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EuroBloodNet

Diagnosis and clinical management of rare forms of Hemochromatosis

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ERN-EuroBloodNet subnetwork Hemochromatosis and other rare genetic disorders of iron metabolism and heme synthesis

Verona – Italy
February 1st 2024

Co-funded by
the Health Programme
of the European Union

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for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

Conflicts of interest - DG

- *SANOFI (Advisory Board)*
- *KEDRION-PHARMACOSMOS(Advisory Board)*
- *VIFOR PHARMA (Advisory Board)*
- *NOVO NORDISK (Advisory Board)*

(not relevant to this presentation)

LEARNING OBJECTIVES

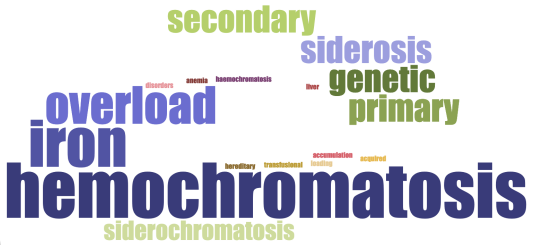
1. Clarify the nomenclature of Hemochromatosis (novel 2022 classification by the BIOIRON Society).

2. When consider the diagnosis of rare forms of H., collectively grouped under the “non-HFE H.” category (very rare / ultra rare conditions as compared to HFE-H.).

3. How to manage rare forms of H..

Terminology

Before hepcidin discovery



The term "hemochromatosis", often used to indicate iron overload generally, should be restricted to the distinct genetic disorder due to (heterogenous) pathogenic mutations in genes regulating hepcidin, thereby leading to hyperabsorption of dietary iron and its progressive accumulation in the body.

Girelli D, Blood 2022
Phatak P and Girelli D, UpToDate® 2024

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Distinct features of Hemochromatosis

blood Special Report

Hemochromatosis classification: update and recommendations by the BIOIRON Society

Domenico Girelli,[1,*] Fabiana Busti,[1,*] Pierre Brissot,[2,*] Ioav Cabantchik,[3] Martina U. Muckenthaler,[4-7] and Graça Porto,[8,9] on behalf of the Nomenclature Committee of the International Society for the Study of Iron in Biology and Medicine (BIOIRON Society)

Table 1. Main clinical, biochemical, and imaging elements for the suspicion of HC

Leading
TSAT >45% (mainstay)
S-Ferritin >200 µg/L (females) or >300 µg/L (males)
Imaging evidence of liver IO (MRI* and/or biopsy†)
Iron deposits in hepatocytes (if biopsy is performed)
Absence of "predominant" acquired risk factors for hepcidin deficiency (eg, alcohol abuse or end-stage liver disease) and iatrogenic IO (eg, regular transfusions)
Absence of hematological signs of a primary RBC disorder, such as anemia‡ and/or reticulocytosis

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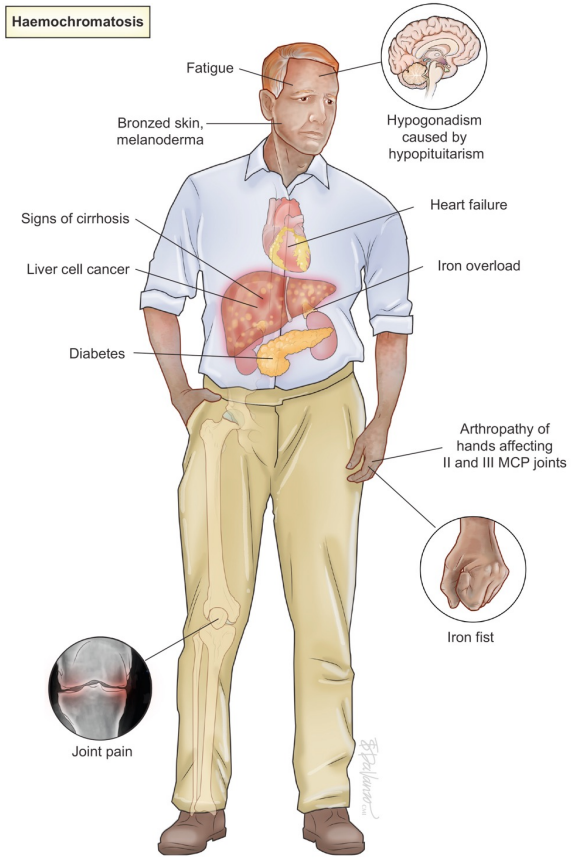
Girelli D, Blood 2022

Clinical manifestations of Hemochromatosis



Not always present

Signs and/or symptoms associated with IO:

- Skin pigmentation, asthenia
- Persistent increase of aminotransferases, hepatomegaly, cirrhosis, hepatocellular carcinoma
- Joint pain, arthritis, chondrocalcinosis, reduced bone mineral density
- Diabetes mellitus, hypopituitarism, hypoparathyroidism, hypogonadotropic hypogonadism
- Cardiomyopathy, heart failure, cardiac arrhythmias

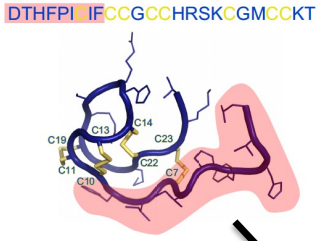
- **Nowadays rarely seen in HFE-H. due to early diagnosis during "routine" biochemical controls.**
- **Can be severe in non-HFE H. forms with early-onset or "juvenile" phenotype.**

EASL Clinical Practice Guidelines on haemochromatosis, J Hepatol 2022

Girelli D, Blood 2022

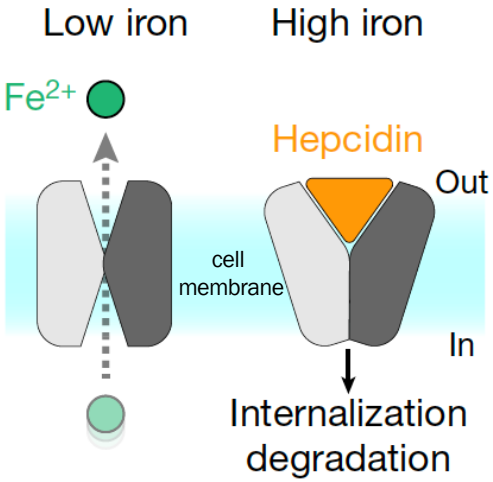
THE HEPCIDIN-FERROPORTIN AXIS

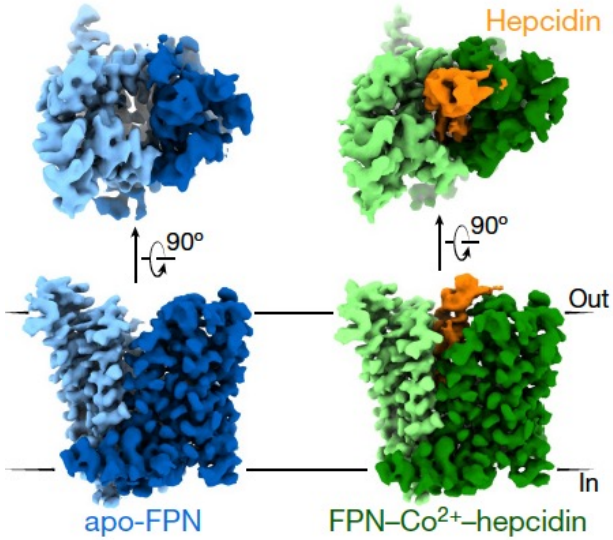
Hepcidin



Ganz T, Physiol Rev 2013

Acts by binding to (and blocking) its membrane **receptor**, **Ferroportin**, the only known iron exporter from the cells, esp. expressed by **duodenal entrocytes (iron absorption)** and **splenic macrophages (iron recycling)**





Billesbolle CB, Nature 2020

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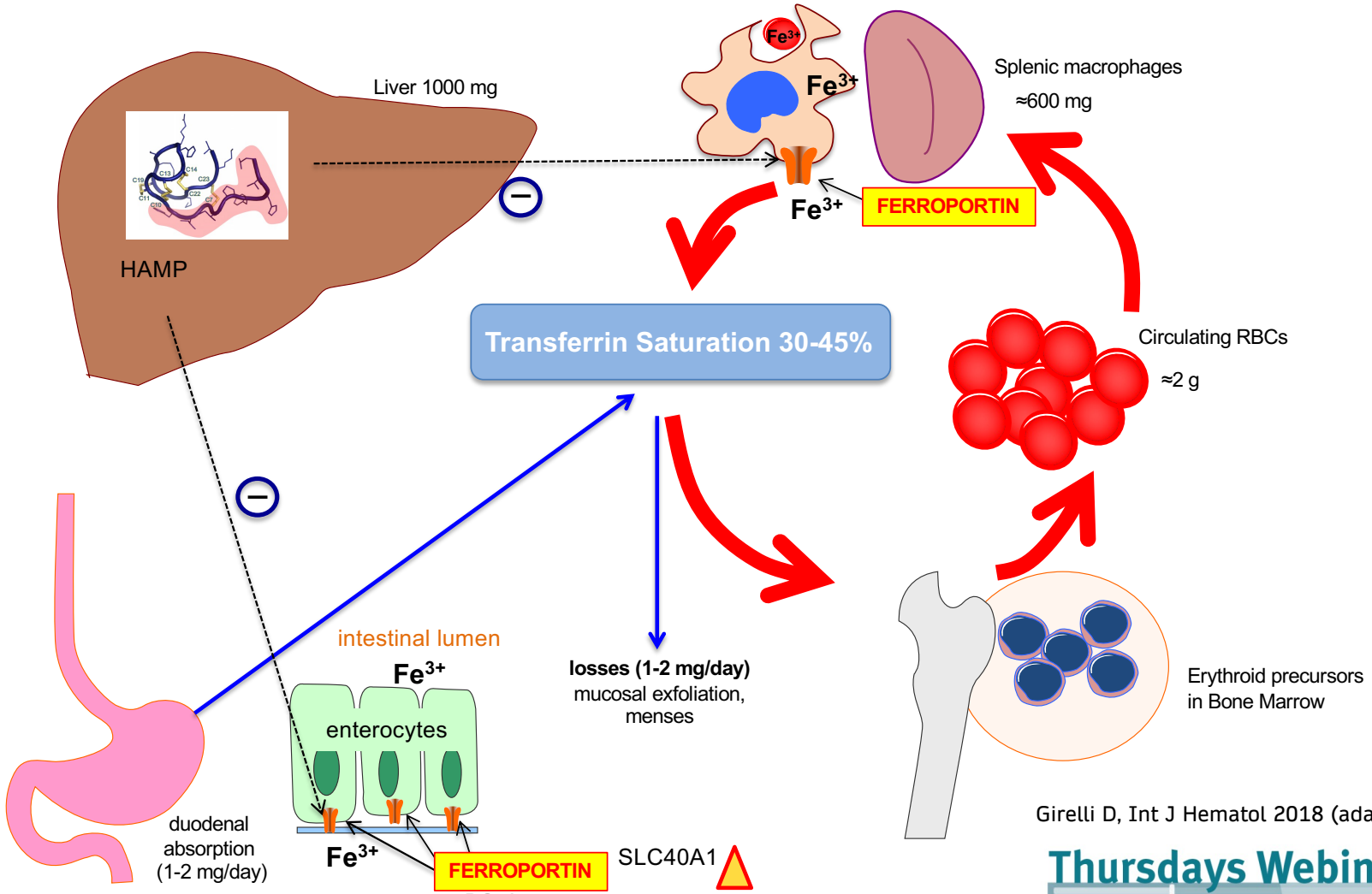
THE UNIQUE PATHOPHYSIOLOGY OF H.

IRON HOMEOSTASIS IS "ECOLOGICAL"

RBC Fe continuously recycled by splenic MΦ with few losses precisely replaced by dietary absorption regulated by hepcidin to keep the balance.



Girelli D, Int J Hematol 2018 (adapted)

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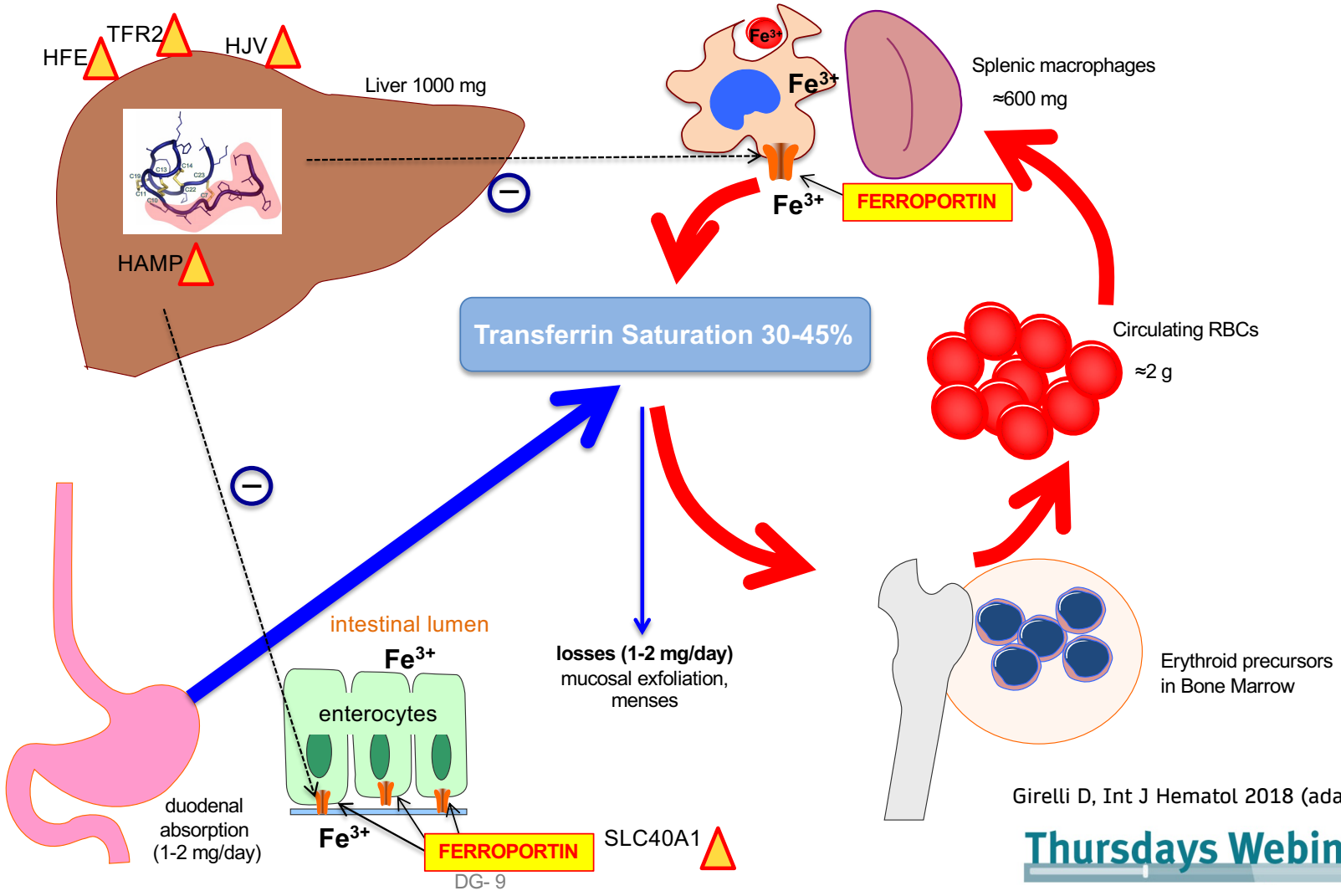
THE UNIQUE PATHOPHYSIOLOGY OF H.

Mutations → ↓↓ Hepcidin



Girelli D, Int J Hematol 2018 (adapted)

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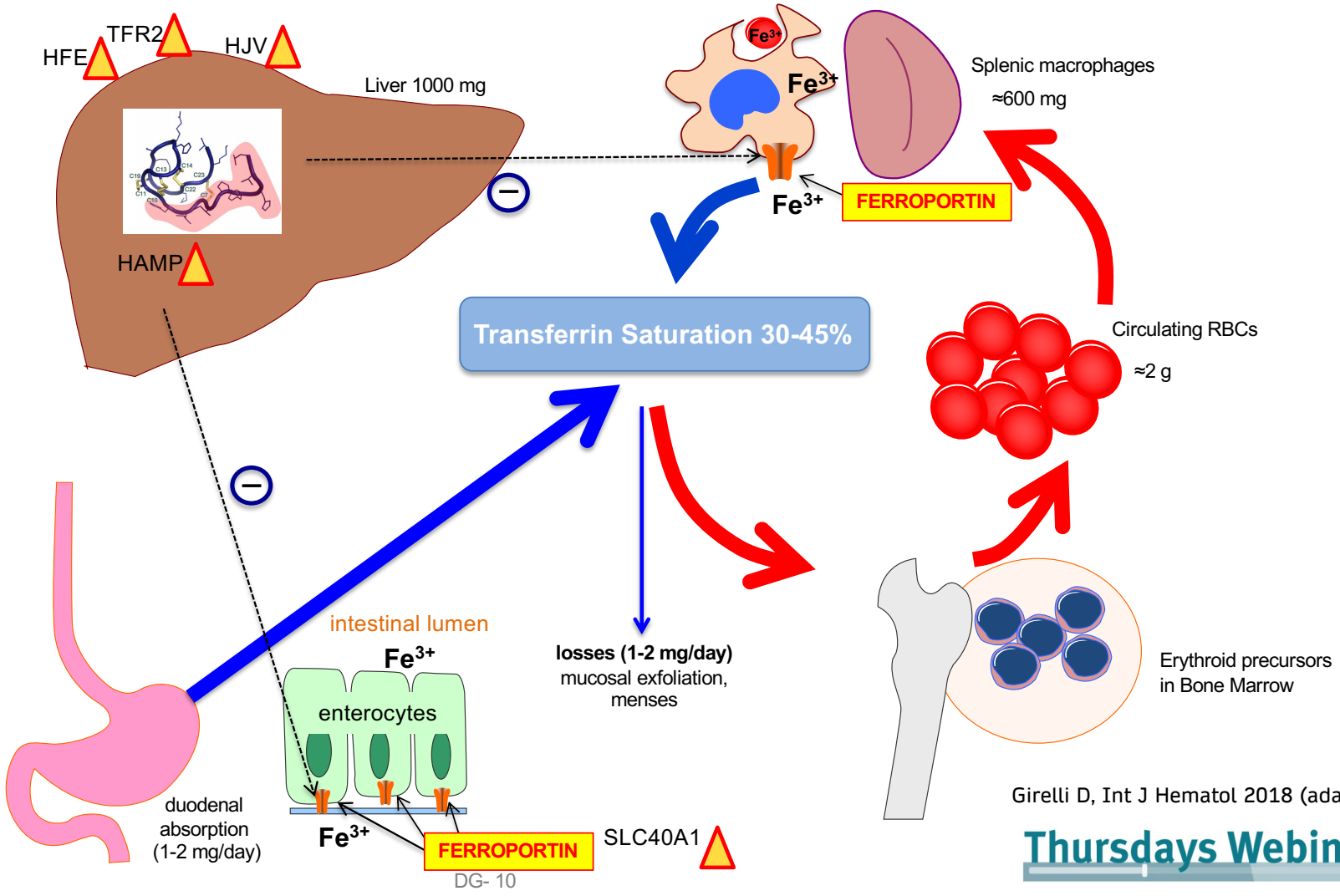

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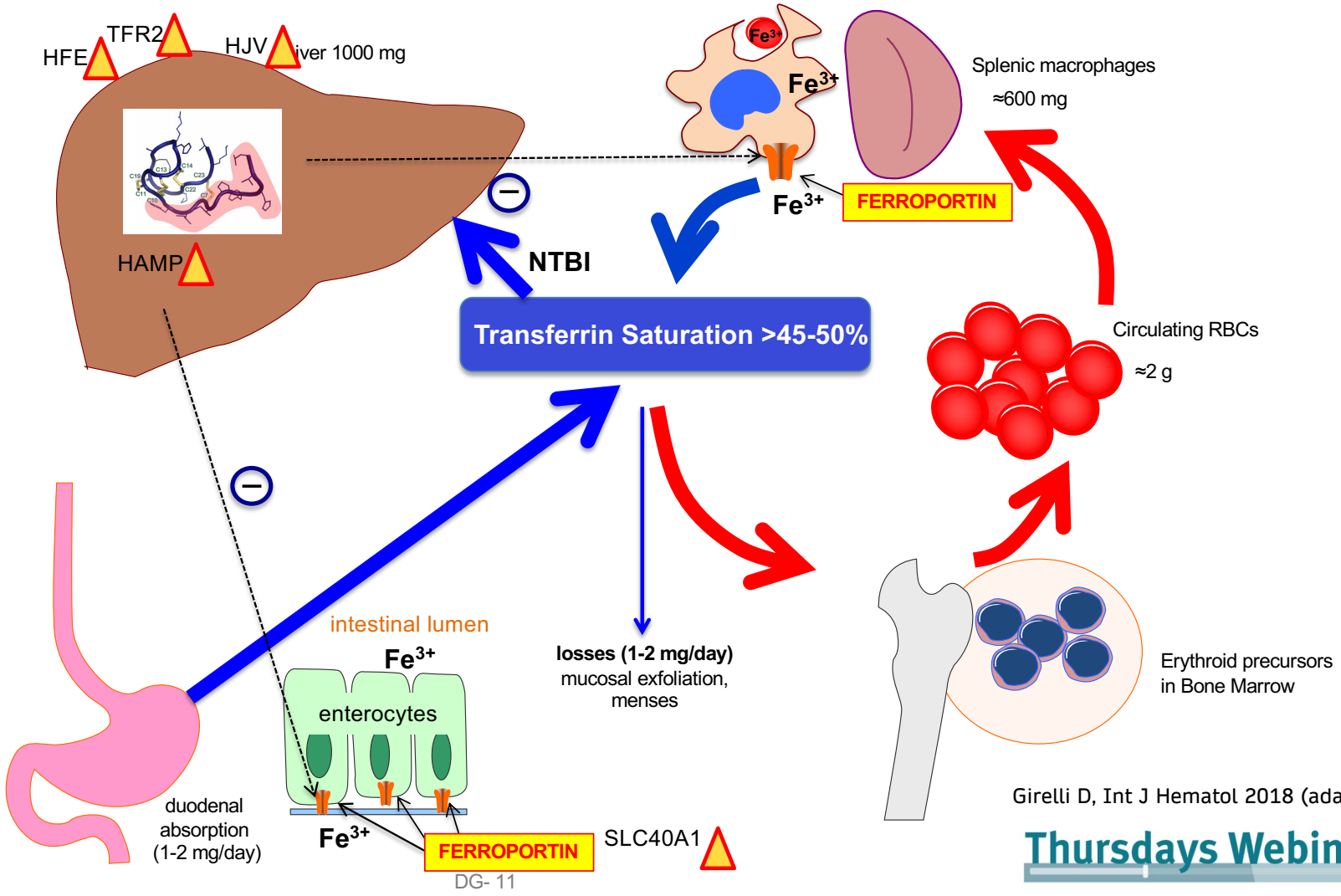

Girelli D, Int J Hematol 2018 (adapted)

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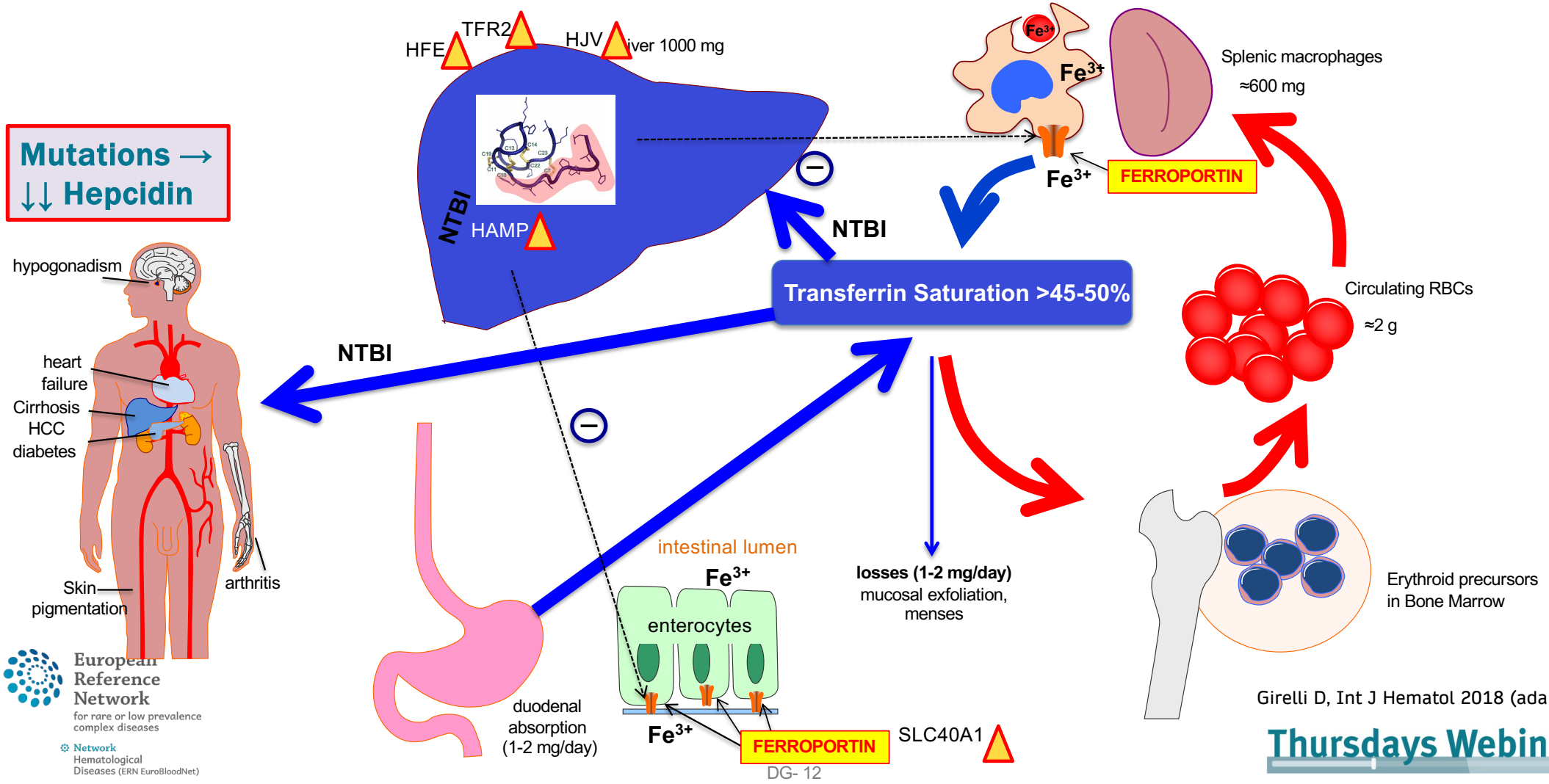

Girelli D, Int J Hematol 2018 (adapted)

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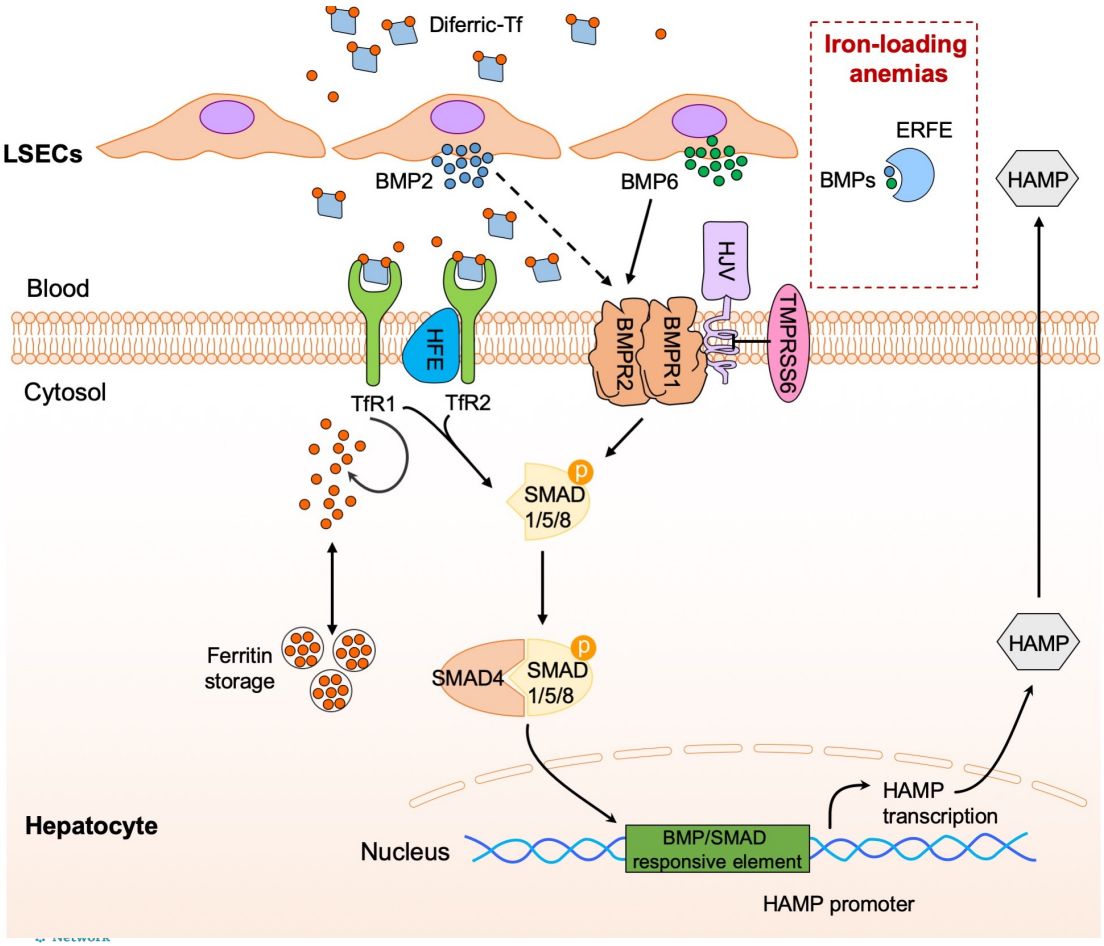
H. is a complex genetic disorder (different genes involved)

Different proteins involved in the homeostatic control of hepcidin synthesis by iron



mutations in **positive upstream hepcidin regulators**:

- ***High Fe (HFE) (most frequent)***
- ***Transferrin Receptor 2 (TFR2)***
- ***Hemojuvelin (HJV)***

or mutations in the genes encoding the key players of the H/F axis:

- **hepcidin (*HAMP*)**
- **ferroportin (*SLC40A1**)**

GoF (very rare) → **hepcidin-resistance**
LoF → different phenotype (**"Ferroportin Disease"**) with ↑↑ ferritin and normal TSAT

Girelli D, Blood 2022

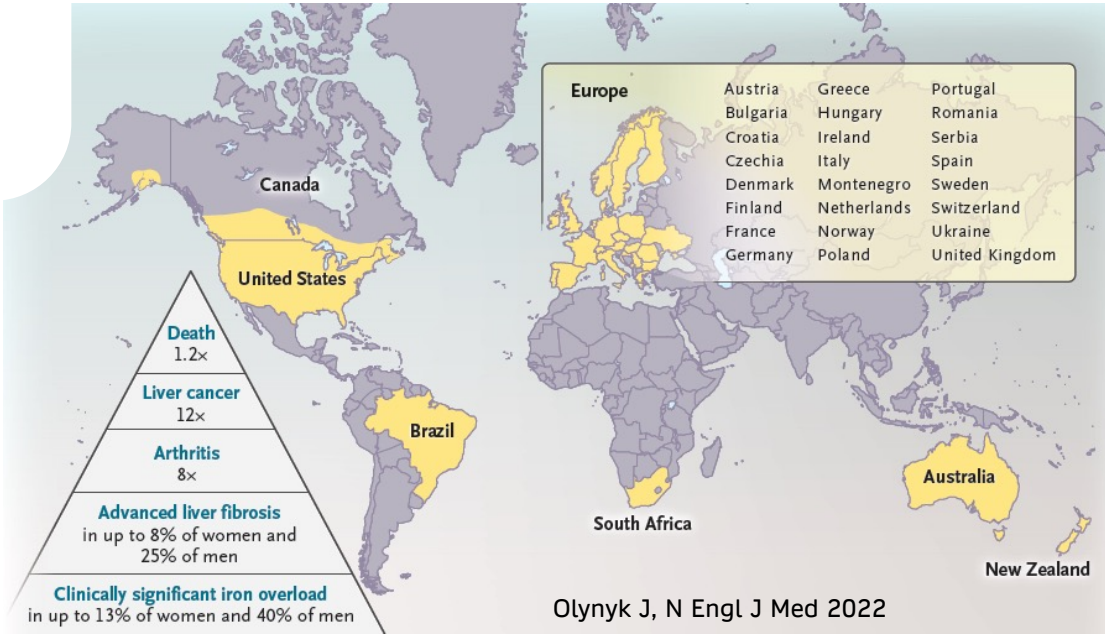
Hematological Diseases (ERN EuroBloodNet)

HFE versus non-HFE H.

Table 2. Combined pathogenic allele frequency for HC genes in the 1000 Genomes Project (1000G), Exome Sequencing Project (ESP), and Exome Aggregation Consortium (ExAc) datasets

Gene	1000G	ESP6500	ExAc	Geographical distribution
HFE (p.Cys282Tyr)	0.013	0.048	0.0324	Highest prevalence in Northern Europe

Common (Northern Europe)
Low penetrance influenced by co-factors (e.g. alcohol)
Middle-age onset
Easy diagnosis (C282Y using widespread 1st level genet



Olynyk J, N Engl J Med 2022

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Common features of rare H. due to mutations in genes other than HFE (collectively "non-HFE H.")

TFR2, HJV, HAMP, SLC40A1, other unknown ? (PIG-A in pediatric patients with neurologic involvement)

1. **Worldwide distribution in different ethnicities (EU immigrants!).**
2. **Possible early-onset with high penetrance and M:F 1:1 ("juvenile" forms).**
3. **Severe phenotype, more often including heart failure, hypogonadism, diabetes.**
4. **Complex molecular diagnosis (mutations in non-HFE genes are typically private, requiring sequencing and adequate interpretation).**

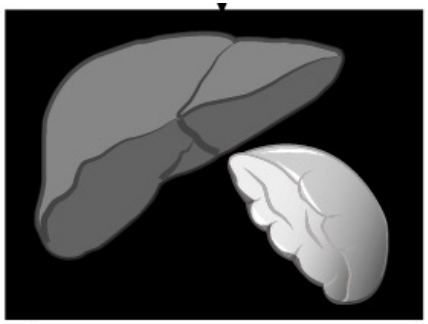
When consider the diagnosis of non-HFE H.

"Diagnosis of exclusion"!

- **Clinical and/or biochemical (↑ Ferritin *and* ↑ TSAT, no anemia) phenotype resembling the classic form, especially in young pts.**

- **Evidence of IO: ↑ LIC documented by MRI ("black liver and white spleen" pattern) or liver biopsy (with prevalent hepatocyte overload) without any alternative explanation**

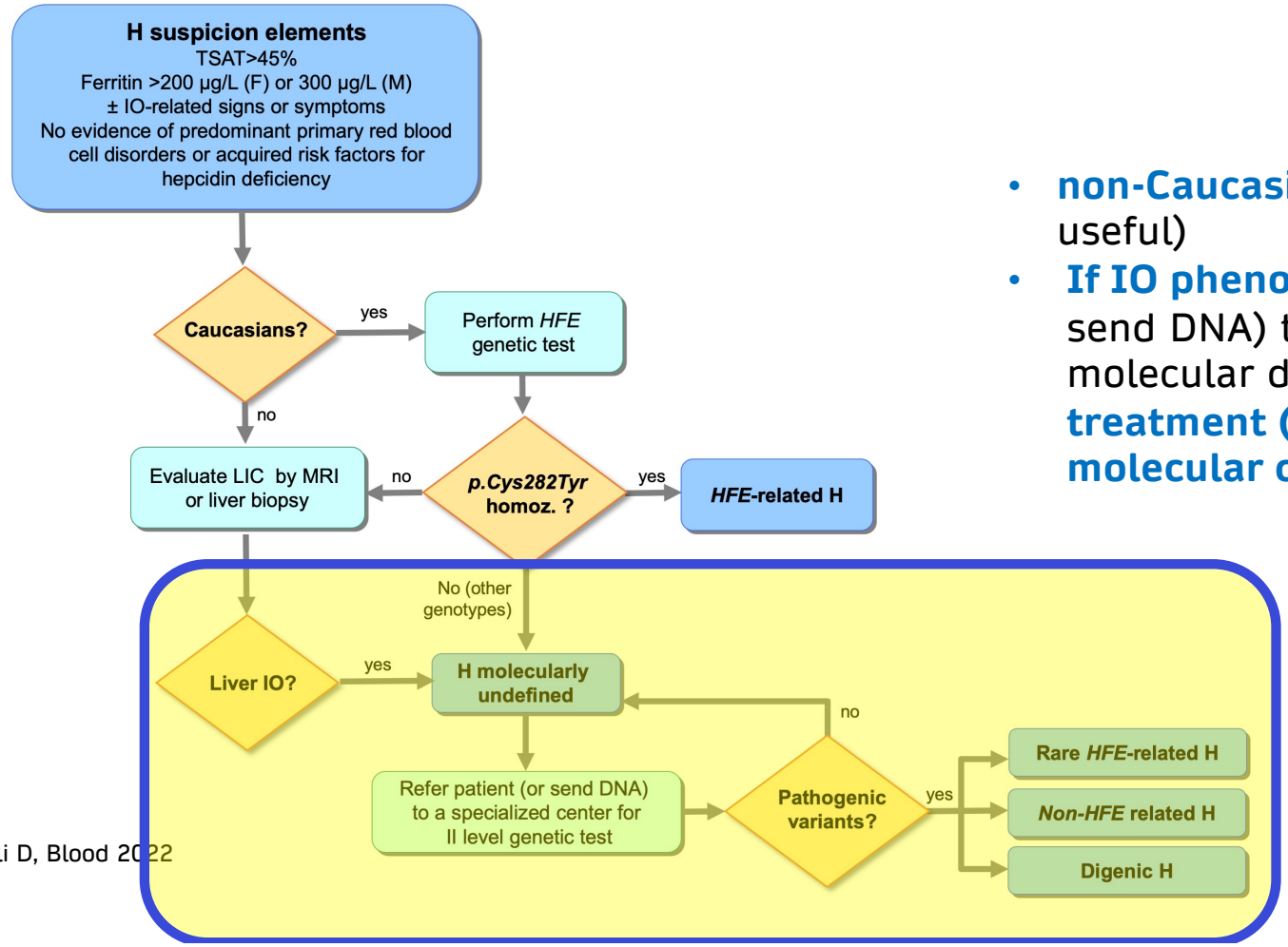
- **Negative (or non-diagnostic, e.g. C282Y +/-) 1st level genetic test for H.**



Brissot P, J Hepatol 2016

Hemochromatosis diagnostic algorithm

from clinical/biochemical and imaging studies to molecular confirmation



- **non-Caucasians (**HFE genetic test rarely useful)
- **If IO phenotype is clear**, refer the patient (or send DNA) to a specialized center for molecular diagnosis by **NGS**, but **start treatment (phlebotomy) without waiting for molecular confirmation**.

Girelli D, Blood 2022

PRACTICAL CLASSIFICATION OF H.

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Girelli et al, page 3018

Hemochromatosis redefined

Chaim Hershko | Shaare Zedek Medical Center

In this issue of *Blood*, Girelli et al propose a new classification for hemochromatosis (HC).[1] The new classification is intended to be of practical help, avoiding delays in diagnosis and treatment even if detailed molecular characterization is not readily available.

Novel classification of HC

Novel classification	Molecular pattern
HFE-related	*p.Cys282Tyr* homozygosity or compound heterozygosity of *p.Cys282Tyr* with other rare *HFE* pathogenic variants or *HFE* deletion
Non-*HFE*–related	Rare pathogenic variants in "non-*HFE*" genes: • *HJV*-related • *HAMP*-related • *TFR2*-related • *SLC40A1* (GOF)-related
Digenic	Double heterozygosity and/or double homozygosity/ heterozygosity for mutations in 2 different genes involved in iron metabolism (*HFE* and/or non-*HFE*)
Molecularly undefined	Molecular characterization (still) not available after sequencing of known genes (provisional diagnosis)

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Hershko C, Blood 2022

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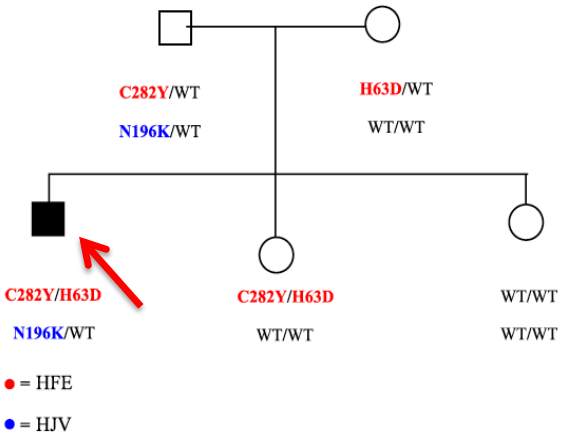
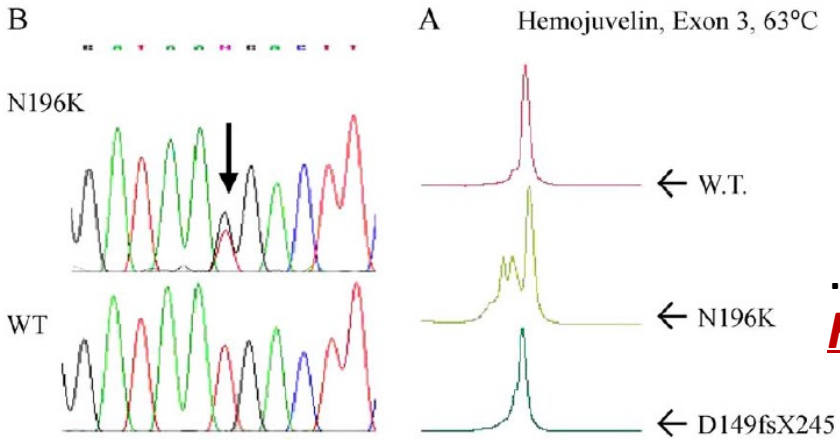
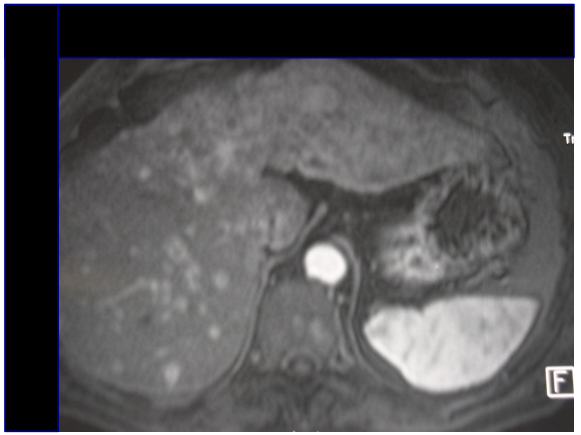
EXAMPLE OF DIGENIC INHERITANCE

41-year-old male presenting with liver cirrhosis and HHC (+ bronze hyperpigmentation and diabetes). 1st level genetic test: ***HFE*** C282/H63D compound heterozygosity…



Re-sequencing for research purposes





Biasiotto G, Blood Cells Mol Dis 2004

… plus
HJV N196K heterozygosity

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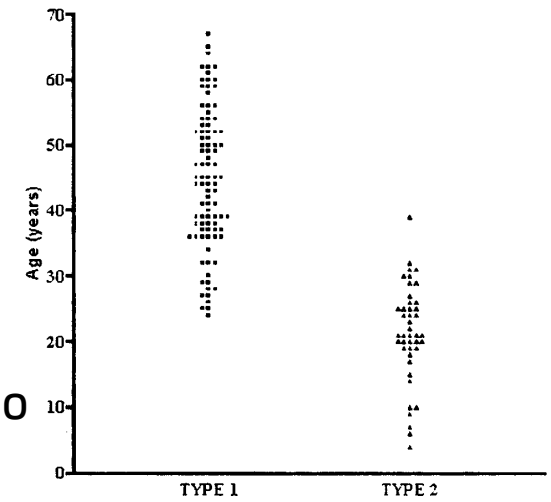
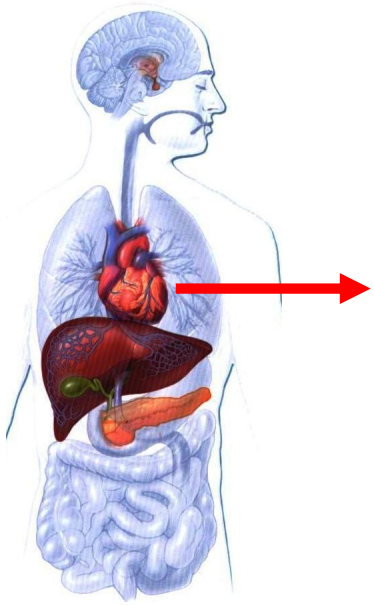
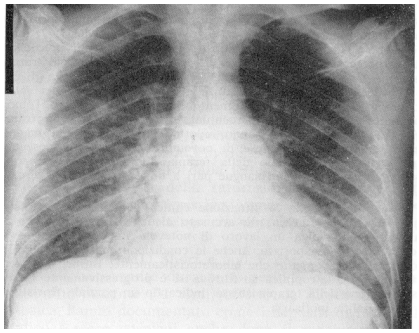
“JUVENILE” HEMOCHROMATOSIS

- ✓ Early onset (usually II decade; exceptions can occur)
- ✓ Full penetrance, severe multi-organ damage
- ✓ Both genders equally affected (no male predominance)
- ✓ Negligible contribute of acquired cofactors



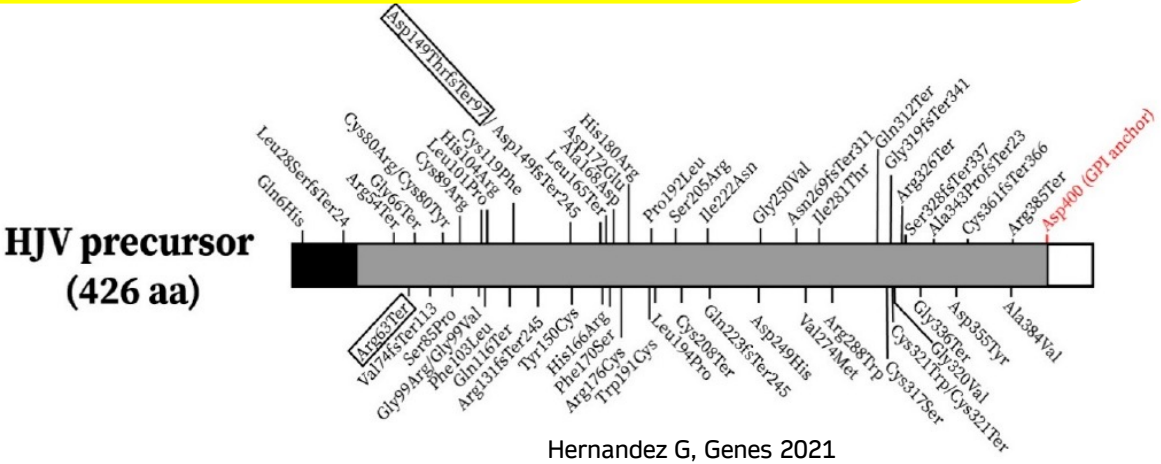
Camaschella C, Semin Hematol 2002





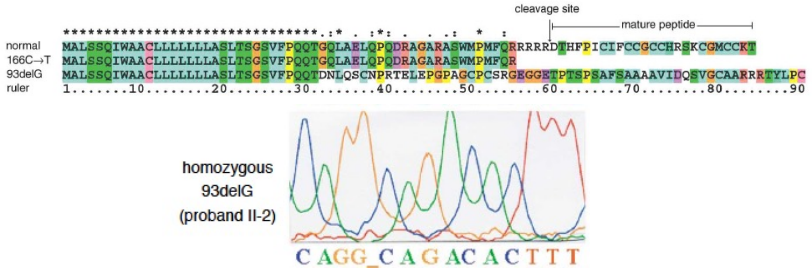
Non-HFE genotypes in JH phenotype

~90% due to ***HJV*** mutations (G320V recurrent in ≈ 50% cases)



Hernandez G, Genes 2021

Very rarely due to mutations of the hepcidin gene (***HAMP***)



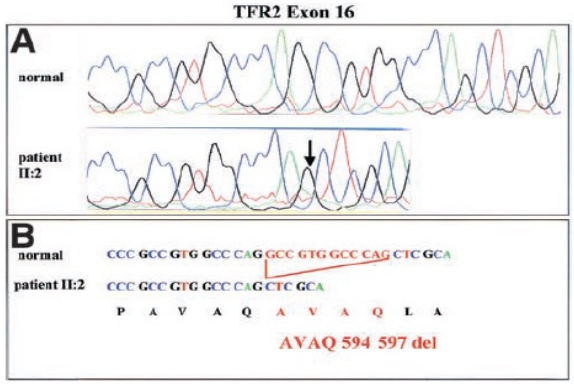
Roetto A, Nat Genet 2003

Other combinations (e.g. ***digenic including TFR2***) possible

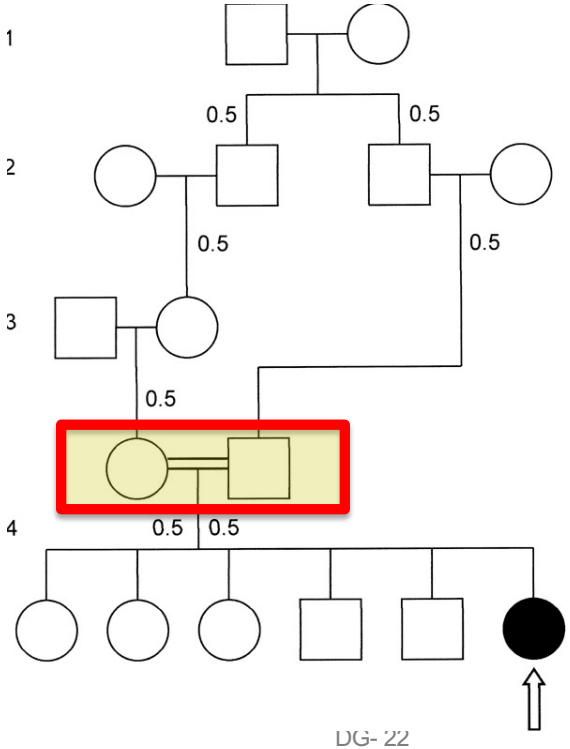
TFR2 related H.

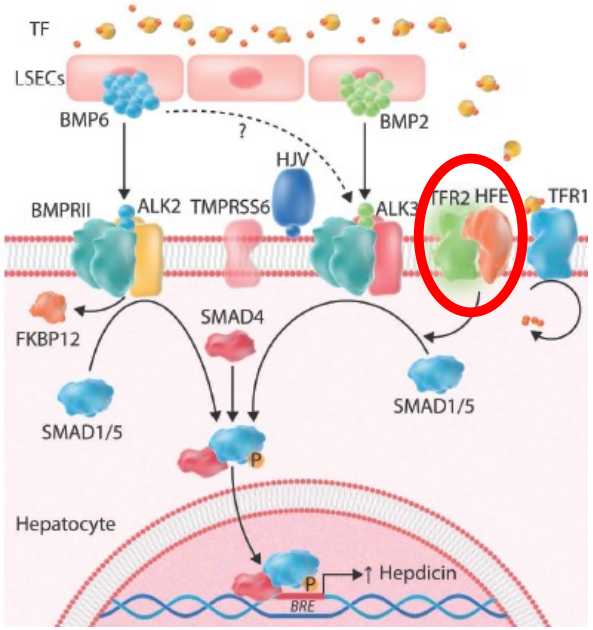
Transmembrane receptor with lower affinity for diferric TF than TFR1, but able to bind HFE in high iron status to promote hepcidin synthesis

Clinical phenotype similar to classical H., but a **tendency to earlier presentation** and often detected in families with **consanguinity**.



Girelli D, Gastroenterology 2002





Camaschella C, Haematologica 2020

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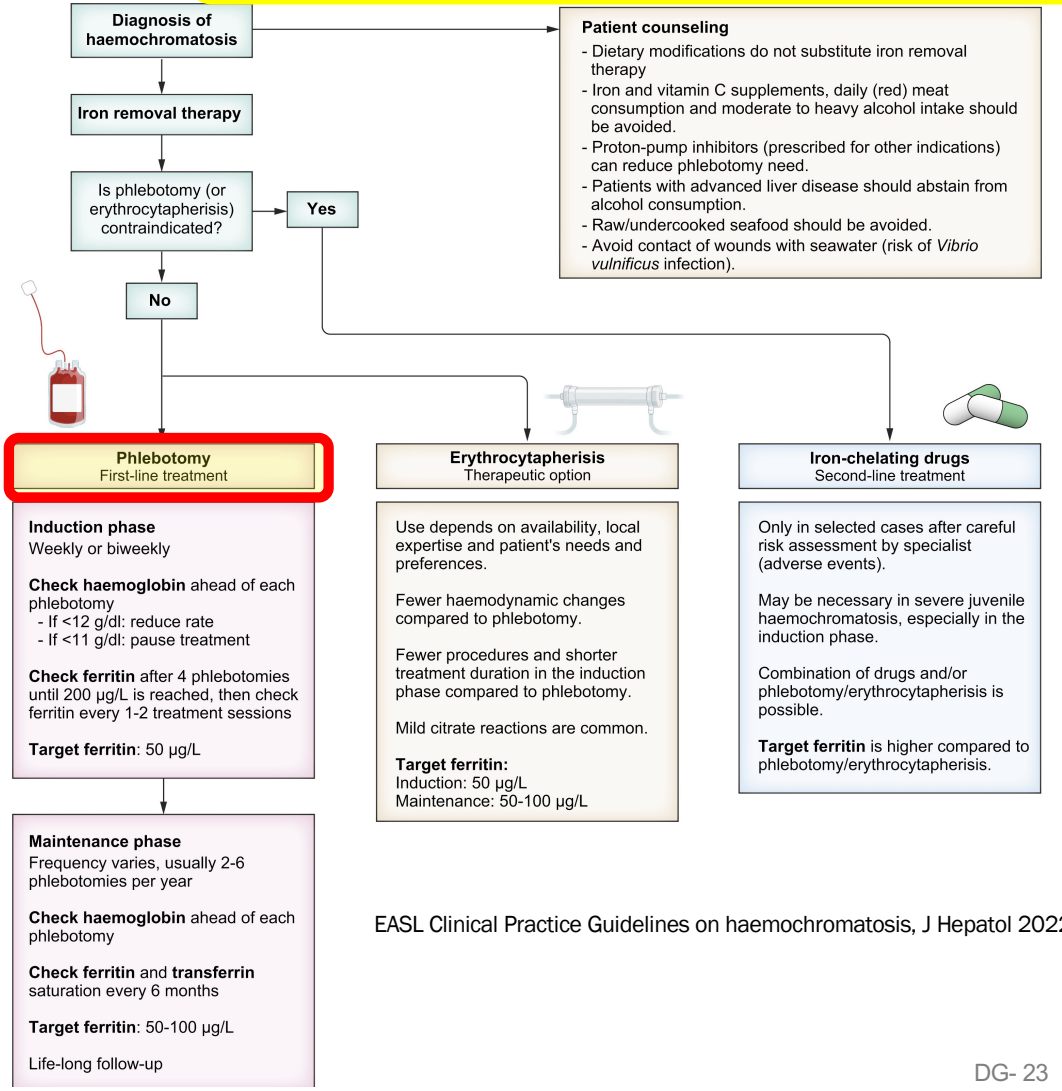
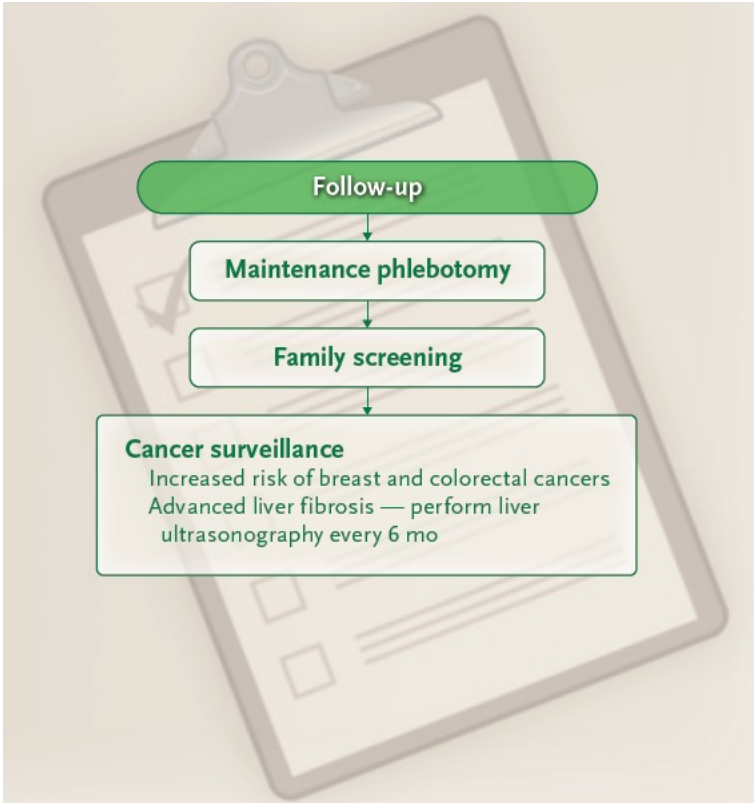
Treatment of hemochromatosis

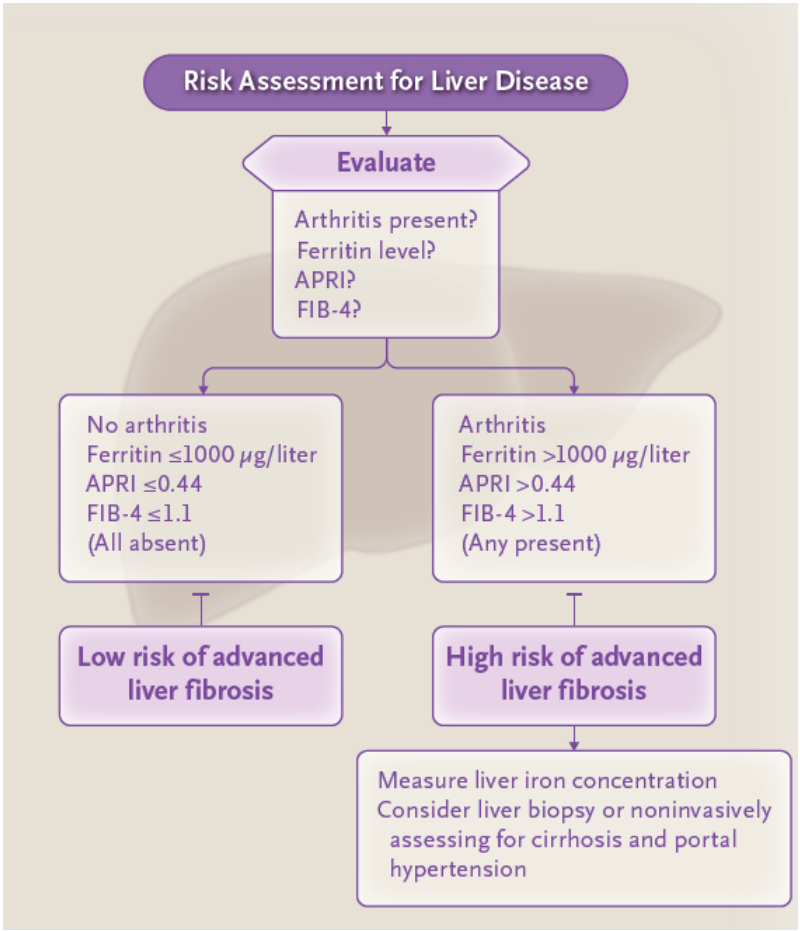


EASL Clinical Practice Guidelines on haemochromatosis, J Hepatol 2022

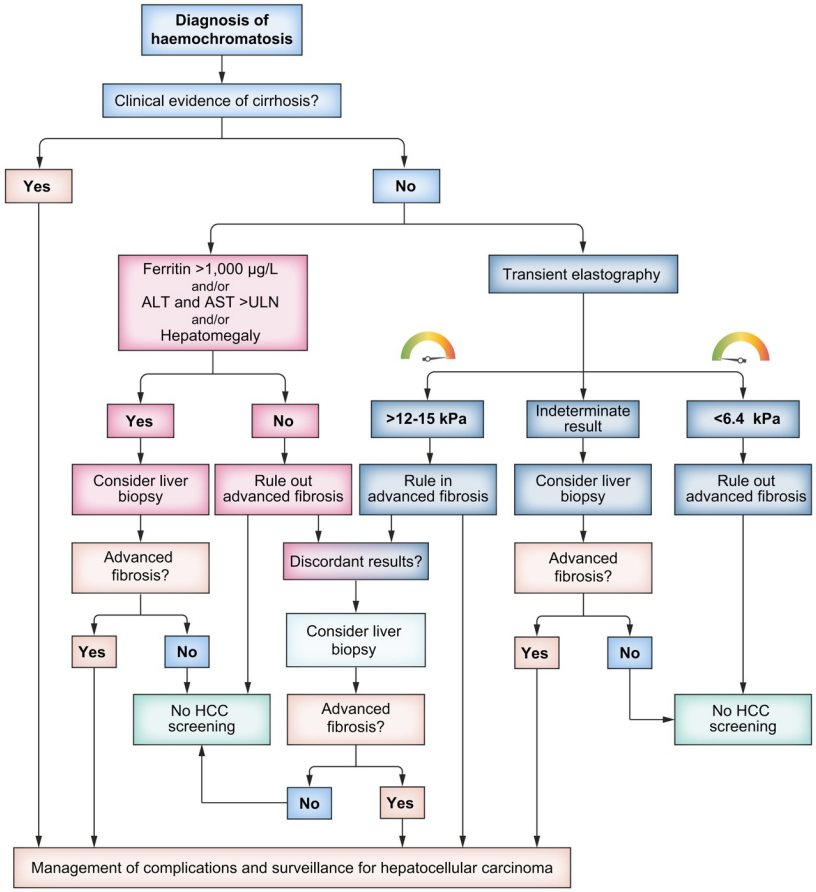


Olynyk J, N Engl J Med 2022

Hemochromatosis risk assessment



Olynyk J, N Engl J Med 2022



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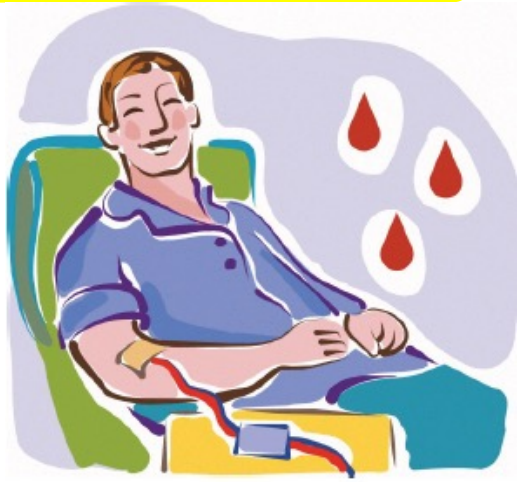
PHLEBOTOMY IN H. (standard of care)

Generally safe and well- tolerated
Morbidity and mortality significantly reduced when treatment is initiated before the development of cirrhosis and/or diabetes.



Caveats:

- Can further suppress hepcidin if ferritin goals is too close to iron deficiency
- “invasive”
- attendance of a health care facility required
- Arthropathy may not ameliorate (even worsen)

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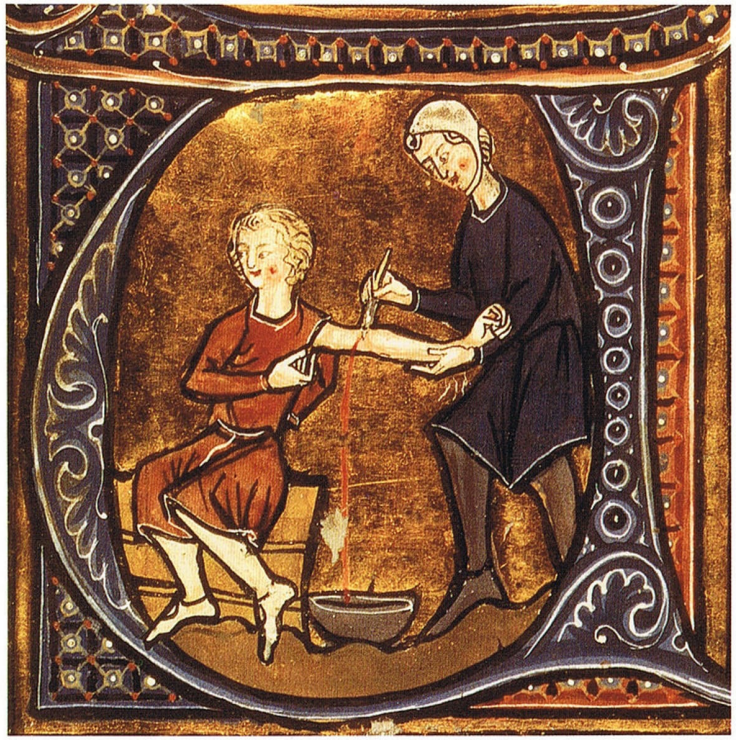
Treatment of H. (with particular reference to severe cases)



Phlebotomies well tolerated

Anemia typically absent in H., unless advanced disease (e.g. portal hypertension with hypersplenism/variceal bleeding) or comorbidities.

1

Phlebotomy induction phase

350-500 mL (according to sex/weight) every 1-2 weeks.

Check Hb (discontinue/delay if <11-12 g/dL) and serum ferritin (SF) every 4 phlebotomies. Goal: SF ~ 50 μg/L (not lower, be flexible!).

2

Phlebotomy maintenance phase

A phlebotomy every 2-3 to 4-6 months to keep SF within the desired range.

Lifelong (reduce or suspend after 70-75 years). Goal: SF ~ 50-100 μg/L.

3

Erythrocytapheresis

Quick and safe, not universally available. Good option in pts. with **severe cardiomyopathy/liver disease** (isovolemic procedure).

4

Iron chelators

Only if phlebotomies contraindicated/unfeasible, or **in combination with phlebotomies in most severe cases**. DFO s.c. 25-40 mg/kg 5-7 days/week. Oral chelators can be used but off-label.

?

Hepcidin agonists

may be of some help in the future.

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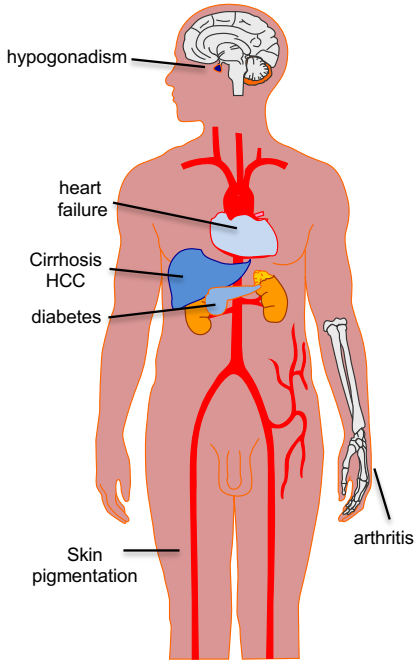
Non-HFE H. patients are often more challenging than classical HFE-H. and need personalized and



- **ADVANCED DISEASE**
- **HF WITH HEMODYNAMIC INSTABILITY**
- **ANEMIA**
  (GI bleeding, hypersplenism)
- **COMORBIDITIES**
  (e.g. renal failure, others)

→

- **ERYTHROCITAPHERESIS**
- **"LOW INTENSITY" PHLEBOTOMIES**
  (e.g. 150-200 ml at close intervals)
- **IRON CHELATORS**
  (alone or in combination with above)
- **HORMONE REPLACEMENT Thx.**
  (e.g. insulin, androgens)
- **MISCELLANEOUS**
  (e.g. bisphosphonates, anti-arthritis)

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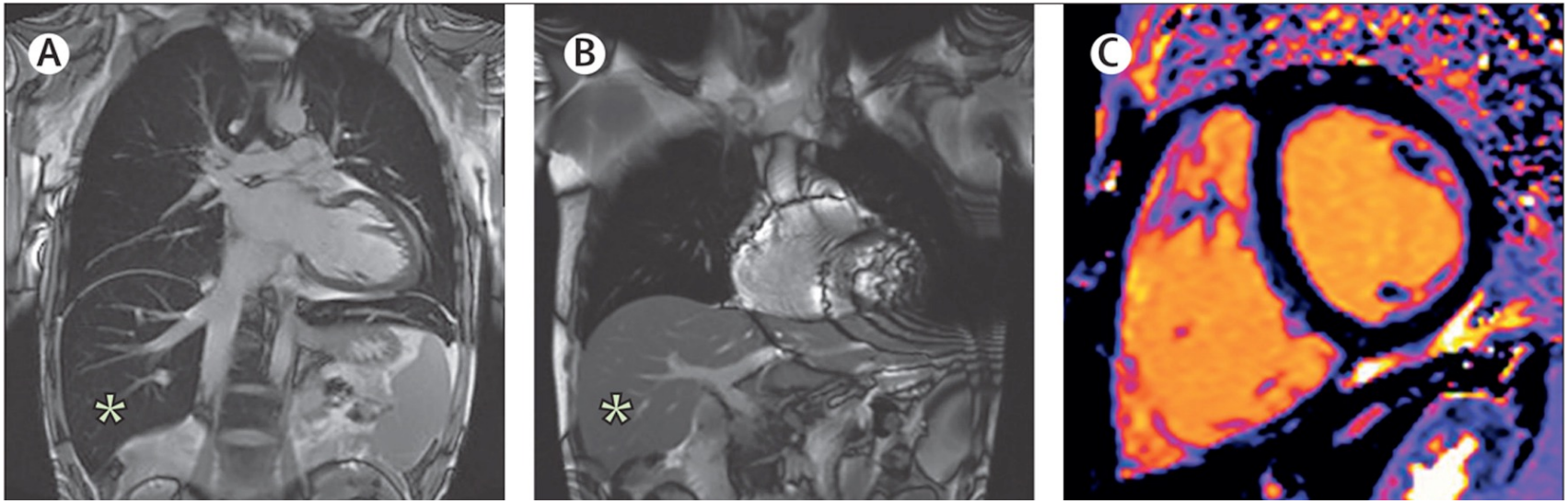
Severe HF in JH
(HJV p.Cys89Arg/1q.21.1 de novo deletion)

39-year-old male admitted for severe heart failure (LVEF 28%).

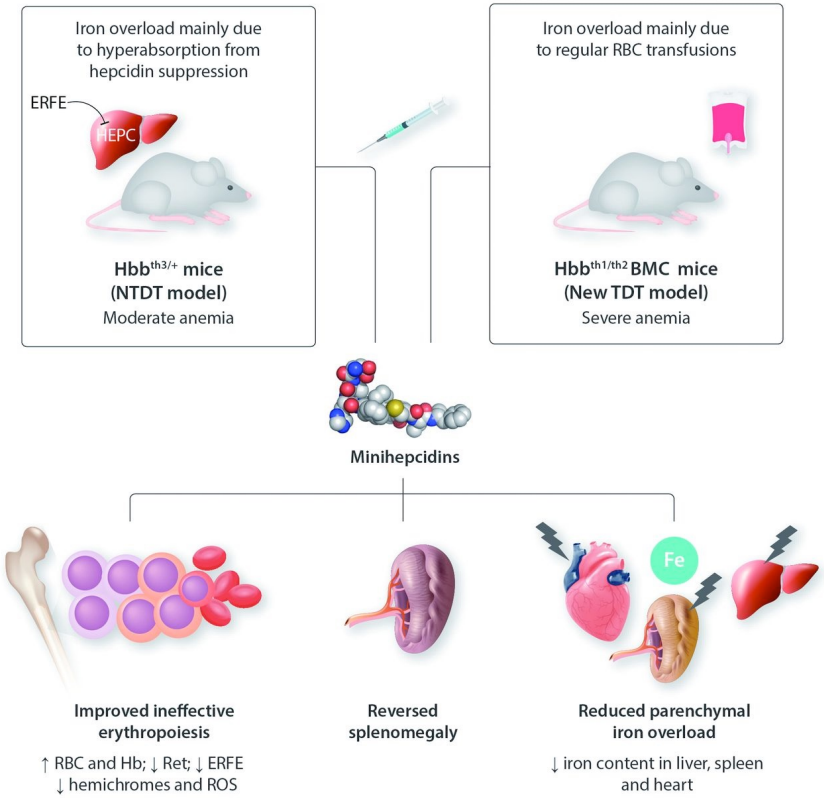
In addition to dobutamine and i.v. furosemide, intensive iron chelation was initiated with a continuous i.v. infusion of deferoxamine (at 40 mg/kg four times a day). After 7 days, oral deferiprone was added at a dose of 75 mg/kg four times a day.



(A) CMR shows hypointense signal—suggestive of iron overload—in the liver (*) and myocardium; by comparison the spleen shows normal signal intensity. (B) liver with normal signal intensity (* -6 months after treatment) (C) CMR shows a severe reduction in T1 mapping (563 ms; n.v. 983 ± 30; shown in black) consistent with severe myocardial iron accumulation.

Cartella I, Lancet 2022

NOVEL DRUGS (HEPCIDIN AGONISTS)



Girelli D & Busti F, Haematologica 2019

Table 1. Classification of hepcidin agonists

Hepcidin agonists	Company	Drug	Target	Clinical trials	Reference
Class 1: hepcidin mimetics	University of California, Los Angeles	MHs (PR65, PR73, M009, M012)	Ferroportin	Validated in preclinical studies	See articles cited in the review
	La Jolla Pharmaceutical Company	LJPC-401 (hepcidin formulation)	Ferroportin	Phase 1: no toxicity reported; expected hypoferremia observed	45
	Protagonist Therapeutics	PTG-300	Ferroportin	Phase 1: no serious adverse events reported; expected hypoferremia observed	58
Class 2: stimulators of hepcidin production	Ionis Pharmaceuticals	Tmprss6-ASO	Tmprss6	Phase 1 ongoing	59
	Alnylam Pharmaceuticals	Tmprss6-siRNA	Tmprss6	Validated in preclinical studies	See articles cited in the review
Class 3: ferroportin inhibitors	Vifor Pharma	VIT-2763	Ferroportin	Phase 1 planned in 2018	60

ASO, antisense oligonucleotide; siRNA, small-interfering RNA.

Casu C, Blood 2018

Table 1. Hepcidin mimetics in clinical development

Drug/Molecule name	Class	Mechanism of action	Clinical trials
Rusfertide	Synthetic hepcidin mimetic peptide	Contains functional ferroportin binding domain, directly binds to and degrades ferroportin	• Preliminary results from two phase 2 trials (NCT04057040, NCT04767802): Rusfertide is highly effective in eliminating phlebotomy, reverses iron deficiency, no major toxicities. • Phase 3 trial underway (NCT05210790)
Sapablursen	Tmprss6 ASO	Downregulation of TMPRSS6 gene product -prevents the degradation of HJV and increases endogenous hepcidin expression	• Ongoing Phase 2a trial in phlebotomy-dependent PV patients (NCT05143957)
SLN124	Tmprss6 siRNA		• Phase 1/2 trial in PV (NCT05499013) planned to begin in 2023 • Ongoing phase 1 study in alpha/beta thalassemia and low risk MDS (NCT04718844)
Vamifeport	Ferroportin inhibitor	Orally bioavailable small molecular directly inhibits ferroportin	• Ongoing phase 2 trial (NCT04817670) in sickle cell disease • Preclinical evidence in PV [67▪▪]

HJV, hemojuvelin; MDS, myelodysplastic syndrome; PV, polycythemia vera; siRNA, small interferring ribonucleic acid; Tmprss6, transmembrane serine protease 6.

Handa S, Curr Opin Hematol 2023

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Rusfertide for the treatment of iron overload in HFE-related haemochromatosis: an open-label, multicentre, proof-of-concept phase 2 trial

Figure 1. A. Liver Iron Concentration measured by MRI prestudy and following rusfertide; B. Phlebotomy rate prestudy and following rusfertide

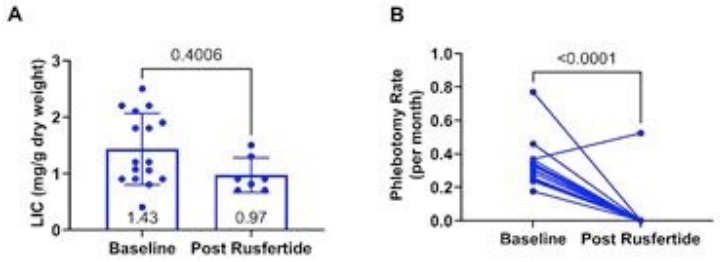
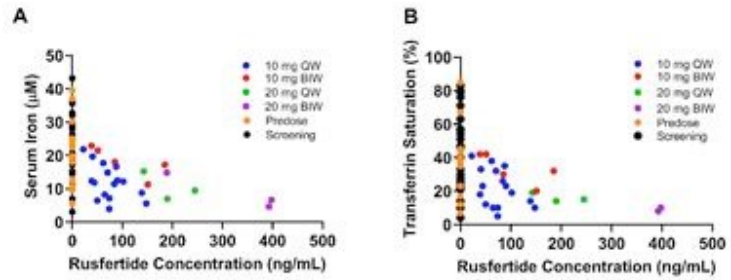


Figure 2. Rusfertide results in a Dose- and Concentration-Dependent decrease in (A) Serum Iron and (B) Transferrin Saturation



16 pts. with HFE-HH who were in the maintenance phase of phlebotomy therapy.

24 weeks of subcutaneous rusfertide 10 mg once weekly.

Proof-of-concept

Kowdley KV, Lancet 2024

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Take-home messages

1. **DIAGNOSIS OF RARE FORMS OF H. IS ESSENTIALLY CLINICAL (based on: ↑ SF and TSAT, plus clinical/imaging/pathological evidence of IO, with no alternative explanation).**

2. **MOLECULAR DIAGNOSIS BY NGS IS USEFUL (consultation with a referral center recommended) BUT NOT REQUIRED FOR STARTING TREATMENT.**

3. **TREATMENT GENERALLY SIMILAR TO CLASSICAL H., but often requires personalization in advanced/challenging cases).**

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