CARENZA ED ECCESSO DI FERRO
nuove conoscenze ed approccio terapeutico
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Emocromatosi genetica

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Hereditary Hemochromatosis diseases, genes and proteins

Disease	Gene	Protein
Hemochromatosis type 1	*HFE*	HFE
Hemochromatosis type 2a/2b (JH)	*HFE2/HAMP*	Hemojuvelin/Hepcidin
Hemochromatosis type 3	*TFR2*	Transferrin receptor 2
Ferroportin disease (HH type 4 A/B)	*SLC40A1*	Ferroportin

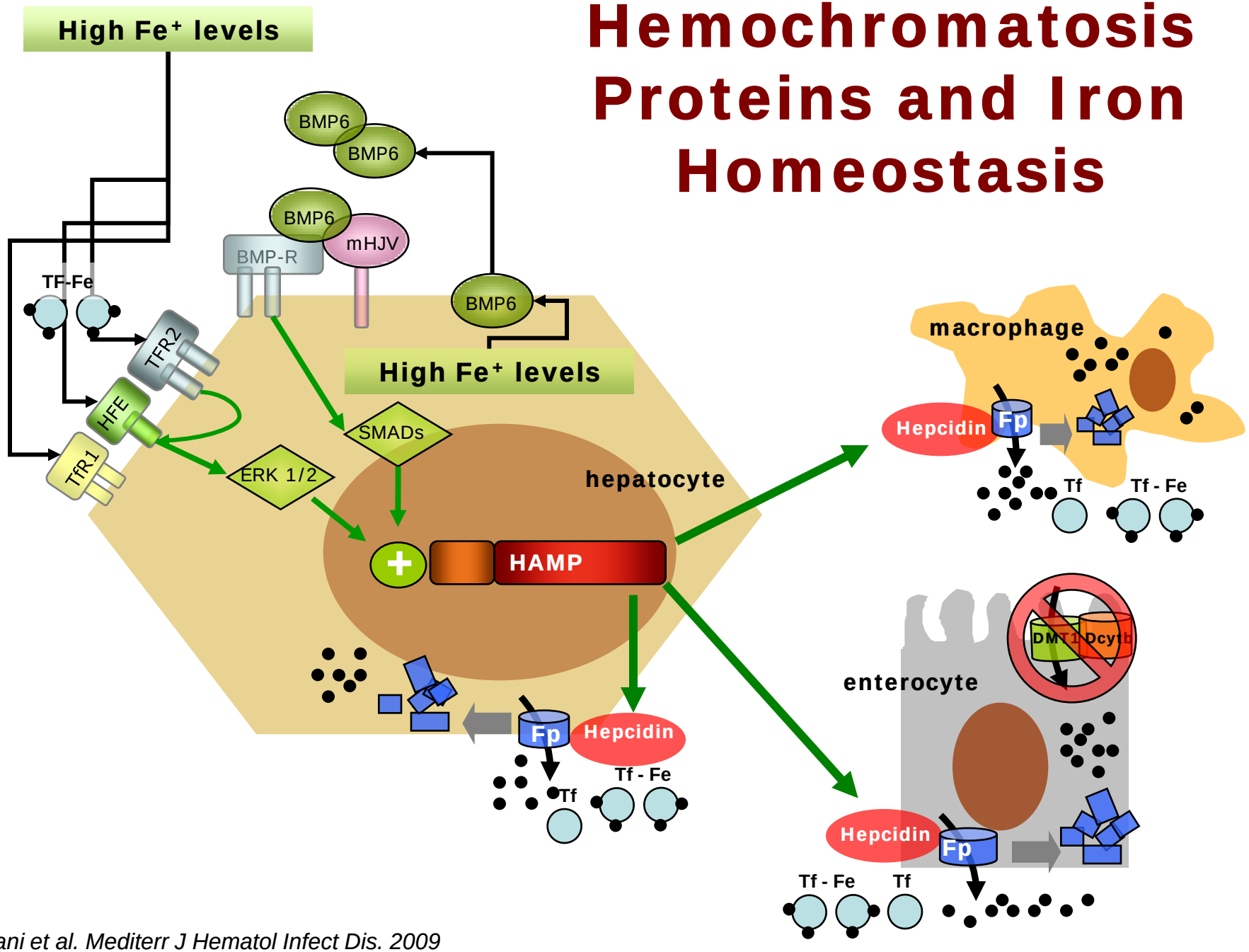
Gene	Allele frequency 1000G	ESP6500	ExAC
HFE	0.014	0.0482	0.0327 (0.0319–0.0339)
HFE (C282Y)	0.013	0.048	0.0324 (0.0315–0.0334)
HFE (non-C282Y)	0.001	0.0002	0.000307 (0.000236–0.000441)
HFE2		0.00074	0.000316 (0.000209–0.000405)
TFR2	0.0004	0.0003	0.000102 (0.000051–0.000173)
HAMP	0.0002		0.0000165
SLC40A1	0.0008	0.0009	0.00034 (0.000292–0.000517)

1000G, 1000 Genomes Project; ESP, Exome Sequencing Project; ExAC, Exome Aggregation Consortium; HH, hereditary hemochromatosis; SNP, single-nucleotide polymorphism.

Genet Med 2016;18:618-626

Figure 3

Hemochromatosis Proteins and Iron Homeostasis

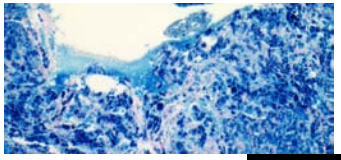


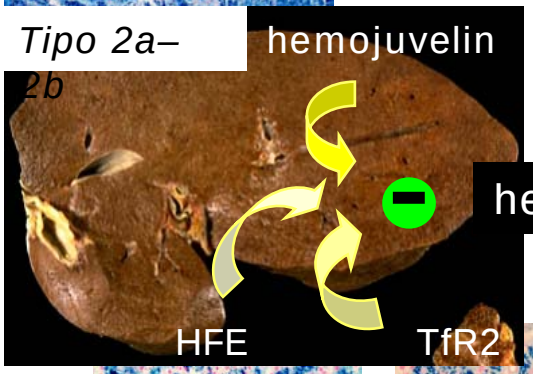
Mariani et al. Mediterr J Hematol Infect Dis. 2009

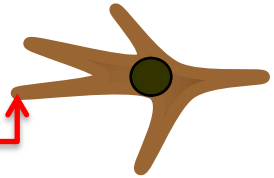
Hemocromatosis

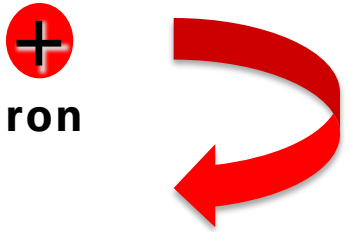
Hepcidin-related pathology

HAMP-HFE2	TFR2	HFE
Absent synthesis	Inadequate synthesis	Inadequate synthesis
Juvenile hemochromatosis	Type 3 hemochromatosis	Adult hemochromatosis

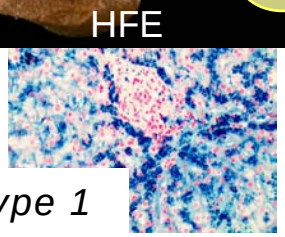






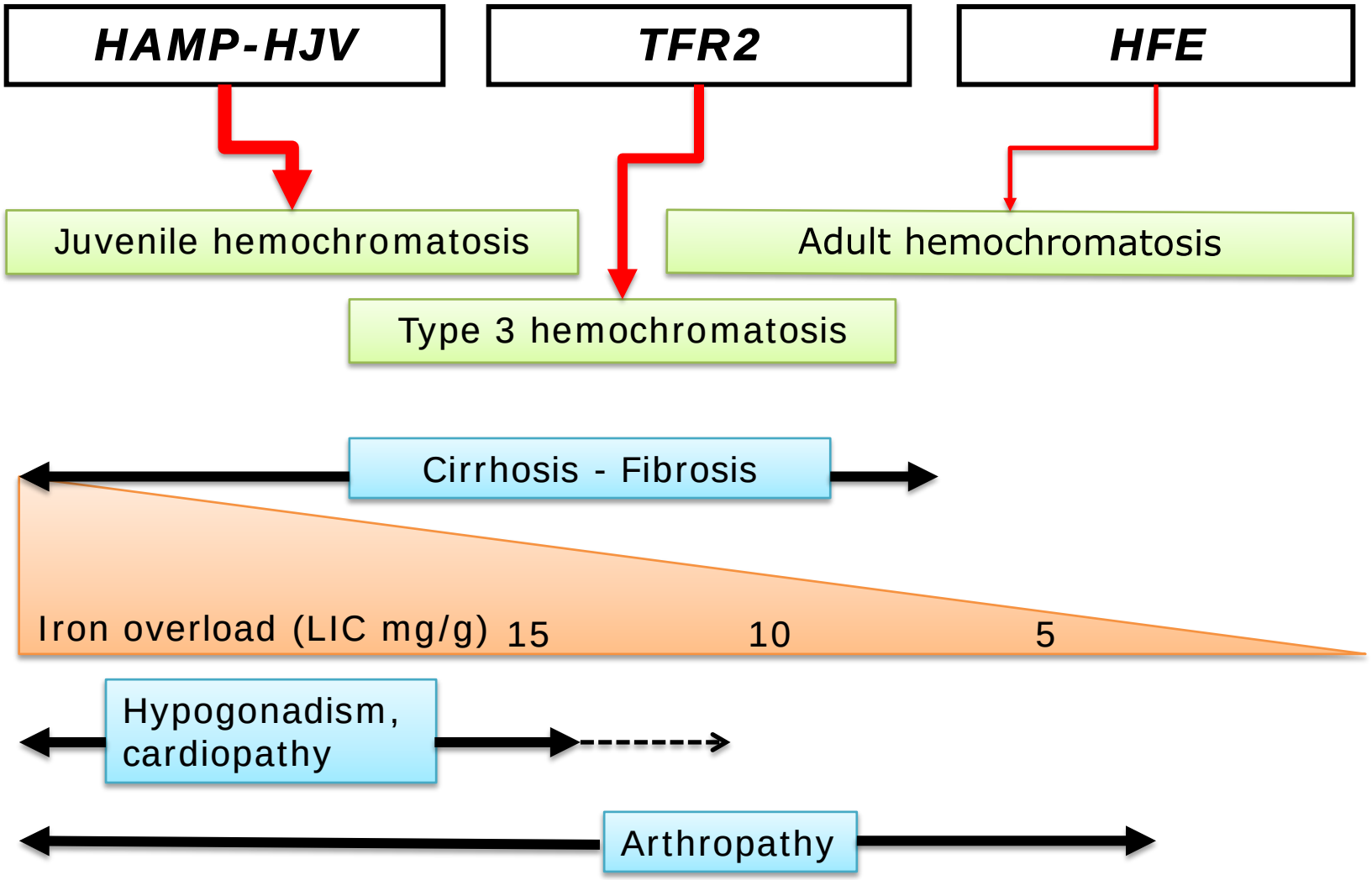


High TF saturation
parenchimal iron overload



Piperno et al. WJG 2009; Pietrangelo A. Gastroenterology 2010

Hemocromatosis



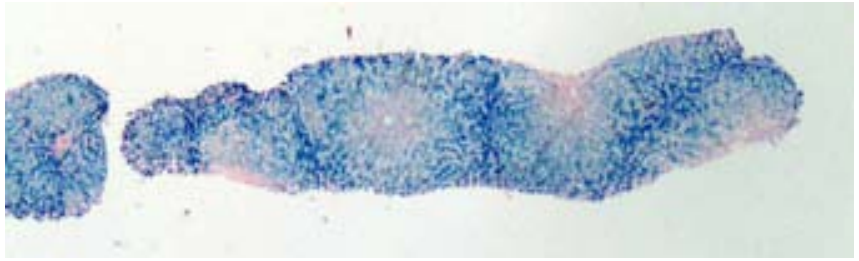
Piperno et al. WJG 2009; Pietrangelo A. Gastroenterology 2010

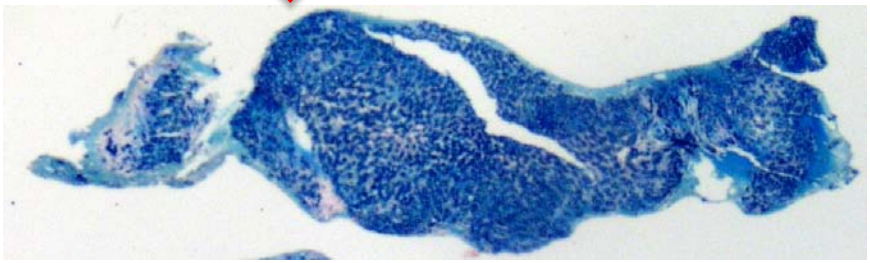
Rate, extent and duration of IOL define phenotype

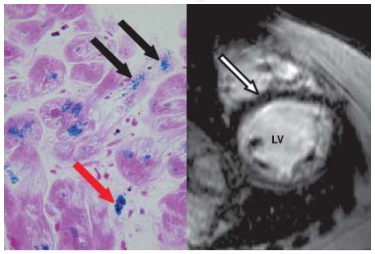
Slower rate of IOL

Faster rate of IOL

Hepatocellular IOL







Liver damage

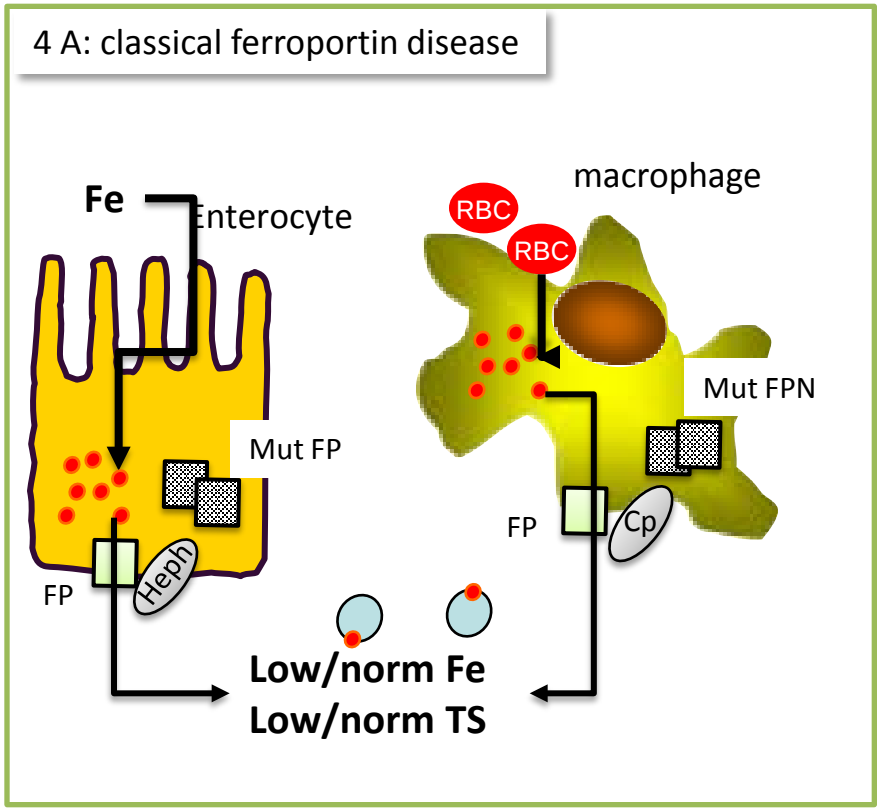
Extrahepatic IOL and damage

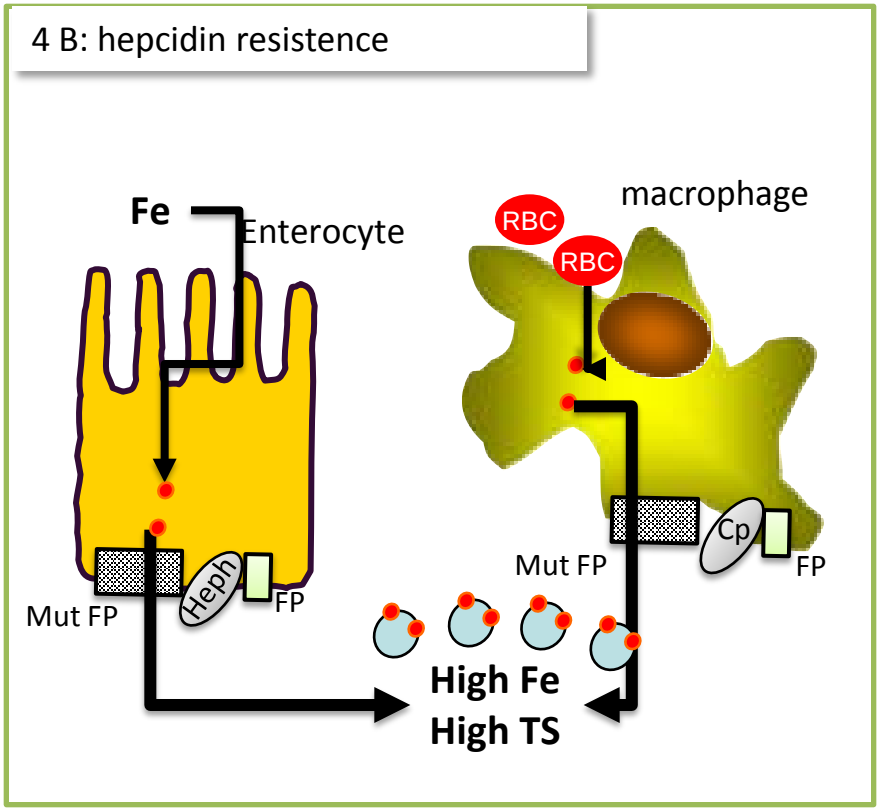
Clinical manifestations help in distinguishing HFE- vs Juvenile-HH

	HFE (n=93)	p value	Type 2 (n=26)	p value	Type 3 (n=11)
Age, years	44.8 ± 10.7	< 0.0001	23.3 ± 6.2	< 0.0001	39.4 ± 7.1
Tf saturation, %	87.7 ± 11.5	NS	88.6 ± 9.7	NS	92.9 ± 11.5
SF, µg/L	2,830 ± 2,239	NS	3,146 ± 1,270	0.003	2,023 ± 1,245
Hypogonadism, %	18.4	< 0.0001	96.1	< 0.0001	27.3
Cardiopathy, %	6.5	< 0.0001	34.6	NS	9.1
Reduced glucose tolerance, %	26.9	0.003	57.7	0.004	9.1
Cirrhosis, %	51.6	NS	42.1	NS	45.4
Arthropathy, %	12	NS	26.9	NS	36.3
IR, g	14.2 ± 8.9	NS	14.0 ± 5.2[a]	NS	14.4 ± 8.9[b]
IR/age	0.32 ± 0.2	< 0.001	0.65 ± 0.3[a]	NS	0.41 ± 0.3[b]
[a] 14 patients. [b] 6 patients.					

De Gobbi M, et al. Br J Haematol. 2002;117:973-9

Hemochromatosis type 4: genetic defects of ferroportin

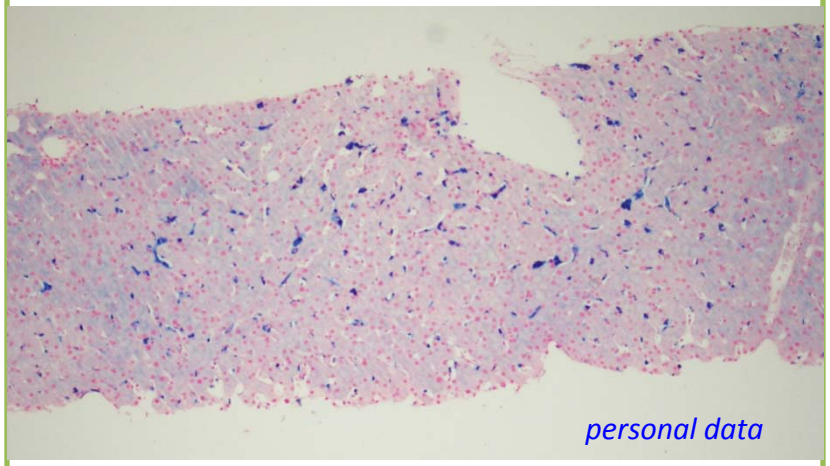
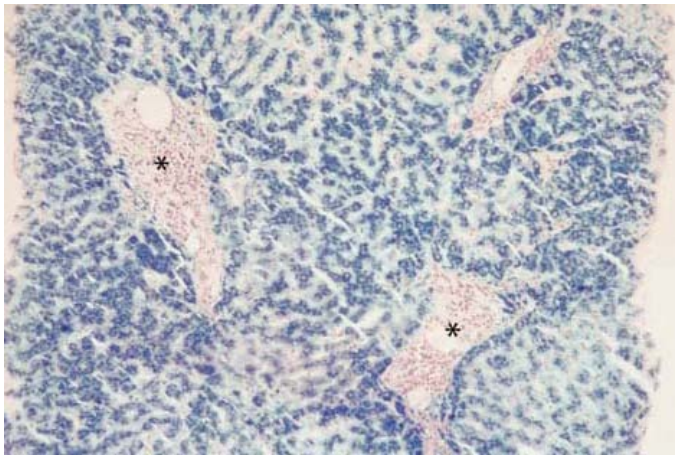




FP: ferroportin; Heph: hephestin; Cp: ceruloplasmin
Fe: s-iron; TS: transferrin saturation

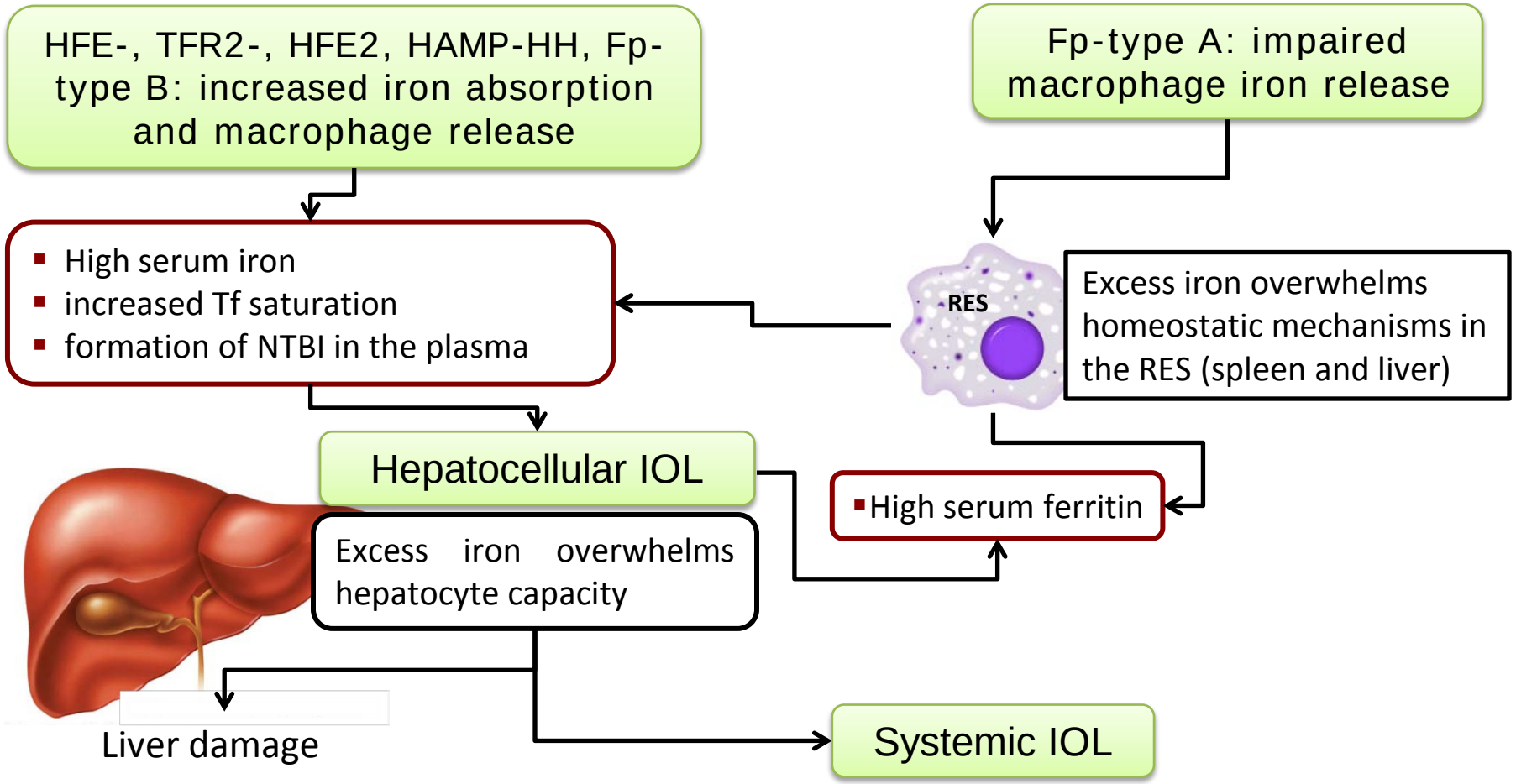
Le Lan et al. Gastroenterology 2011;140:1199–1207

Hemochromatosis type 4A and 4B have distinct phenotypes

Ferroportin disease type A	Ferroportin disease type B
22-year-old woman Tf saturation 24%; SF 3,200 µg/L; LIC 3.9 mg Fe/g dry wt	30-year-old men Tf saturation 88%; SF 5600 µg/L; LIC 14.5 mg Fe/g dry wt
	
Kupffer cell IOL	Hepatocellular IOL

Pietrangelo et al. N Engl J Med 1999;341:725-32;unpublished data

Physiopathology of iron overload defines iron phenotypes



NTBI, non-transferrin-bound iron;
RES, reticuloendothelial system; Tf, transferrin.

Hemochromatosis classification according to transferrin saturation

Increased serum ferritin

Transferrin saturation

<45%

No iron overload
Inflammation
Hepatocellular necrosis
High alcohol intake
Metabolic disorders (DHF)
Hereditary hyperferritinemia-cataract
Benign hyperferritinemia

Iron overload (mostly mild to moderate)
Chronic liver diseases (viral, ASH, NASH)
Metabolic disorders (DIOS)
Hemolytic anemias
Transfusional iron overload (early stages)
Parenteral iron administration (inadequate)
Ferroportin disease (HH type 4A)
Aceruloplasminemia

>45%

Primary iron overload
Hemochromatosis (type 1, 2 and 3)
Ferroportin disease (HH type 4b)

Secondary iron overload
Iron-loading anemias
Transfusional iron overload
End-stage liver disease
Porphyria cutanea tarda
Chronic liver diseases

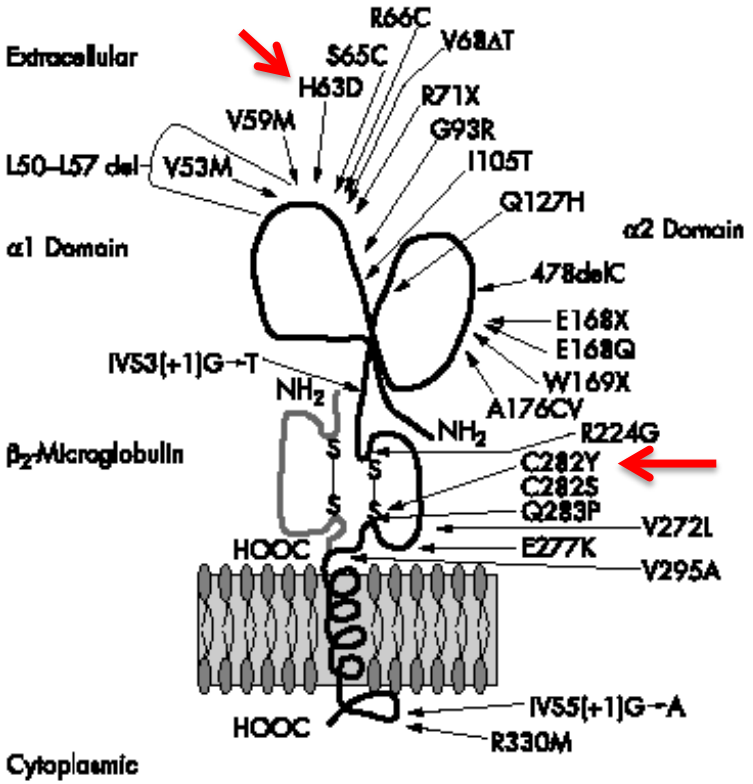
Other iron overload diseases
DMT1 deficiency
Hypotransferrinemia
Neonatal hemochromatosis
African iron overload

Piperno A. Expert Opin. Med. Diagn. 2013;7:161

Genetic testing in hemochromatosis

C282Y/C282Y
C282Y/H63D
H63D/H63D

HFE



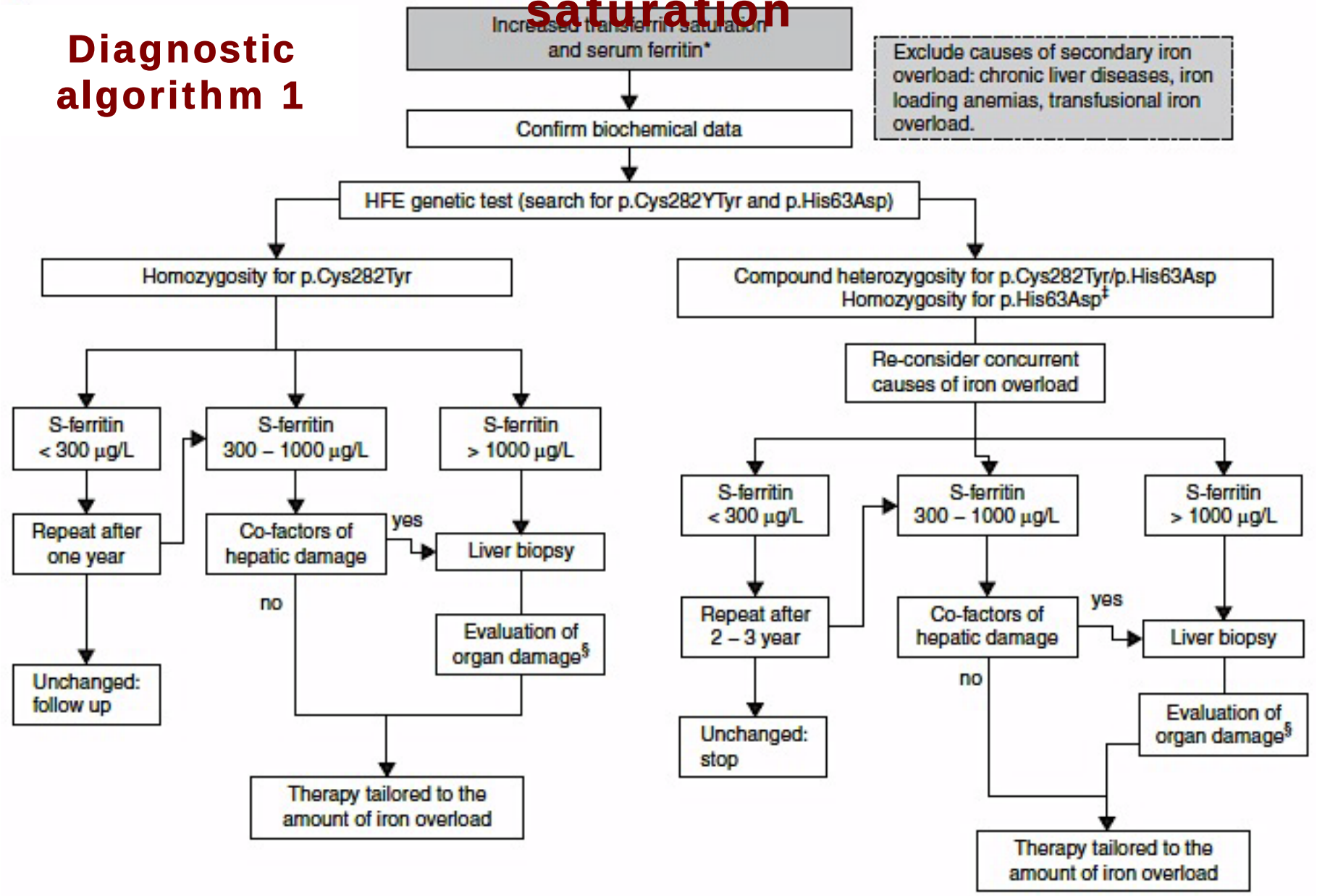
HFE-2
HAMP
TFR2
SLC40A1

Often private mutations.
Need gene sequencing

Piperno et al. Gastroenterology 2000;119:441-5; Robson et al. J Med Genet 2004;41:721; Piperno A. Expert Opin. Med. Diagn. 2013;7:161

Hyperferritinemia and high transferrin saturation

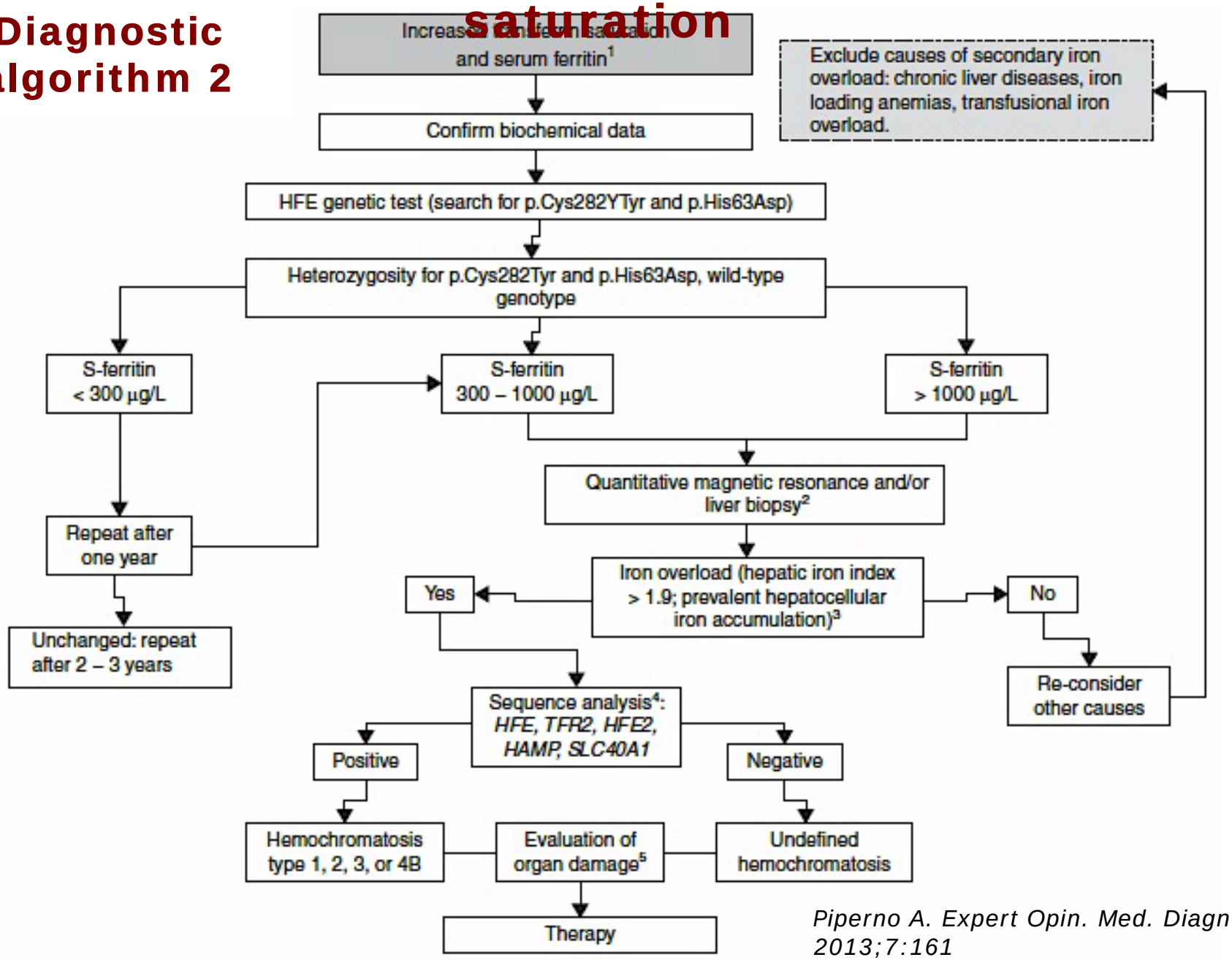
Diagnostic algorithm 1



Piperno A. Expert Opin. Med. Diagn. 2013;7:161

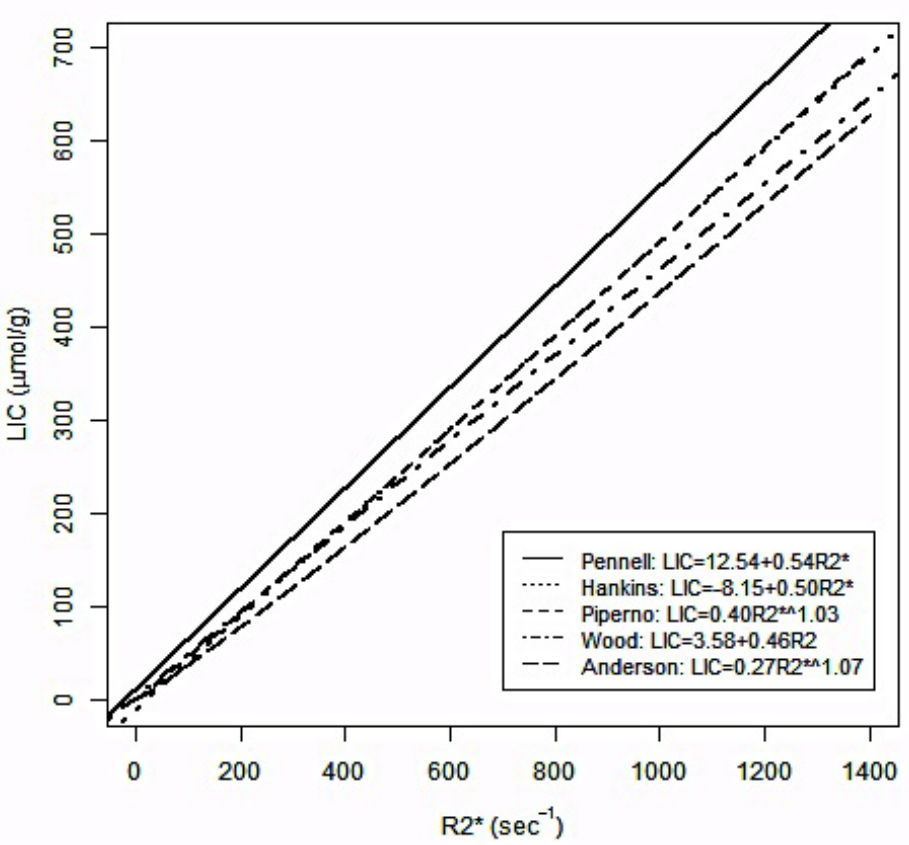
Hyperferritinemia and high transferrin saturation

Diagnostic algorithm 2



Piperno A. Expert Opin. Med. Diagn. 2013;7:161

Define the amount of iron overload
Quantitative MRI: Liver



Calibration equations for the prediction of LIC based on R2* proposed by different authors.

- Highly accurate noninvasive estimates of hepatic iron over the entire clinically relevant range.
- Interstudy variability is low, making it a good tool for serial evaluation of chelation efficacy, leading to closer monitoring.
- It is relatively inexpensive and can be performed at the same time for cardiac iron evaluation (T2*).

p.C282Y heterozygotes with HH-phenotype may carry rare HFE mutations

Table 2
Serum and hepatic iron data of patients with hemochromatosis due to compound heterozygosity for p.Cys282Tyr and rare HFE mutations published to date.

Genotype	Gender (M/F) Age (years)	Transferrin saturation (%)	Ferritin (μg/L)	Iron Overload (hepatic iron and/or iron removed)	Reference
p.[Cys282Tyr] + [Arg71X]	M; 62 years	93	745	HIC: 210.8 μmol/g	5
	M; 65 years	100	543	-	
p.[Cys282Tyr] + [Leu183Pro]	M; 44 years	72.5	2070	Liver MR: heavy iron deposition, IR: 8.5 g	8
	M; 34 years	83	544	IR: 2.25 g	
p.[Cys282Tyr] + [Gln283Pro]	M; 30 years	90	395	IR: 3 g	9
	F; 37 years	74	99	-	
p.[Cys282Tyr] + [Gly93fs]	M; 62 years	88	845	Perls' staining: 4+	10
p.[Cys282Tyr] + [Tyr138X]	M; 63 years	90	1000	-	11
p.[Gly43Asp; His63Asp] + [Cys282Tyr]	M; 61 years	94	1900	Liver MR: 160 μmol/g IR: > 6 g	12
p.[Cys282Tyr] + c.[616+1G>T]	M; 52 years	-	-	HIC: 260 μmol/g , IR: 14 g	6
	F; 55 years	73	386	-	
p.[Cys282Tyr] + [Gly93Arg]	M; 40 years	78	861	Perls' staining: 4+	19
	F; 37 years	-	-	Perls' staining: 4+	
p.[His63Asp;Cys282Ser] + [Cys282Tyr]	M; 60 years	100	3958	Perls' staining: 3+	14
	M; 22 years	74	276	Perls' staining: 3+ ; HIC: 127.6 μmol/g	
p.[Cys282Tyr] + [Glu168X]	M; 48 years	96	694	HIC: 235.2 μmol/g, IR: 4g	4
	M; 45 years	78	774	HIC: 176.4 μmol/g	
	M; 47 years	79	1206	HIC: 357.2 μmol/g, IR: 23g	
p.[Cys282Tyr] + [Trp169X]	M; 42 years	86	608	HIC: 143 μmol/g, IR: 3.6g	4
	M; 50 years	106	2740	HIC: 425 μmol/g, IR: 21g	
	M; 66 years	100	1351	HIC: 356.4 μmol/g	

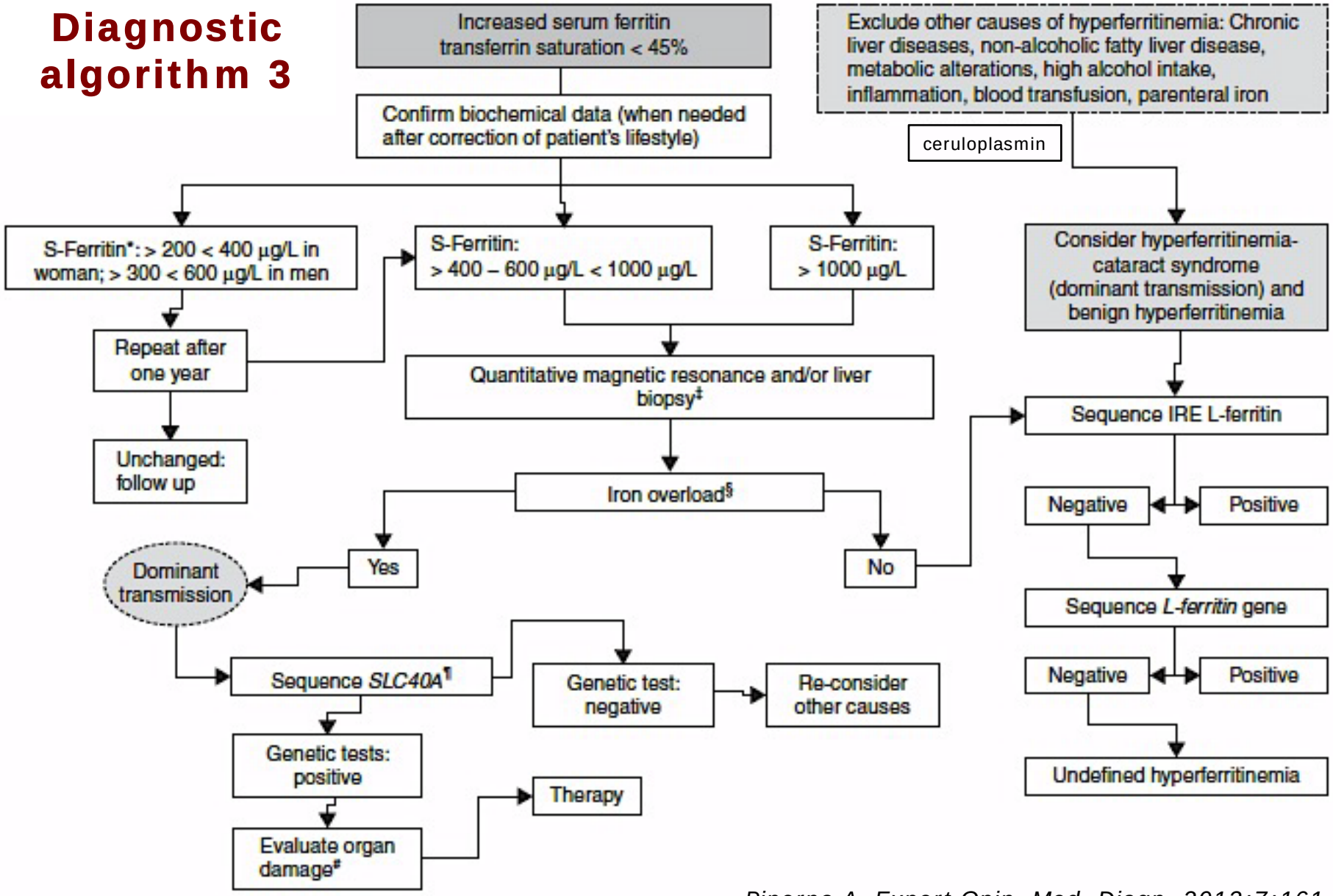
HIC: Hepatic iron concentration; MR: magnetic resonance; IR: iron removed, Perls' staining 3+: > 50–75% positive hepatocyte staining, 4+ : diffuse strong positive hepatocyte staining.. Genotypes are reported according to the guideline for mutation nomenclature of Human Genome Variation Society (www.hgvs.org/mutnomen/refseq.html).

All of the patients with compound genotypes had transferrin saturation largely higher than the usual cut-off for HH (45%), and had a prevalent iron accumulation in hepatocytes, thus presenting with a full expressed iron overload phenotype.

Mariani et al. BCMD 44 (2010) 159–163

Hyperferritinemia and normal TS

Diagnostic algorithm 3



Piperno A. Expert Opin. Med. Diagn. 2013;7:161

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2007.00950.x

Novel mutations of the ferroportin gene (*SLC40A1*): analysis of 56 consecutive patients with unexplained iron overload

No causal mutations in HFE, HJV, HAMP, TfR2

Table 1. Iron status of the patients[a]

	Men ($n = 44$)	Women ($n = 12$)	Total ($n = 56$)
Age (years)	56 (28–73)	48 (22–70)	54 (22–73)
Hb (g/dl)	15 (11.9–17.3)	13.3 (11.1–15.3)	14.7 (11.1–17.3)
TS (%)	36 (18–118)	56 (28–99)	37 (18–118)
TS > 45%	10 (23)	8 (67)	18 (32)
SF (μg/l)	1059 (425–3354)	1742 (529–3987)	1173 (425–3987)
SF > 1000 μg/l	24 (55)	10 (83)	33 (59)
TIS	18 (12–40)	36 (14–46)	19 (12–46)
TIS/age	0.3 ± 0.1 (0.2–1)	0.7 (0.3–1.2)	0.4 (0.2–1.2)

Hb, hemoglobin; TS, transferrin saturation; SF, serum ferritin; TIS, total iron score (biopsy performed in 55 patients).
[a]Data are reported as median and (range) and *n* (%).

Fp mutations

2/56 (3.6%)

Solo probandi con familiari affetti: 2/25 (8%)
Con alterazioni maggiori degli indici del ferro: 2/11 (18.2%)

Lo studio familiare dovrebbe precedere il test genetico in pazienti candidati. La presenza di alterazioni maggiori degli indici del ferro nei familiari dei probandi può essere un criterio aggiuntivo per migliorare il rapporto costo-efficacia dell'analisi genetica.

Pelucchi et al, Clin Genet 2008

NGS as a new tool for genetic diagnosis of patients with HH phenotype with a negative first level test (p.C282Y and p.H63D)

Identification of novel mutations in hemochromatosis genes by targeted next generation sequencing in Italian patients with unexplained iron overload

Am. J. Hematol. 91:420–425, 2016.

Sadaf Badar,[1] Fabiana Busti,[1] Alberto Ferrarini,[2] Luciano Xumerle,[2] Paolo Bozzini,[1] Paola Capelli,[3] Roberto Pozzi-Mucelli,[4] Natascia Campostrini,[1] Giovanna De Matteis,[5] Sergio Marin Vargas,[2] Alejandro Giorgetti,[2] Massimo Delledonne,[2] Oliviero Olivieri,[1] and Domenico Girelli[1,6]*

Case Report

EASL JOURNAL OF HEPATOLOGY

http://dx.doi.org/10.1016/j.jhep.2015.06.027

Next-generation sequencing: Application of a novel platform to analyze atypical iron disorders

Cameron J. McDonald[1], Lesa Ostini[1], Daniel F. Wallace[1,3], Alison Lyons[2], Darrell H.G. Crawford[3], V. Nathan Subramaniam[1,3,*]

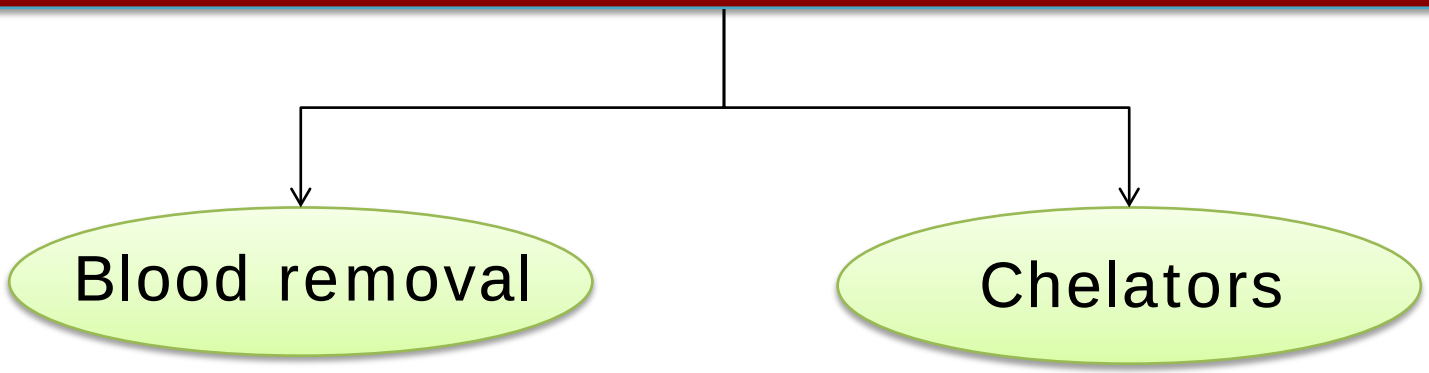
[1]*Membrane Transport Laboratory, QIMR Berghofer Medical Research Institute, Australia;* [2]*Gosford Hospital, Australia;* [3]*Faculty of Medicine and Biomedical Sciences, The University of Queensland, Brisbane, Australia*

Iron removal has to be TAILORED according to

- Causes and pathogenesis of iron accumulation
- Severity of iron load
- Presence of organ damage
- Clinical status
- Goals of iron removal
  (prevention, rescue, maintenance)

in order to choose the best therapeutic regimen: blood removal or iron chelation therapy and their frequency, dosage and duration of the therapy.

Management of iron overload



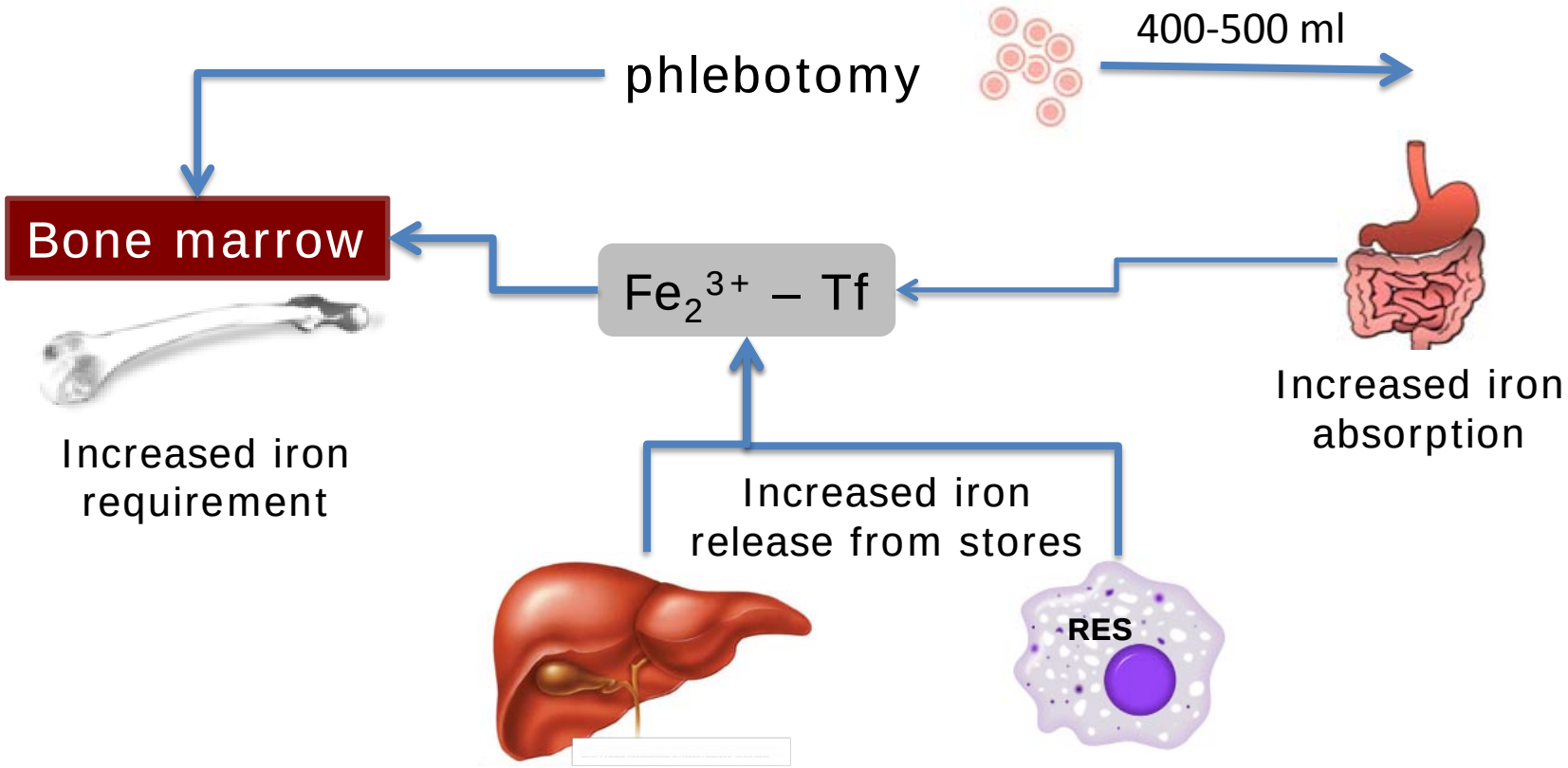
Phlebotomy
- Hemochromatosis w/o anemia
- Secondary IOL w/o anemia (long term survivors of HSCT; IOL associated with CLDs)

Erythrocyto-Aferesis
- Selected cases

DFO, DFP, DFX
- Contraindication to phlebotomy
- Primary or secondary IOL with anemia
- Iron removal from brain in aceruloplasminemia and neurological focal iron disorders

Blood removal

- Iron depletion through removal of RBC (1 ml of RBC ≅ 1 mg of iron; 500 ml of whole blood ≅ 250 mg of iron).
- Erythropoiesis activation and mobilization of iron from stores.



Tf, transferrin.

Blood removal

Treatment	Advantages	Compliance	Disatavantages	Adverse effects
Phlebotomy	Much experience; effective on the part of clinician; available, safe, inexpensive	Excellent for iron depletion; good for maintenance*	Repeated visits to healthcare facility; requires normal erythropoiesis; some patients report intolerance	Transient hypovolemia; fatigue; increases iron absorption; iron deficiency if monitoring inadequate or inappropriate
Erythrocyto apheresis	Rapid, safe; may be preferred for patients with severe iron overload; hysovolemia	Excellent in selected patients	Limited clinical experience; requires special apparatus and facility, limited availability; Expensive°	fatigue; increases iron absorption; citrate reaction; iron deficiency if monitoring inadequate or inappropriate

*In some countries asymptomatic HH patients are accepted as donors. In general, the policies regarding blood donation from patients remain widely variable (Pauwels NS et al. Vox Sang 2013)
°A recent study suggested that total apheresis treatment costs are not significantly higher because fewer treatment procedures are needed (Rombout-Sestrienkova E et al. Tranfusion 2012).

Modified from Adams PC & Barton JC. Blood 2011; Mariani et al. Haematologica 2005; 90:717-718

Blood removal: phlebotomy

Guidelines recommend starting therapy in in HH patients with SF >200 μg/L in women, >300-350 μg/L in men)

Induction phase

- 375 to 500 ml according to sex, height and weight.
- Phlebotomies every 1-3 weeks according to ferritin level.
- Ferritin every 4-8 phlebotomies according to its baseline and follow-up levels.
- If Hb <12 g/dl in men or 11 g/dl, in women, discontinue or delay phlebotomies.
- For HH type 4A, there is a risk of anemia during treatment.
- The goal is to achieve iron depletion: serum ferritin around 50 μg/L.

Maintenance phase

- The goal of the maintenance phase is to keep ferritin level between 50-100 μg/L. (MRI for residual tissue iron excess, if needed)
- Usually, one phlebotomy every 2–4 months is sufficient, but sometimes can be every six.
- Ferritin is checked after every 6 or 12 months, according to phlebotomy frequency.
- In the absence of a physiological iron removal mechanism, phlebotomies are performed lifelong.

Absolute contraindications: anemia, severe cardiopathy not linked to HH. Temporary or relative contraindications: BP<100 mmHg, lower limb arteriopathy, stroke, heart beat <50 bpm or >100 bpm, pregnancy, insufficient venous network and an impaired general condition.

Blood removal

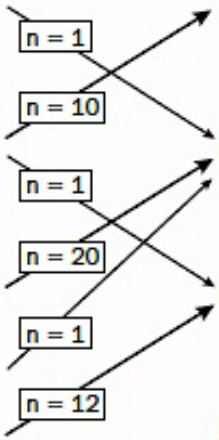
Efficacy on signs and symptoms

- Early diagnosis and treatment reduce morbidity and mortality in hemochromatosis
- It reverses asthenia and pigmentation, normalizes transaminases.
- May significantly improve cardiac function.
- May favour regression of hepatic fibrosis / cirrhosis.
- Improvement of diabetes is partial and inconstant
- Can improve early stages of hypogonadism, induce partial and inconstant improvement in established hypogonadism.
- It has variable, unpredictable effect on arthropathy.

AASLD Guidelines, Hepatology 2011; Niederau K et al. NEJM 1996; Cecchetti et al. Eur Heart J 1991; Falize et al. Hepatology 2006

Regression of severe liver fibrosis after phlebotomies in hemochromatosis

Fibrosis stage	At diagnosis (%)	After documentation of iron removal (%)	Improved	Worsened	Unchanged	Total
0	21/185 (11.4)	30/185 (16.2)	0	1	20	21
1	32/185 (17.3)	42/185 (22.7)	10	1	21	32
2	39/185 (21.8)	32/185 (17.3)	20	0	19	39
3	93/185 (50.2)	81/185 (43.8)	12	0	81	93
Total			42	2	141	185



NOTE. $\chi^2 = 33.19$; $P = 0.000009625$.

13% improvement

Niederau K et al. Gastroenterology 1996

47% improvement

	Second Liver Biopsy					
Fibrosis Assessment	**F 0**	**F 1**	**F 2**	**F 3**	**F 4**	**Total**
First liver biopsy						
F 3	3	6	2	0	2	13
F 4	1	4	3	2	13	23
Total	4	10	5	2	15	36

Falize et al. Hepatology 2006

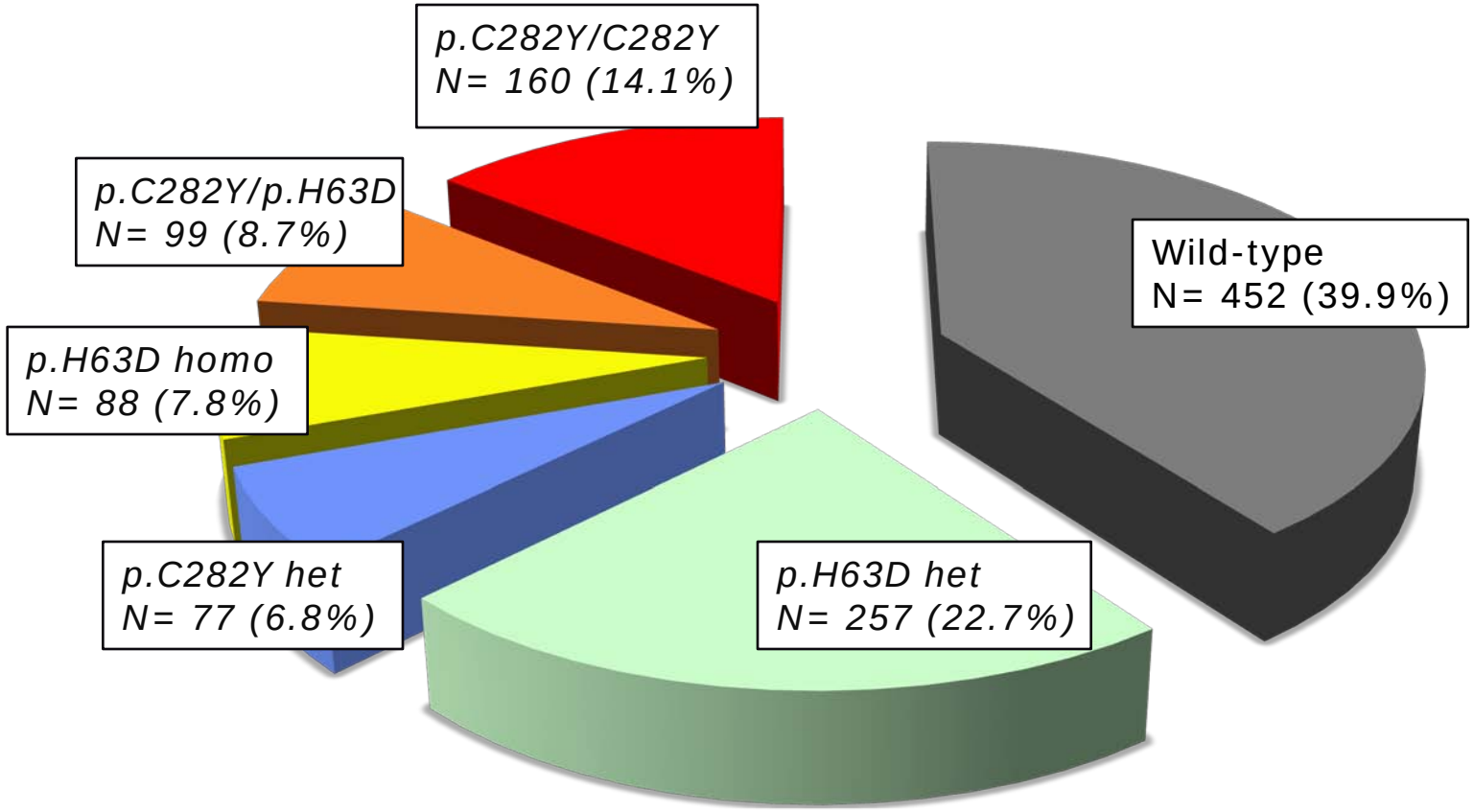
Hemochromatosis: key points

- Molecular tests have acquired a relevant role in the diagnostic setting of hemochromatosis. *HFE* genotyping has clinical validity and utility, and is cost-saving in the diagnosis of probands and affected relatives if rigorous criteria of phenotype characterization are used.
- A careful clinical and biochemical evaluation of the index case has to be done before performing DNA testing that should be used in conjunction with testing iron parameters when there is a clear clinical indication of suspicion of being at risk for HH or when being at familial risk for HH.
- There will be a small group of patients who have iron overload with no other explanation who are negative for the common mutations in *HFE*. Further genetic testing can be impractical in a routine clinical setting but can be performed in referral centers according to strict criteria of phenotype characterization that should include non-invasive or invasive evaluation of liver iron.
- Liver biopsy is no more needed for the diagnosis of type 1 HH, but is still necessary for prognostic purposes. It can be still a useful diagnostic tool in specific circumstances.
- Treatment of HH should be tailored according to the type of HH, *HFE* genotype, and disease expression.

Piperno A. Expert Opin. Med. Diagn. 2013;7:161

Clinical validity of HFE testing

evaluation of 1133 patients presenting with hyperferritinemia



69.4% no significant genotype, mostly with normal transferrin saturation

Coletti & Piperno, thesis unpublished

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Iron Metabolism Diseases
Hereditary Anemias
Hereditary Metabolic Diseases

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