Le emocromatosi rivisitate

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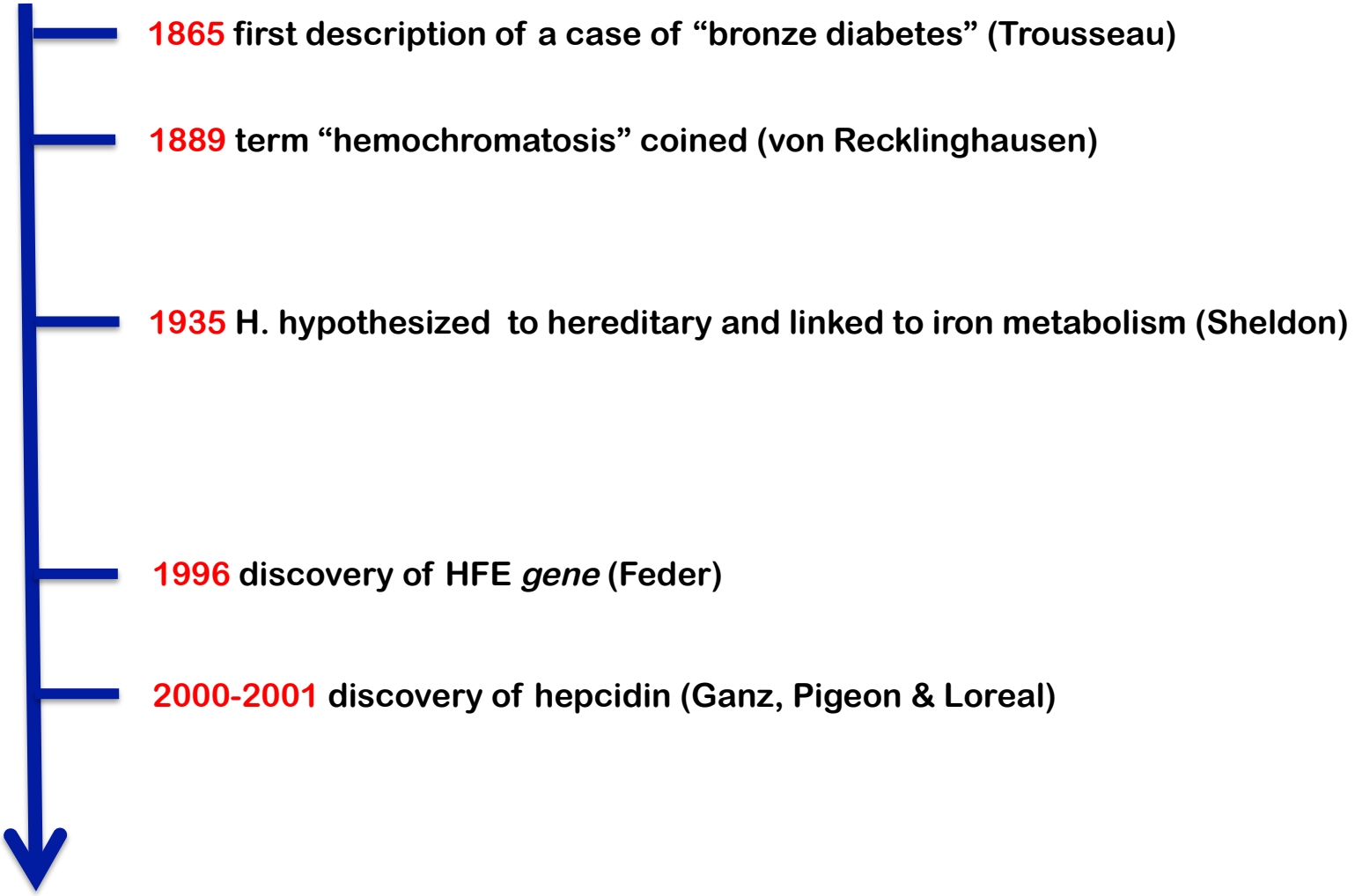
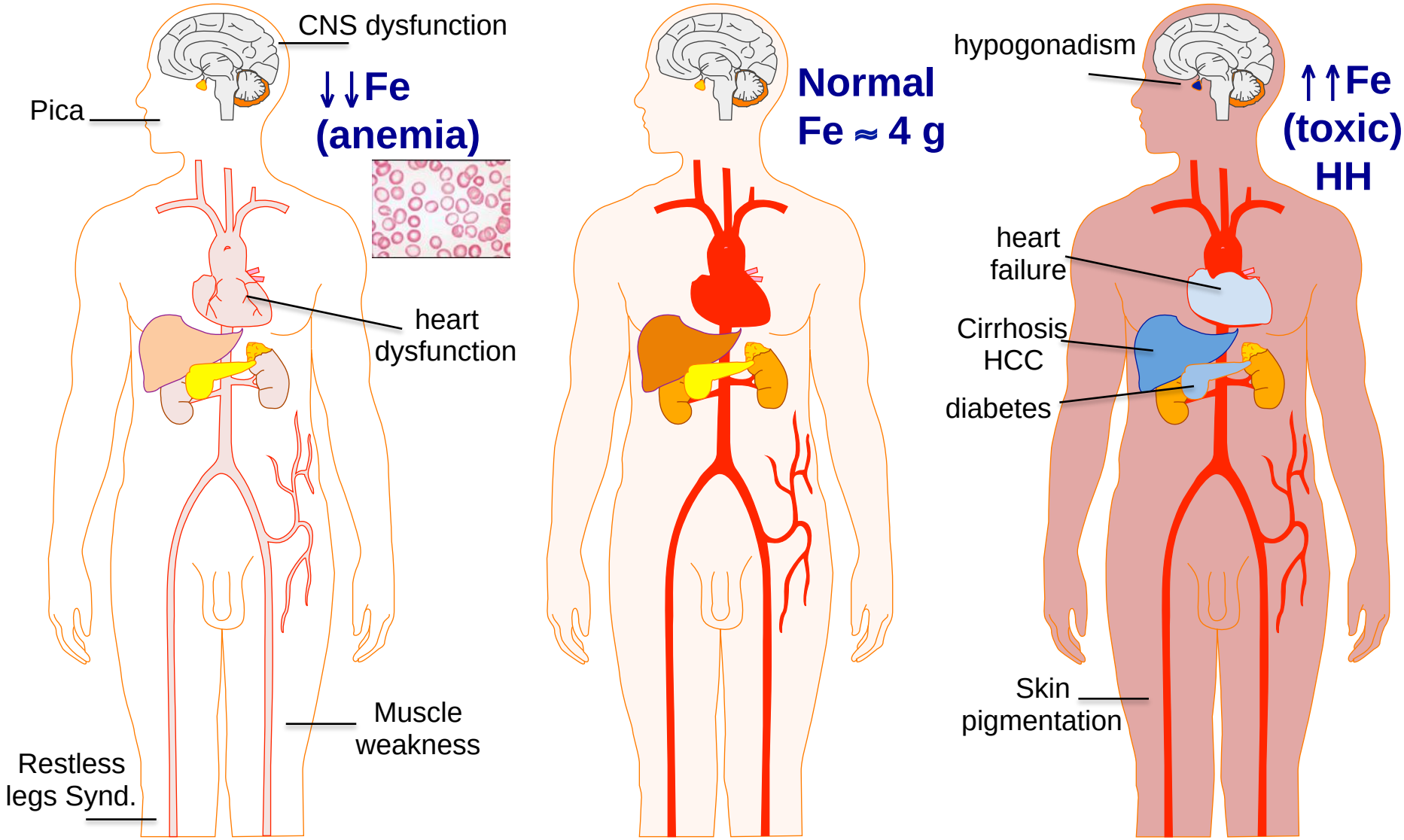
Conflict of interest

Nothing to disclose

Timeline of Hemochromatosis research



Iron: an essential micronutrient that needs strict regulation

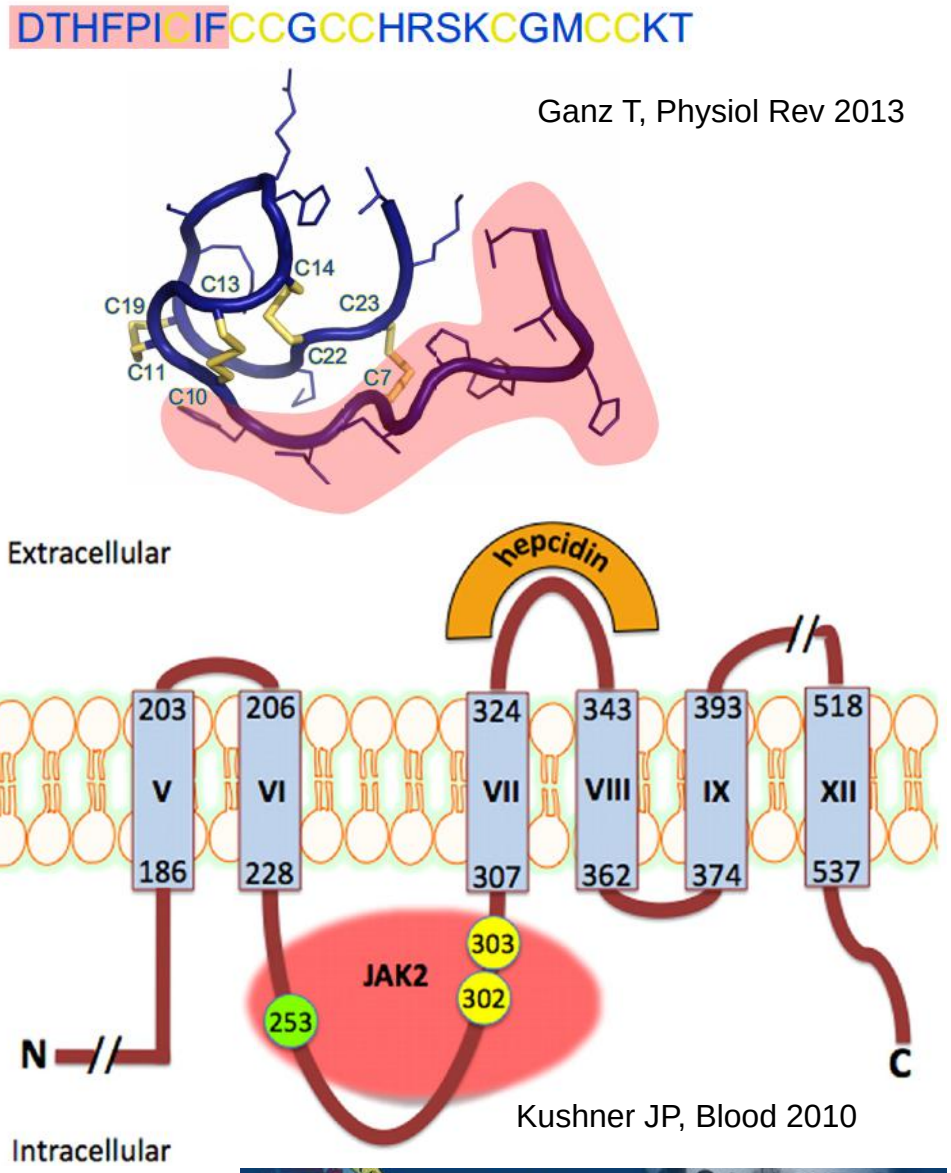


HEP-(atic) CIDIN (antimicrobial)

- small (25 aa) peptide mainly produced by the **liver**

- defensin-like (innate immunity-related peptides with natural antimicrobial activity)

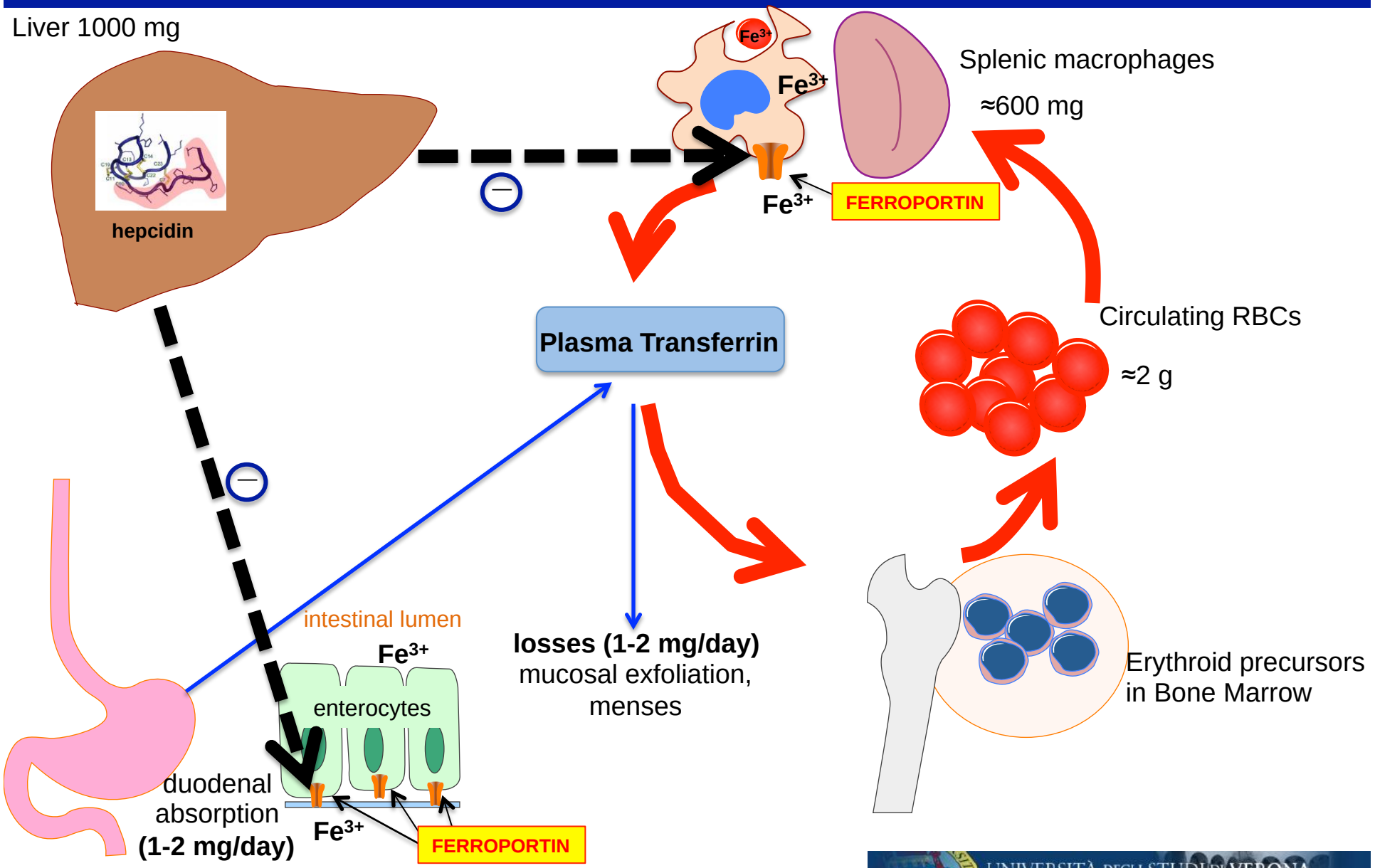
- interact with **its receptor** (**Ferroportin**, the only known iron exporter from the cells, ubiquitous but highly expressed in **duodenal cells**, **macrophages**, **hepatocytes**)



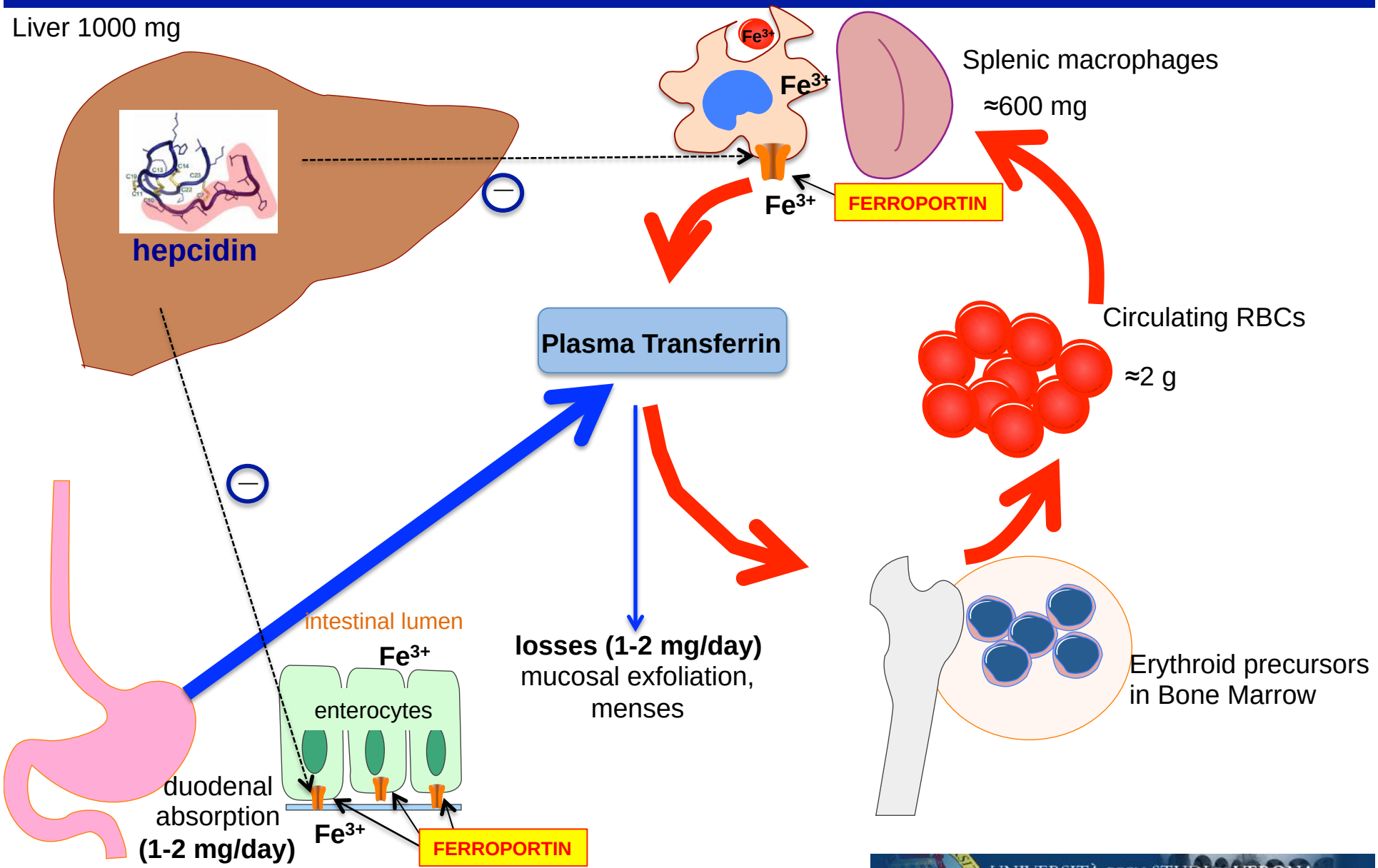
Ganz T, Physiol Rev 2013

Kushner JP, Blood 2010

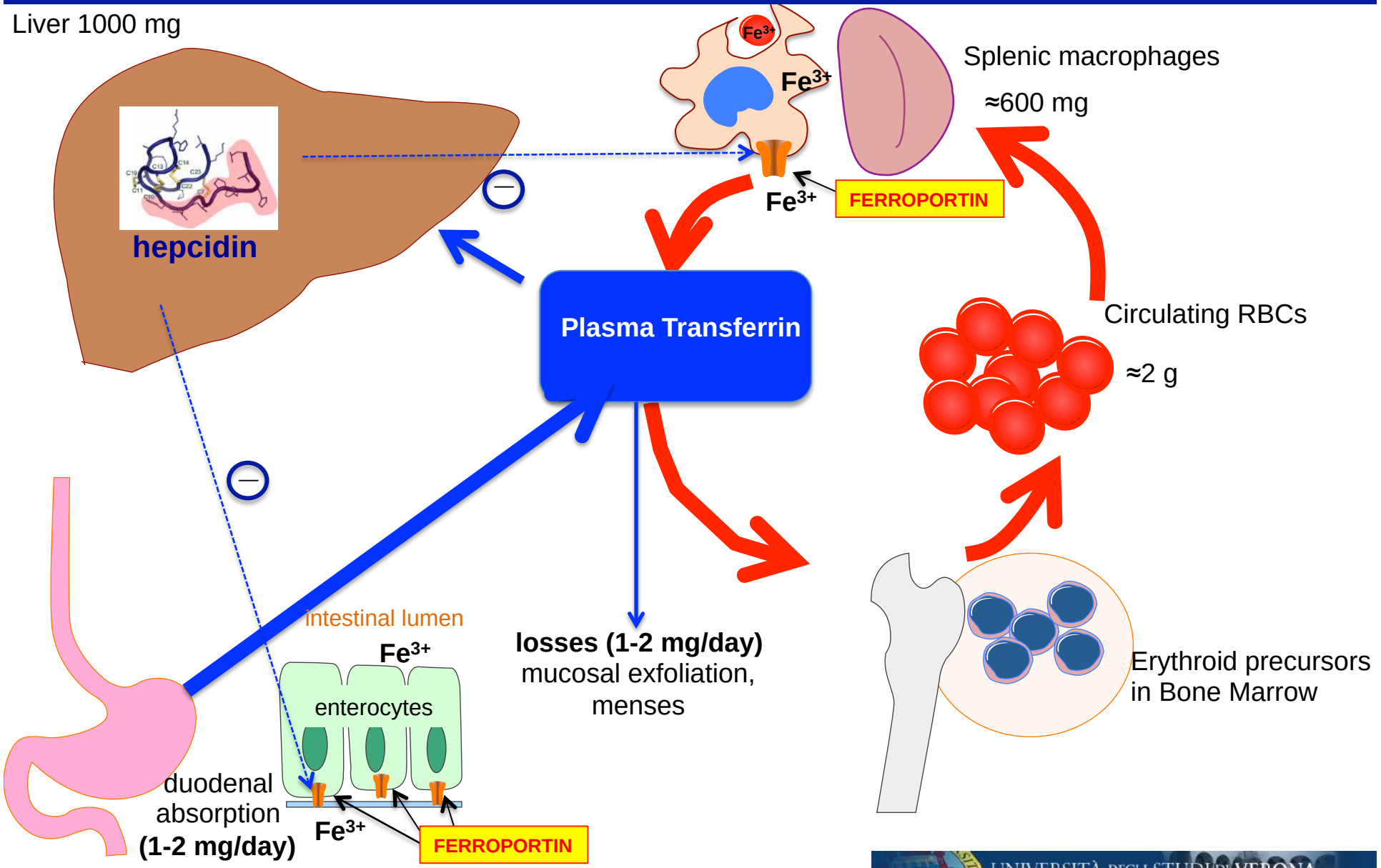
IRON "ECOLOGY"



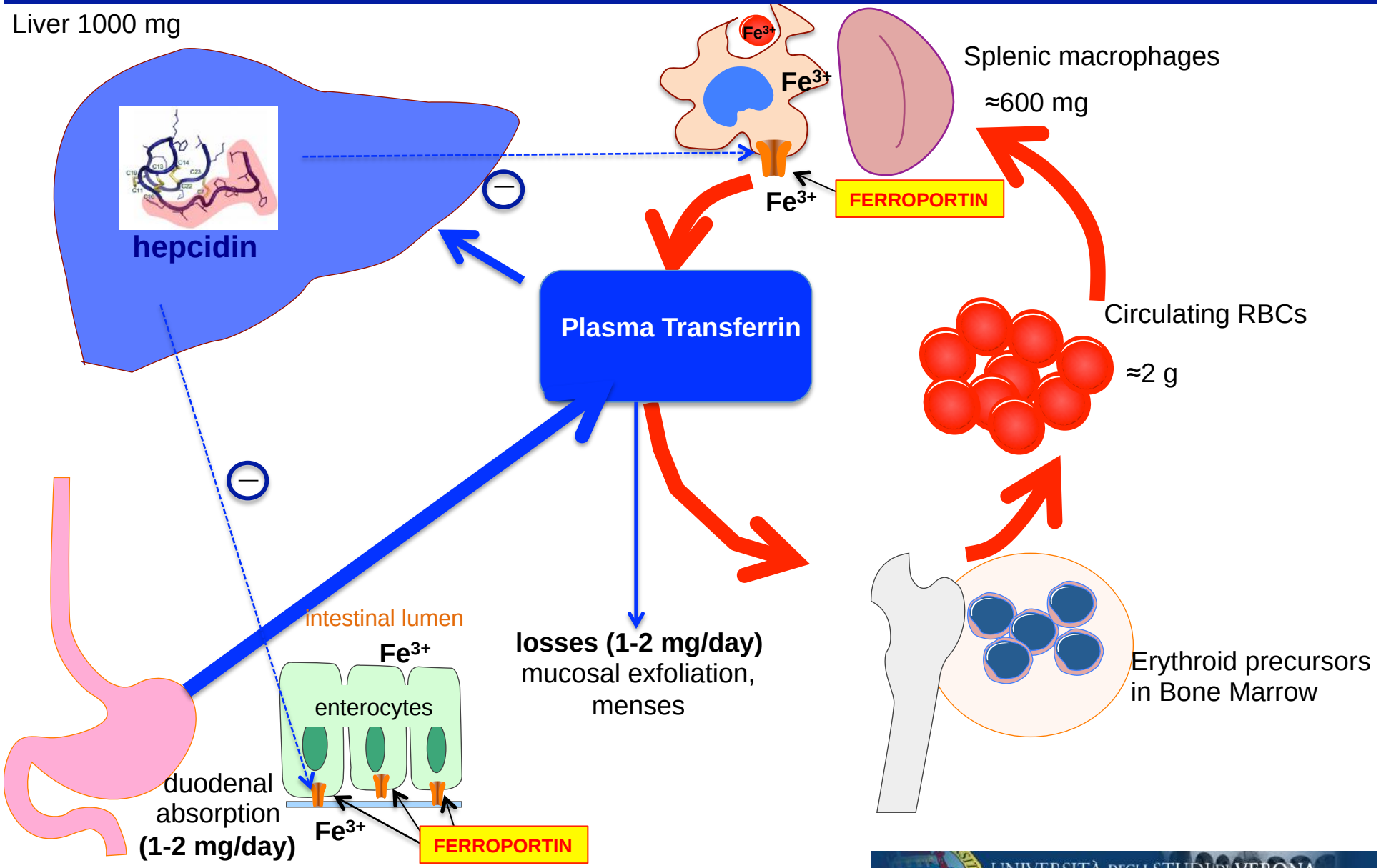
(genetically determined) Hepcidin deficiency leads to hemochromatosis



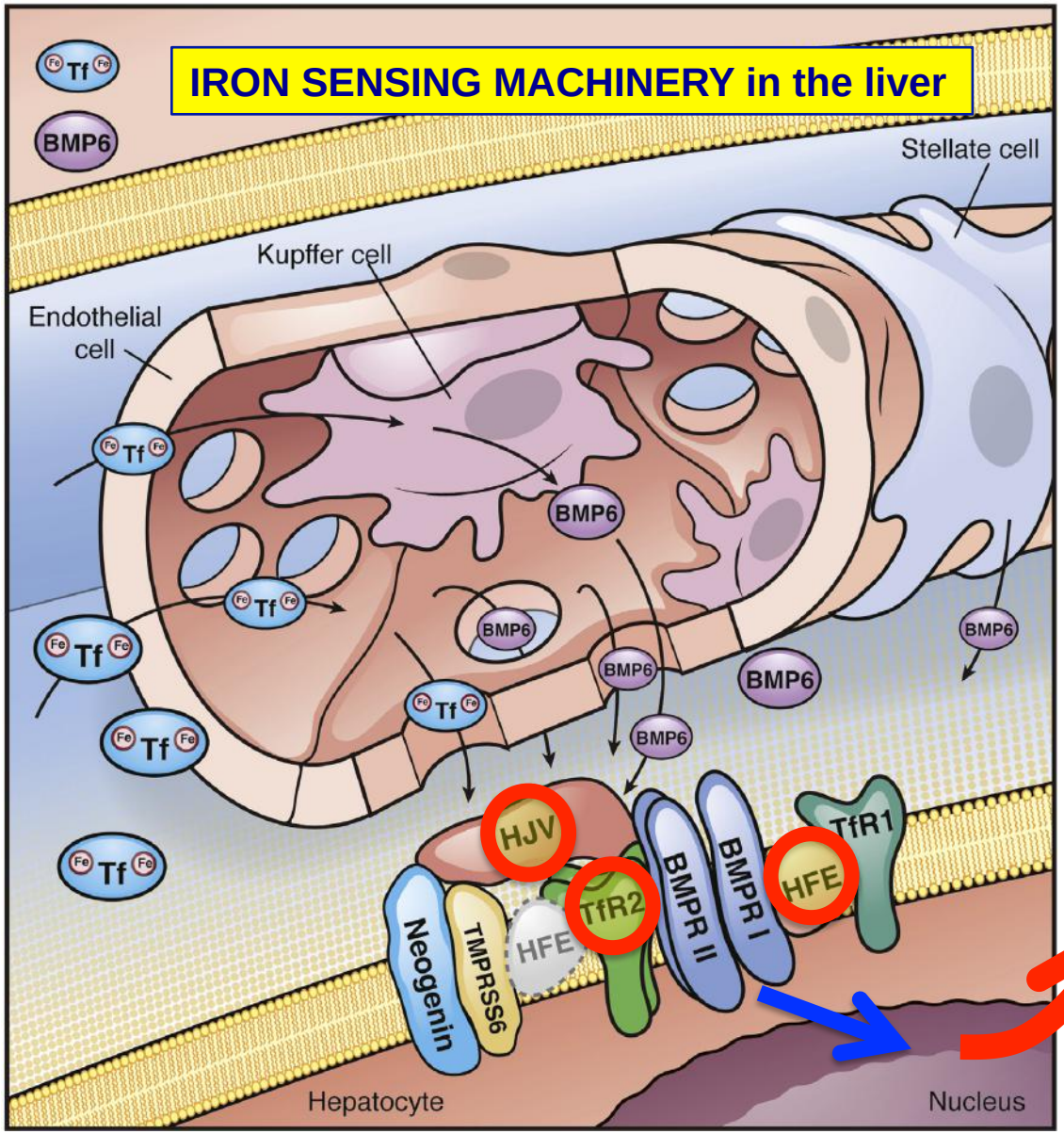
Genetic defects of hepcidin leads to hemochromatosis



Genetic defects of hepcidin leads to hemochromatosis



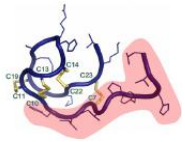
Mechanisms of hepcidin deficiency (or resistance)



Mutations in any of the genes encoding proteins involved in iron sensing (***HFE***, ***TFR2***, ***HJV***)…

…but also in the genes encoding for hepcidin (***HAMP***) or its receptor ferroportin (***GoF*** mut. on ***SLC40A1**** → hepcidin-**resistance**).

hepcidin transcription



Pietrangelo A, Gastroenterology 2015

HH: a genetically heterogenous disorder

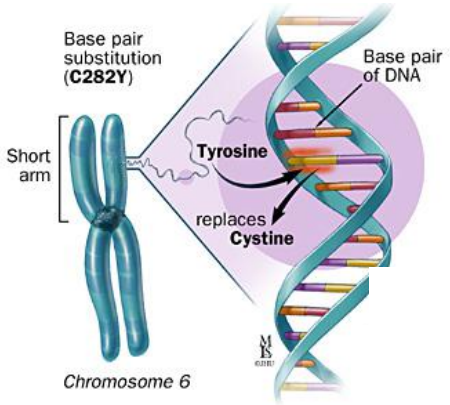
Table 1. Heritable Forms of Systemic Iron Overload According to the Pathophysiological Defect.*

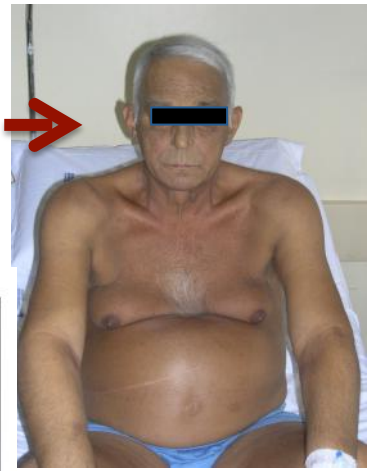
Disorder	Gene and Inheritance	Age at Presentation	Neurologic Symptoms	Anemia	Transferrin Saturation
Impaired hepcidin–ferroportin axis					
HH type I	*HFE*, AR	Adult	No	No	High
HH type IIA	*HFE2*, AR	Child to young adult	No	No	High
HH type IIB	*HAMP*, AR	Child to young adult	No	No	High
HH type III	*TFR2*, AR	Young adult	No	No	High
HH type IVA (atypical HH)	*FP* (LOF), AD	Adult	No	Variable	Low initially
HH type IVB	*FP* (GOF), AD	Adult	No	No	High
Impaired iron transport					
Inadequate release to erythron: aceruloplasminemia	*CP*, AR	Adult	Yes	Yes	Low
Inadequate uptake by erythron					
DMT1 mutations	*DMT1*, AR	Child	No	Yes	High
Hypotransferrinemia	*TF*, AR	Variable	No	Yes	High
Ineffective erythropoiesis					
Thalassemia	*Globin*, AR	Child	No	Yes	High
Congenital sideroblastic anemia	*ALAS2*, XL; *SLC25A38*, AR; *GLRX5*, AR; *ABCB7*, XL	Variable	*ALAS2* and *SLC25A38*: no; *GLRX5* and *ABCB7*: yes	Yes	High
Congenital dyserythropoietic anemia					
Type I	*DAN1*, AR	Child	No	Yes	High
Type II	*SEC23B*, AR	Child	No	Yes	High
Type III	Unknown, AD	Child	No	Yes	High

Fleming RE, N Engl J Med 2012

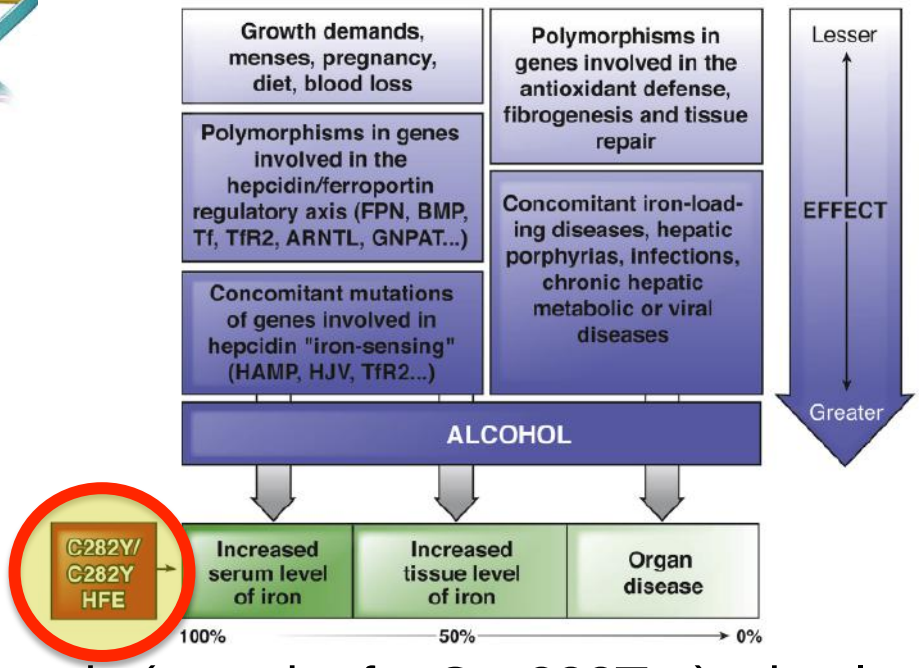
Type 1 (*HFE*-related, “classic”) HH

The commonest genetic disorder in European populations (carriers ≈ 1:200)





Recessive T
> Males
IV-V decades

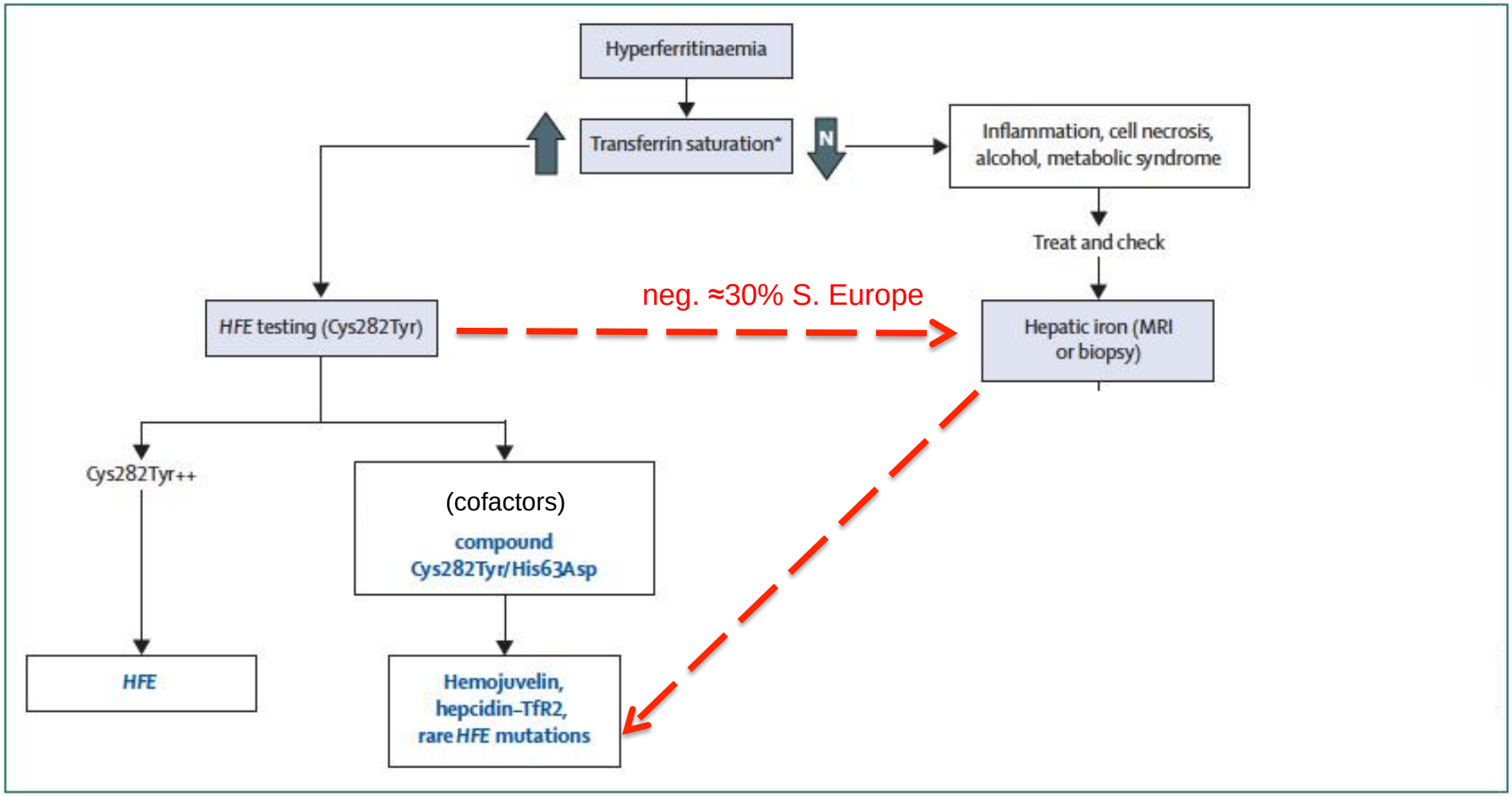


↓↓ clinical penetrance influenced by environmental/genetic (largely unknown) factors

Pietrangelo A, Gastroenterology 2015

Molecular diagnosis (search of p.Cys282**Tyr**): simply through a widely available 1st level genetic test

Diagnostic algorithm for hemochromatosis



Powell LW, Lancet 2015 (adapted)

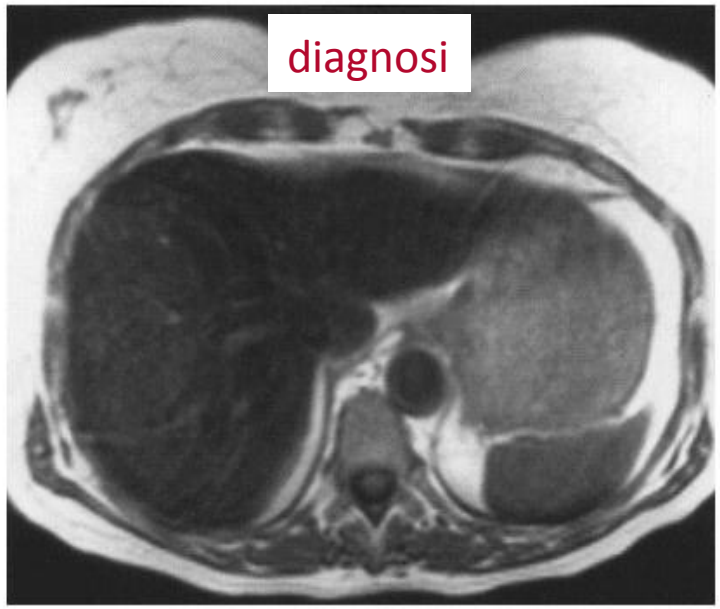
Imaging (determinazione Liver Iron Content)

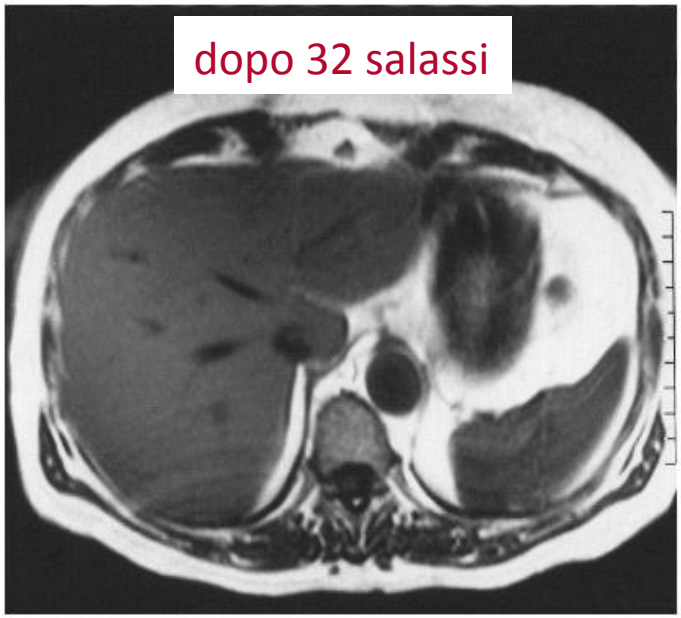
ECOGRAFIA

- aspecifica (↑ ecogenicità spesso attribuita a “steatosi”)

RISONANZA MAGNETICA

- ↓ segnale T_1-T_2 , “biopsia magnetica”.





calcolo del LIC con protocollo di Gandon (Lancet 2004), oppure con T2*
Sovraccarico significativo (tipico dell’EE): ≥100 μM/g.
Valori tipicamente inferiori nel DIO. V.N. 30±30 μM/g

Biopsia Epatica

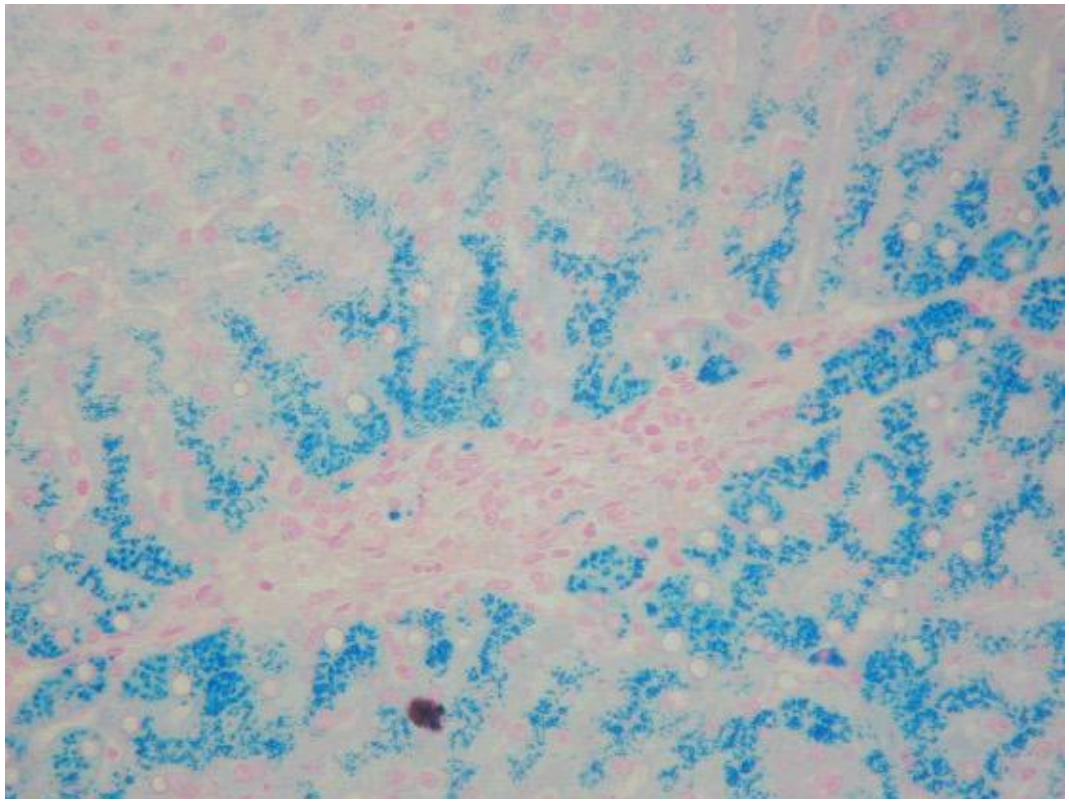
Gold Standard, in quanto valuta:

• [Fe] epatico/età = Hepatic Iron Index)

• grading istologico (Perl's): da 0 a 4+

• distribuzione del ferro (epatociti **periportali**, biliociti, SRE)

• presenza di fibrosi, cirrosi e/o lesioni associate
→ stratificazione prognostica



Girelli D, Gastroenterology 2002

INDICAZIONI (consensus):

• **ferritina ≥1.000 μg/l** anche in omozigoti C282Y(↑ probabilità fibrosi/cirrosi)

• **ferritina < 1000 μg/l** senza spiegazione alternativa (e test genetico di I livello non diagnostico.), sopr. se **↑ persistente AST e/o ALT**

Morrison, Ann Intern Med 2003

"Non-*HFE* HH": common features

Diagnosis of exclusion *in pts. with a consistent IO phenotype and negative 1st level genetic test*

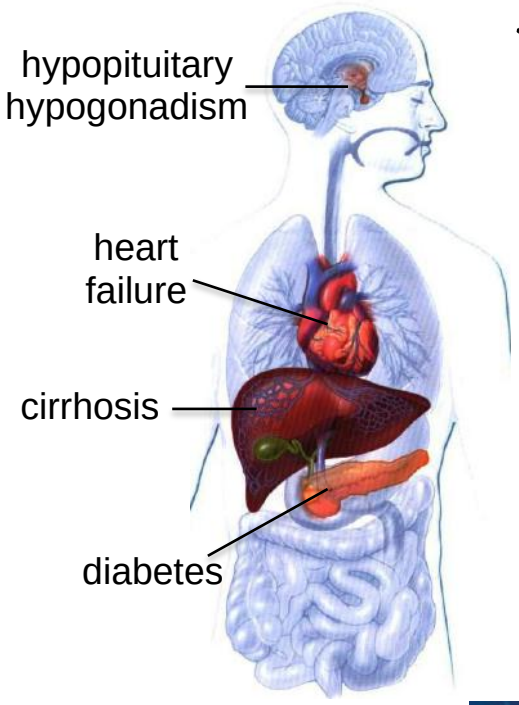
- ✓ Far more rare than type 1 (*HFE*-related) HH
- ✓ Worldwide distribution (not restricted to Northern EU descent - immigrants)
- ✓ Mostly private mutations in at least 4 other genes (*HJV*, *HAMP*, *TFR2*, *SLC40A1**) → difficult molecular diagnosis

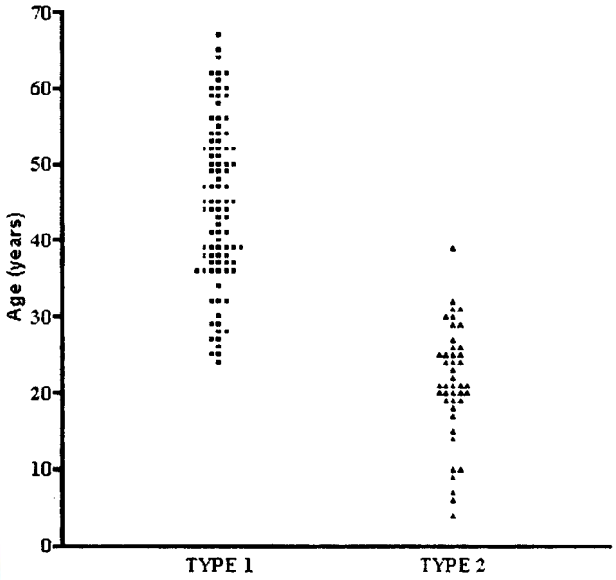
**Gain of Function*

Type 2 HH: “Juvenile” hemochromatosis (JH)

well-defined clinical entity (known since 1950’s)* characterized by:

- ✓ Early onset (usually **II decade**)
- ✓ **Equally high penetrance in M and F** , → **severe** multi-organ damage. Negligible influence of acquired cofactors.





Camaschella C, Semin Hematol 2002

*Nussbaum T, J Genet Hum 1951

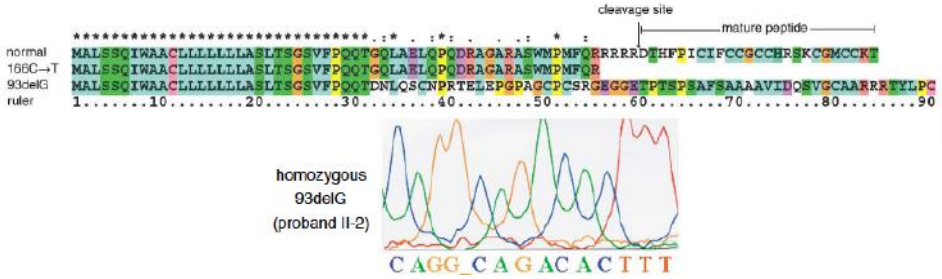
Molecular basis of JH: *HJV or HAMP* mutations

- ✓ most frequently due to *HJV* mutations (Type 2A HH).

  More than 50 pedigree reported.

- ✓ One recurrent mutation G320V (present in ≈ 50% cases)

- ✓ Type 2B HH (*HAMP* mutations): much more rare (consanguinity).



Roetto A, Nat Genet 2003

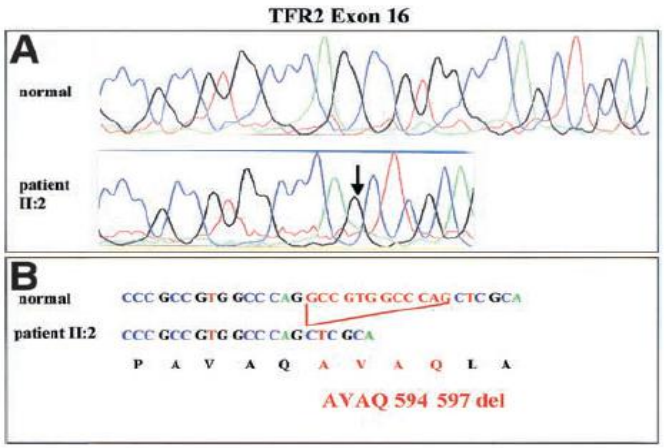
Table 1 | Mutations of the *HJV* gene linked to JH.

Residue mutation	Exon	Type of mutation	Nucleotide change	Family origin	References
Q6H	2	Missense	18G > C	Asian	Huang et al., 2004
L27fsX51	2	Frame shift	81delG	English/Irish	Wallace et al., 2007
R54X	3	Nonsense	160A > T	African American	Murugan et al., 2008
G66X	3	Nonsense	196G > T	Romanian	Jánosi et al., 2005
V74fsX113	3	Frame shift	220delG	English	Lanzara et al., 2004
C80R	3	Missense	238T > C	Caucasian	Lee et al., 2004
S85P	3	Missense	253T > C	Italian	Lanzara et al., 2004
G99R	3	Missense	295G > A	Albanian	Lanzara et al., 2004
G99V	3	Missense	296G > T	Multiple	Papanikolaou et al., 2004; Silvestri et al., 2007
L101P	3	Missense	302T > C	Albanian	Lanzara et al., 2004; Lee et al., 2004
G116X	3	Nonsense			Santos et al., 2012
C119F	3	Missense	G356 > T	German	Gehrke et al., 2005; Silvestri et al., 2007
R131fsX245	3	Frame shift	391-403del	Italian	Lanzara et al., 2004
D149fsX245	3	Frame shift	445delG	Italian	Lanzara et al., 2004
L165X	3	Nonsense	494T > A		van Dijk et al., 2007
A168D	3	Missense	503C > A	Australian /English	Lanzara et al., 2004
F170S	3	Missense	509T > C	Italian	De Gobbi et al., 2002; Lanzara et al., 2004; Silvestri et al., 2007
D172E	3	Missense	516C > G	Italian	Lanzara et al., 2004
R176C	3	Missense	526C > T	European	Aguilar-Martinez et al., 2007; Ka et al., 2007
W191C	3	Missense	573G > T	Italian	De Gobbi et al., 2002; Lanzara et al., 2004; Silvestri et al., 2007
N198K	3	Missense	586T > G		Santos et al., 2012
S205R	3	Missense	615C > G	Italian	Lanzara et al., 2004
I222N	4	Missense	665T > A	Canadian	Papanikolaou et al., 2004
K234X	4	Nonsense	700-703AAG del	European	Santos et al., 2012
D249H	4	Missense	745G > C	Asian	Santos et al., 2012
G250V	4	Missense	749G > T	Italian	Lanzara et al., 2004
N269fsX311	4	Frame shift	806 > 807insA	English	Lanzara et al., 2004
I281T	4	Missense	842T > C	Multiple	Huang et al., 2004; Papanikolaou et al., 2004
C282Y	4	Missense		Caucasian	Le Gac et al., 2004
R288W	4	Missense	863C > T	French	Lanzara et al., 2004
R288Y	4	Missense	862C > T		Wallace et al., 2007
E302K	4	Missense	904G > A	Brazilian	Santos et al., 2011
A310G	4	Missense	929C > G	Brazilian	de Lima Santos et al., 2010; Santos et al., 2011
Q312X	4	Nonsense	934C > T	Asian	Nagayoshi et al., 2008
G319fsX341	4	Frame shift	954-955insG	Italian	Lanzara et al., 2004
G320V	4	Missense	959G > T	Multiple	Lanzara et al., 2004; Papanikolaou et al., 2004; Gehrke et al., 2005; Silvestri et al., 2007; Santos et al., 2011
C321W	4	Missense	963C > G	European	Wallace et al., 2007
C321X	4	Nonsense	962G > A, 963C > A	Asian	Huang et al., 2004; Santos et al., 2012
R326X	4	Nonsense	976C > T	Asian	Huang et al., 2004; Papanikolaou et al., 2004
S328fsX337	4	Frame shift	980-983 delTCTC	Slovakian	Gehrke et al., 2005
R335Q	4	Missense	1004G > A		Wallace et al., 2007
C361fsX366	4	Frame shift	1080delC	European	Papanikolaou et al., 2004
N372D	4	Missense	1114A > G		Wallace et al., 2007
R385X	4	Nonsense	1153C > T	Italian	Lanzara et al., 2004; Santos et al., 2012

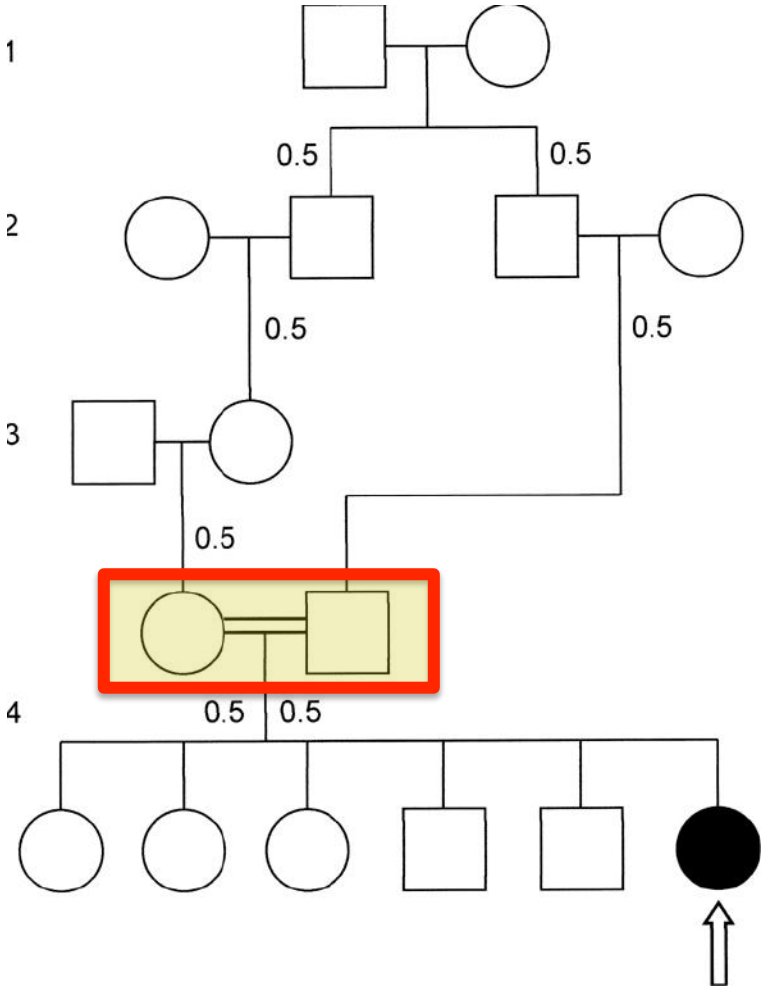
Core AB, Front Pharmacol 2014

TFR2-related type 3 HH

- ✓ Similar to **adult** onset, HFE-HH
- ✓ Tendency toward **earlier** presentation than type 1 HH
- ✓ Often detected in offspring of inbred parents (**consanguinity**).

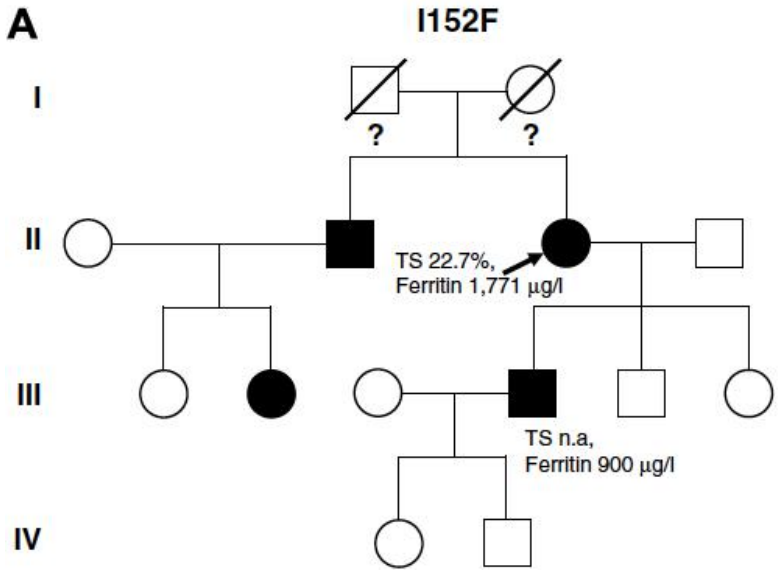


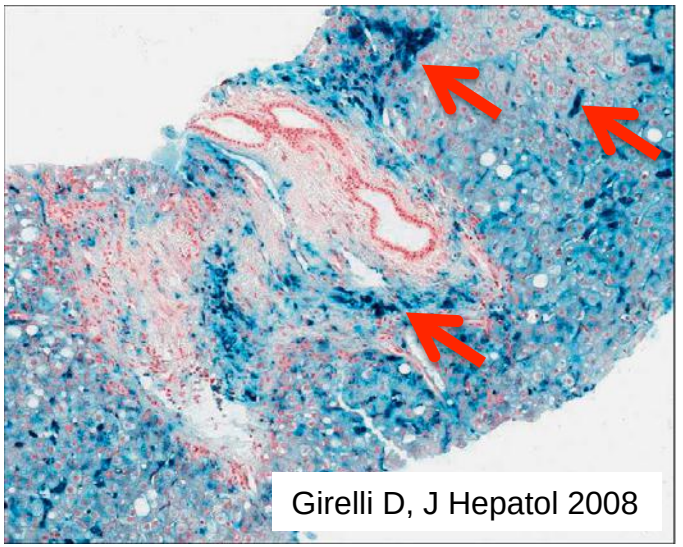
Girelli D, Gastroenterology 2002



Ferroportin Disease (FD)

- ✓ **Most common non-HFE** hereditary iron loading disorder
- ✓ Autosomal **dominant** transmission (critical clue to right diagnosis)
- ✓ Type 4A (most common) with **distinct biochemical phenotype (↑↑↑ ferritin and normal TS%)**.
- ✓ Early/Predominant iron deposition in **Kupffer cells**
- ✓ **Standard phlebotomies** sometimes **poorly tolerated (personalization needed)**
- ✓ Tendency toward **milder** clinical course, i.e. low risk of overt cirrhosis and HCC (debated)

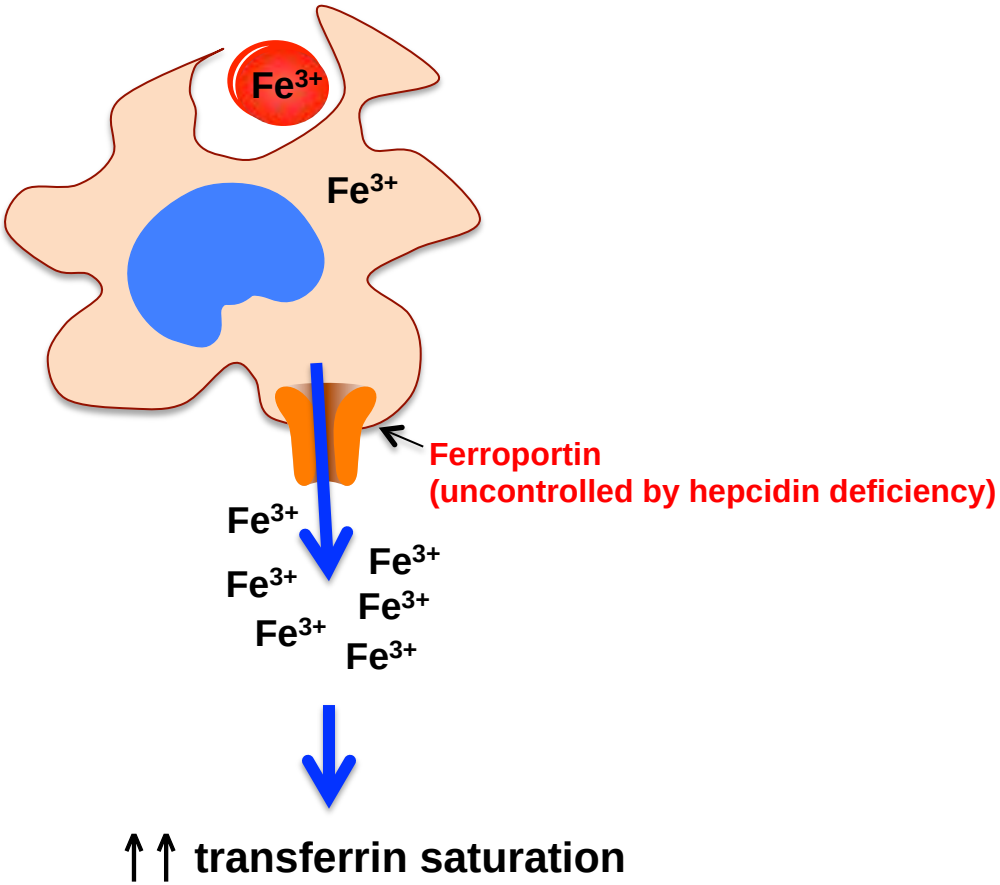




Girelli D, J Hepatol 2008

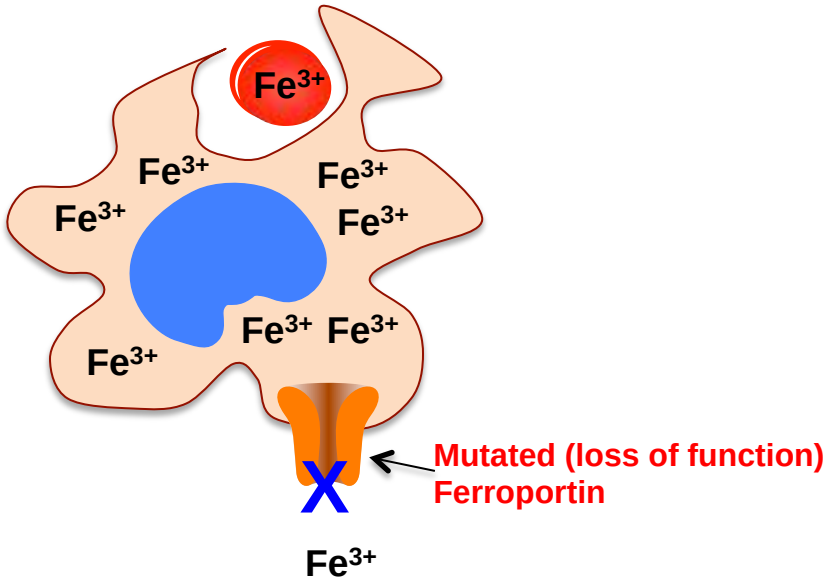
Macrophages in HFE-related HH vs FD

Type 1 (HFE) HH



↑↑ transferrin saturation

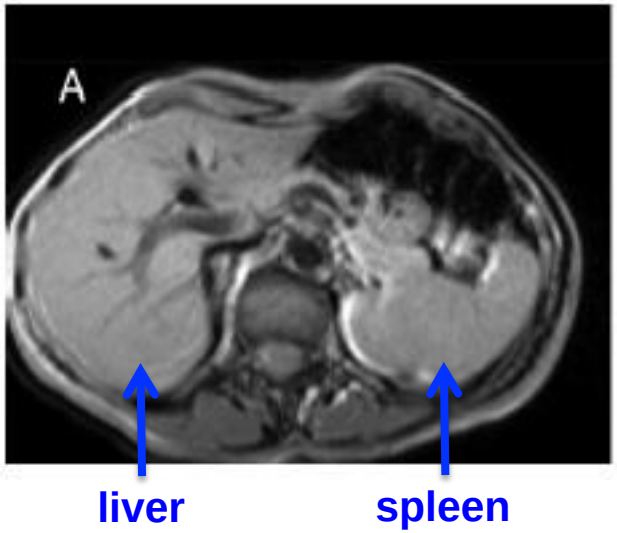
Type 4A HH (FD)



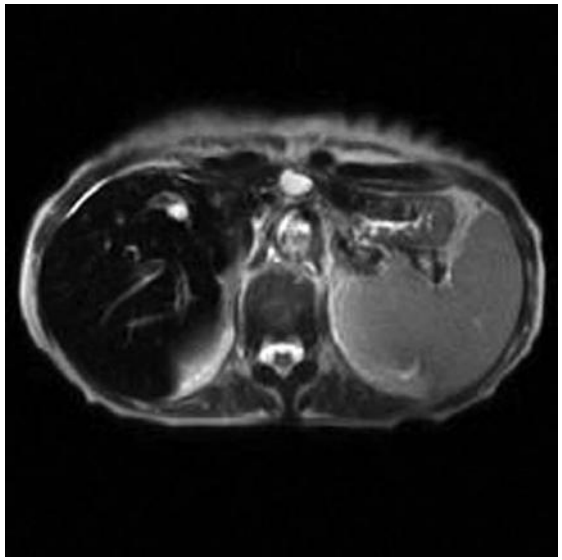
Normal to ↓ transferrin saturation

MRI as a clue for Type 4A HH

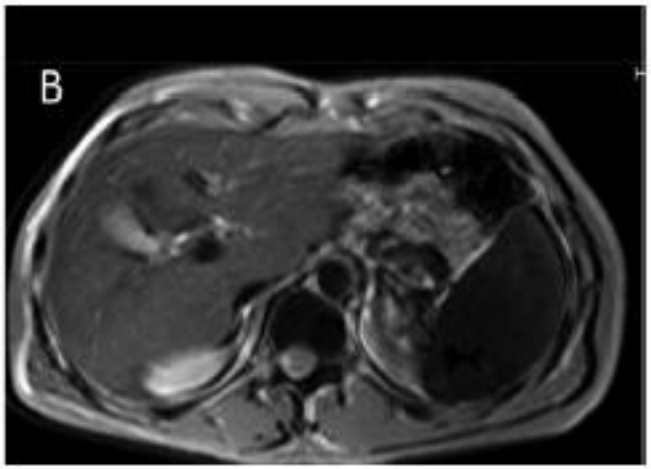
(T2 weighted gradient echo sequences)



Control subject: normal signal in both liver and spleen



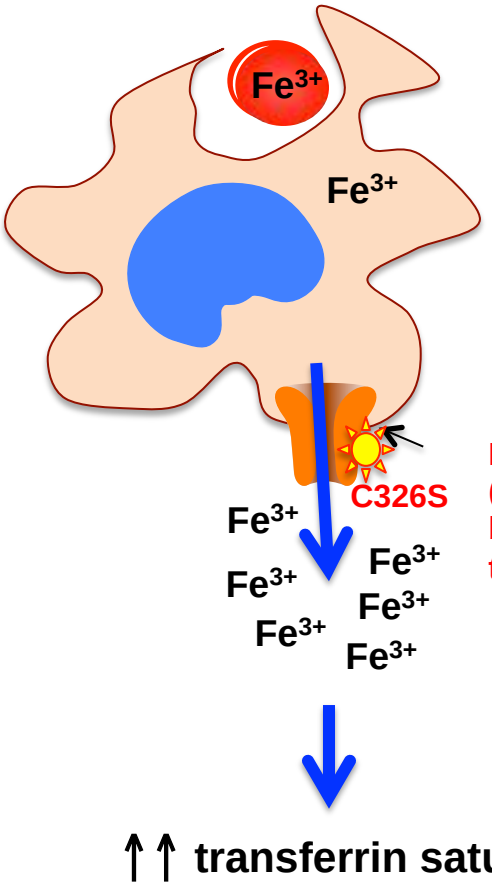
HFE-related HH: markedly attenuated signal (corresponding to IO) in the **liver**, but not in the spleen



Type IV HH (classic **Ferroportin Disease**): markedly attenuated signal in the **spleen**, but only moderately attenuated signal in the liver.

Pietrangelo A, BCMD 2006 (adapted)

Type 4B HH (hepcidin resistance)



Ferroportin
(distinct GoF mutations in critical hepcidin binding sites leading to insensitivity to internalization/degradation)

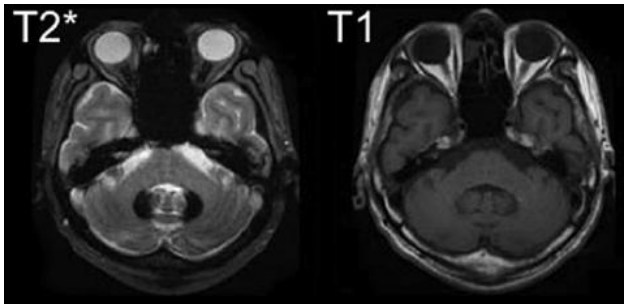
- ✓ **Clinical, biochemical and pathological features similar to typical HH* (hyperabsorption!).**
- ✓ **Hepcidin levels ↑ rather than ↓**

Sham RL, Blood 2009

“Atypical” iron overload disorders

Ferroportin Disease (*SLC40A1*)

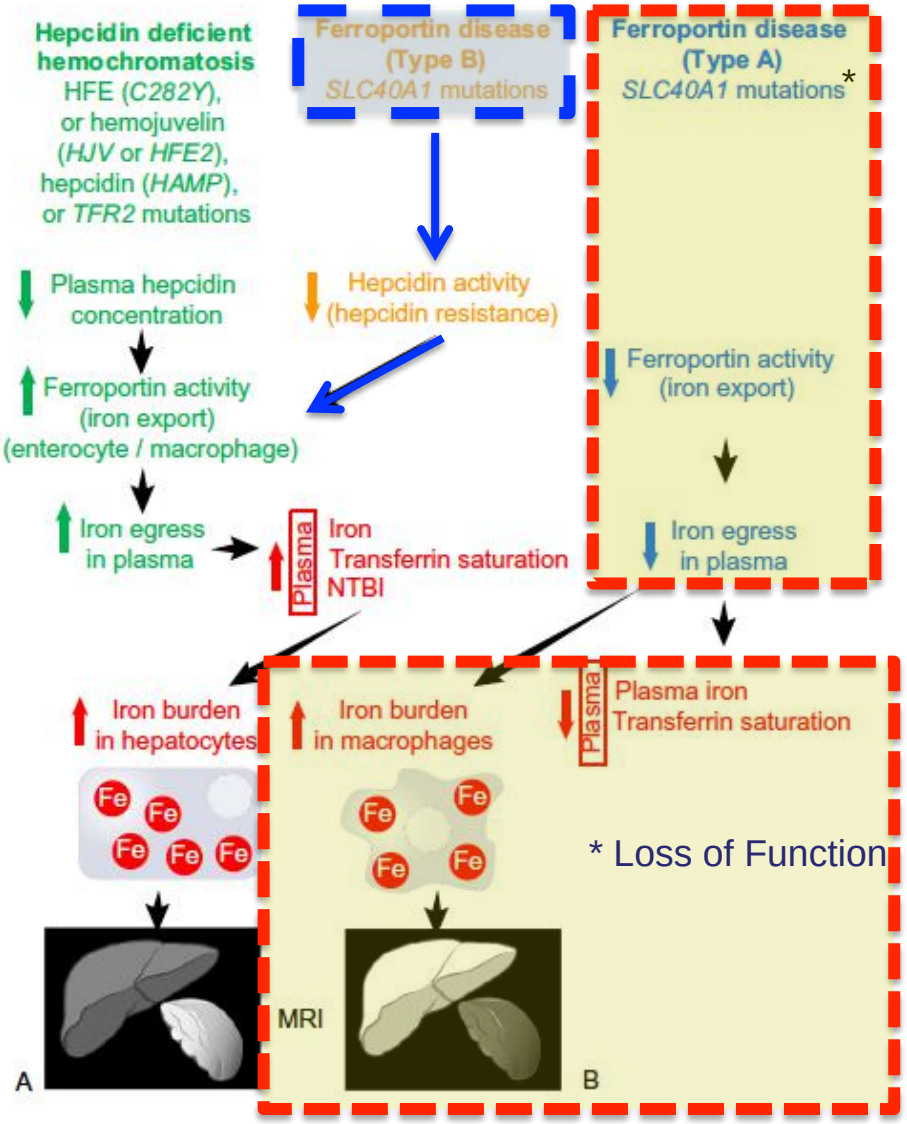
(Wallace DF, Genet Med 2015)



Miyajima H, Neuropathology 2015

✓ Aceruloplasminemia (*CP*)

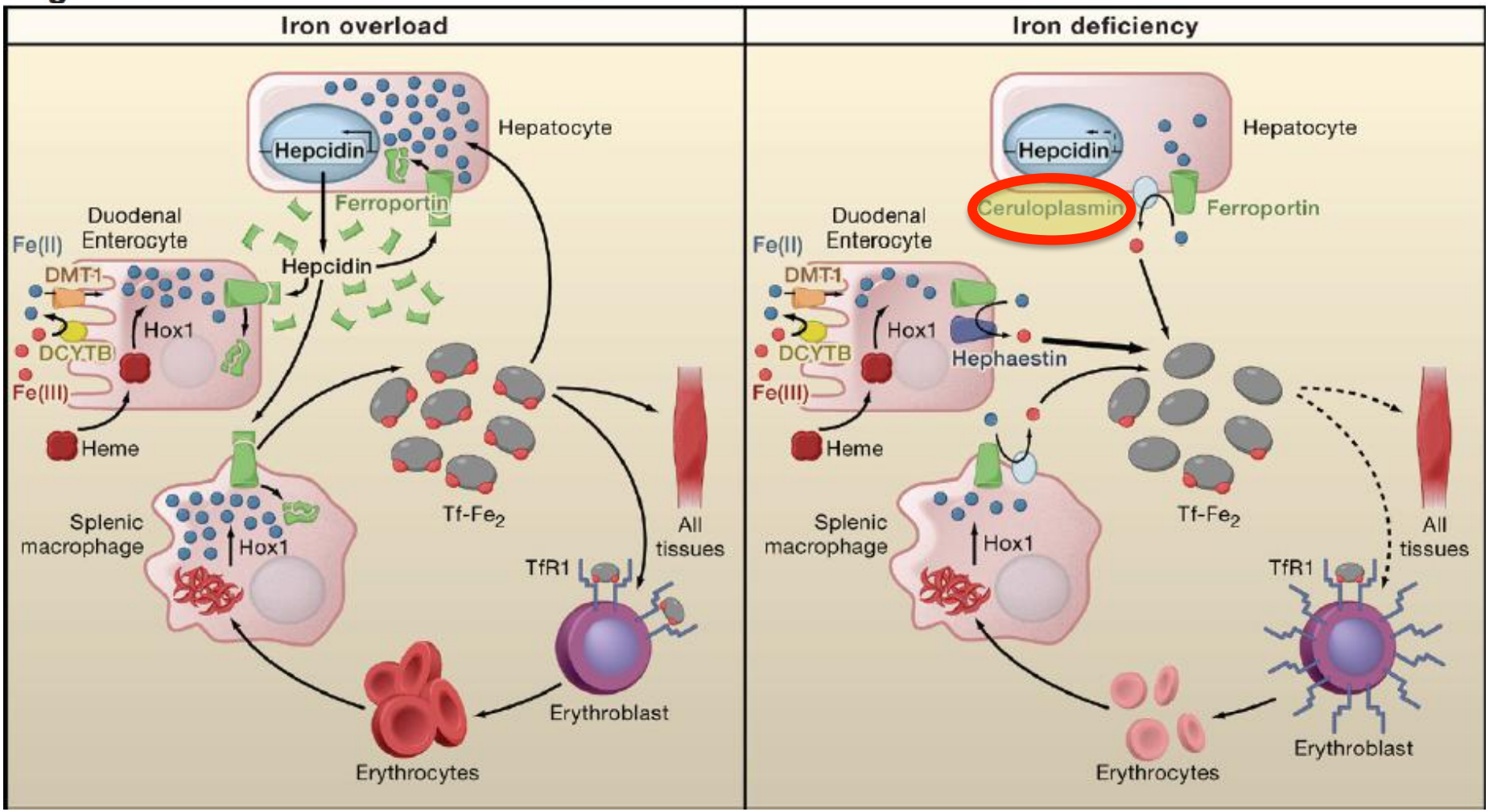
-**NBIA**
-retinitis
-diabetes
-**microcytic anemia**



Brissot P, J Hepatol 2016

Iron regulation at systemic level: the hepcidin/ferroportin axis

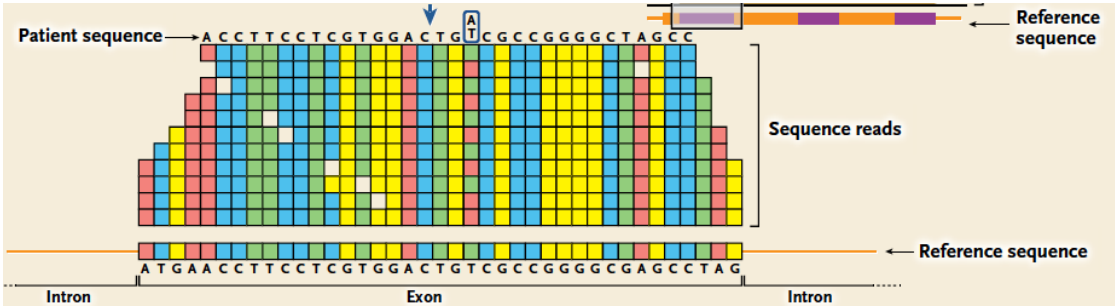
Two to Tango:
Regulation of Mammalian Iron Metabolism



Hentze MW, Cell 2010

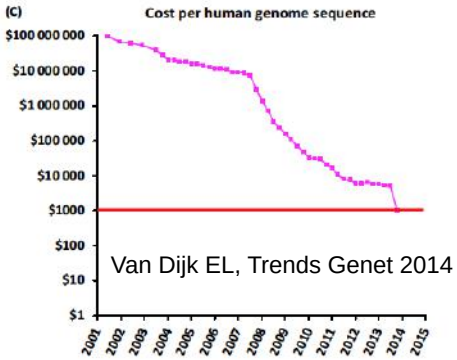
The advent of NGS: a revolution in molecular diagnosis

PROS-1: rapid, simultaneous high-coverage sequencing of target genes



Biesecker LG, N Engl J Med 2014

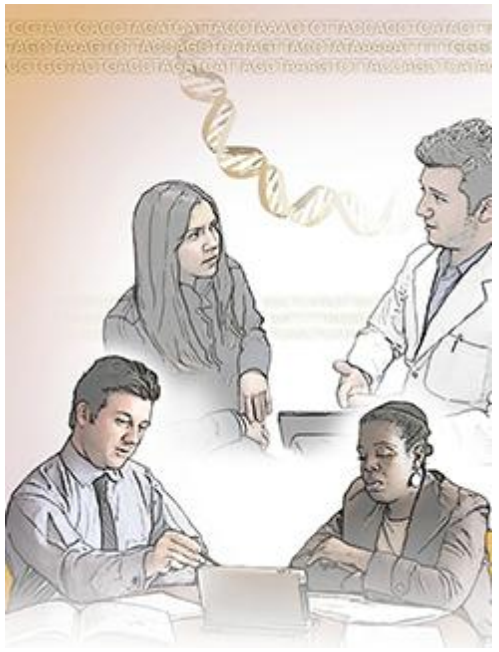
CONS-1: costs (though constantly ↓)



Van Dijk EL, Trends Genet 2014

CONS-2: lot of variants of uncertain pathogenic significance found → difficult interpretation!

Close/continuous interaction between geneticists, bioinformatics and clinicians needed.



Diagnosing non-*HFE* HH by NGS: recent experiences

Identification of novel mutations in hemochromatosis genes* AJH

*HAMP, HJV, HFE, TFR2

Pt. ID	1stlevel genetic test	TS, Ferritin	NGS	Reference	Clinical-molecular interpretation
#01	Wild-type[b]	80%, 1,450 µg l^{-1}	-	-	Remains unexplained
#02	H63D +/−	61%, >1,000 µg l^{-1}	HFE W163X +/−	Novel	Atypical Type 1 HH (HFE-related)
#03	H63D +/−	73%, >1,000 µg l^{-1}	HAMP R59X +/−	Novel	Digenic HH (HFE/HAMP)?
#04	Wild-type[b]	60%, 1,786 µg l^{-1}	-	-	Remains unexplained
#05	H63D +/+	n.a., 1,089 µg l^{-1}	TFR2 D555N +/−	Novel	Digenic HH (HFE/TFR2)?
#06	Wild-type[b]	100%, n.a.	TFR2 N241I +/+	Bardou-Jacquet E et al.[36]	Type 3 HH
#07	Wild-type[b]	48%, 2,352 µg l^{-1}	-	-	Remains unexplained
#08[c]	H63D +/−	95%, 6,242 µg l^{-1}	SLC40A1 A69T +/−	-	Type 4 HH

BMP6 in Humans: first data in Iron Overload Patients

BASIC AND TRANSLATIONAL—LIVER

Heterozygous Mutations in BMP6 Pro-peptide Lead to Inappropriate Hepcidin Synthesis and Moderate Iron Overload in Humans

- **Late onset, moderate iron overload patients**
- **Presence of co-factors**
- **Negative for mutations in the 5 HH genes**
- **(inappropriate) Normal or slightly high serum hepcidin**

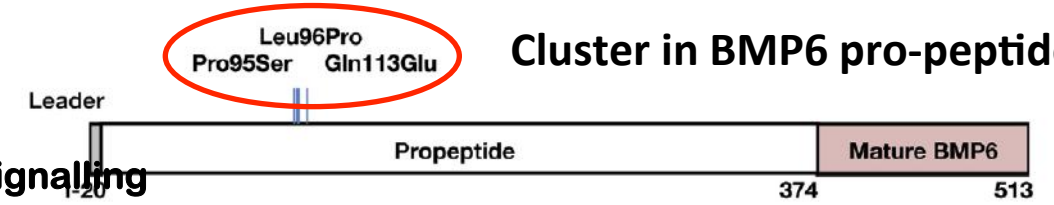
Table 1. Clinical and Biological Data of the Patients With BMP6 Mutations

	Initial cohort						Replication cohort	
BMP6 mutation	p.Pro95Ser (1 proband)	p.Leu96Pro (3 probands)		p.Gln113Glu (2 probands)			p.Leu96Pro (R1)	p.Gln113Glu (R2)
Sex	M	M	F	M	M	M	F	M
Weight, *kg*/height, *m*	73/1.73	68/1.73	55/1.64	106/1.73	89/1.71	71/1.78	83/1.5	78/1.71
Age at diagnosis, *y*	56	58	56	46	53	52	77	60
Transferrin saturation, %	26	38	40	99	92	41	70	51
LIC,[a] *µmol/g*	170	55	70	230	220	200	250	160
Serum ferritin level before/after phlebotomies, *µg/L*	900/55	700/150	481/148	4000/680	2358/808	800/117	1430/ND	830/ND
Serum hepcidin level by LC-MSMS before/after phlebotomies, *µg/L*	ND/16.4	32.9/ND	ND/7.6	30.6/ND	31.9/16.9	25/ND		
Serum hepcidin by ELISA before/after phlebotomies, *µg/L*							38/ND	62/ND
Phlebotomies, *mL*/iron removed, *g*	ND	10 × 300 /1.5	10 × 250/1.25	20 × 500/5	8 × 500/2	14 × 500 /3.5	ND	ND
Clinical symptoms, other factors	Arthralgia	None	None	None overweight	Alcohol	Arthralgia	Diabetes, porphyria cutanea tarda	Dyslipidemia

- **3 mutations in pro-peptide BMP6**

***In vitro* functional analysis:**

- **reduced BMP6 secretion**
- **Reduced Hepc production and SMAD signalling**
- **Dominant negative effect**



Cluster in BMP6 pro-peptide

Daher et al. *Gastroenterology* 2016

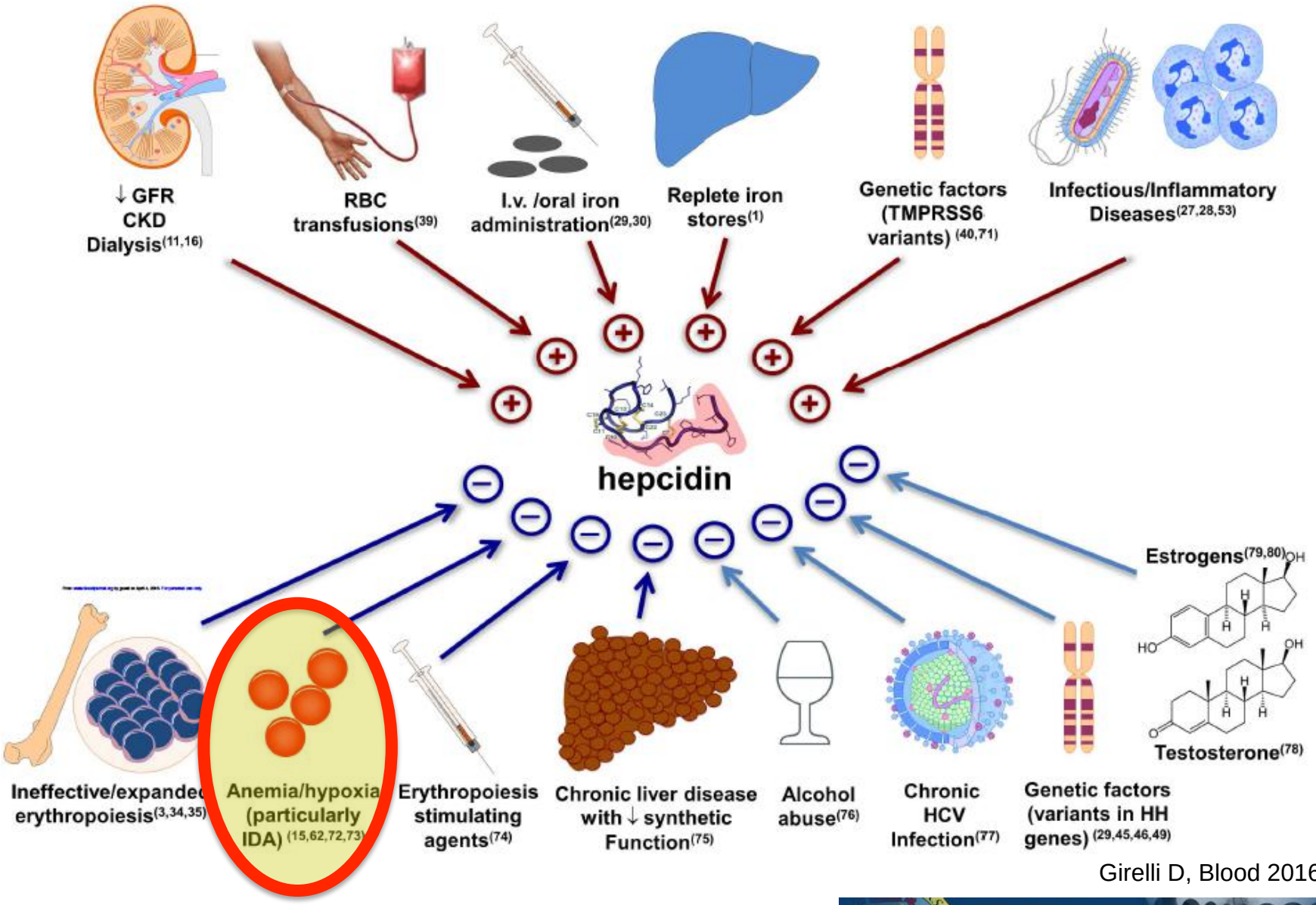
Main international recommendations on venesection therapy in HFE HH

	Initial therapy			Maintenance therapy
	Criteria for testing	Phlebotomy	Endpoint	Goal
France (2005)	SF >300 µg/L in men; SF >200 µg/L in women	5–7 mL/kg (<550 mL) once a week	SF <50 µg/L	SF <50 µg/L
Netherlands (2007)	Not precise	500 mL once a week	SF <300 µg/L	SF within the reference values
EASL (2010)	SF above the normal range	400–500 mL every 1 or 2 weeks	SF <300 µg/L	SF <50 µg/L
AASLD (2011)	Increased SF with or without clinical symptoms	500 mL once or twice a week	SF 50–100 µg/L	SF 50–100 µg/L

EASL=European Assocation for the Study of the Liver. AASLD=American Association for the Study of Liver Diseases. SF=serum ferritin.

Powell LW, Lancet 2015

Clinical conditions influencing circulating hepcidin levels



Girelli D, Blood 2016

Take-home messages

Le Emocromatosi rivisitate nell'era dell'epcidina:

- ✓ (almeno) 5 sotto-tipi
- ✓ diagnosi differenziale possibile (clinico-anamnestica + MRI/biopsia + NGS)
- ✓ Implicazioni pratiche (trattamento)

The Verona Interdisciplinary group on Iron Disorders

GRUPPO INTERDISCIPLINARE MALATTIE DEL FERRO

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http://www.gimferverona.org

Participants Units

1. Internal Medicine
2. Clinical Chemistry & Molecular Biology
3. Blood Bank / Transfusional Service
4. Radiology
5. Pathology
6. Gastroenterology

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Telethon

Ministero della Salute

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