Prof.dr.Hans Van Vlierberghe
Maag-, Darm- en Leverziekten

Diagnostiek en behandeling van metabole leverziekten.

UZ GENT

UNIVERSITEIT GENT

Disclosures.

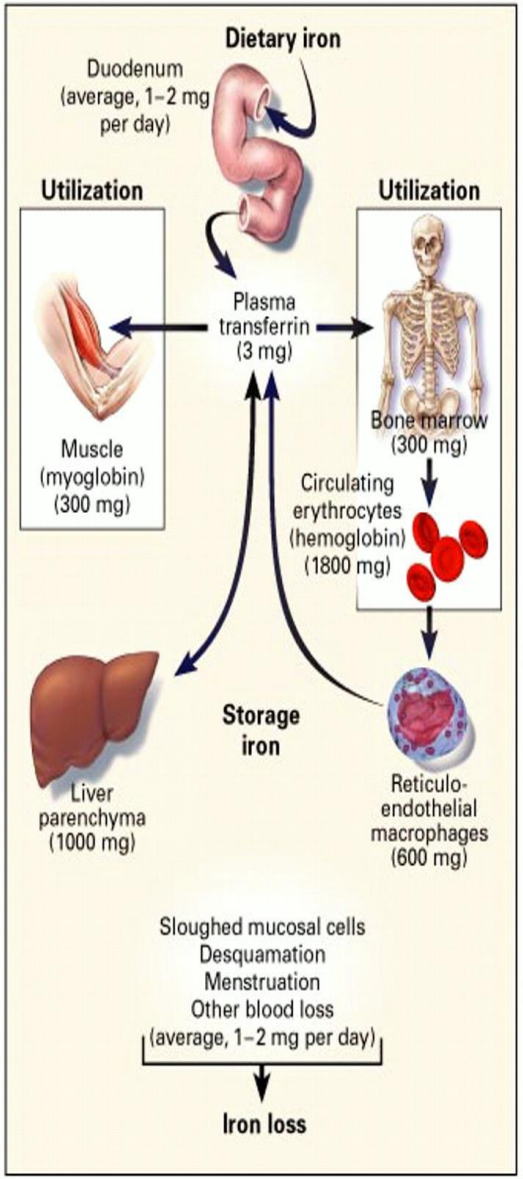
- Betaald door de Vlaamse overheid.
- Verder niets te melden.

- Hemochromatose
- Ziekte van Wilson
- Alfa 1 antitrypsine deficiëntie
- ….

Table 2. Classification of Iron Overload Syndromes

- Hereditary Hemochromatosis
  - *HFE*-related
    - C282Y/C282Y
    - C282Y/H63D
    - Other *HFE* mutations
  - Non–*HFE*-related
    - Hemojuvelin (*HJV*)
    - Transferrin receptor-2 (*TfR2*)
    - Ferroportin (*SLC40A1*)
    - Hepcidin (*HAMP*)
    - African iron overload
- Secondary Iron Overload
  - Iron-loading anemias
    - Thalassemia major
    - Sideroblastic
    - Chronic hemolytic anemia
    - Aplastic anemia
    - Pyruvate kinase deficiency
    - Pyridoxine-responsive anemia
  - Parenteral iron overload
    - Red blood cell transfusions
    - Iron–dextran injections
    - Long-term hemodialysis
  - Chronic liver disease
    - Porphyria cutanea tarda
    - Hepatitis C
    - Hepatitis B
    - Alcoholic liver disease
    - Nonalcoholic fatty liver disease
    - Following portocaval shunt
  - Dysmetabolic iron overload syndrome
- Miscellaneous
  - Neonatal iron overload
  - Aceruloplasminemia
  - Congenital atransferrinemia

Iron metabolism



- NEJM 1999;341:1986.

Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice

Gaël Nicolas*, Myriam Bennoun*, Isabelle Devaux†, Carole Beaumont†, Bernard Grandchamp†, Axel Kahn*, and Sophie Vaulont*‡

*Institut National de la Santé et de la Recherche Médicale 129, Departement Genetique Developpement et Pathologie Moléculaire, Institut Cochin de Génétique Moléculaire.

PNAS,July 2001.

Molecule structure of human synthetic hepcidin-25

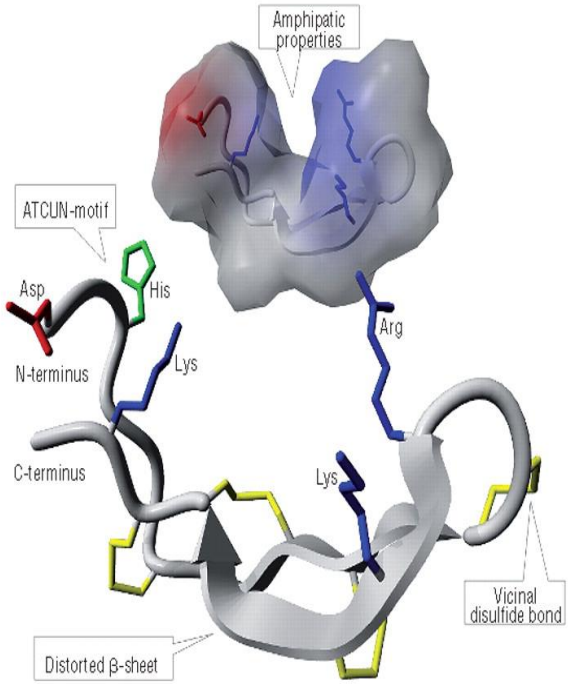


Figure 2. Molecule structure of human synthetic hepcidin-25. *Front*: overview of the structure of hepcidin-25. Distorted β-sheets are shown as grey arrows, and the peptide backbone is colored gray. The disulfide bonds are colored yellow, highlighting the position of an unusual vicinal bond between adjacent cysteines at the hairpin turn. Positive residues of Arginine (Arg)and Lysine (Lys) are pictured in blue, the negative residue of Aspartic acid (Asp) in red, and the Histidine containing amino terminal Cu^{2+}- Ni^{2+} (ATCUN)-binding motif in the N-terminal region is colored green. *Background*: hepcidin-25 molecule displayed with solvent accessible surface that illustrates the amphipathic structure of the molecule. The molecule is colored gray, except for the side-chains of positive (blue) and negative (red) residues. Molecular graphics created with YASARA[16] (*www.yasara.org*) and PovRay (*www.povray.org*), with coordinates and factors obtained from the Protein Data Bank (*www.rcsb.org*; PDB file code 1M4F).

Kemna, E. H.J.M. et al. Haematologica 2008;93:90-97

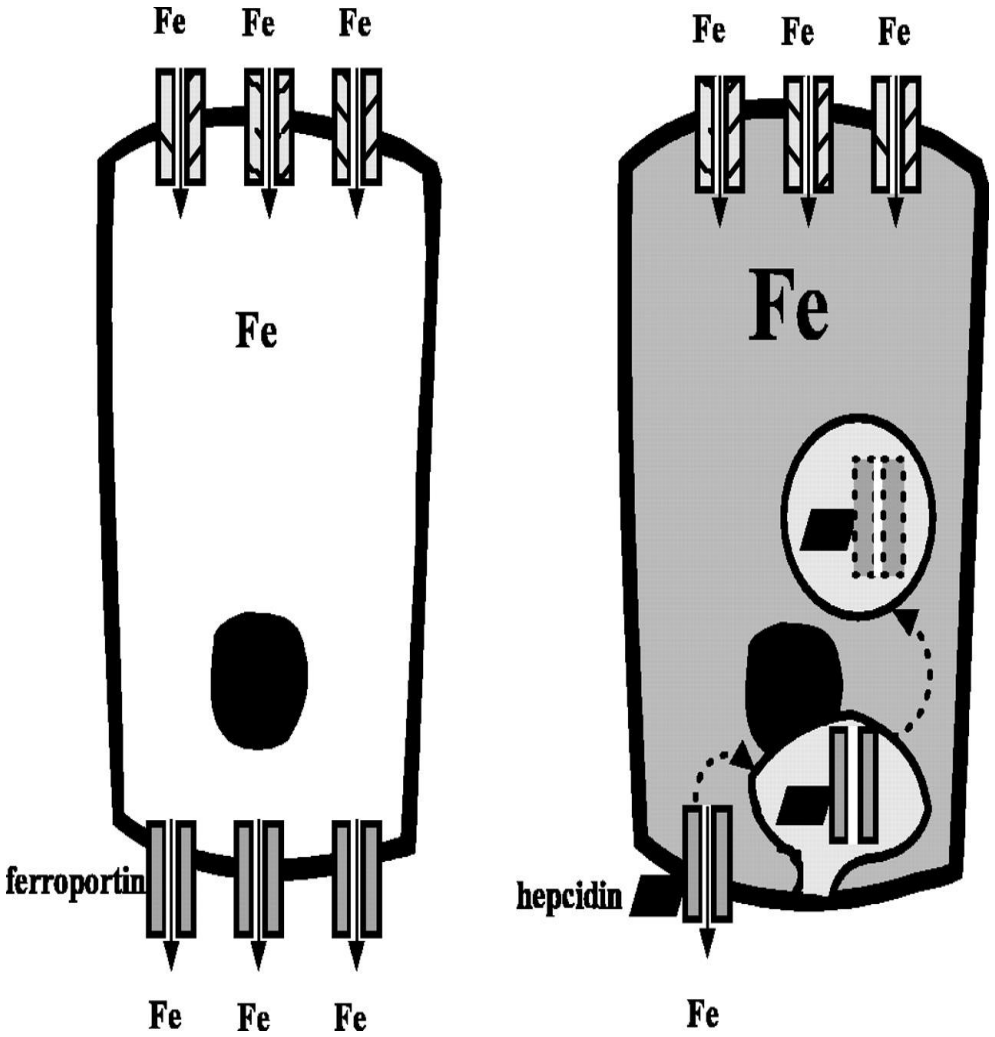
haematologica
the hematology journal

Sequences of verterbrate hepcidins

**Ganz, T. et al. Am J Physiol Gastrointest Liver Physiol 290: G199-G203 2006;
doi:10.1152/ajpgi.00412.2005**

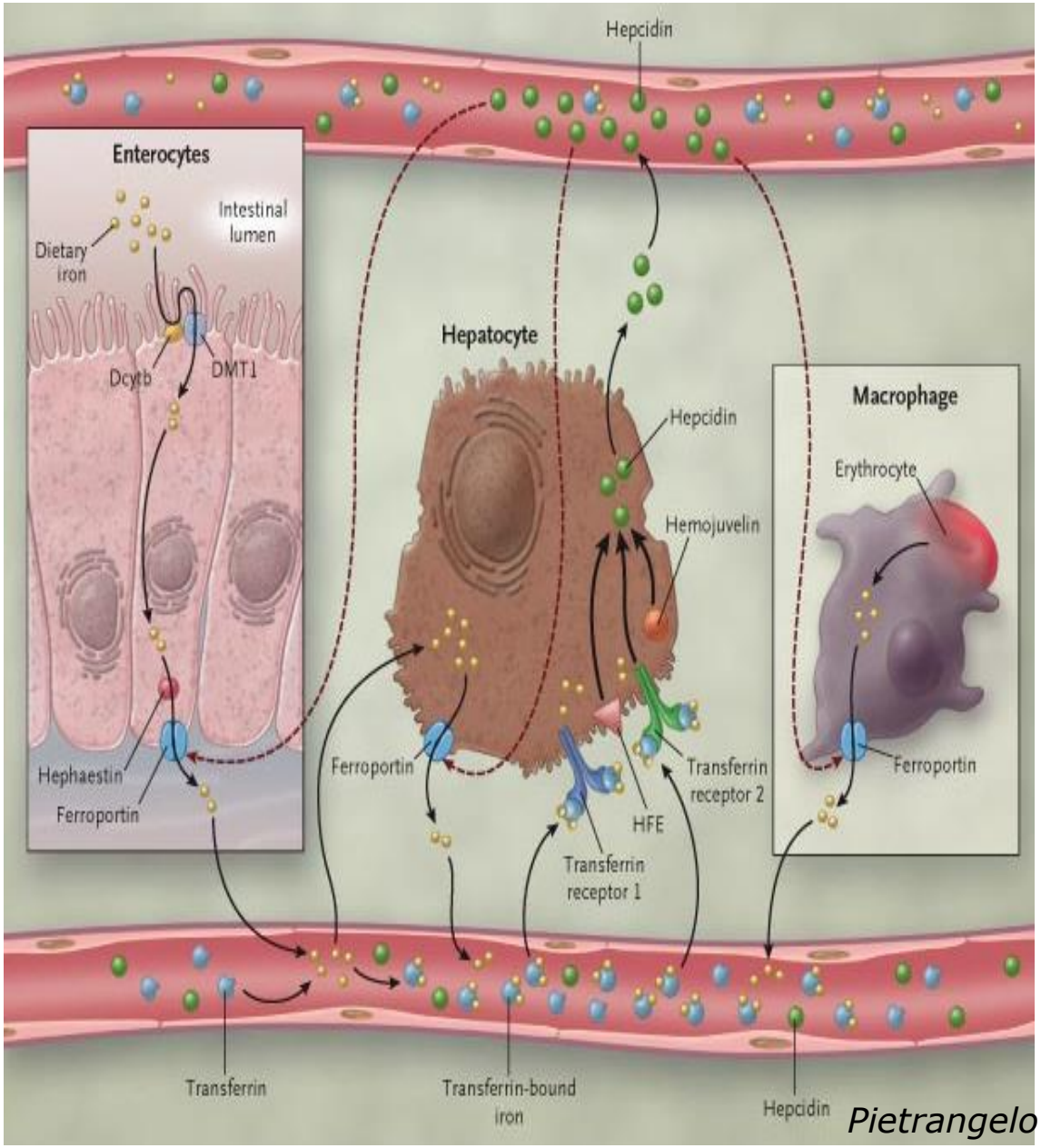
AJP - Gastrointestinal and Liver Physiology

Hepcidin regulates ferroportin expression on the basolateral membrane of enterocytes



Ganz, T. et al. Am J Physiol Gastrointest Liver Physiol 290: G199-G203 2006;
doi:10.1152/ajpgi.00412.2005

AJP - Gastrointestinal and Liver Physiology



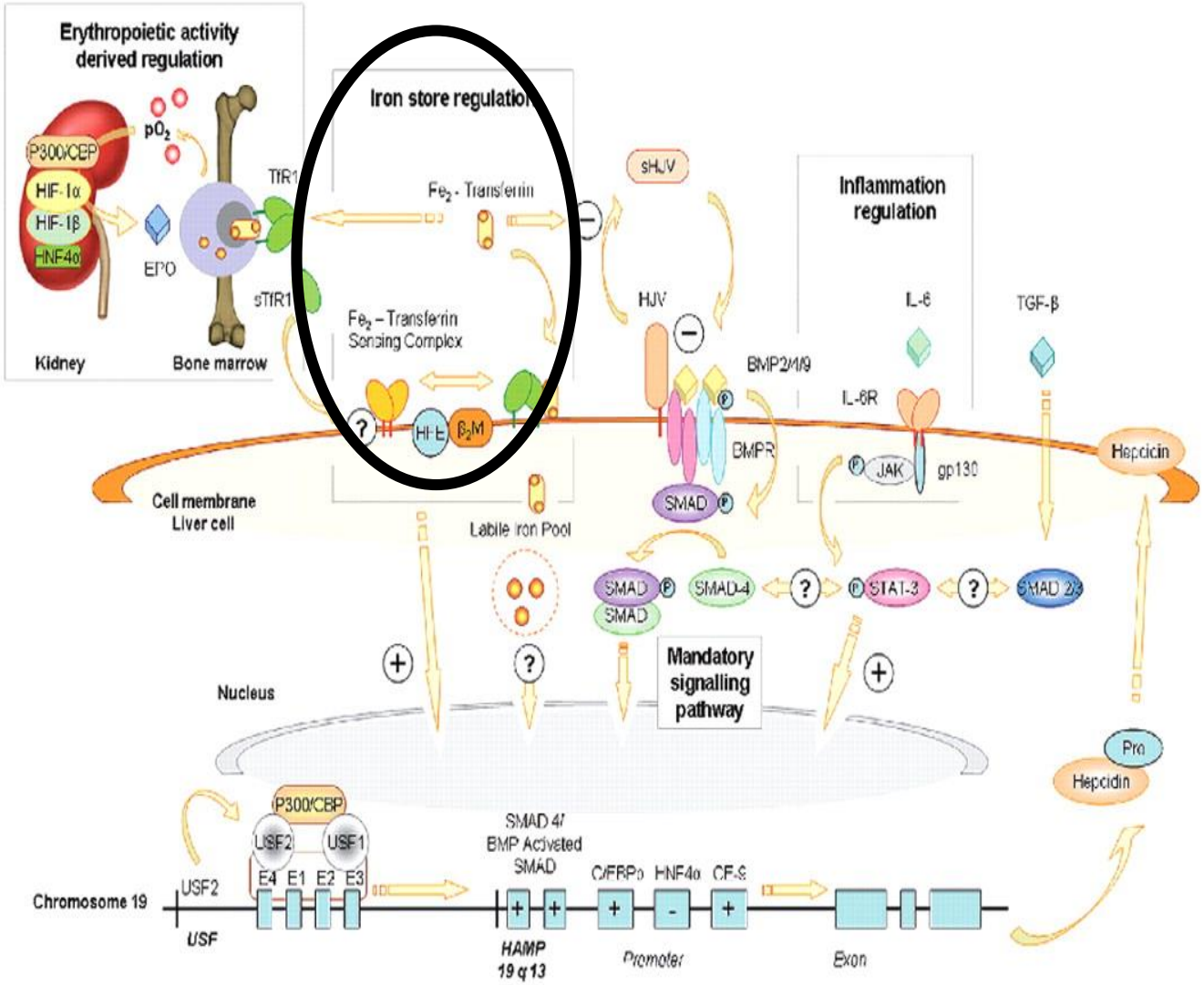
Pietrangelo NEJM 2004

Key points 1. Essentials on hepcidin

- Structure: 84 amino acid prepropeptide containing N-terminal 24 amino acid endoplasmic reticulum targeting signal sequence, and a 35 amino acid proregion with a consensus furin cleavage site followed by the C-terminal 25 amino acid bioactive iron-regulatory hormone
- Metabolism:
  - Synthesis: mainly in the hepatocytes
  - Serum concentration: 20-200 ng/ml
  - Excretion: kidney
- Regulation:
  - Stimulators: high serum/hepatic iron, inflammatory cytokines, bone morphogenetic proteins (BMPs), ER stress
  - Inhibitors: low serum/hepatic iron, anemia-hypoxia, bone-marrow derived factors (GDF15, TWSG1), erythropoietin
- Activity: N-terminus interacts with the iron-exporter ferroportin in macrophages, enterocytes, hepatocytes and placental cells and causes its internalization and degradation, leading to decreased cell iron efflux

GDF15: growth differentiation factor 15; TWSG1: twisted gastrulation protein homolog 1.

Model of pathways involved in hepcidin regulation



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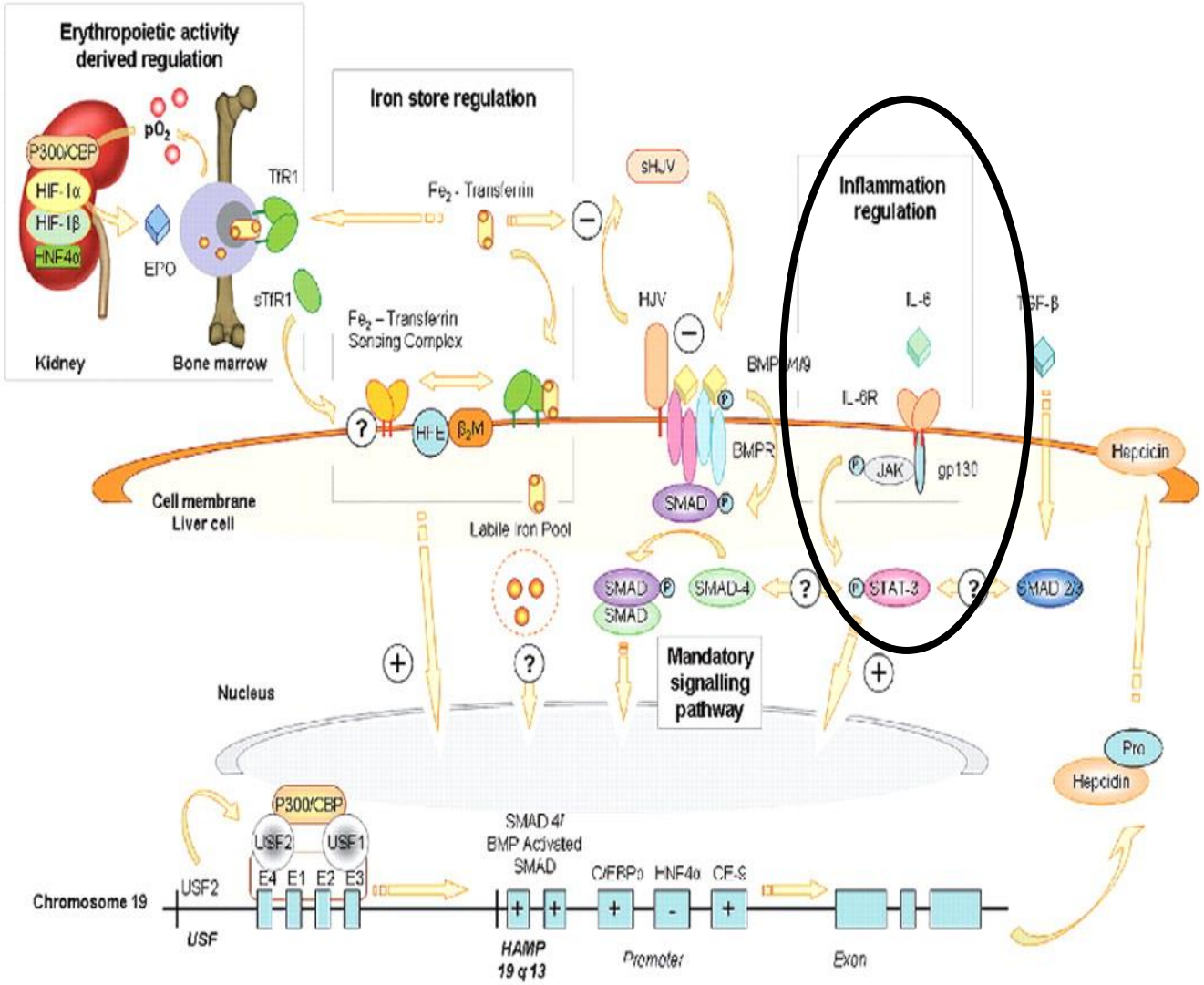
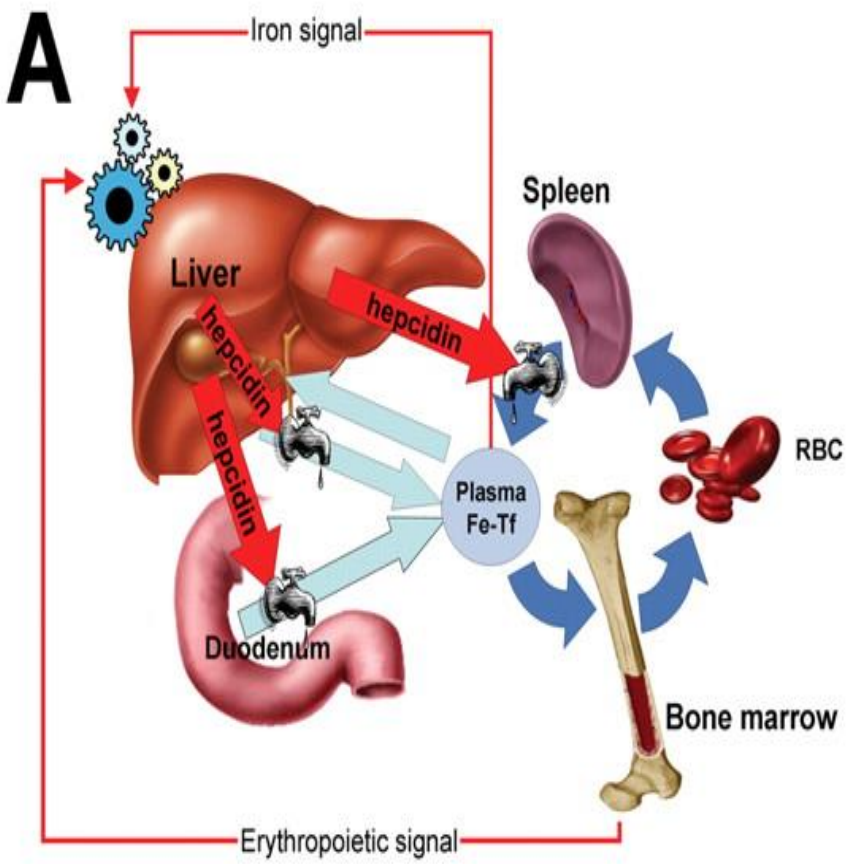


HEMATOLOGY
ASH Education Program Book

Regulation of systemic iron flows in normal subjects (A), patients with hereditary hemochromatosis (B) and patients with anemia of inflammation (C)



Hematology 2006;2006:505-51

HEMATOLOGY
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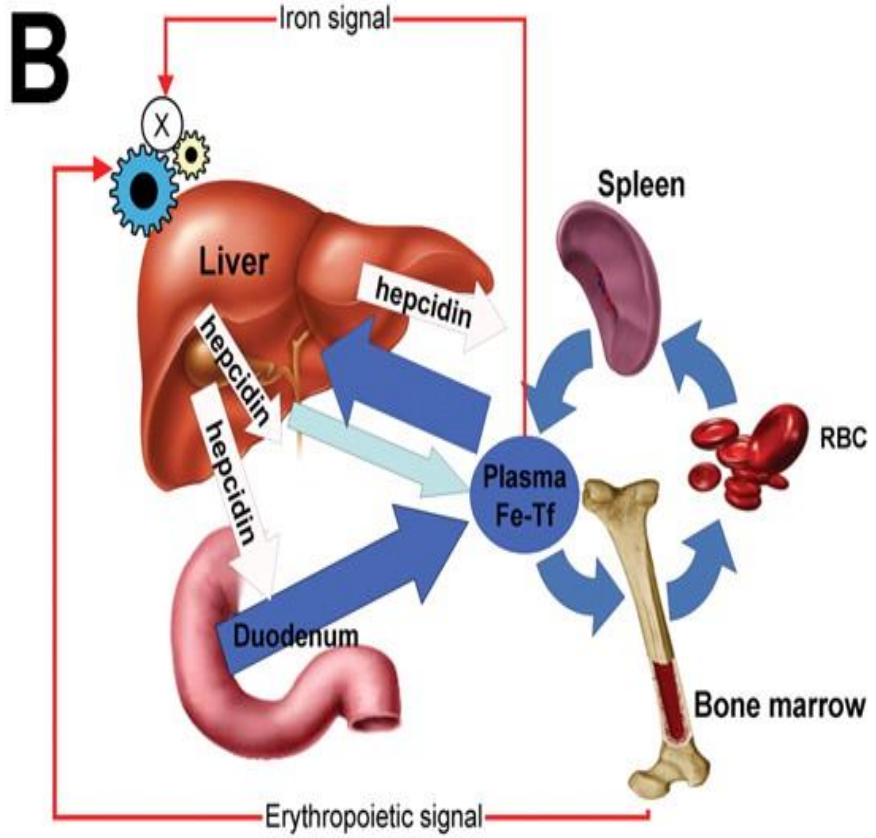
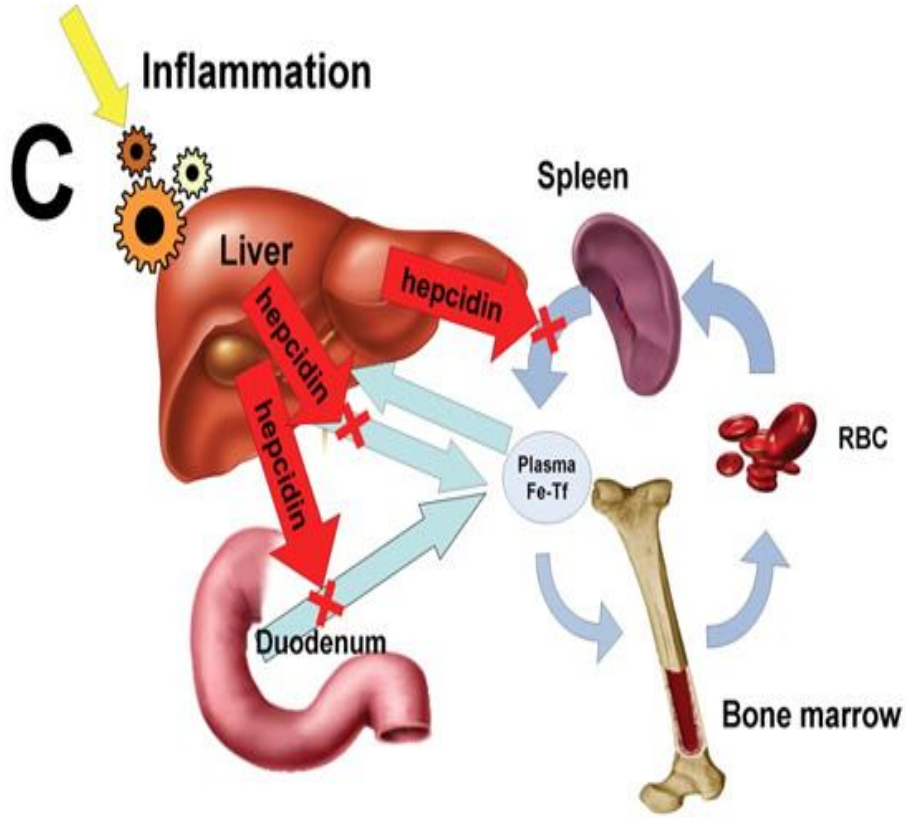


Hematology 2006;2006:505-516

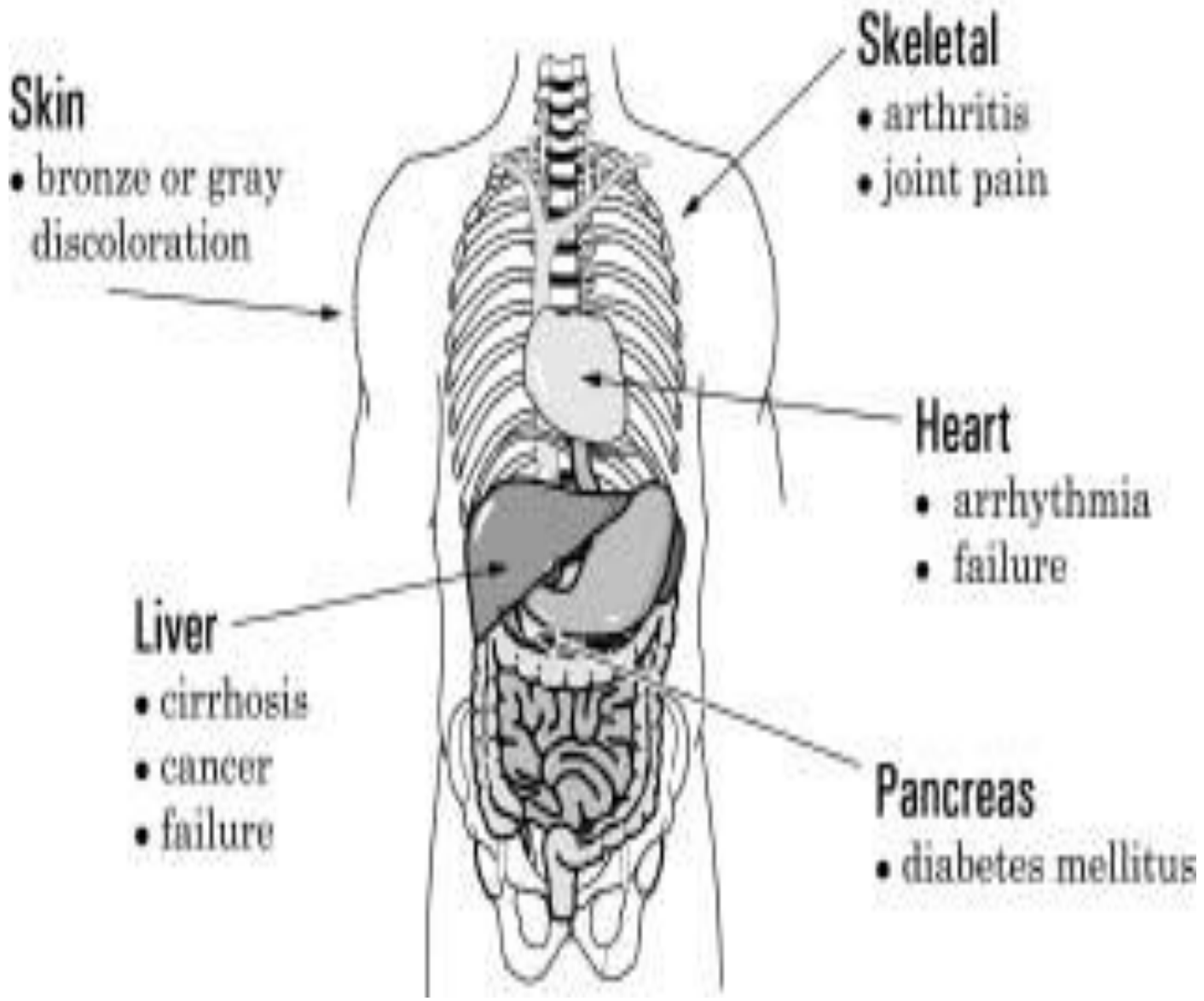
HEMATOLOGY
ASH Education Program Book



Hematology 2006;2006:505-516

Historiek

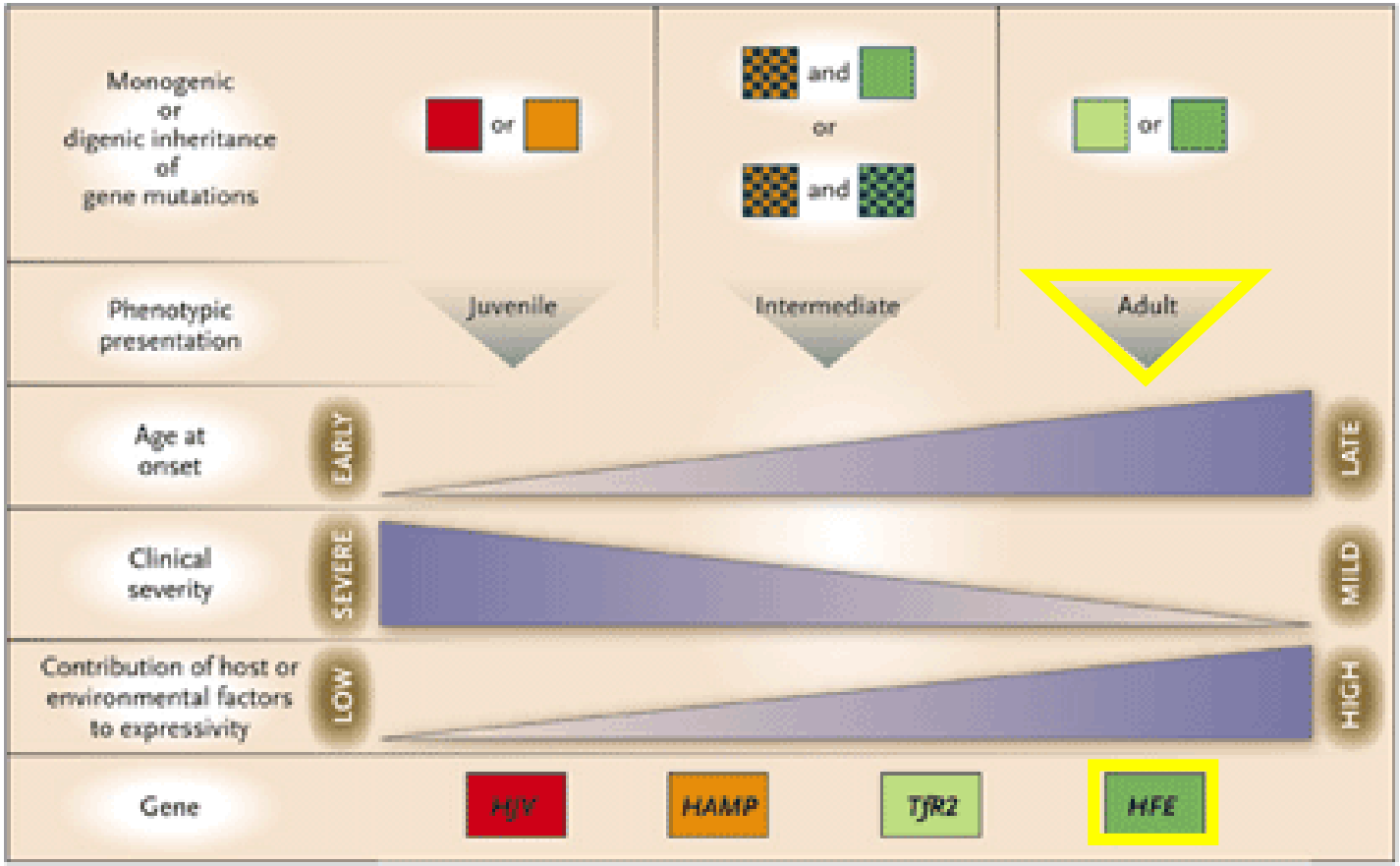
- 1865 – Trousseau
  - "bronsdiabetes"
- 1889 – Von Recklinghausen
  - "haemochromatosis"
- 1935 – Sheldon
  - erfelijke aandoening
- 1976 - Simon et al.
  - autosomaal recessieve ziekte, gekoppeld aan korte arm chromosoom 6 (HLA-A3)
- 1996 - Feder et al.
  - identificatie causale gen: initieel *HLA-H*
  - huidige naam: *HFE* gen


Skin
• bronze or gray discoloration
Skeletal
• arthritis
• joint pain
Heart
• arrhythmia
• failure
Liver
• cirrhosis
• cancer
• failure
Pancreas
• diabetes mellitus

- Variabele expressie

  - biochemische en klinische heterogeniteit

  - omgevingsfactoren, modifier genen => “multifactorieel”



Pietrangelo NEJM 2004

HH type 1 of *HFE*-HH

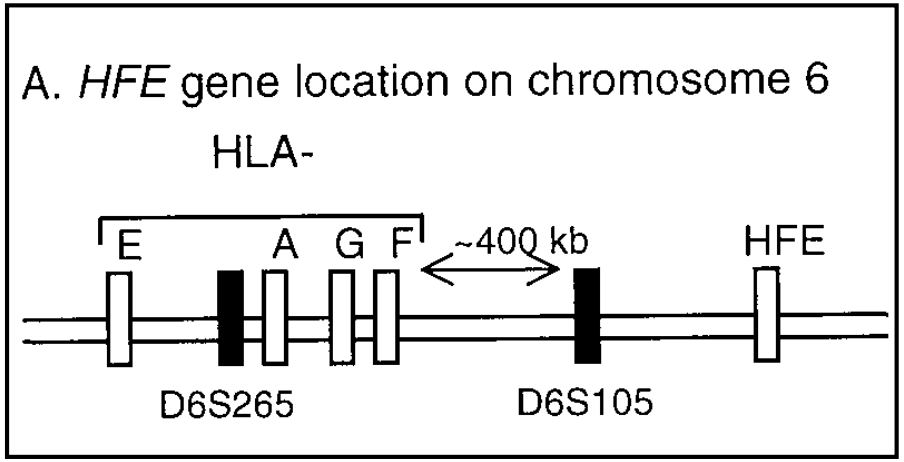
Gut, 1976, **17**, 332-334

Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis

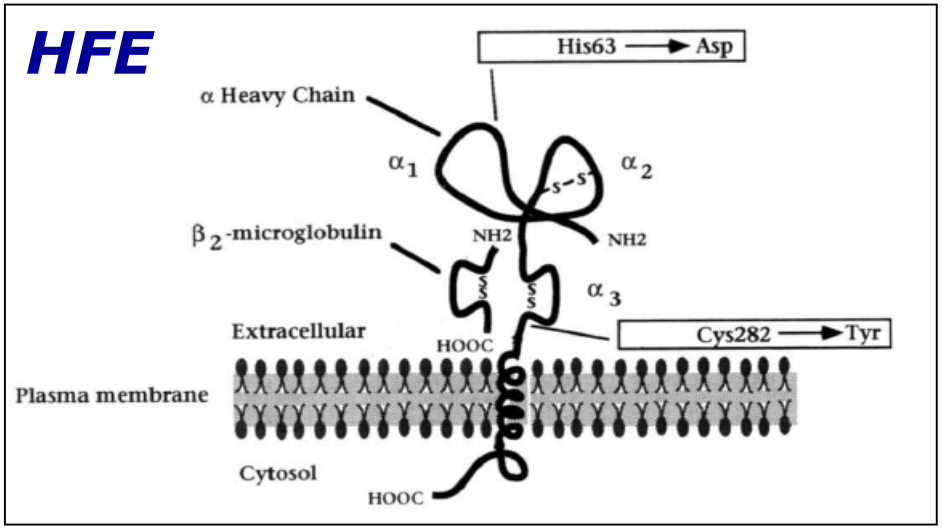
M. SIMON[1], M. BOUREL, R. FAUCHET, AND B. GENETET

From the Clinique Médicale A, Unité de Recherche U 49 (INSERM), and Centre Régional de Transfusion Sanguine, Hôpital Pontchaillou, Rennes, France

SUMMARY The frequency of HLA-A3 and HLA-B14 antigens was significantly higher in a series of 51 patients with idiopathic haemochromatosis than in a control group, being respectively 78·4 versus 27·0% and 25·5 versus 3·4%. This finding strongly supports the suggestion that idiopathic haemochromatosis is a genetic disease and suggests that the gene(s) responsible for the disease may be linked to the histocompatibility genes.



Simon et al. 1976



H63D

C282Y

Feder et al. 1996

Epidemiologie *HFE* mutaties

Table 1. Prevalence of *HFE* C282Y and H63D Genotypes According to Race or Ethnic Group.*

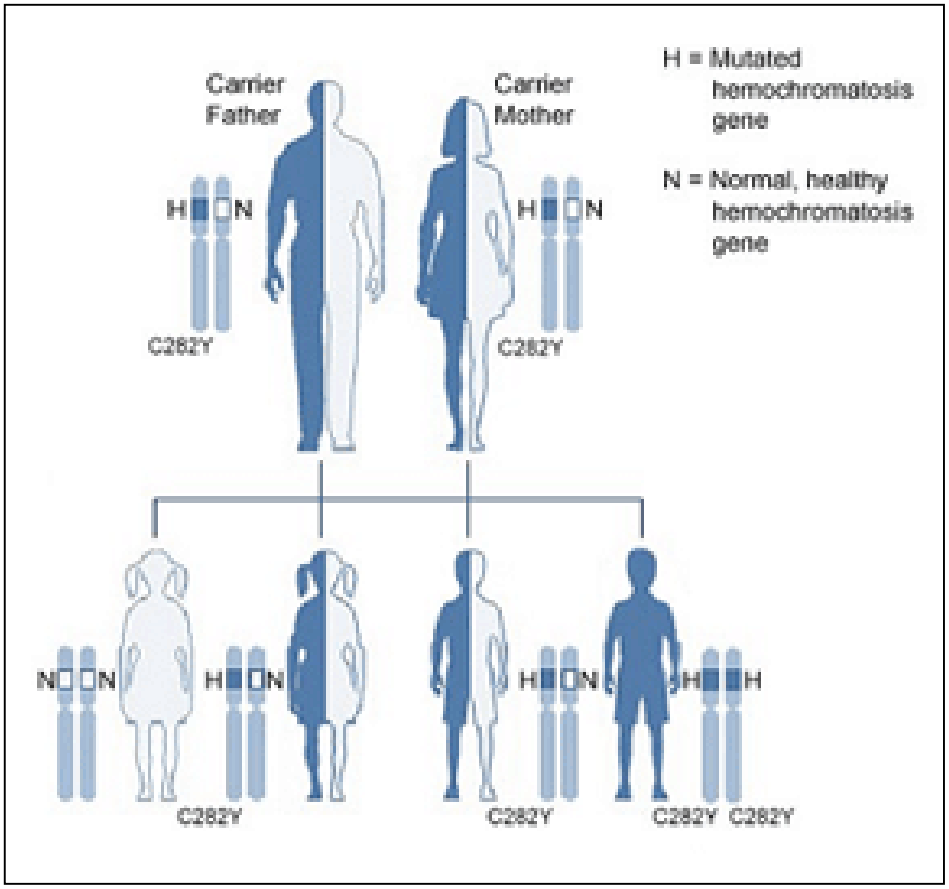
Race or Ethnic Group	Total No. of Participants	C282Y/C282Y		C282Y/H63D		H63D/H63D	
		No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %
White	44,082	281	0.44 (0.42–0.47)	908	2.0 (2.0–2.1)	1029	2.4 (2.3–2.4)
Native American	648	1	0.11 (0.061–0.20)	7	0.77 (0.56–1.1)	7	1.3 (0.98–1.8)
Hispanic	12,459	7	0.027 (0.022–0.032)	48	0.33 (0.30–0.37)	154	1.1 (0.98–1.1)
Black	27,124	4	0.014 (0.012–0.017)	35	0.071 (0.065–0.078)	30	0.089 (0.081–0.097)
Pacific Islander	698	0	0.012 (0.0043–0.032)	0	0.096 (0.055–0.17)	0	0.20 (0.12–0.32)
Asian	12,772	0	0.000039 (0.000015–0.00010)	0	0.0055 (0.0029–0.0093)	29	0.20 (0.17–0.22)
Multiple/unknown	1928	6	—	19	—	21	—
All	99,711	299	—	1017	—	1270	—

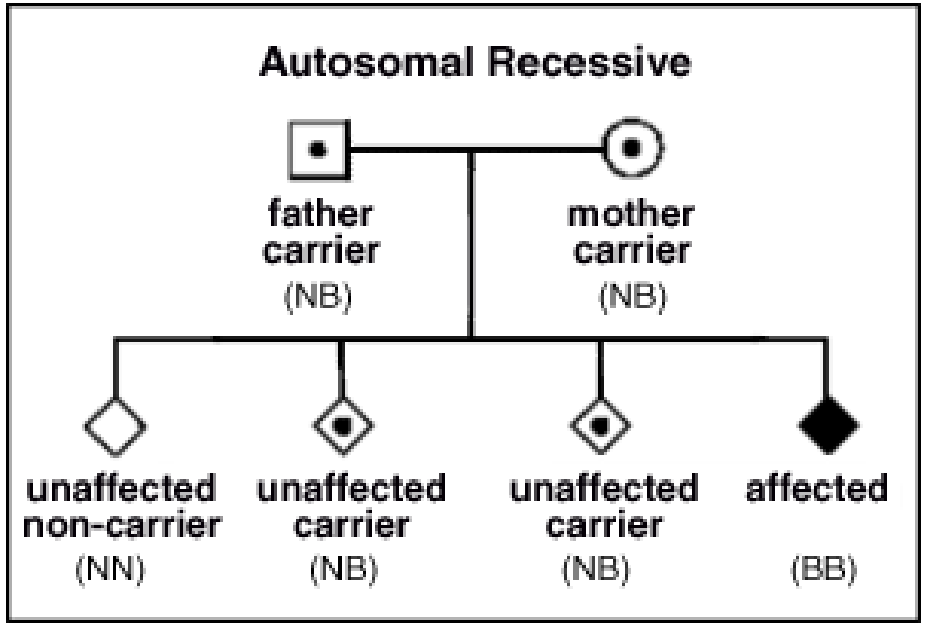
Race or Ethnic Group	Total No. of Participants	C282Y/+		H63D/+		+/+	
		No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %
White	44,082	4548	10 (10–11)	10,537	24 (24–24)	26,779	61 (60–61)
Native American	648	35	5.7 (4.2–7.7)	128	20 (17–22)	470	72 (69–76)
Hispanic	12,459	351	2.9 (2.6–3.2)	2199	18 (18–19)	9700	78 (77–78)
Black	27,124	605	2.3 (2.1–2.5)	1520	5.7 (5.4–6.0)	24,930	92 (92–92)
Pacific Islander	698	15	2.0 (1.2–3.4)	62	8.4 (6.6–11)	621	89 (87–91)
Asian	12,772	16	0.12 (0.074–0.19)	1070	8.4 (8.0–8.9)	11,657	91 (91–92)
Multiple/Unknown	1928	111	—	313	—	1458	—
All	99,711	5681	—	15,829	—	75,615	—

Pietrangelo NEJM 2004

Genetische counseling: wie testen ?

- Eerstegraadsverwanten indexpatient: afhankelijk van risico's
- Presymptomatisch in sibs: risico M/M is 25% als ouders beiden N/M (obligate dragers)






Iceland
Sweden
Finland
Norway
Estonia
United Kingdom
Denmark
Latvia
Russia
Lithuania
Ireland
Neth.
Germany
Poland
Belarus
Bel.
Lux.
France
Czech Rep.
Slovakia
Ukraine
Switz.
Austria
Hungary
Slo.
Croatia
Romania
Moldova
Bosnia Herz.
Bulgaria
Yugoslavia
Mace.
Portugal
Albania
Spain
Italy
Greece

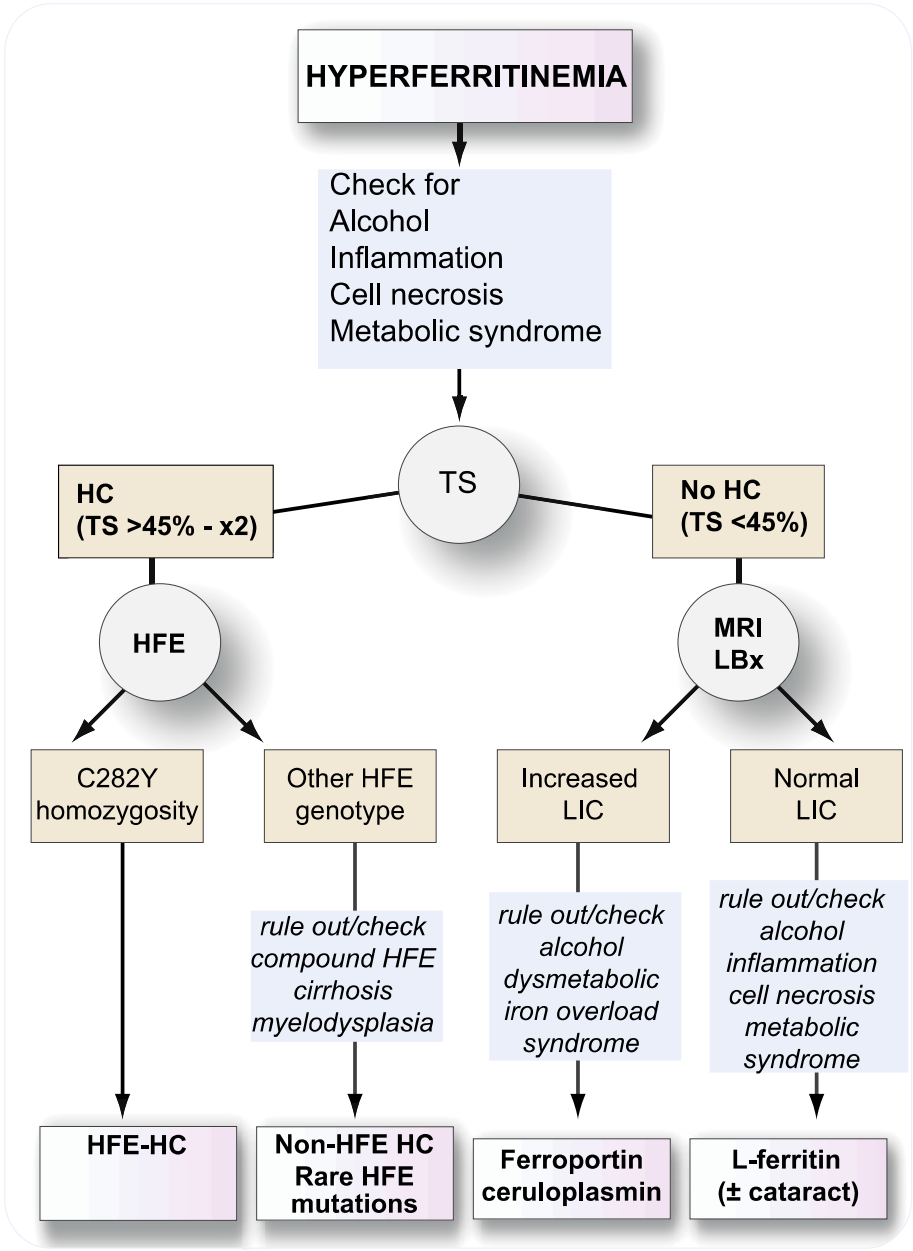
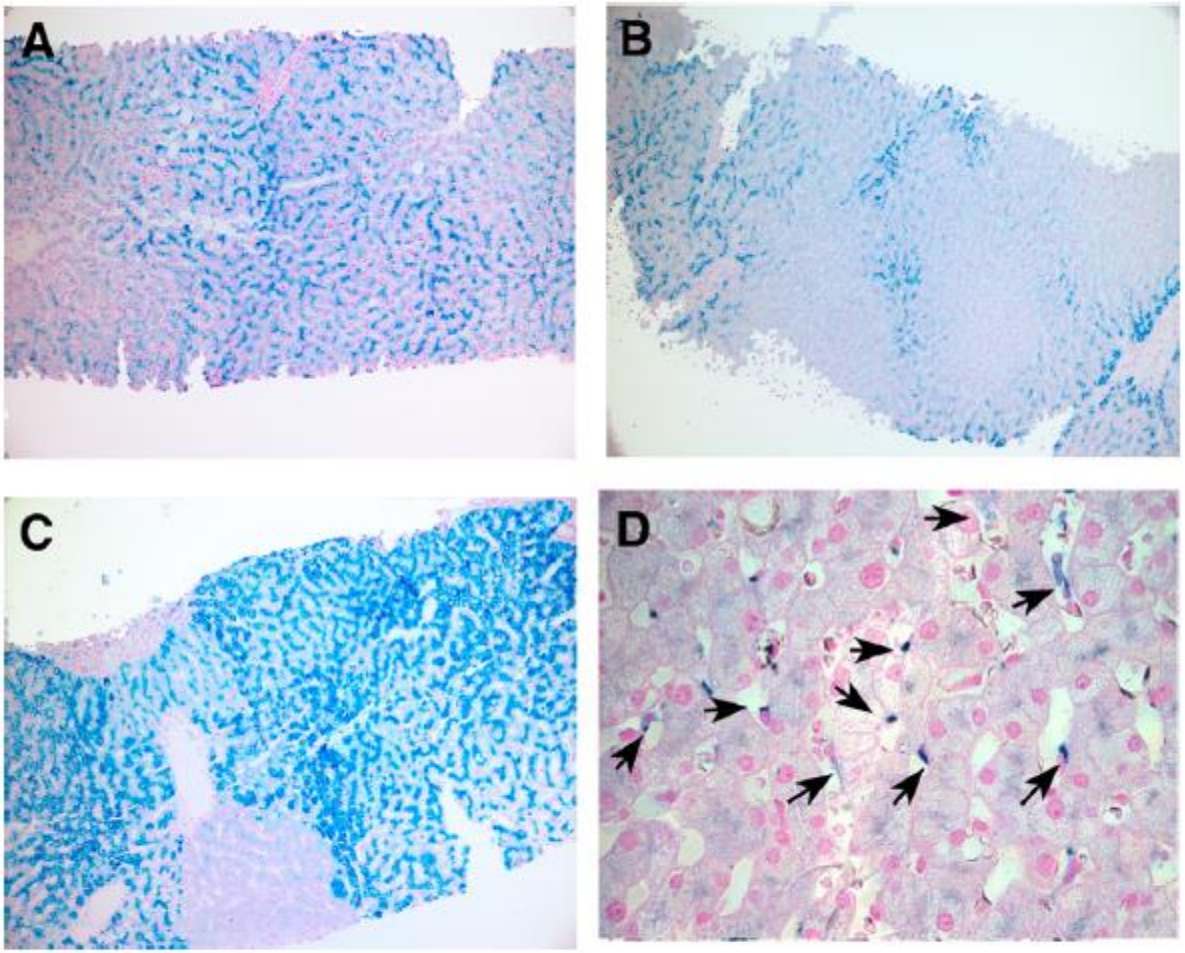


Fig. 3. Proposed algorithm for the diagnosis of genetic causes of hyperferritinemia.

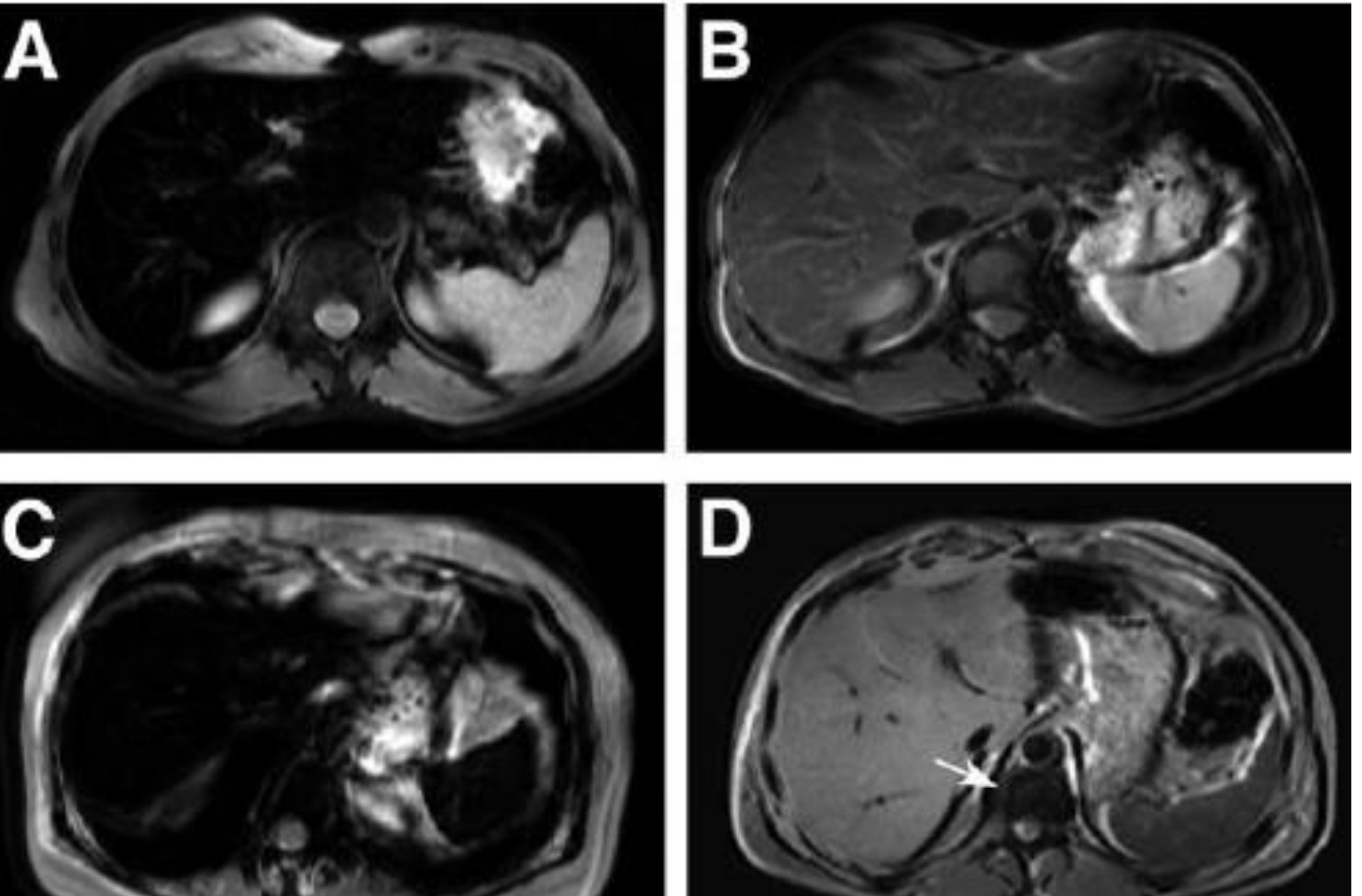


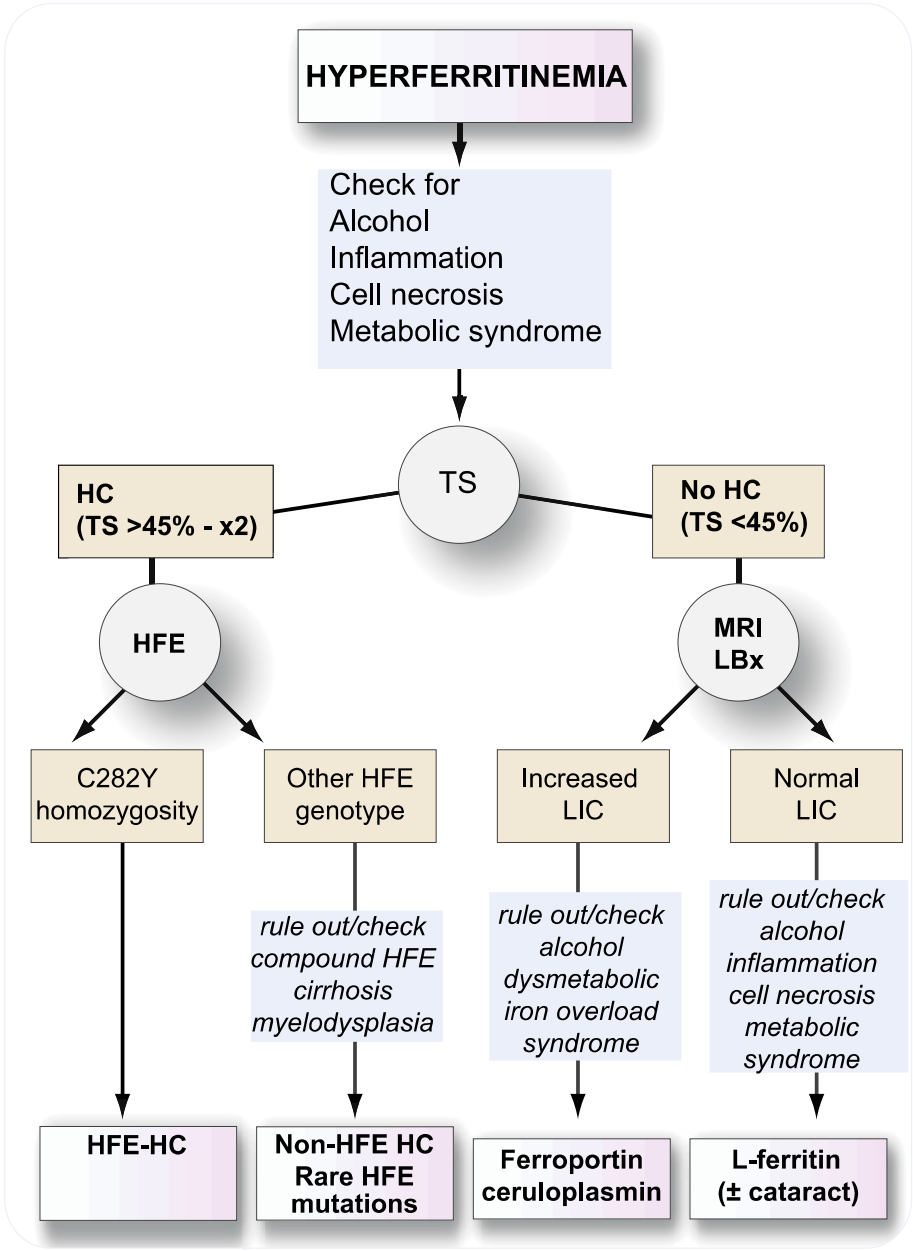
Supplementary Figure 1. Liver histology in patients with hemochromatosis. Perls' Prussian blue stain for iron. (*A*) HFE-related hemochromatosis is characterized by purely parenchymal iron overload that is heaviest in the periportal areas and less intense in the centrolobular areas. (*B*) TfR2-related hemochromatosis. The histopathologic picture is identical to HFE-related hemochromatosis with iron accumulation in periportal parenchymal cells. (*C*) HJV-related juvenile-onset hemochromatosis: massive pan-lobular parenchymal iron overload. (*D*) Classic ferroportin disease. Unlike the previous 3 cases, this liver displays iron overload that predominantly affects the Kupffer cells (*arrows*).

BEFORE
phlebotomy

AFTER
phlebotomy

- MRI





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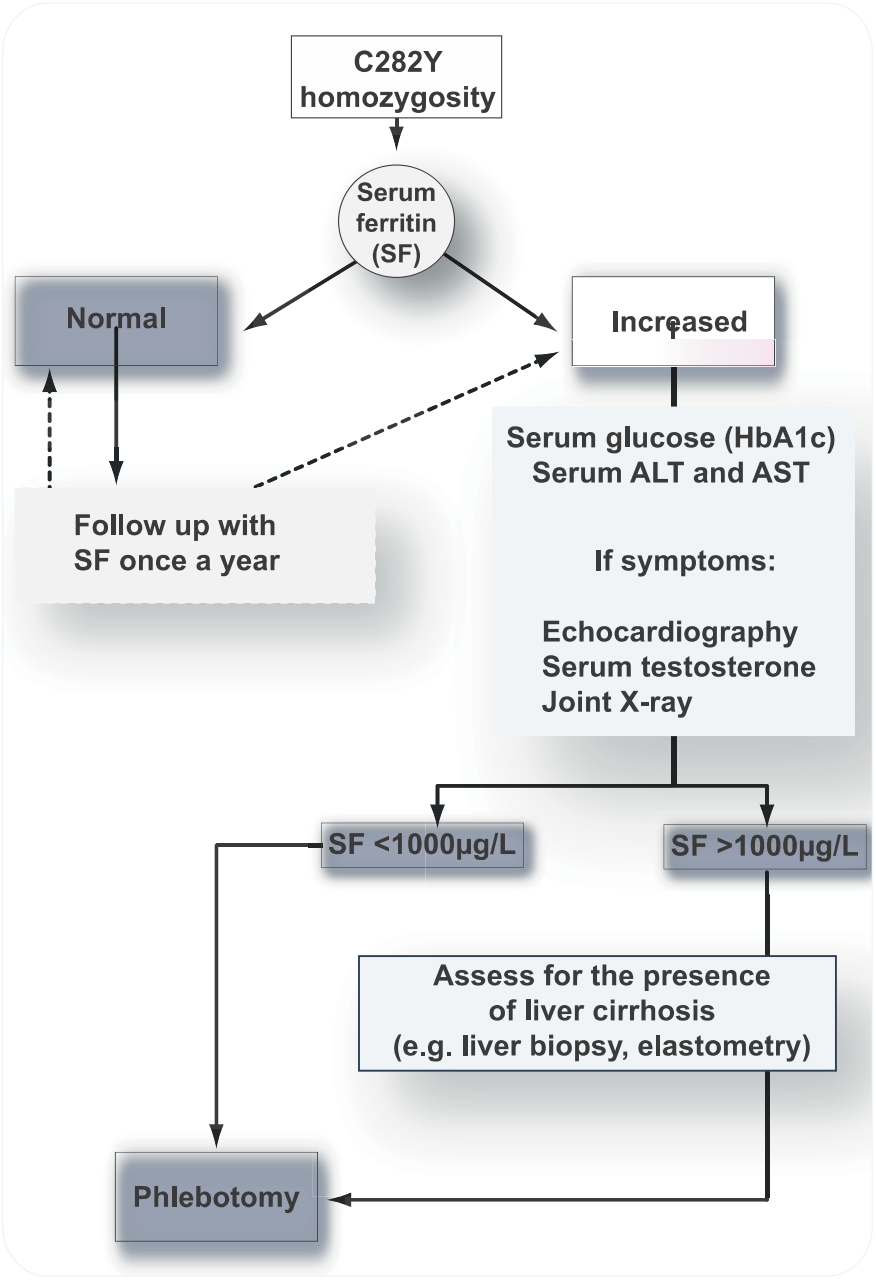


Fig. 4. Proposed algorithm for the diagnostic management of patients with C282Y homozygosity.

Behandeling

- Aderlatingen
- Chelatoren
- Erythrocytaferese

HFE Cys282Tyr Homozygotes With Serum Ferritin Concentrations Below 1000 μg/L Are at Low Risk of Hemochromatosis

Katrina J. Allen,[1,2,3] Nadine A. Bertalli,[1,4] Nicholas J. Osborne,[1,2,4] Clare C. Constantine,[4,5] Martin B. Delatycki,[1,2,6] Amy E. Nisselle,[1,2] Amanda J. Nicoll,[7] Dorota M. Gertig,[8] Christine E. McLaren,[5] Graham G. Giles,[9] John L. Hopper,[4] Gregory J. Anderson,[10] John K. Olynyk,[11,12] Lawrie W. Powell,[10,13] Lyle C. Gurrin,[4] and for the HealthIron Study Investigators*

Effects of treatment

Complication	Expected outcome
None	Prevention complications, N life expectancy
Weakness, fatigue	Resolution or improvement
Elevated liver tests	Resolution or improvement
Hepatomegaly	Resolution or improvement
Cirrhosis	No change
Arthropathy	Almost no improvement
Secondary hypogonadism	Resolution rare > 40y
Diabetes	Sometimes ↓ insulin need
Cardiomyopathy	Resolution rare
Hyperpigmentation	Resolution usually

Screening

- Familieleden
- Populatie
- Risicopopulatie

Casus

- Man 45 jaar.
- Ferritine 4000 ng/ml
- Ijzersaturatie 80%
- ALT: 55 IU/L

Casus

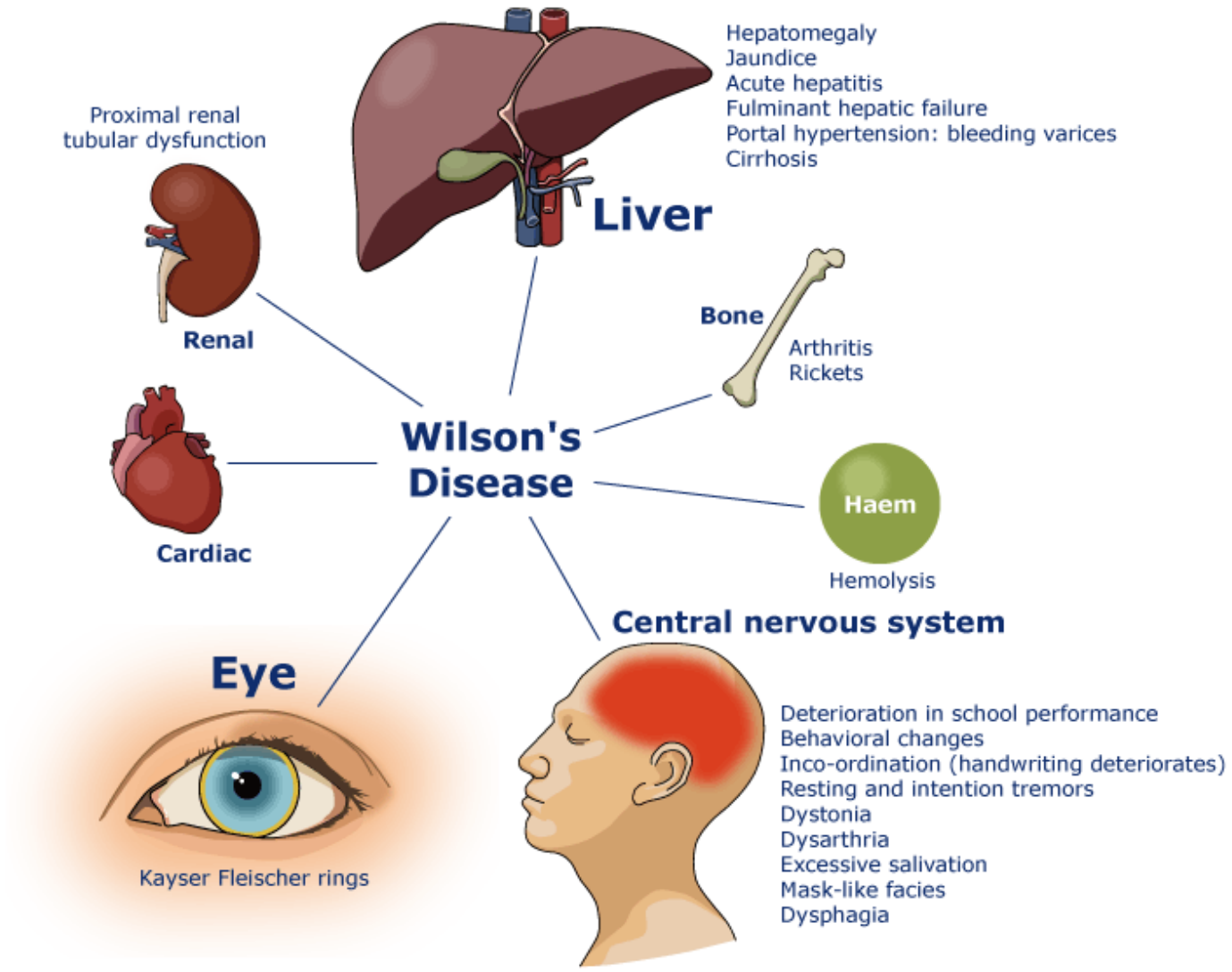
- Man 45 jaar.
- Ferritine 1000 ng/ml
- Ijzersaturatie 43%
- ALT: 55 IU/L

Casus

- Man 45 jaar.
- Ferritine 4000 ng/ml
- Ijzersaturatie 80%
- ALT: 3000 IU/L

- Hemochromatose
- Ziekte van Wilson
- Alfa 1 antitrypsine deficiëntie
- ….

- Wilson disease (WD; also known as hepatolenticular degeneration) was first described in 1912 by Kinnear Wilson as “progressive lenticular degeneration,”
- pattern of inheritance was determined to be autosomal recessive.
- In 1993, the abnormal gene in WD was identified. This gene, *ATP7B*, encodes a metal-transporting P-type adenosine triphosphatase (ATPase), which is expressed mainly in hepatocytes and functions in the transmembrane transport of copper within hepatocytes.


Wilson's Disease
Liver
Hepatomegaly
Jaundice
Acute hepatitis
Fulminant hepatic failure
Portal hypertension: bleeding varices
Cirrhosis
Renal
Proximal renal tubular dysfunction
Bone
Arthritis
Rickets
Cardiac
Haem
Hemolysis
Central nervous system
Deterioration in school performance
Behavioral changes
Inco-ordination (handwriting deteriorates)
Resting and intention tremors
Dystonia
Dysarthria
Excessive salivation
Mask-like facies
Dysphagia
Eye
Kayser Fleischer rings

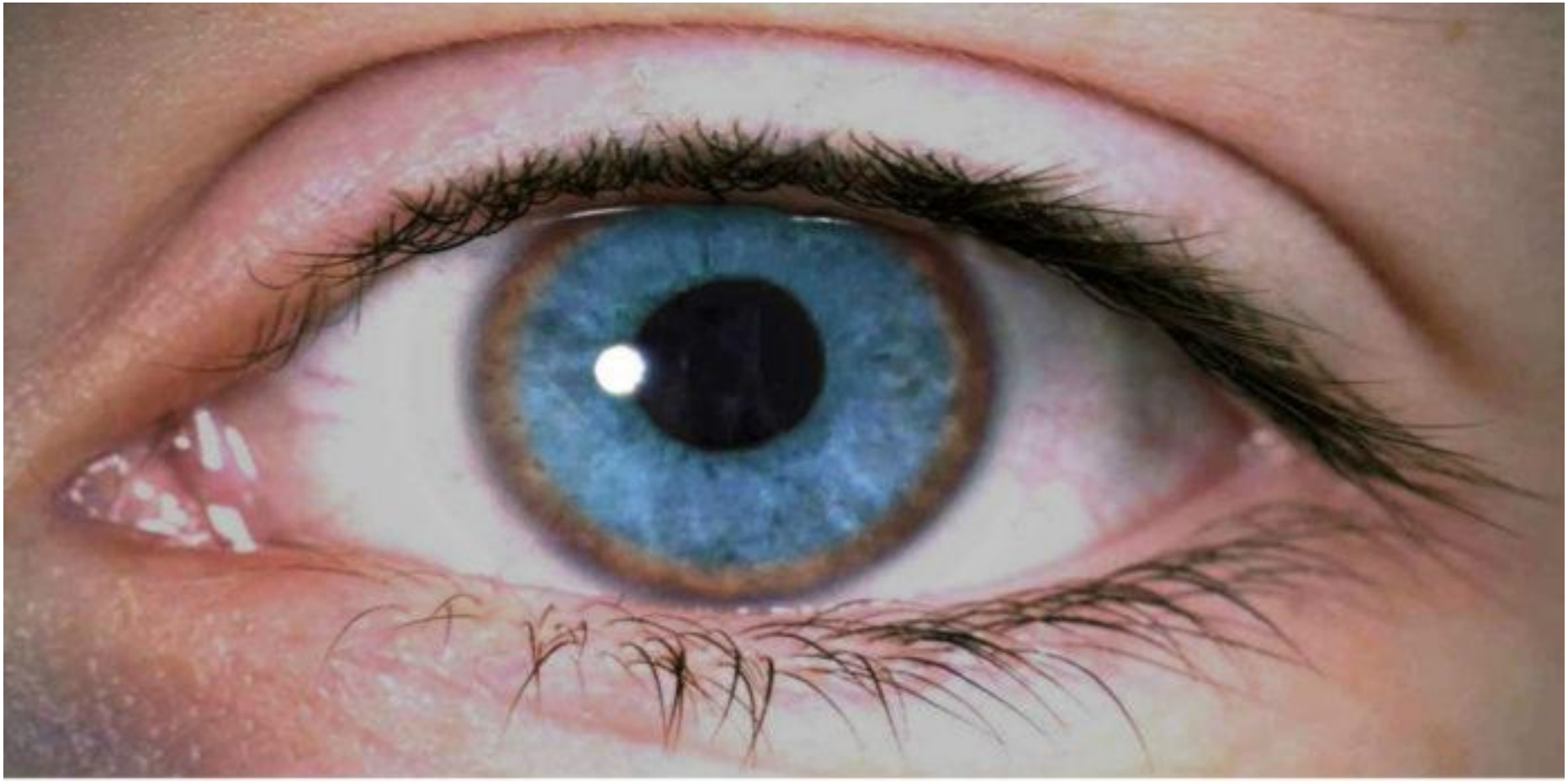


Table 4. Routine tests for diagnosis of Wilson's disease.

Test	Typical finding	False "negative"	False "positive"
Serum ceruloplasmin	Decreased by 50% of lower normal value	Normal levels in patients with marked hepatic inflammation Overestimation by immunologic assay Pregnancy, estrogen therapy	Low levels in: - malabsorption - aceruloplasminemia - heterozygotes
24-hour urinary copper	>1.6 µmol/24 h >0.64 µmol/24 h in children	Normal: - incorrect collection - children without liver disease	Increased: - hepatocellular necrosis - cholestasis - contamination
Serum "free" copper	>1.6 µmol/L	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	>4 µmol/g dry weight	Due to regional variation - in patients with active liver disease - in patients with regenerative nodules	Cholestatic syndromes
Kayser-Fleischer rings by slit lamp examination	Present	Absent - in up to 50% of patients with hepatic Wilson's disease - in most asymptomatic siblings	Primary biliary cirrhosis

Table 5. Scoring system developed at the 8th International Meeting on Wilson's disease, Leipzig 2001 [44].

Typical clinical symptoms and signs	
KF rings	
Present	2
Absent	0
Neurologic symptoms**	
Severe	2
Mild	1
Absent	0
Serum ceruloplasmin	
Normal (>0.2 g/L)	0
0.1-0.2 g/L	1
<0.1 g/L	2
Coombs-negative hemolytic anemia	
Present	1
Absent	0

Other tests	
Liver copper (in the absence of cholestasis)	
>5x ULN (>4 μmol/g)	2
0.8-4 μmol/g	1
Normal (<0.8 μmol/g)	-1
Rhodanine-positive granules*	1
Urinary copper (in the absence of acute hepatitis)	
Normal	0
1-2x ULN	1
>2x ULN	2
Normal, but >5x ULN after D-penicillamine	2
Mutation analysis	
On both chromosomes detected	4
On 1 chromosome detected	1
No mutations detected	0

TOTAL SCORE	Evaluation:
4 or more	Diagnosis established
3	Diagnosis possible, more tests needed
2 or less	Diagnosis very unlikely

*If no quantitative liver copper available, **or typical abnormalities at brain magnetic resonance imaging. KF, Kayser–Fleischer; ULN, upper limit of normal.

Table 3. Prognostic index in Wilson's disease [40], modified by Dhawan *et al.* [41].

	1*	2*	3*	4*
Serum bilirubin (μmol/L)	100-150	151-200	201-300	>300
AST (U/L)	100-150	151-300	301-400	>400
INR	1.3-1.6	1.7-1.9	2.0-2.4	>2.4
WBC [10^9/L]	6.8-8.3	8.4-10.3	10.4-15.3	>15.3
Albumin [g/L]	34-44	25-33	21-24	<21

*= score points, upper limit of normal for AST = 20 IU/ml (at King's College). A score $\geqslant 11$ is associated with high probability of death without liver transplantation.

Fulminant hepatitis due to Wilson's disease

- Peculiarities
  - Alk fosfatase
  - Hemolysis
- Treatment
  - Liver transplantation

Table 3. Pharmacological Therapy for Wilson Disease

Drug	Mode of Action	Neurological Deterioration	Side Effects	Comments
D-Penicillamine	General chelator induces cupruria	10%-20% during initial phase of treatment	• Fever, rash, proteinuria, lupus-like reaction • Aplastic anemia • Leukopenia • Thrombocytopenia • Nephrotic syndrome • Degenerative changes in skin • Elastosis perforans serpingosa • Serous retinitis • Hepatotoxicity	Reduce dose for surgery to promote wound-healing and during pregnancy Maximum dose 20 mg/kg/day; reduce by 25% when clinically stable
Trientine	General chelator induces cupruria	10%-15% during initial phase of treatment	• Gastritis • Aplastic anemia rare • Sideroblastic anemia	Reduce dose for surgery to promote wound-healing and during pregnancy Maximum dose 20 mg/kg/day; reduce by 25% when clinically stable
Zinc	Metallothionein inducer, blocks intestinal absorption of copper	Can occur during initial phase of treatment	• Gastritis; biochemical pancreatitis • Zinc accumulation • Possible changes in immune function	No dosage reduction for surgery or pregnancy Usual dose in adults: 50 mg elemental Zn three times daily; *minimum* dose in adults: 50 mg elemental Zn twice daily
Tetrathiomolybdate	Chelator, blocks copper absorption	Reports of rare neurologic deterioration during initial treatment	• Anemia; neutropenia • Hepatotoxicity	Experimental in the United States and Canada

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