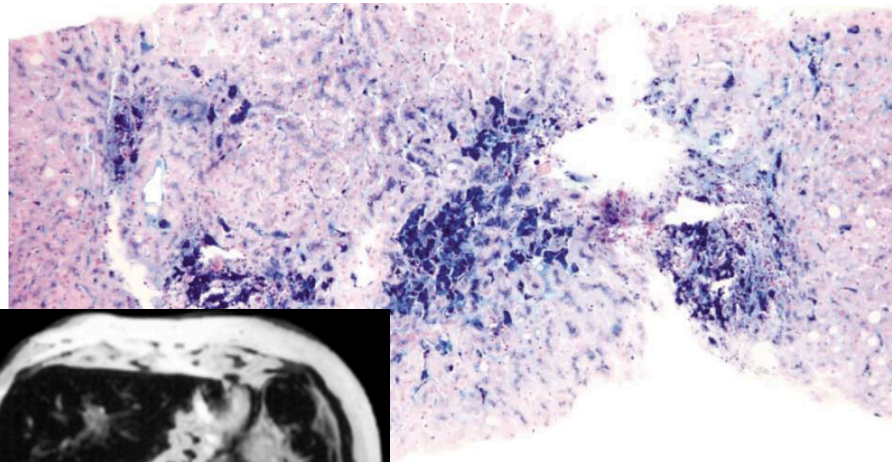
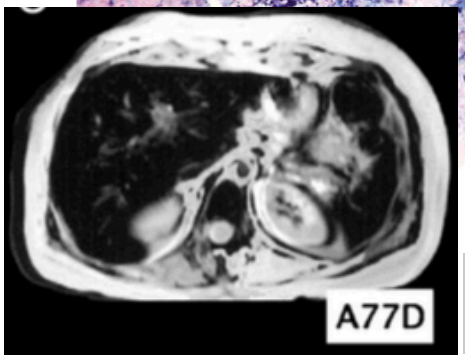
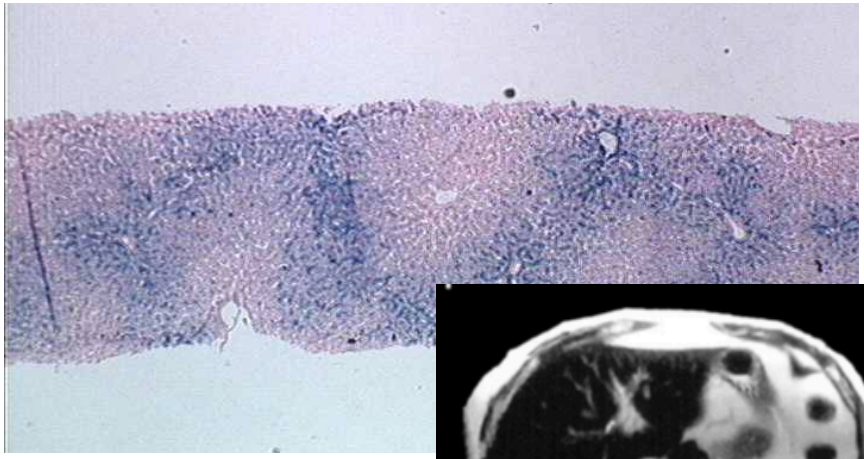
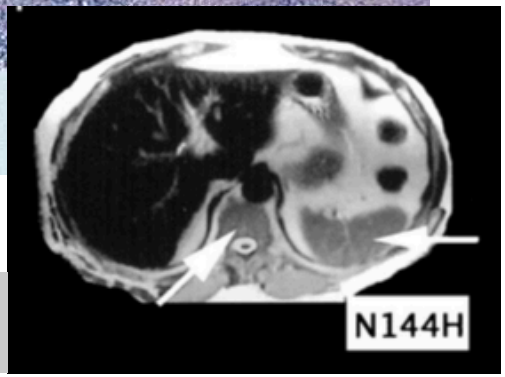




Gain of Function mutations:
affects hepcidin binding site, reducing sensitivity to hepcidin

- Elevated serum ferritin and serum iron
- Hepatocellular iron loading pattern
- "White" spleen and BM
- Classic HH phenotype: liver fibrosis, DM, cardiomyopathy, arthralgia, skin hyperpigmentation



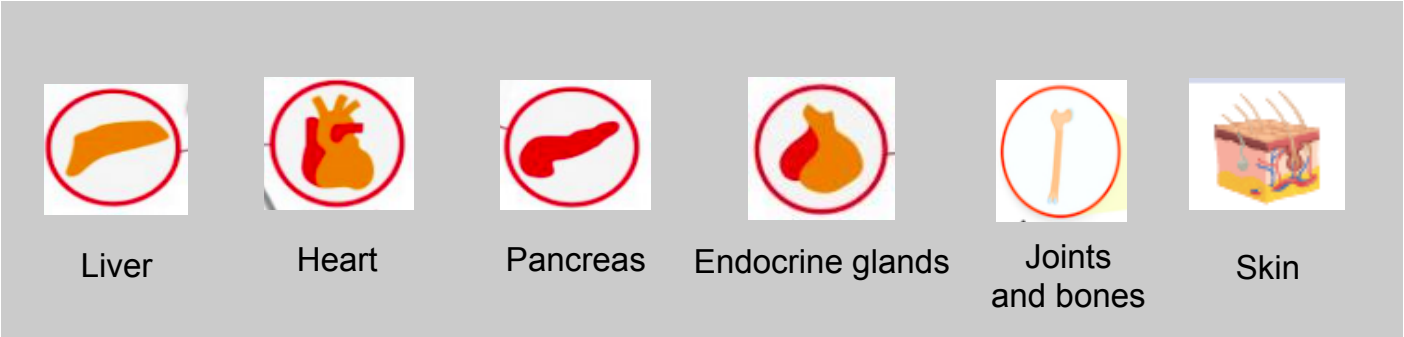


Reviewed in
Pietrangelo et al. SLD 2011 and Pietrangelo. Haematologica 2017

When to suspect hemochromatosis (HFE-, TfR2-, FPN-, HJV-, HAMP-related)?

- In presence of **hyperferritinemia** with concomitant **increase of Transferrin saturation** (>45%)

- **+/- clinical signs and symptoms**



Clinical Practice Guidelines

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

EASL clinical practice guidelines for HFE hemochromatosis

European Association for the Study of the Liver*

- Patients with suspected iron overload should first undergo measurements of **fasting transferrin saturation and serum ferritin** *(1B)*

- **HFE testing should be performed only in those with increased transferrin saturation** *(1A)*

EASL CPG on HFE Hemochromatosis. J Hepatol. 2010.

Clinical Practice Guidelines

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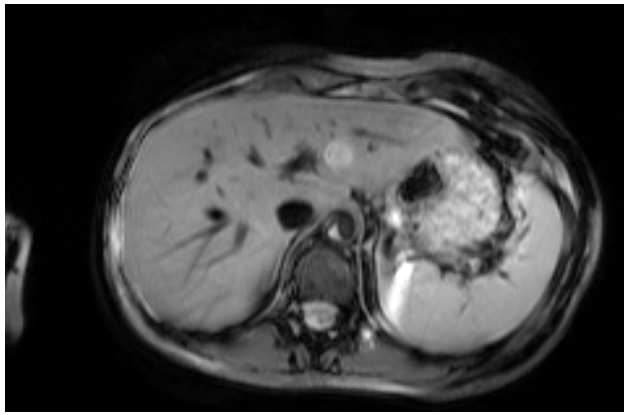
- **Diagnosis of HFE-HH** should not be based on **C282Y** homozygosity alone, but **requires** **evidence of increased iron stores** *(1B)*:

  - ✓ **Serum ferritin**
  - ✓ **MRI**
  - ✓ **Liver biopsy**
  - ✓ *(SQUID not widely available, not specifically validated)*
  - ✓ *(Iron removed)*

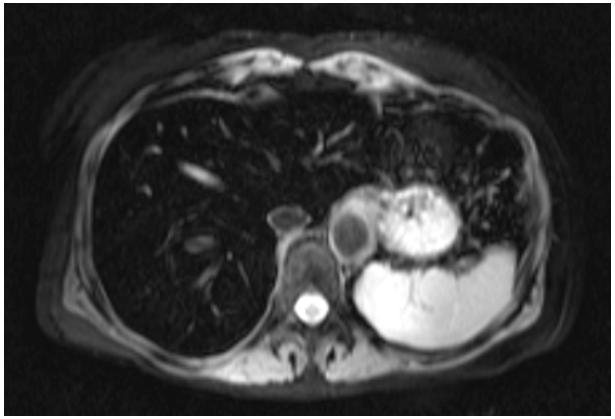
EASL CPG on HFE Hemochromatosis. J Hepatol. 2010;

MRI detection and quantification of liver iron

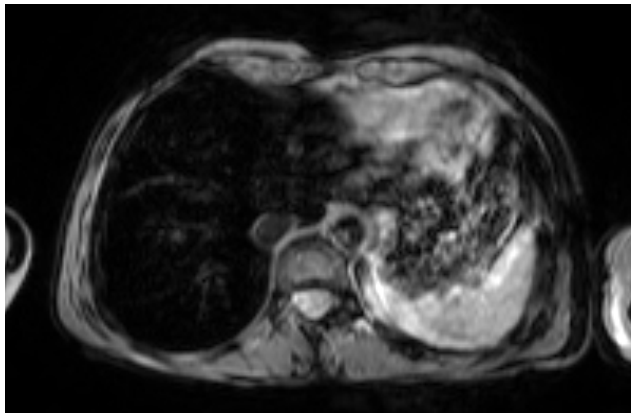
Pietrangelo et al. BCMD 2006 and personal data and



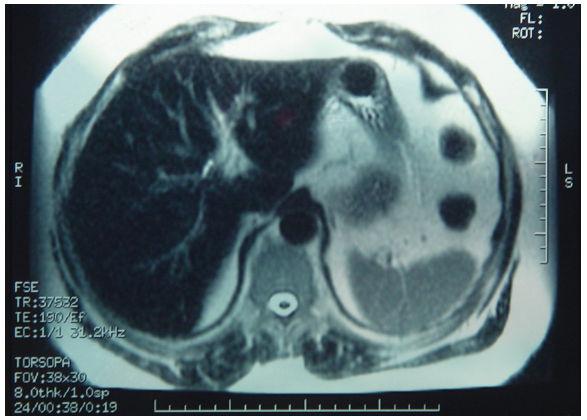
Control patient



HFE-hemochromatosis



HJV-hemochromatosis

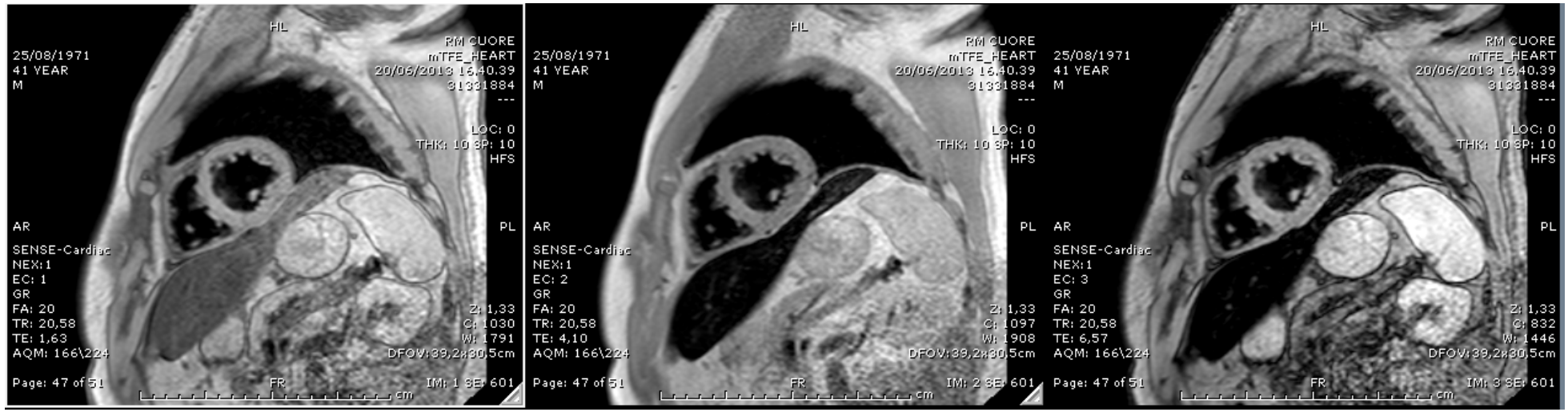


FPN-hemochromatosis

- detection of hepatic iron excess (50-350 umol/g)
- 84-91% Se and 80-100% Sp, according to LIC cut-off 37 to 60 umol/g

Non-invasive assessment of hepatic iron stores by MRI. Gandon Y. et al. Lancet. 2004

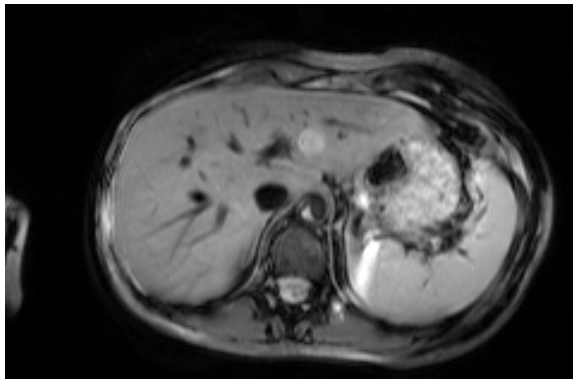
MRI detection and quantification of heart iron



Multi-center validation of the transferability of the magnetic resonance T2* technique for the quantification of tissue iron. Tanner MA, He T, Westwood MA, Firmin DN, Pennell DJ; Thalassemia International Federation Heart T2* Investigators. Haematologica. 2006

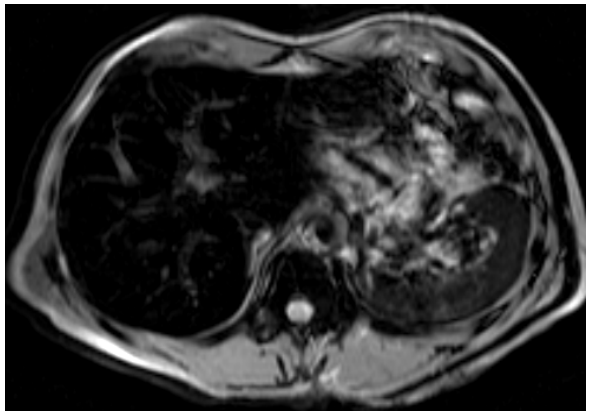
MRI detection and distribution of iron

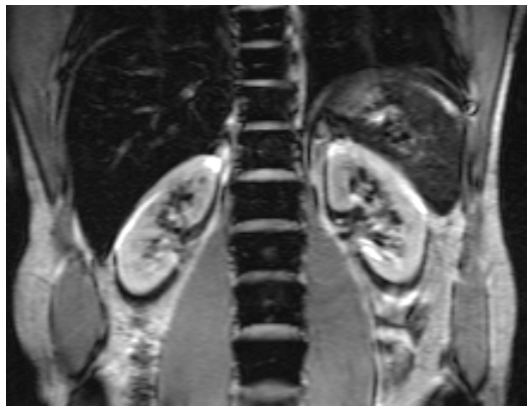
Control patient



Pietrangelo et al. BCMD 2006 and personal data and

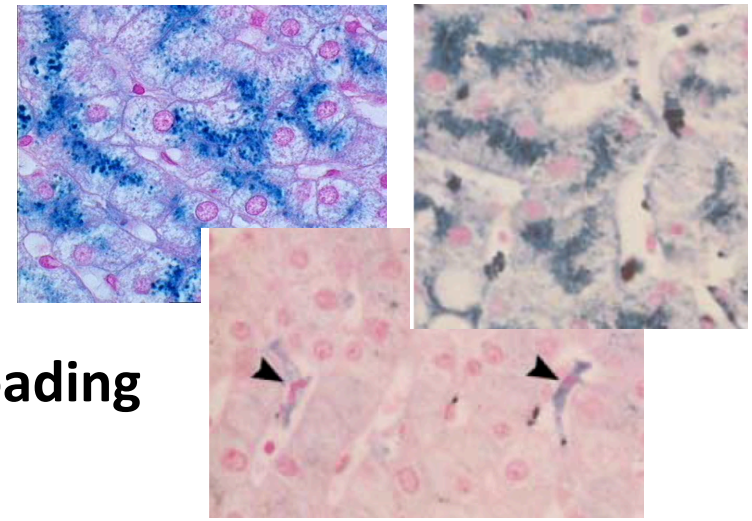
Ferroportin Disease





Pietrangelo et al. BCMD 2006. Reviewed in: Pietrangelo et al. SLD 2011 and Pietrangelo. Haematologica 2017

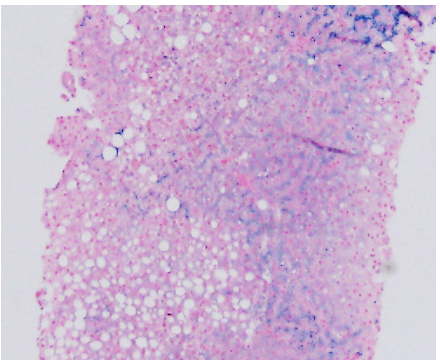
Liver Biopsy



- To **detect, quantify, and characterize iron loading**

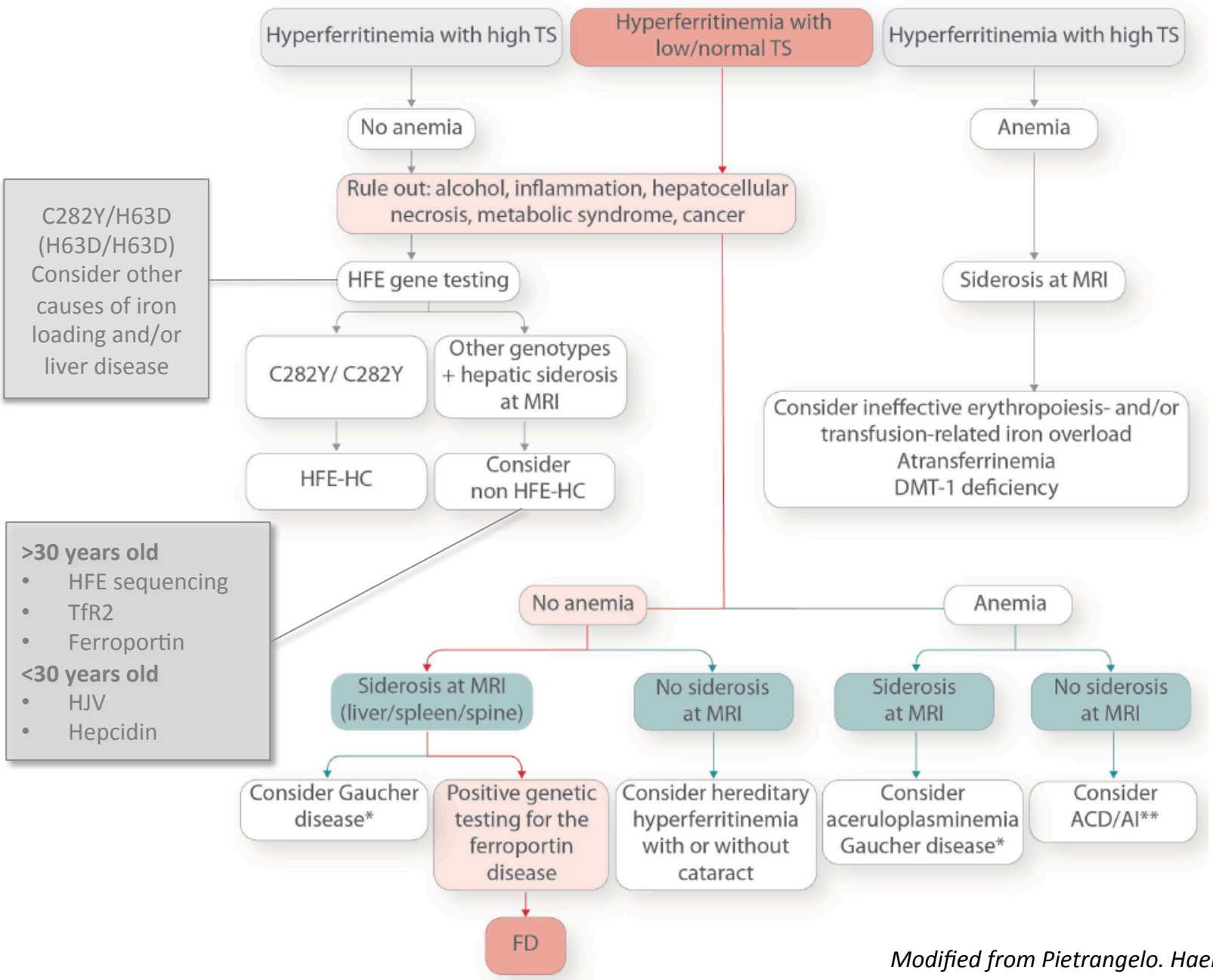
- To detect liver **fibrosis/cirrhosis**
  - If Ferritin < 1000 ng/ml, no enlarged liver and no AST increase = never significant fibrosis
  - If Ferritin < 1000 ng/ml, no AST increase, and PLT >200.000: high negative predictive value (~100%) for high-degree fibrosis
  - Liver elastometry

- To **diagnose different or/and concomitant liver disease**



Reviewed in Reviewed in EASL CPG on HFE Hemochromatosis. J Hepatol. 2010; AASLD guidelines 2011; Deugnier et al. SLD 2011, Legros et al. Liver Int. 2015

Diagnostic approach



Modified from Pietrangelo. Haematologica 2017

Next Generation Sequencing in Iron Overload Disorders

- Identification of novel pathogenic mutations in “hemochromatosis-genes”
- Rapid and cost-effective identification of digenic/polygenic disease
- Identification of mutations in unexpected “hemochromatosis-genes” *(e.g. HJV-HH in patients with adult phenotype)*
- Potential for identification of pathogenic mutations in “new” genes in patients with iron overload of unknown origin.

Badar et al. AJH2015, Mc Donald et al. J Hepatol 2015, De Tairac et al. J Hepatol 2015, Faria et al. BCMD 2016, Lanktree et al. Eur J of Hematol 2016, Piubelli et al Am J Hematol 2017, and personal data *(manuscript in preparation)*

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- Potential for identification of pathogenic mutations in "new" genes in patients with iron overload of unknown origin.
- Helpful "second level" tool for molecular diagnosis after "first level test" (HFE)
- Lots of VUS (variants of uncertain or unknown significance)

Badar et al. AJH2015, Mc Donald et al. J Hepatol 2015, De Tairac et al. J Hepatol 2015, Faria et al. BCMD 2016, Lanktree et al. Eur J of Hematol 2016, Piubelli et al Am J Hematol 2017, and personal data *(manuscript in preparation)*

Hemochromatosis management 1/3

Therapeutic phlebotomy is the mainstay of treatment:

- *Iron depletion endpoint*: ferritin level </close to 50 ng/ml
- *Maintenace phase*: 50-100 ng/ml; 2-4 phleb/y

EASL Guidelines. J Hepatol. 2010; AASLD Guidelines. Hepatology 2011; Adams et al. Blood 2011; Piperno. Expert Opin Med Diagn 2013; Roumbout-Sestienkova et al. BMJ 2016; Buzzetti et al. Cochrane Database Syst Rev 2017

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Erythrocytoapheresis**:**

- effective but not widely practiced
- requires special equipment and trained staff
- insufficient evidence to compare to venesection
- for selected cases

EASL Guidelines. J Hepatol. 2010; AASLD Guidelines. Hepatology 2011; Adams et al. Blood 2011; Piperno. Expert Opin Med Diagn 2013; Roumbout-Sestienkova et al. BMJ 2016; Buzzetti et al. Cochrane Database Syst Rev 2017

Hemochromatosis management 2/3

- Fatigue, skyn hyperpigmentation, transaminases improve.
- **Regression of biopsy-proven liver fibrosis has been reported in 13% to 50% of subjects**
- **Endocrinological and cardiological abnormalities varies,** related to the degree of tissue damage
- Hypogonadism, cirrhosis, destructive arthritis, and IDDM are usually irreversible
- **In the absence of cirrhosis or diabetes, the life expectancy of treated patients is normal**

EASL CPG on HFE Hemochromatosis. J Hepatol. 2010.; AASLD Guidelines. Hepatology 2011; Niederau et al. Gastro 1996; Falize et al. hepatol 2006

Hemochromatosis management 3/3

- ✓ **Therapeutic phlebotomy**

- ✓ **Iron chelators** for selected cases:
  - *deferoxamine*
  - *deferasirox*
  - *deferiprone*

- ✓ **Future directions**
  - Hepcidin-stimulating agents or Hepcidin replacement therapy
  - Asymptomatic patients with mild iron burden benefit from iron-depletion?
  - Role of %Tf. saturation in patient follow-up?
  - Revision of optimal ferritin target?

TAKE-HOME MESSAGES

Definition	Iron-overload disease caused by a genetically determined failure to prevent unneeded iron from entering the circulatory pool
Distinguishing features	1. Hereditary (usually autosomal recessive) trait 2. Early and progressive expansion of the plasma iron compartment 3. Progressive parenchymal iron deposition that can cause severe damage and disease involving the liver, endocrine glands, heart, and joints 4. Nonimpaired erythropoiesis and optimal response to therapeutic phlebotomy 5. Defective hepcidin synthesis or activity
Postulated pathogenic basis	Gene mutations leading to inappropriately low hepatic synthesis or impaired peripheral activity of hepcidin
Recognized genetic causes	Pathogenic mutations of *HFE*, *TfR2*, *HJV*, or *HAMP* and certain *ferroportin* mutations

From Pietrangelo. Gastroenterology 2010

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In European populations **HFE-HH** is

- the most common form of HH
- the commonest genetic disease

Searching for C282Y (and H63D) is the "**first levels test**" **in adults**

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- **NGS is a new tool for genetic diagnosis of "first-test negative" patients**
- **MRI is a useful tool for diagnosis** (detection, quantification, distribution of iron)
- Hepcidin replacement/stimulating therapy may represent a future therapeutic option