Winter School 2014
Saas Fee January 12 – 18

Iron deficiency and…….
Iron Refractory Iron Deficiency Anemia (IRIDA): New insights and diagnosis

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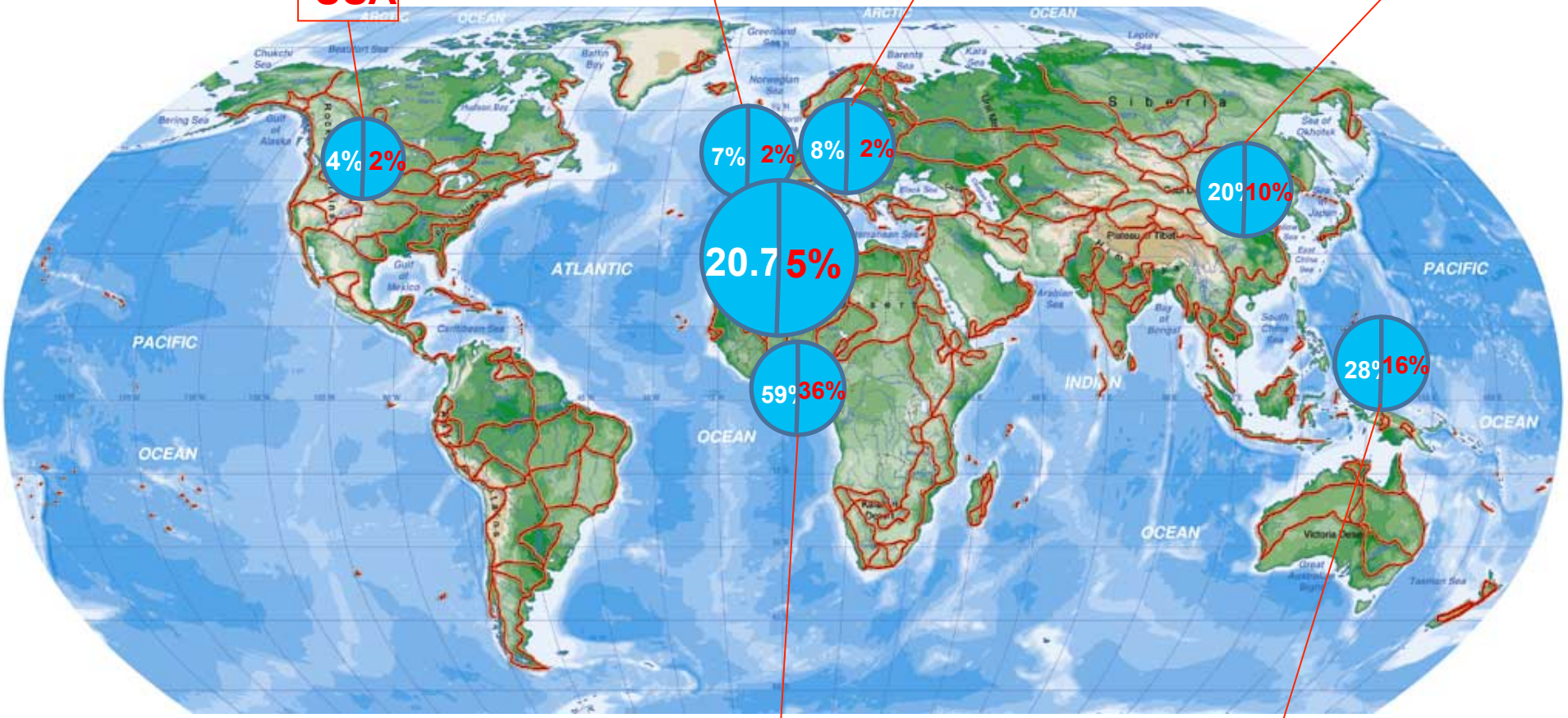
Prevalence of Iron Deficiency and Iron Deficiency Anemia



Iron content in the body in different age

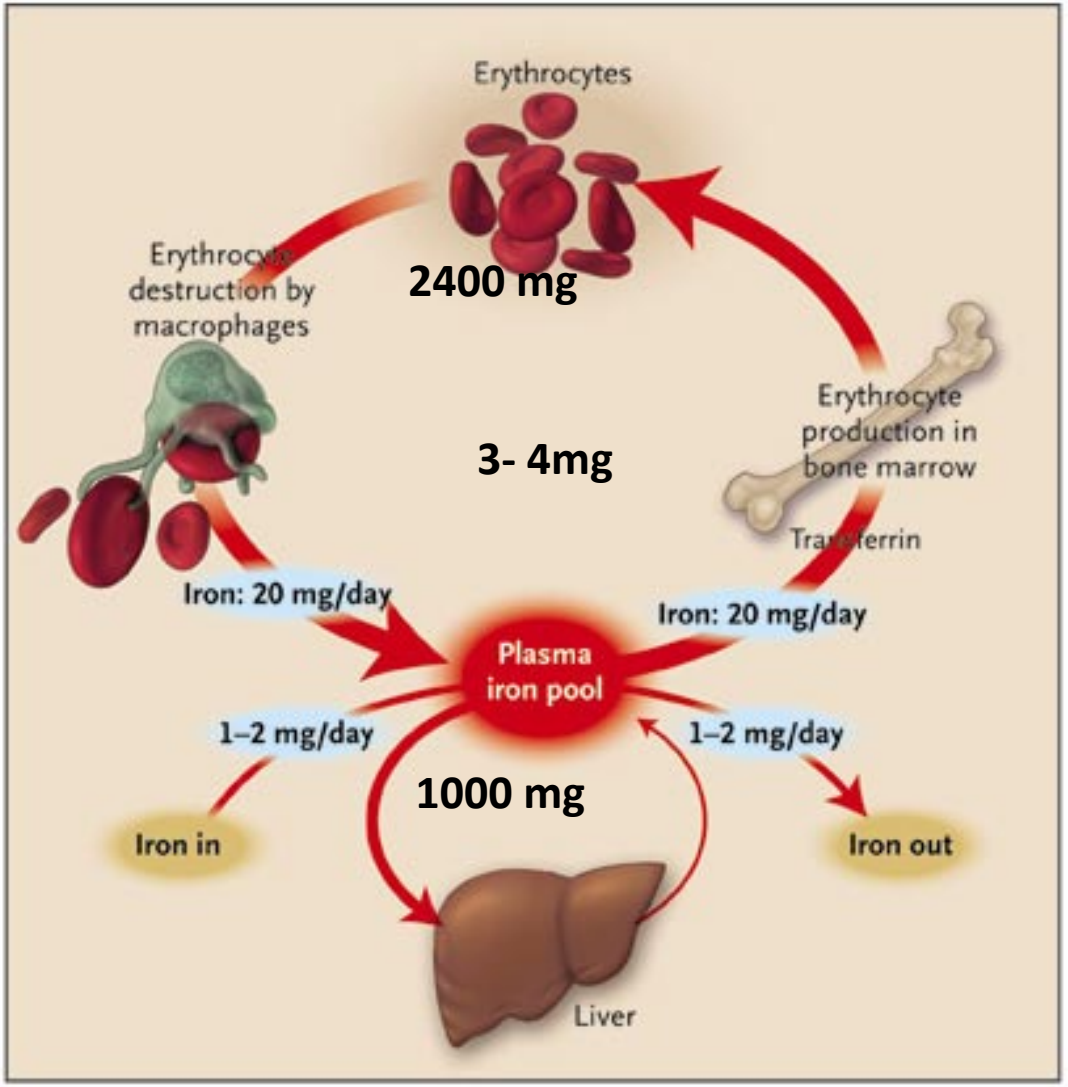


1kg body weight=
50 mg Fe

	Newborn (3,300 Kg)	*Children(35 Kg)*	*Adult (75 Kg)*
Total iron	**240-250 mg**	**1,5 – 2 g**	**3 -4 g**
HB	132 – 137,5 mg (55%)	1 – 1,4 g (68%)	2,04 – 2,72 g (68%)
Ferritin	101 – 105 mg (42%)	400 – 500 mg (27%)	0,81 -1,08 g (27%)
Myoglobin	7 -7,5 mg (3%)	60 – 80 mg (4%)	120 – 160 mg (4%)
Enzyme		9 – 12 mg (0,6%)	18 – 24 mg (0,6%)
Transferrin		15 – 20 mg (0,1%)	3 – 4 mg (0,1%)

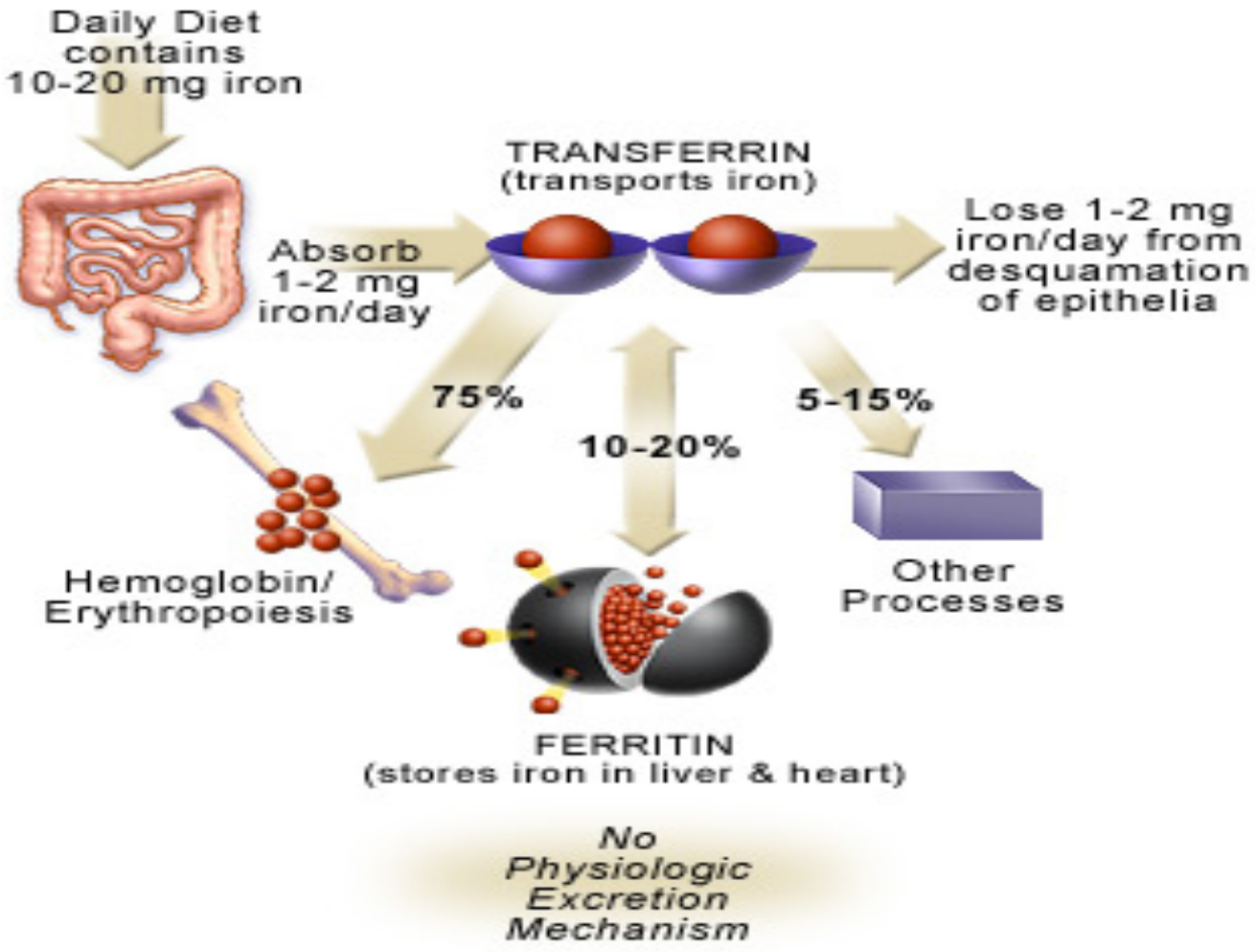
Iolascon A et al.,2013

Iron Economy



Pietrangelo A. *N Engl J Med*. 2004;350:2383-2397

Iron metabolism



Iron balance is maintained by the meticulous regulation of iron absorption from the intestine because there is no regulated pathway for iron excretion

(Andrews, NEJM, 1999)

Factors that modify the bioavailability of oral iron

Cysteine
Low gastric pH
Vitamin A,B,C
Copper
Manganese

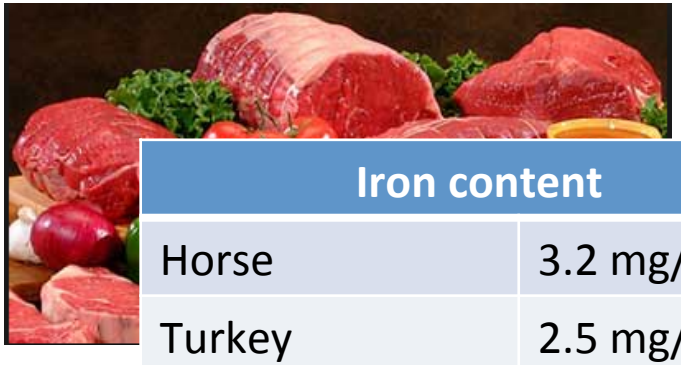
Tannin
Fibers
Some drugs

Iolascon A et al.,2013

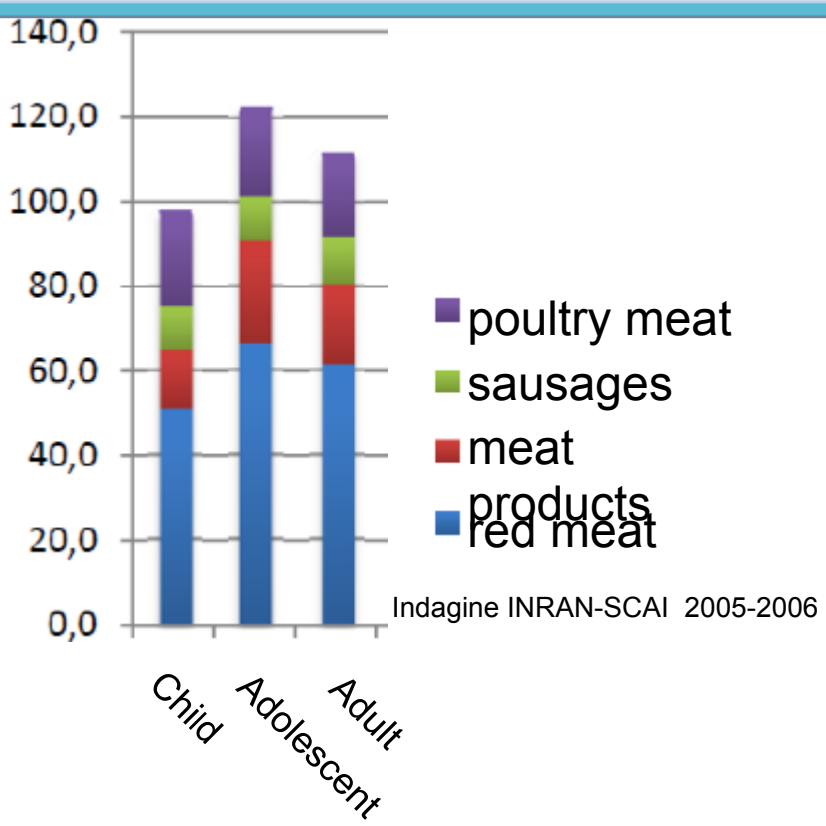
Meat and Iron

Red Meat: -beef, horse , heep, duck…

White Meat: - rabbit, chicken, turkey, pig…



Iron content	
Horse	3.2 mg/100g
Turkey	2.5 mg/100g
Beef	2.1 mg/100g
Pig	1.5 mg/100g
Chicken	1.5 mg/100g



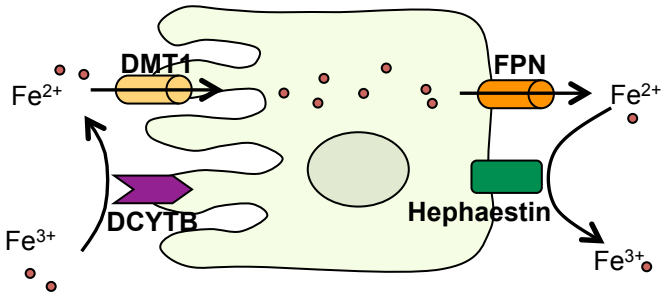
Indagine INRAN-SCAI 2005-2006

The iron cycle

Iron absorption

Enterocyte

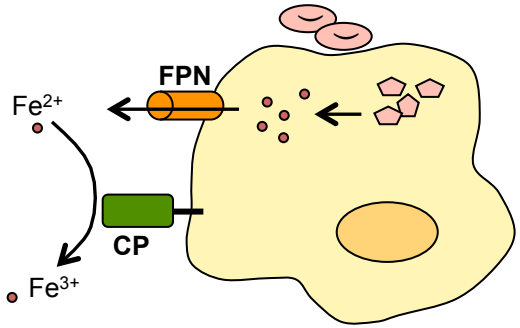
- 1–2 mg/day
- Hepcidin-regulated
- Balanced by iron losses (1–2 mg/day)
- Reduced in inflammation
- Increased in iron deficiency



Iron recycling

Macrophage

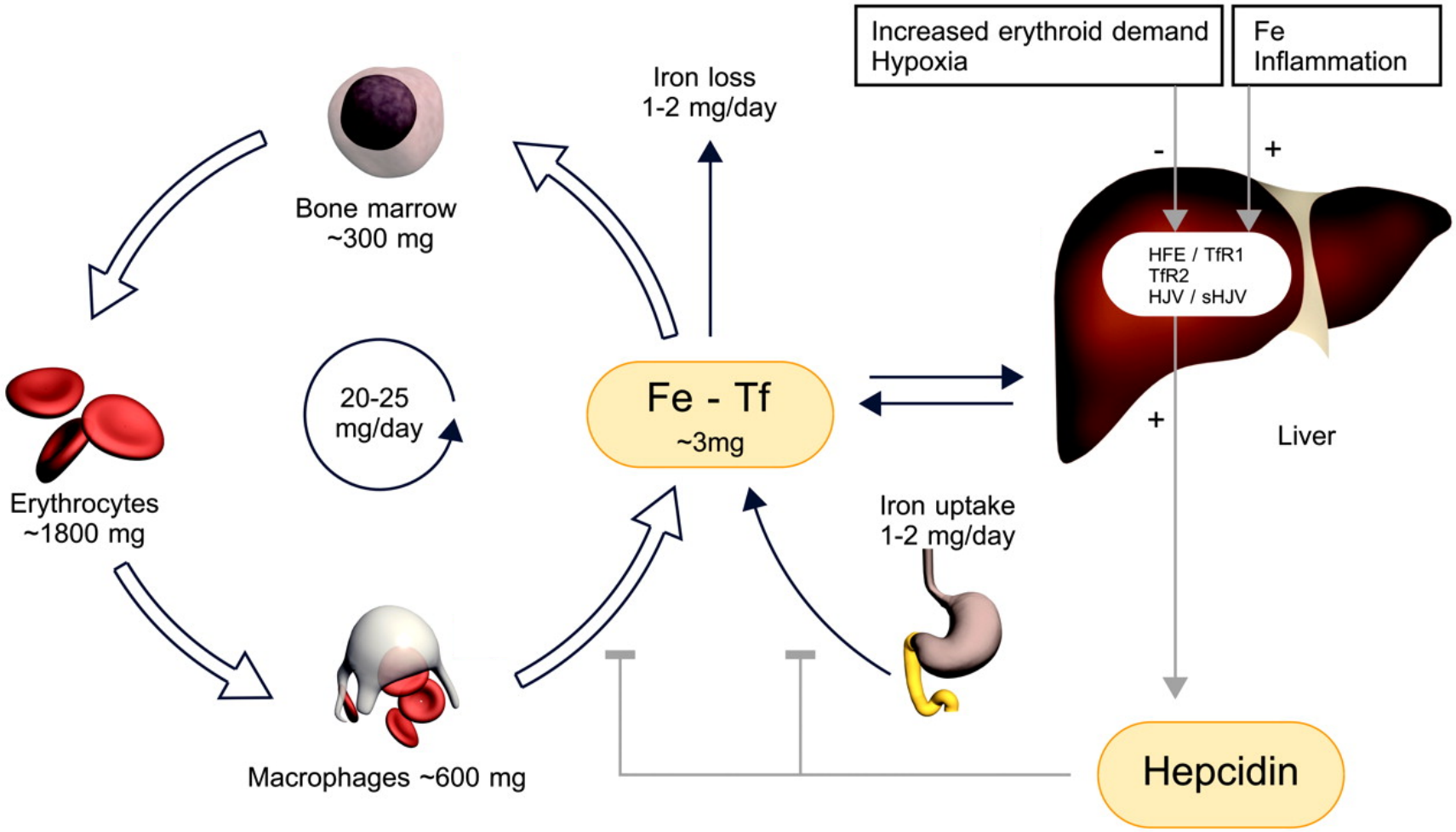
- 20–30 mg/day
- Hepcidin-regulated
- Balanced by erythroid request
- Reduced in inflammation
- Increased in iron deficiency



CP = ceruloplasmin; DCYTB = duodenal cytochrome B;
DMT1 = divalent metal transporter 1; FPN = ferroportin.

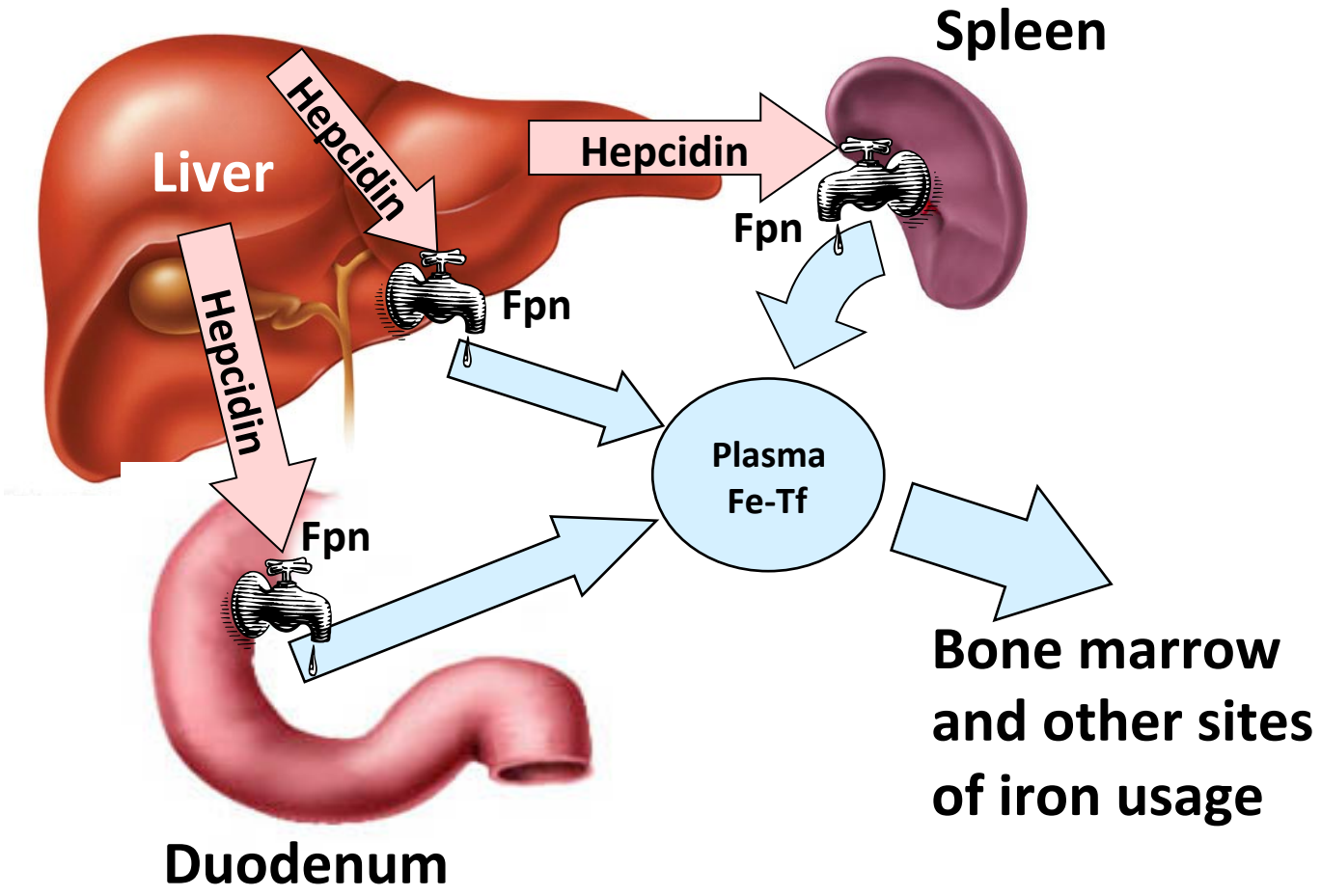
Finch C. Blood. 1994;84:1697-702. Andrews NC. Blood. 2008;112:219-30.

PATHWAY OF IRON EXCHANGE



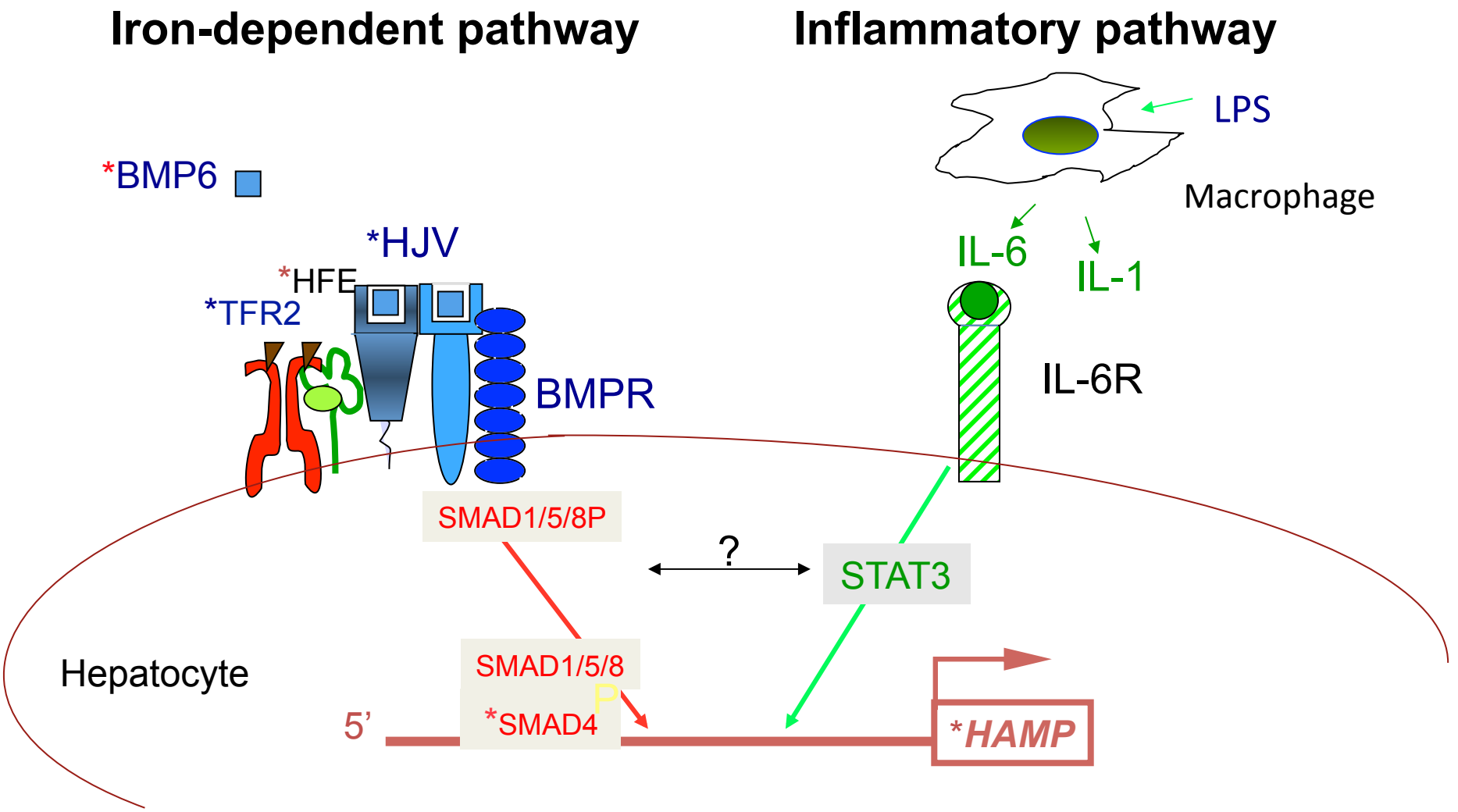
Swinkels DW *et al*. *Clin Chemistry* 2006;52:950-68.

How Hepcidin Regulates Iron



Nemeth E, et al. *Science.* 2004;306:2090-2093

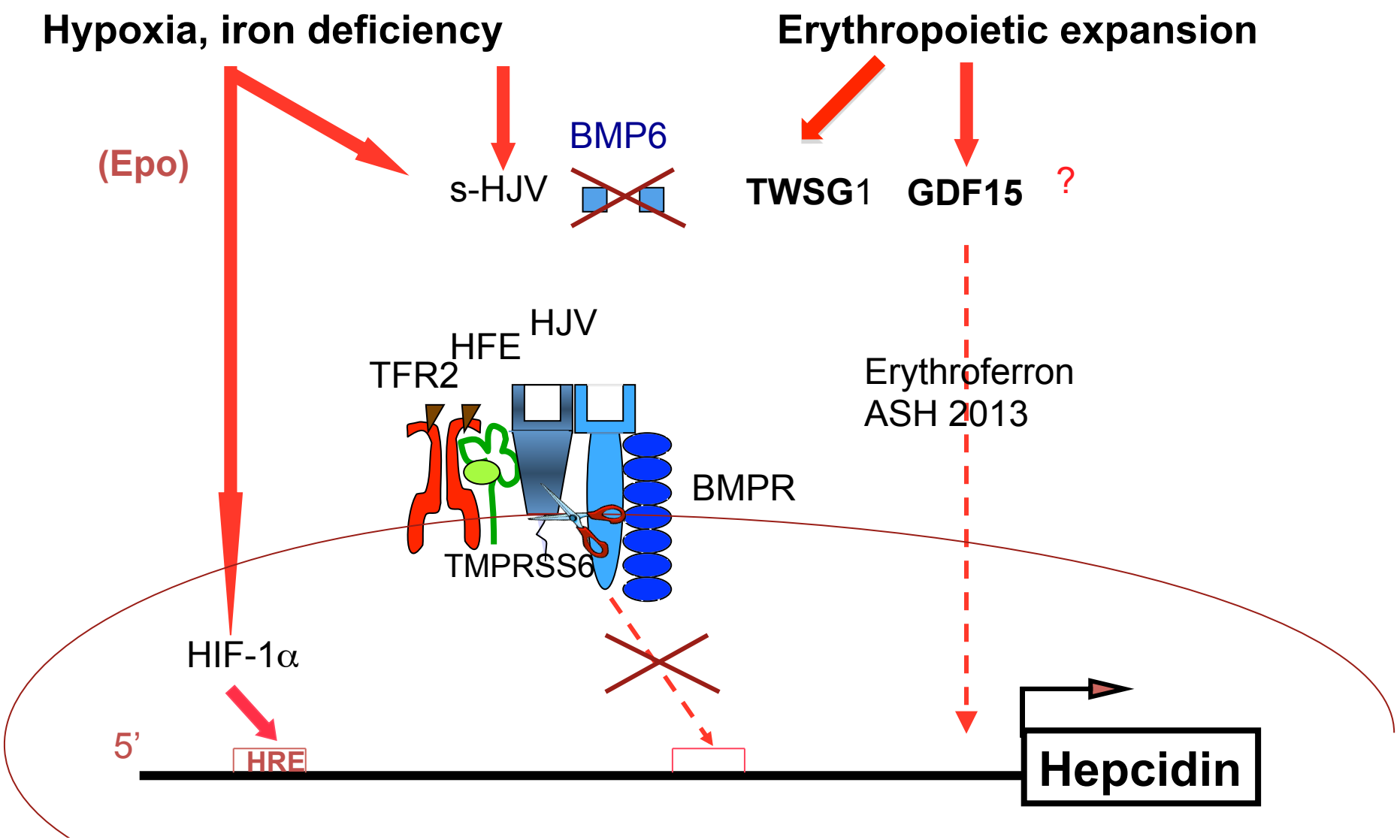
Hepcidin upregulation: two pathways



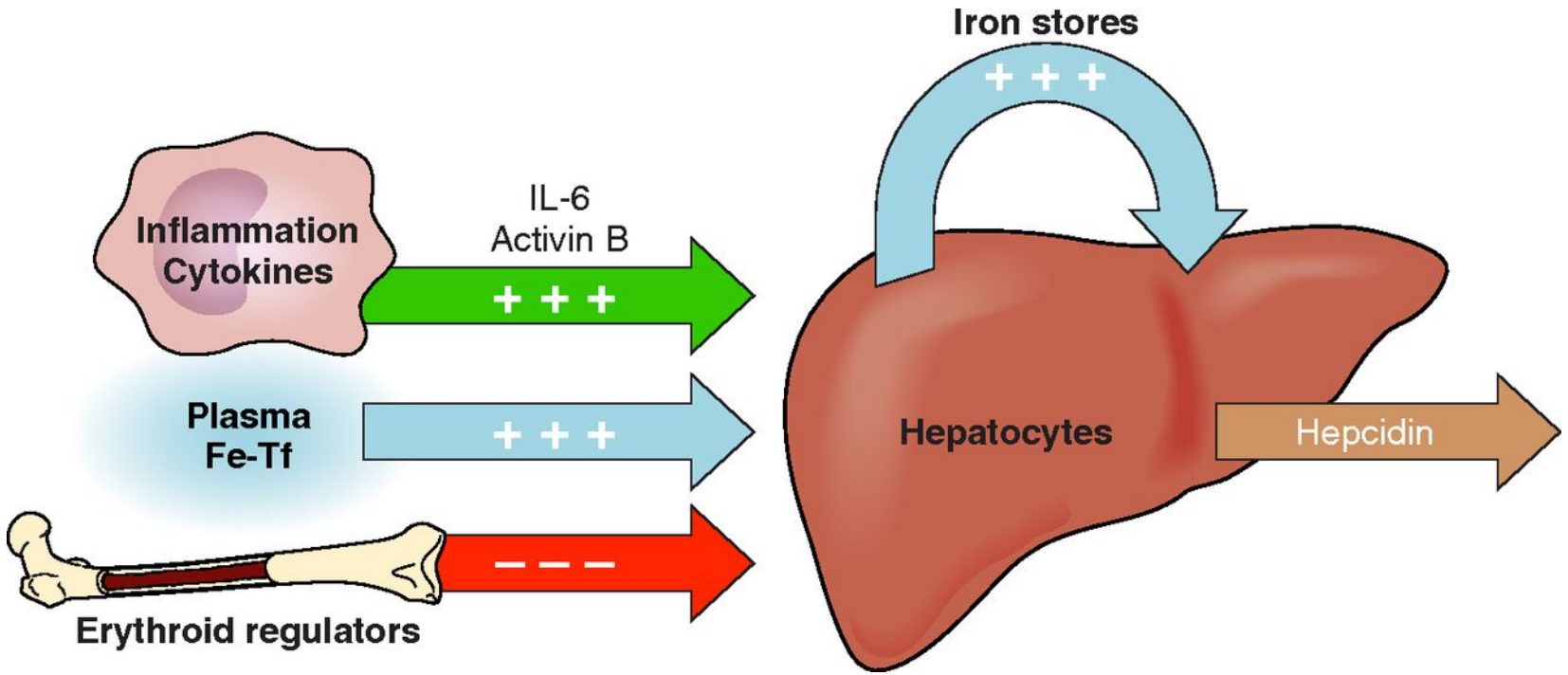
LPS = lipopolysaccharide.

* Indicates proteins whose inactivation causes iron overload.

Hepcidin downregulation: multiple pathways

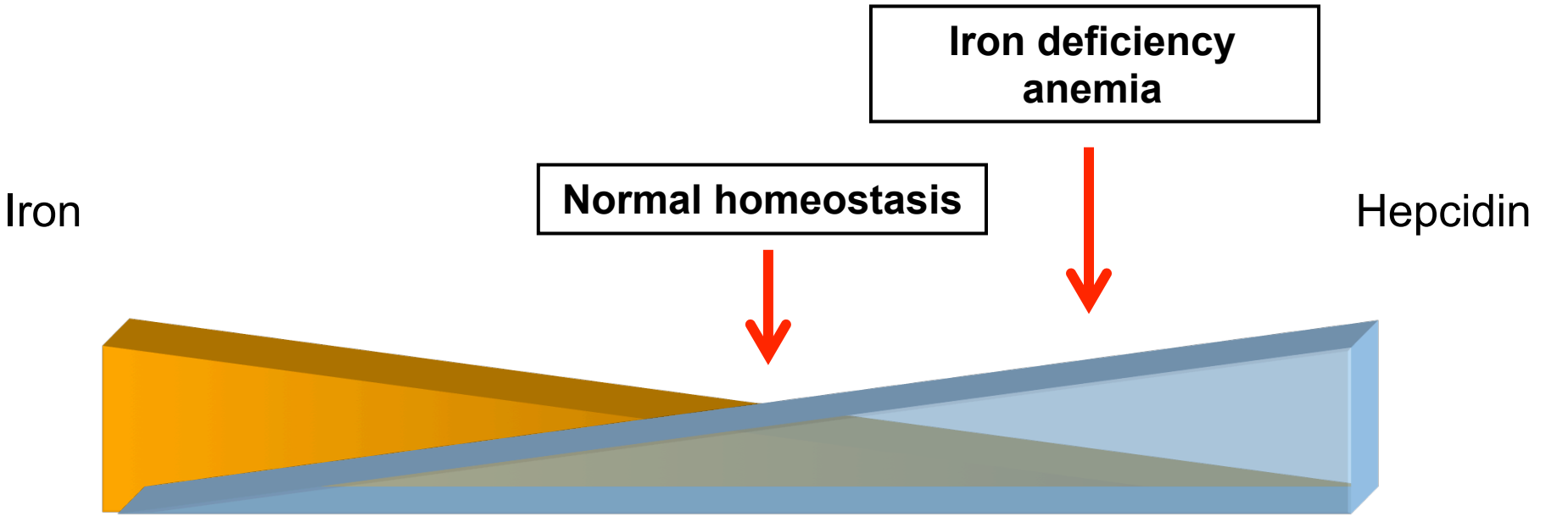


Regulation of hepcidin synthesis in hepatocytes.



Ganz T Physiol Rev 2013;93:1721-1741

Diseases of Hepcidin Dysregulation

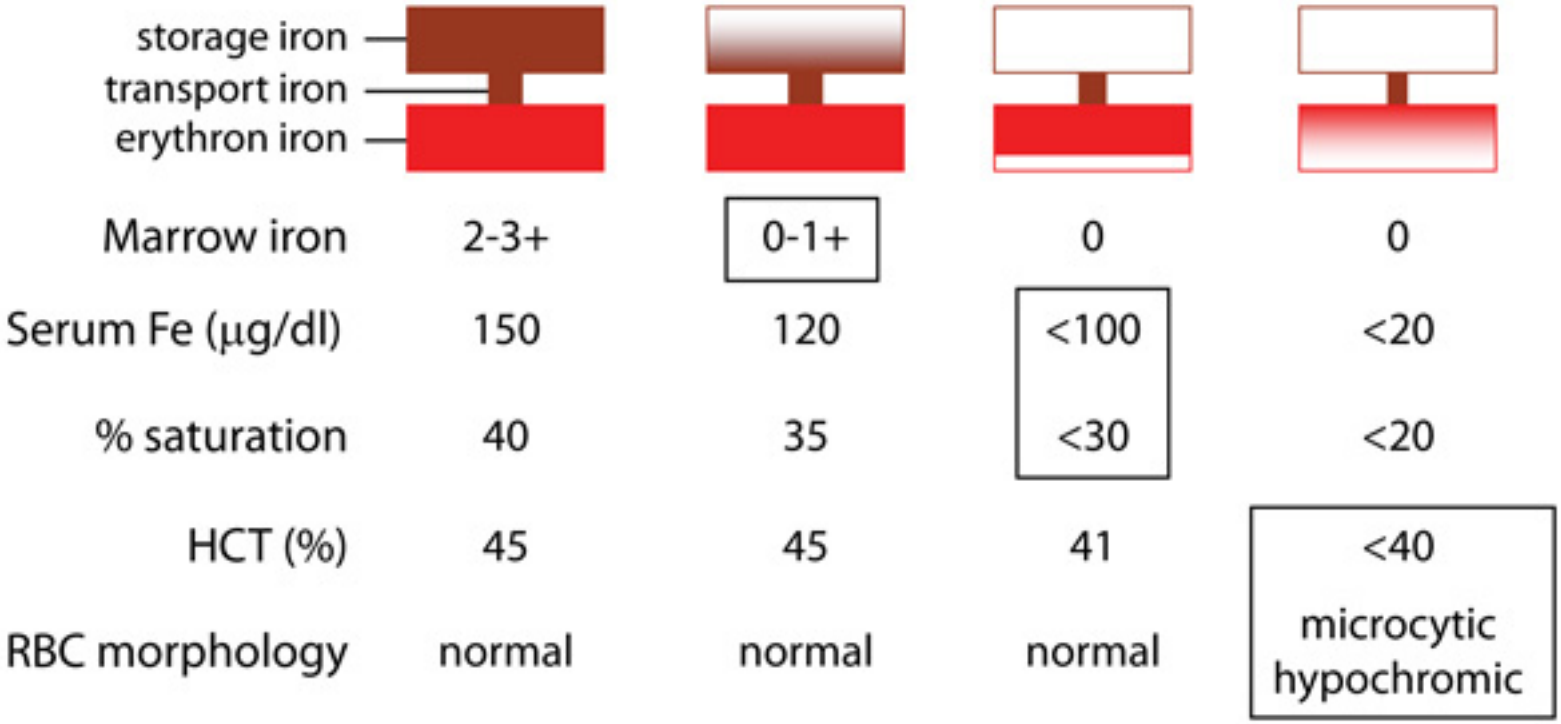


Hereditary haemochromatosis
Iron-loading Anaemias

Anaemia of Inflammation
Iron-refractory iron-deficiency anaemia
Hepcidin-secreting tumors

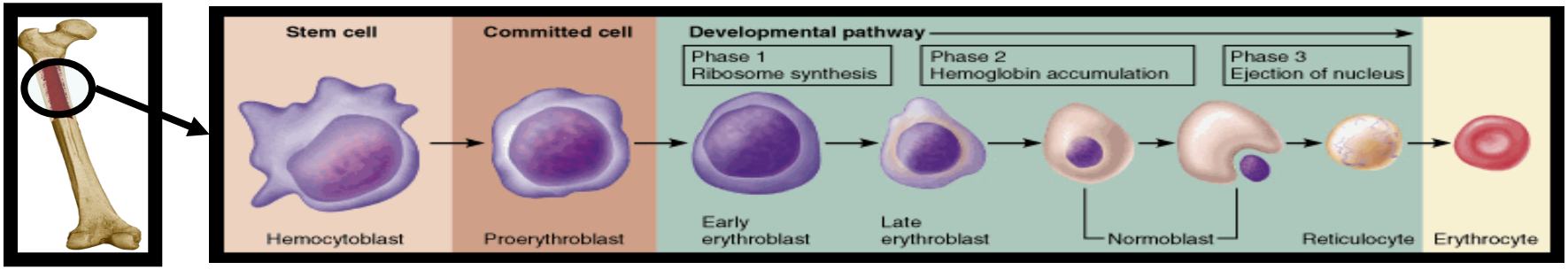
Ganz T. *J Am Soc Nephol*. 2007;18:394-400.
Ganz T, Nemeth E. *Am J Physiol Gastrointest Liver Physiol*. 2006;290:G199-G203.
Courtesy of Tomas Ganz, PhD, MD.

Iron deficiency and iron deficiency anemia

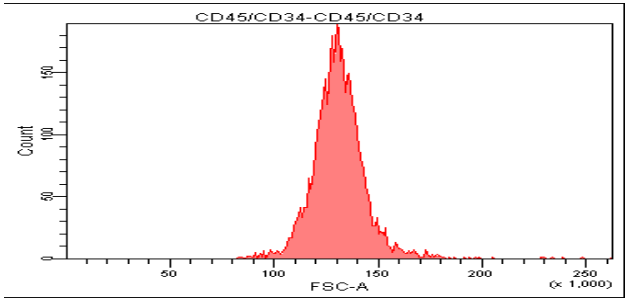


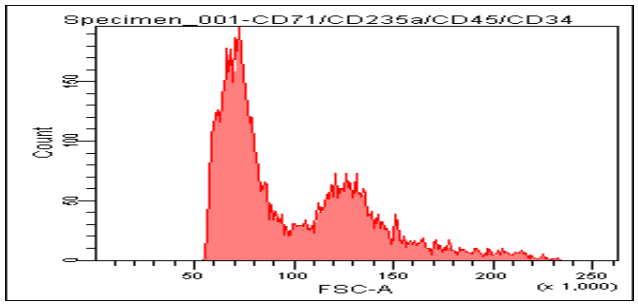
Iolascon A et al.,2013

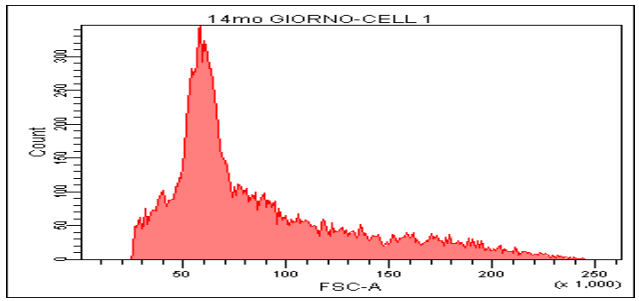
MCV During Differentiation



Globins, iron, and heme play a critical role in MCV determination

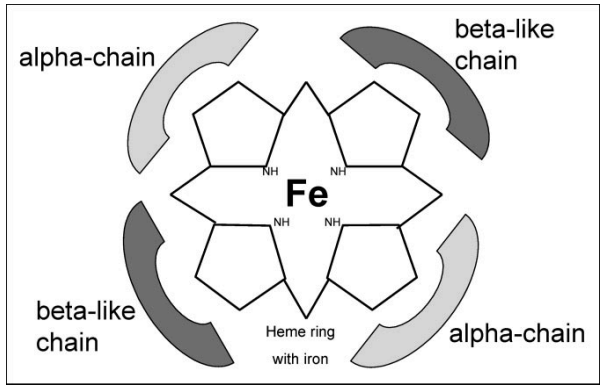






Mean MCV

0 day	131 fL
7 day	99 fL
14 day	86 fL



Data obtained from erythroid cultures (Drs. Iolascon and De Falco).
Graphics courtesy of Prof. Achille Iolascon.

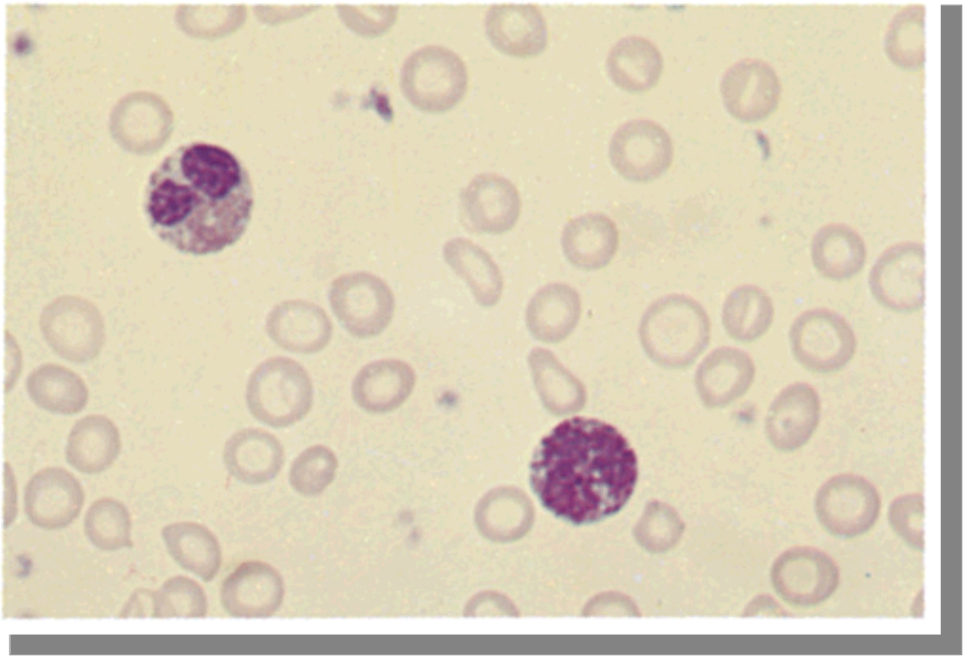
Characteristics of Microcytosis

✓**RBC:** Microcytosis hypochromia
reduced size and reduced Hb content of red blood cells,
as inferred by erythrocyte indexes

Normal values for age

Age	MCV (fl)
At born	110-128
5-24 months	80-85
2-6 years	75-90
6-12 years	78-95
>12 years	80-100

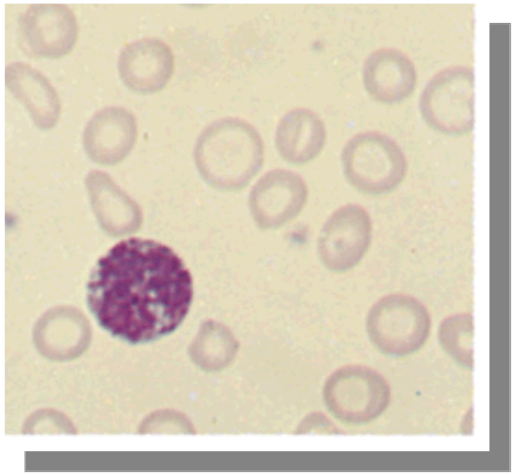
MCH: <26 pg (n.v 27-30)
MCHC: <30 g/dl (n.v.31-37)



Peripheral blood smear

Parameters to evaluate anemia

- ✓**RDW:** red cell distribution width
  (measure of anysocytosis, e.g. dual populations)
- ✓**HRC:** % hypochromic red cells
- ✓**CHr:** reticulocyte Hb content
- ✓ **Serum iron**
- ✓**Transferrin**
- ✓**Transferrin saturation**
- ✓**Serum ferritin**
- ✓**Soluble transferiin receptor**
- ✓**Hepcidin assay**



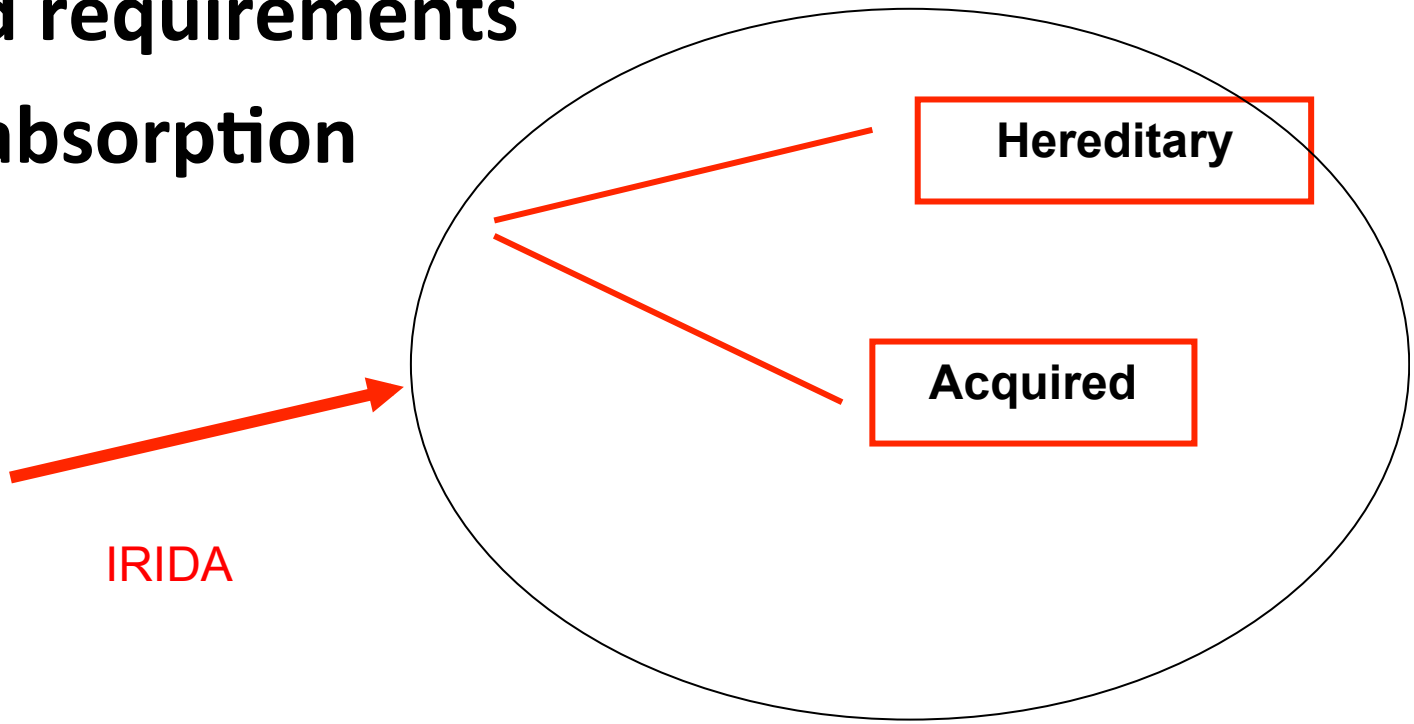
Differential diagnosis of the most common forms of microcytosis

	Nutritional deficiency	Deficit of absorption	Thalassemia heterozygotes	ACD	ACD+iron deficiency
Hb	-	-	= / -	-	--
MCV	-	-	-	-	-
GR	-	-	+	-	--
RDW	=	=	= / +	= / +	+
Reticulocytes	-	-	= / +	= / +	= / + / -
IS	- / --	- / --	=	= / -	-
Ferritin	= / -	= / +	=	=	= / -
FEP	= / +	= / +	=	=	= / +
sTfR	+	+	+	=	= / +
CHr	-	-	= / -	-	--
Oral response	YES	NO	NO	Not to be expected	Partial
Iv response	YES	YES	NO	Not to be expected	Partial
Inheritance	Acquired	Acquired / multifactorial	AR	Multifactorial	Multifactorial
Suggested therapy	Oral iron	Etiological therapy / iv injection if severe anemia	Not required	Etiological therap yif possible (EPO, iv iron)	Etiological therap + oral iron

Iolascon A et al.,2013

Causes of Iron Deficiency Anaemia

- **Blood loss**
- **Limited supply (poor diet)**
- **Increased requirements**
- **Iron malabsorption**



Unexplained or Refractory Acquired Iron-Deficiency Anaemia (IRIDA)

- ***Helicobacter pylori***
- **Celiac disease**
- **Autoimmune atrophic gastritis**

Helicobacter pylori Infection

- In recent years, *H. pylori* has been implicated in several studies as a cause of IDA refractory to oral iron treatment[1]
  - –Favorable response to *H. pylori* eradication
- Mechanisms: Occult GI bleeding? Alterations in intragastric pH and ascorbic acid concentration? Induction of IL-1β and TNF-α, (inhibitors of parietal cell function)? Induction of parietal cell apoptosis?[2]
- Diagnosis: IgG antibody screening, urease breath test[1]

1. Hershko C, et al. *Semin Hematol.* 2009;46:339-350.
2. Hershko C, et al. *Blood Cells Mol Dis.* 2007;38:45-53.

Celiac Disease

- Celiac disease is a common nonbleeding gastrointestinal condition that may result in refractory IDA[1]
  - Celiac disease accounts for 5%–6% of unexplained IDA cases
  - Approximately 50% of patients with subclinical celiac disease develop IDA
- Diagnosis: Anti-tissue transglutaminase antibodies and/or anti-endomysial antibodies

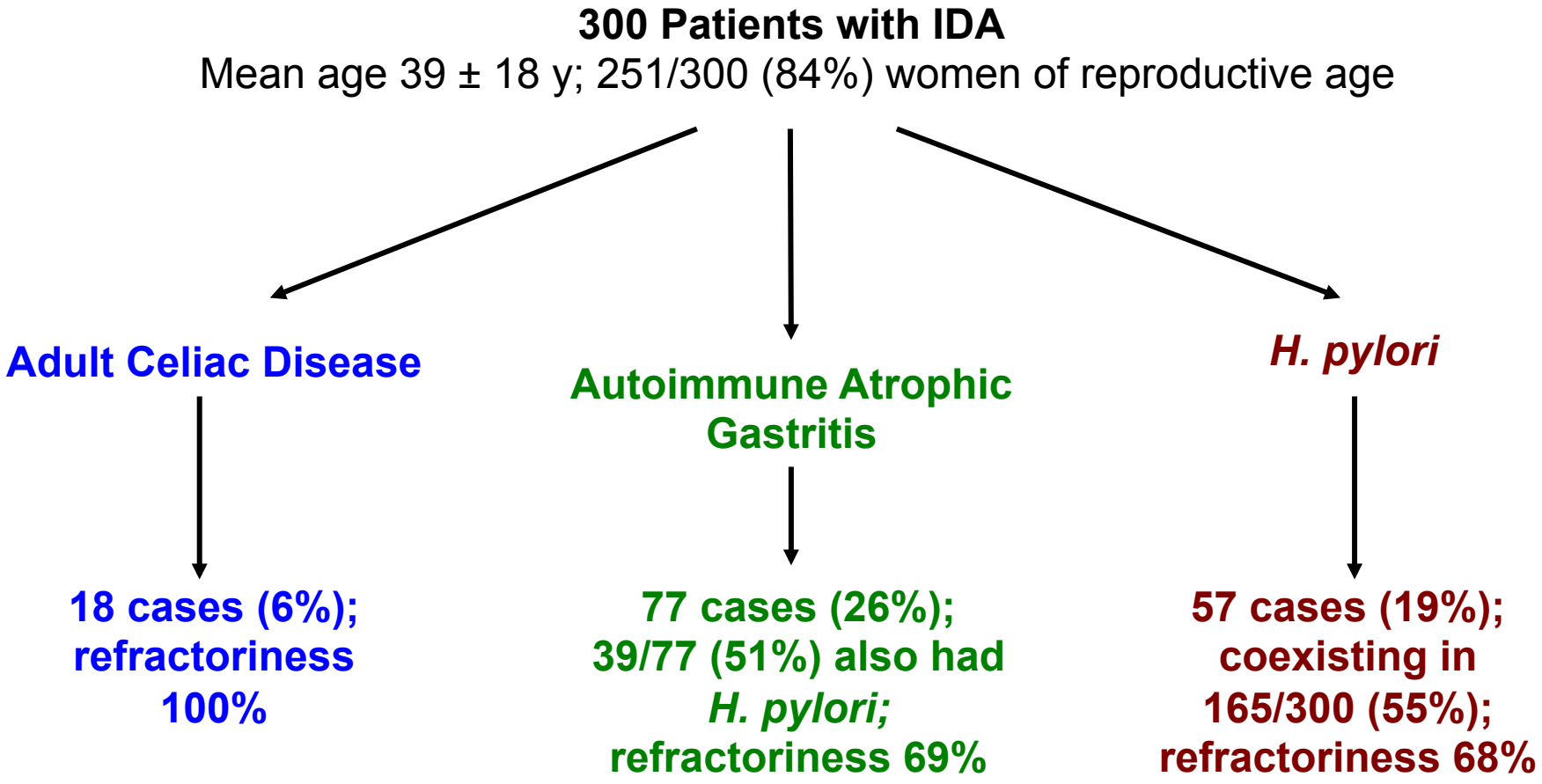
Hershko C, et al. *Semin Hematol.* 2009;46:339-350.

Autoimmune Atrophic Gastritis

- Autoimmune atrophic gastritis, or atrophic body gastritis, is associated with chronic idiopathic IDA with no evidence of gastrointestinal blood loss
- Iron deficiency may develop many years before the depletion of vitamin B_{12} stores
- Possible role of *H. pylori* in the pathogenesis of autoimmune gastritis due to antigenic mimicry of H^+K^+-ATPase
- Diagnosis: Serum gastrin, parietal cell antibodies

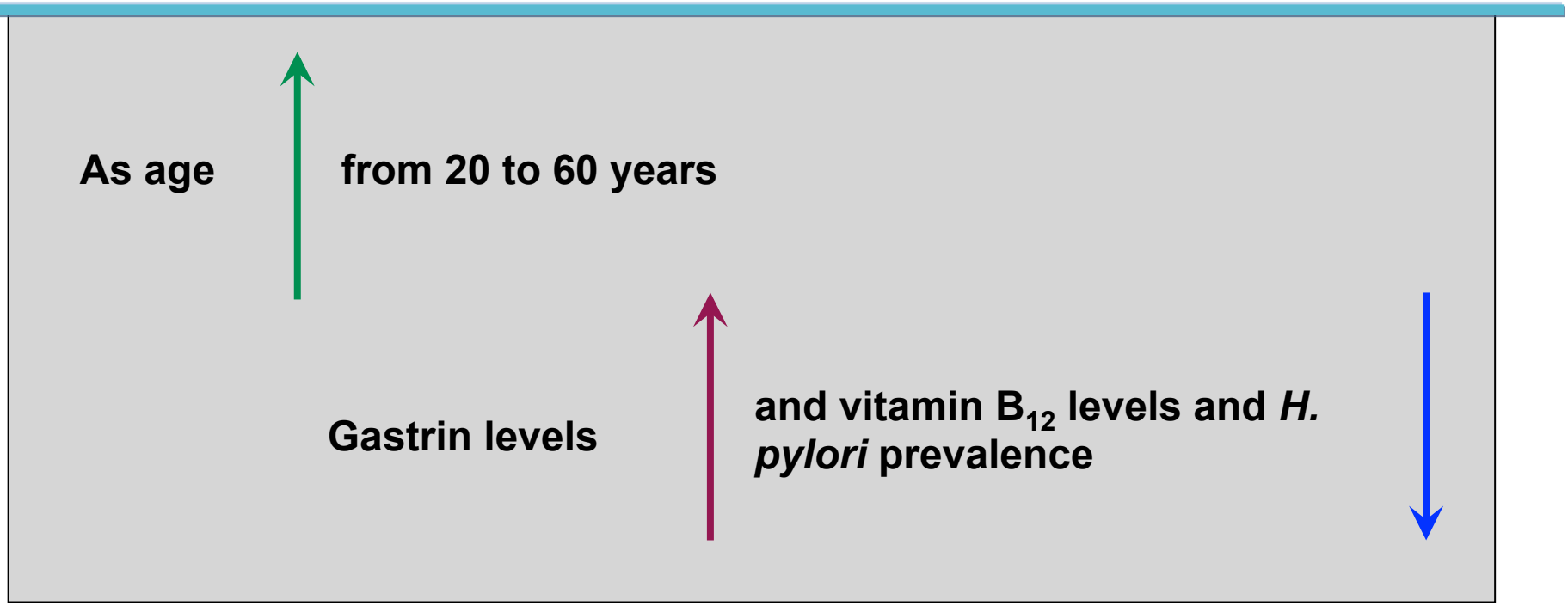
Hershko C, et al. *Semin Hematol.* 2009;46:339-350.

Unexplained or Refractory Acquired IRIDA



Hershko C, et al. *Blood Cells Mol Dis.* 2007;39:178-183. Graphic courtesy of Dr. Photis Beris.

Effect of Age on Autoimmune Gastritis[1,2]



- **With increasing age from <20 to >60 years, gastrin progressively increases and B12 decreases[1]**
- **HP infection decreases from 87.5% at age <20 years to 12.5% at age >60 years[1]**

1. Hershko C, et al. *Blood.* 2006;107:1673-1679. 2. Hershko C, et al. *Blood Cells Mol Dis.* 2007;39:178-183.

Treatment

- Cure the underlying disease
- In many cases, *H. pylori* eradication will cure the anaemia without iron therapy
- IV iron therapy is indicated in autoimmune atrophic gastritis due to malabsorption
- When IV iron is used, always calculate the precise amount of iron needed to correct anaemia and to replenish iron stores

Hershko C, et al. *Semin Hematol.* 2009;46:339-350.

Recommendations for the Diagnostic Work-Up of Unexplained or Refractory Acquired IRIDA

Screening for celiac disease, autoimmune atrophic gastritis and for *H. pylori* should be performed in the following populations:

- Males and postmenopausal females with IDA and negative endoscopic and radiologic studies
- Fertile females and children/adolescents refractory to oral iron treatment

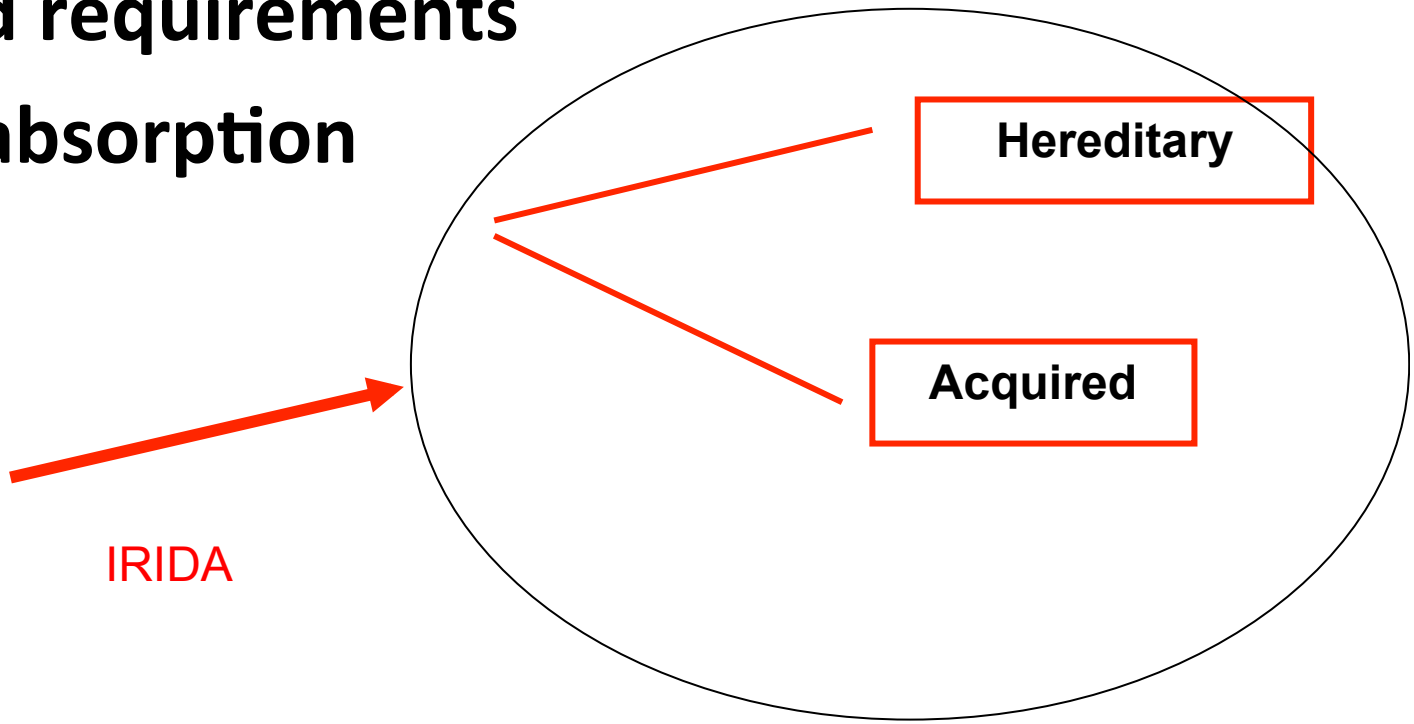
Hershko C. In: *Disorders of Iron Homeostasis, Erythrocytes, Erythropoiesis.* Forum service editore: Genoa, Italy; 2006.

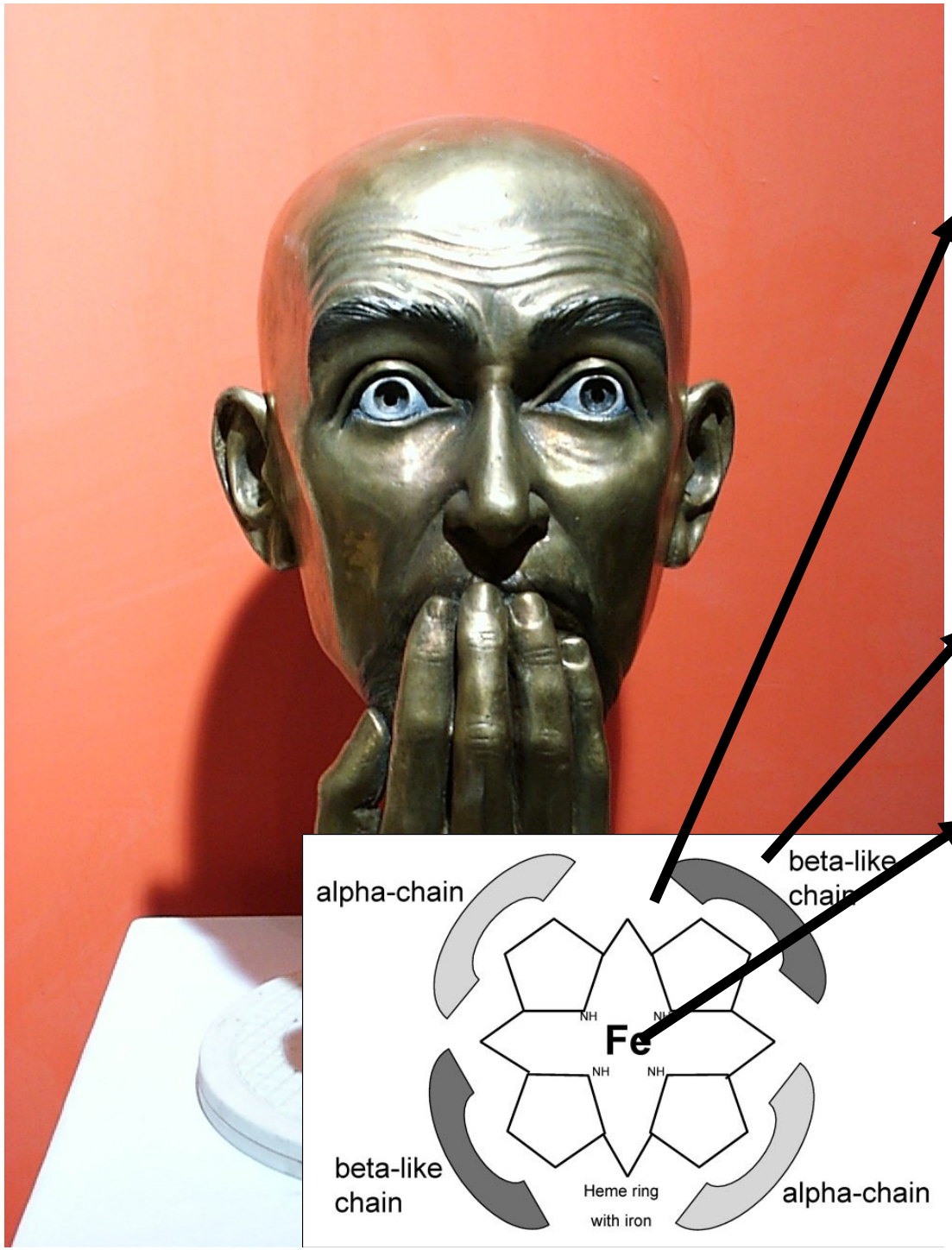
Acquired IRIDA
Conclusions

- Blood loss, insufficient dietary iron intake, and increased iron requirements are the main causes of iron deficiency anaemia.
- Acquired decreased iron absorption has recently been recognized in patients with unexplained or refractory IDA
- Celiac disease, autoimmune atrophic gastritis, and *H. pylori* infection are increasingly diagnosed in such patients
- In some cases, *H. pylori* may be directly implicated in the genesis of autoimmune gastritis
- We strongly recommend a diagnostic work-up for these conditions in case of acquired refractory or obscure IDA

Causes of Iron Deficiency Anaemia

- **Blood loss**
- **Limited supply (poor diet)**
- **Increased requirements**
- **Iron malabsorption**





Inherited Microcytic Anaemias

Heme synthesis

- Porphyrias
  - Erythropoietic porphyria
- Sideroblastic anaemias
  - X-linked
  - X-linked with ataxia
  - Autosomal recessive (due to glutaredoxin 5 or to Gly transporter deficiency)

Globin synthesis

- Thalassaemias
- Haemoglobinopathies

Iron metabolism

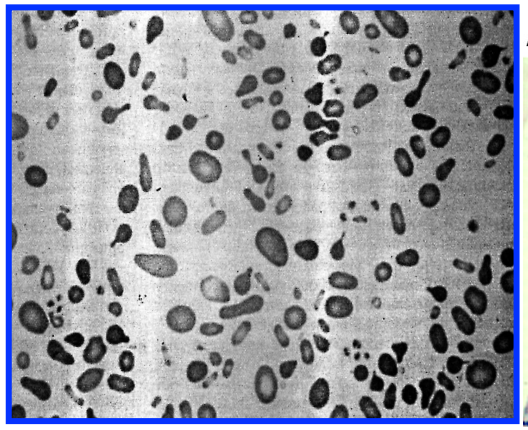
- Hereditary hypotransferrinaemia
- Aceruloplasminaemia
- Divalent metal transporter 1 (DMT1) disease
- Ferroportin disease
- TMPRSS6 deficiency

Graphics courtesy of Dr. Achille Iolascon.

DMT1 Deficiency

delCTT intron 4 /

R416C



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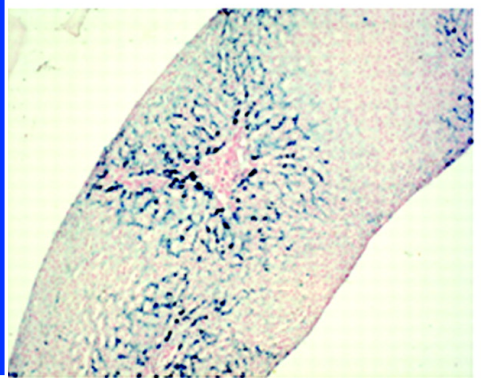

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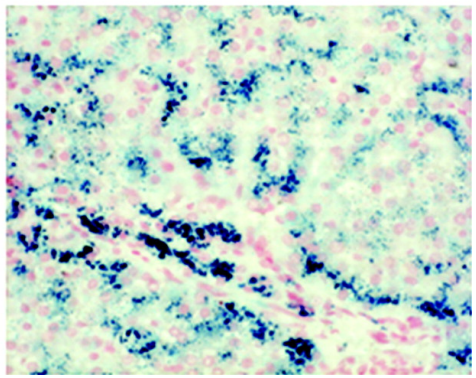


Severe microcytic anaemia with high transferrin saturation
Severe hypochromia with liver iron overload and normal ferritin levels

	Father, I-1	Mother, I-2	Proband, II-1							Normal values (range)
Age	35 y	32 y	Birth	2 mo	3 mo	6 mo	1 y	3 y	5 y	2-3 y
Body weight, percentile	NA	NA	< 3rd	3rd	5th	10th	15th	15th	25th	NA
Hb, g/L	149	128	40	74	76	82	98	90	85	130 (120-150)
MCV, fL	84	79.6	71	75	69	50	50	48	51	80
MCH, pg	28.8	27	14	14	15	15.3	14	13.5	15	26
Serum iron, µM	14.3	12.9	ND	29.7	28.6	30.4	26.5	34.7	36.5	14.3 (10.6-21.5)
Transferrin saturation, %	28	35	ND	85	100	80	63	80	90	7-30
Ferritin, µg/L	110	133	ND	256	864	110	70	26	34	7-140
FEP, µg/g Hb	ND	ND	ND	4.7	ND	ND	ND	ND	5.3	< 3
Treatment	None	None	18 mL PRBCs	25 mL PRBCs	30 mL PRBCs	sc rEpo	sc rEpo	sc rEpo	sc rEpo	NA

Graphics A, B, and Table with permission from Iolascon, A. et al. *Blood.* 2006;107:349-354.
Top left graphic courtesy of Dr. Achille Iolascon.

Clinical and Laboratory Findings of DMT1 Mutations[1,2]

MCV	45–55 fL
Serum iron	++
Tf saturation	++
sTfR	++
BM sideroblasts	-
FEP	+
Liver iron	+++
Neonatal appearance	+
Effect oral/IV Fe	-/-
Serum or urinary hepcidin	-
Inheritance	AR
Therapy	Epo

- DMT1 is essential in erythropoiesis
- DMT1 is not essential for liver iron uptake
- DMT1 is not essential for duodenal iron absorption
  - Alternative pathways?
  - Heme absorption?
- Increased iron absorption occurs in the presence of iron overload because of low hepcidin levels
- Partial response of anemia to erythropoietin treatment

1. Iolascon A, et al. *Blood.* 2006;107:349-354. 2. Iolascon A, et al. *J Pediatr.* 2008;152:136-139.
Graphic courtesy of Dr. Achille Iolascon.

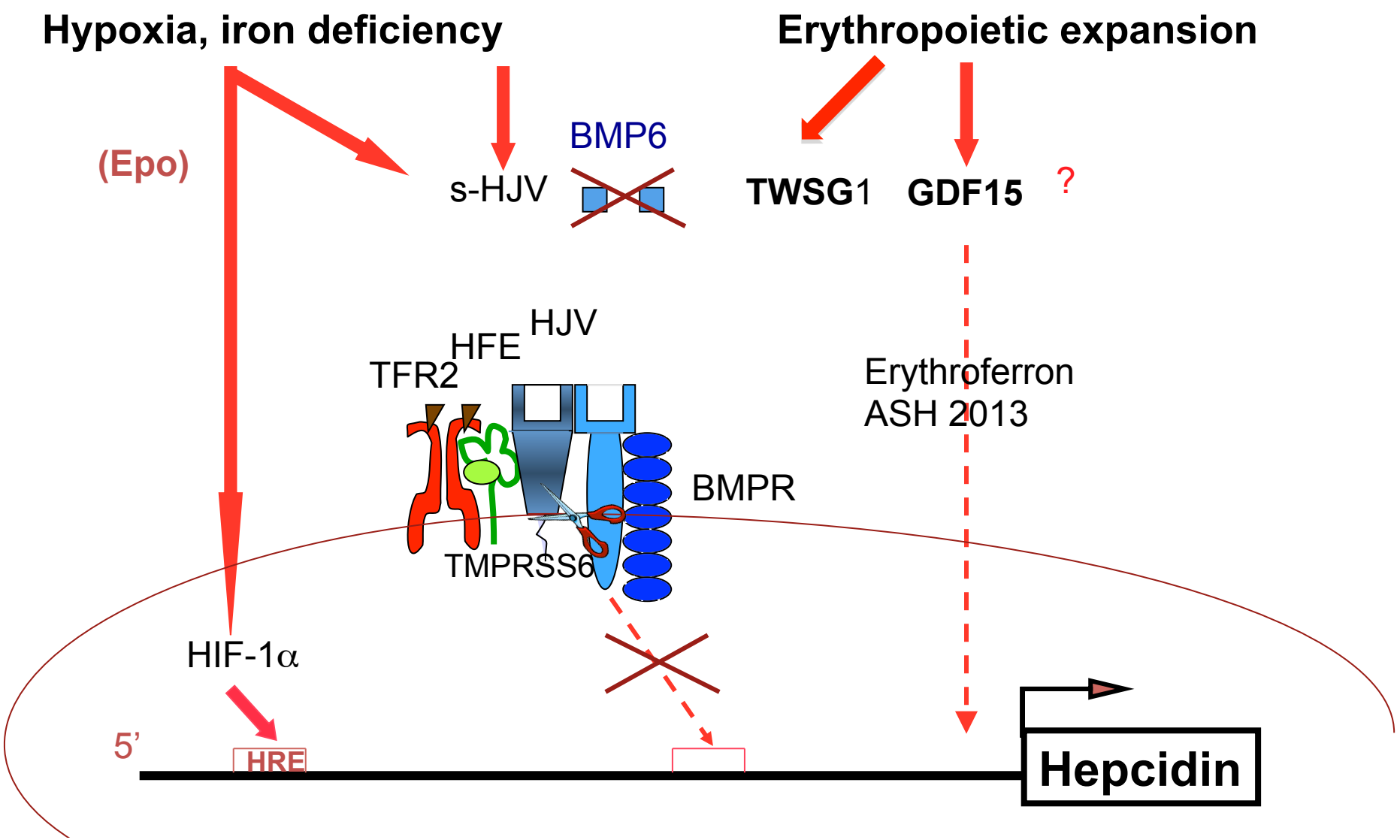
Genetic defects of iron absorption

IRIDA = iron refractory-iron deficiency anemia
(OMIM #206200)

Autosomal recessive

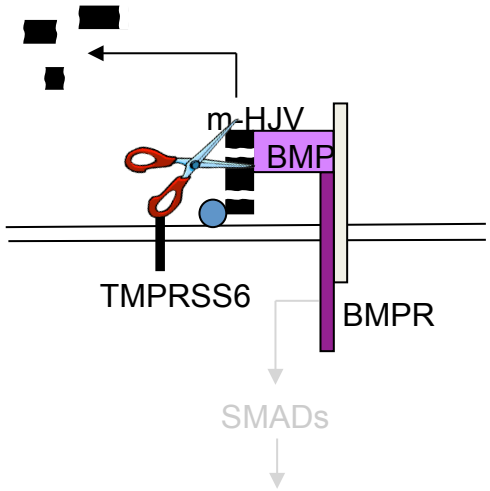
Caused by inappropriately high hepcidin production

Hepcidin downregulation: multiple pathways



Hepcidin regulation in Iron Deficiency and deregulation in IRIDA

Iron Deficiency



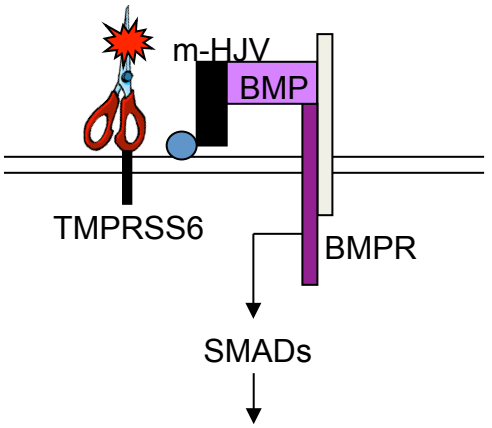
↓HAMP

↑ serum iron

⇩

Normal Erythropoiesis

IRIDA



↑HAMP

↓ serum iron

⇩

Iron Deficiency

Matriptase-2 features

TMPRSS6:
18 exon gene on chromosome 22
RNA expression: liver (kidney, olfactory epitelium)

Matriptase-2 protein:
811aa type II transmembrane serine protease
(TTPS family: enteropeptidase, hepsin, corin, matriptase 1…)

Structure:
cytoplasmic tail, transmembrane, SEA, CUB, LDLR, serine protease domains

Function?
cleavage activity indispensable for function

nature genetics

Mutations in *TMPRSS6* cause iron-refractory iron deficiency anemia (IRIDA)

Karin E Finberg[1,2,14], Matthew M Heeney[2,3,15], Dean R Campagna[4,15], Yeşim Aydınok[5], Howard A Pearson[6], Kip R Hartman[7], Mary M Mayo[8], Stewart M Samuel[9], John J Strouse[10], Kyriacos Markianos[11,12], Nancy C Andrews[2,12,14,16] & Mark D Fleming[4,13,16]

blood

2008 112: 2089-2091
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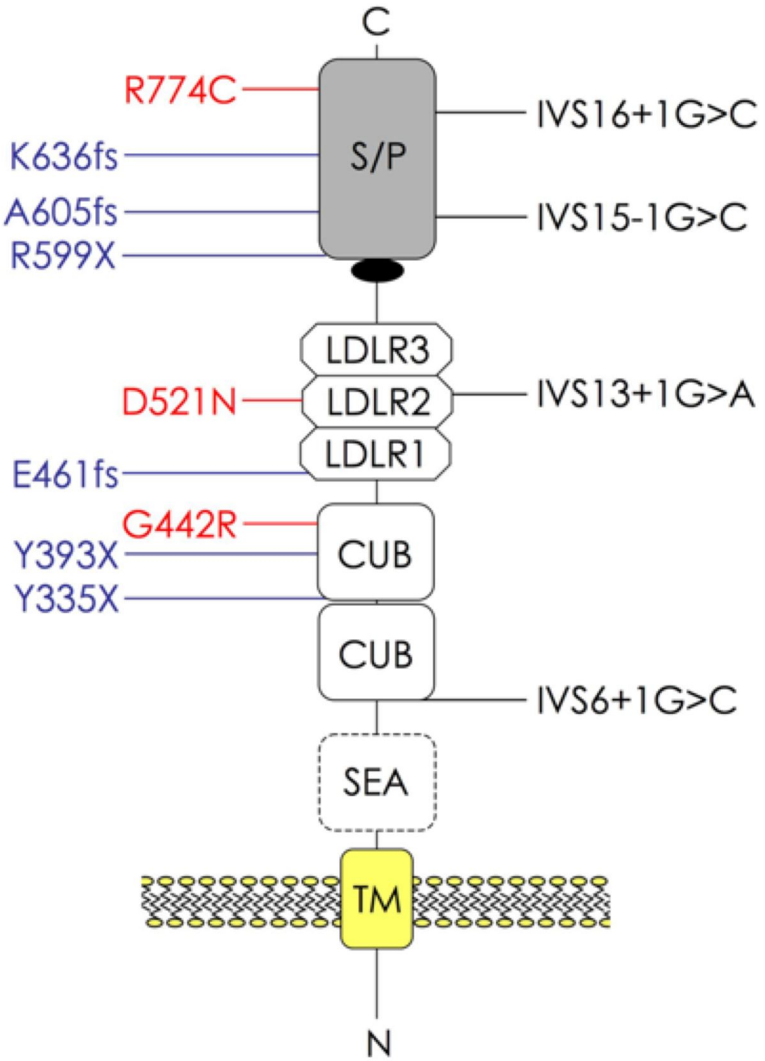
doi:10.1182/blood-2008-05-154740

Two nonsense mutations in the TMPRSS6 gene in a patient with microcytic anemia and iron deficiency

Flavia Guillem, Sarah Lawson, Caroline Kannengiesser, Mark Westerman, Carole Beaumont and Bernard Grandchamp



A mutation in the *TMPRSS6* gene, encoding a transmembrane serine protease that suppresses hepcidin production, in familial iron deficiency anemia refractory to oral iron

Maria Antonietta Melis,[1] Milena Cau,[1] Rita Congiu,[1] Gabriella Sole,[2] Susanna Barella,[2] Antonio Cao,[2] Mark Westerman,[4] Mario Cazzola[5] and Renzo Galanello[1,2]

All patients have high hepcidin levels!

Mutations of TMPRSS6 in IRIDA

Most patients are homozygous or compound heterozygous for two mutations,

Different types of mutations (non sense, missense, frameshift ecc..).
Most private, few recurrent.

Missense: in the serine protese, but also in other domains
(CUB, LDLR)

In few patients a single mutation is identified (?)

Hypothesis: may common TMPRSS6 polymorphisms cause susceptibility to or iron deficiency?

Clinical and laboratory features of IRIDA

Microcytic hypochromic anemia diagnosed in infancy

Extremely low iron and transferrin saturation

Increased serum transferrin

Normal-high serum ferritin

High/normal serum/urinary hepcidin levels

More severe anemia in infancy than in adulthood
(Males less affected)

Hematological parameters of the probands

n.	sex	age	Hb	MCV	% TF saturation	Serum Ft	Serum hepc
A	m	6	8.8	58	2	50	↑
B	f	13m	9.2	65	10	37	↑
C	m	17m	7.0	49	5	40	↑
D	f	11	8.2	56	3		↑
E	m	7	7.5	49	4	27	↑
F	f	3	9.7	61	4		-
G	m	15m	7.9	53	2	59	↑

(Finberg et al, Nature Genetics 2008;40:569-71)

Cell
PRESS

Cell Metabolism
Article

The Serine Protease Matriptase-2 (TMPRSS6) Inhibits Hepcidin Activation by Cleaving Membrane Hemojuvelin

Laura Silvestri,[1] Alessia Pagani,[1] Antonella Nai,[1] Ivana De Domenico,[2,3] Jerry Kaplan,[3] and Clara Camaschella[1,*]

(Cell Met 2008: 8:502-11)

IRIDA: differential diagnosis with other genetic disorders causing microcytic anemia

	Atransferrinemia	DMT1 mutations	Tmprss6 mutations
Hb	low	low	low
MCV	low	low	low
Fe	low	high	low
Tf	Low/absent	low	high
Tf sat	high	high	low
ferritin	high	high	normal/high
hepcidin	low	low/normal	high

Differential diagnosis of the less common forms of microcytosis

	IRIDA	Erythropoietic protoporphyria	Sideroblastic anemia X-linked	Sideroblastic anemia X-linked with ataxia	Microcytic anemia sideroblastic-like (GLRX5)	Deficiency of DMT1	Hypotransferrinemia	Aceruloplasminemia	Deficiency of Steap3
Hb	- /--	-	-	-	--- (età dipendente)	--	-	-	---
MCV	--	--	-	-	--	---	--	-	-
GR	--	-	-	-	-	-	-	-	--
RDW	=	=	=	=	=	=	=	=	=
Reticulocytes	-	-	-	-	-	-	-	-	---
SI	-- /---	+	+	+	+	++	100%	+	++
Ferritin	= / -	=	=	=	=	+	=	+	+++
FEP	++	+++	= / -	= / -	=	+	=	=	+
Oral response	NO	NO	NO	NO	NO	NO	NO	YES	NO
Iv response	YES, not long-lasting	NO	NO	NO	NO	NO	NO	YES	NO
Inheritance	AR	AD/AR	X- linked	X- linked	AR	AR	AR	AR/AD	AR
Suggested therapy	not possible	β-carotene	Vit B6	Vit B6	Iron chelation	EPO	Plasma / apotransferrin	Iron chelation	EPO, iron chelation

Iolascon A et al.,2013

Answer to iron administration

	Patient I-1 (age: 3 years) *Intravenous Iron*		**Patient II-1** (age: 3 years) *Intravenous Iron*		**Patient II-2** (age: 5 years) *Intravenous Iron*		**Patient III-1** (age: 3 years) *Intravenous Iron*	
	Before Treatment	*After Treatment*	*Before Treatment*	*After Treatment*	*Before Treatment*	*After Treatment*	*Before Treatment*	*After Treatment*
Hb, g/dL	8.3 →	9.5	9.8 →	11	10.4 →	11.6	9.1 →	10.7
MCV, fL	52	58	65	66.4	68	71.8	60	60
MCH, pg	15	16	16.7	18	18	19	17	18
Serum Ferritin, µg/L	15	74	50	113	32	133	26	25
Serum iron, µg/dL	12	14	22	34.2	48	48	14	18
Transferrin saturation, %	3	3.7	6.2	10.2	9.4	15.8	3.7	4.5

Iolascon et al.2010

Laboratory findings of IRIDA-TMPRSS6 mutations

MCV	47-60 fL
Serum Iron	-
Tf saturation	-
sTfR	++
BM sideroblasts	-
FEP	+
Liver Iron	n
Neonatal appearance	+/-
Effect oral /iv Fe	**+/-**
Serum or urinary Hepcidin	+
Inheritance	AR
Therapy	-

Iolascon A et al.,2013