UNIVERSITA' degli Studi di Napoli FEDERICO II
Scuola di Specializzazione in Genetica Medica

Napule è...
PEDIATRIA PREVENTIVA E SOCIALE

SIPPS
SOCIETÀ AFFILIATA ALLA SIP

L' età del ferro: le nuove acquisizioni

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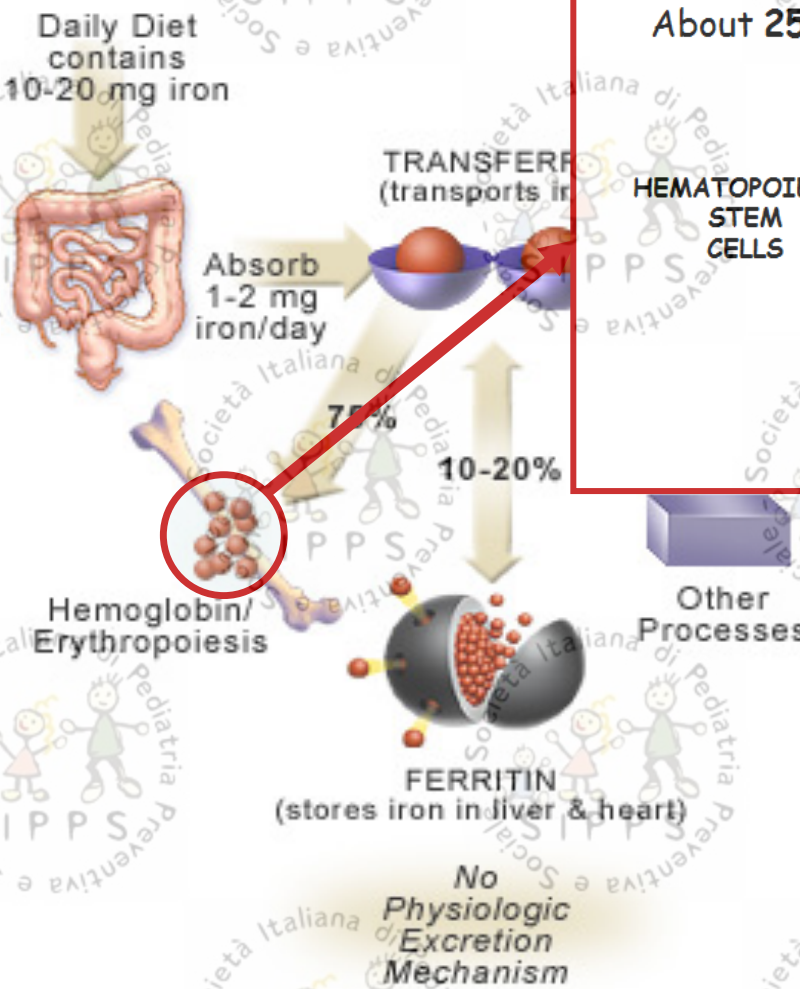
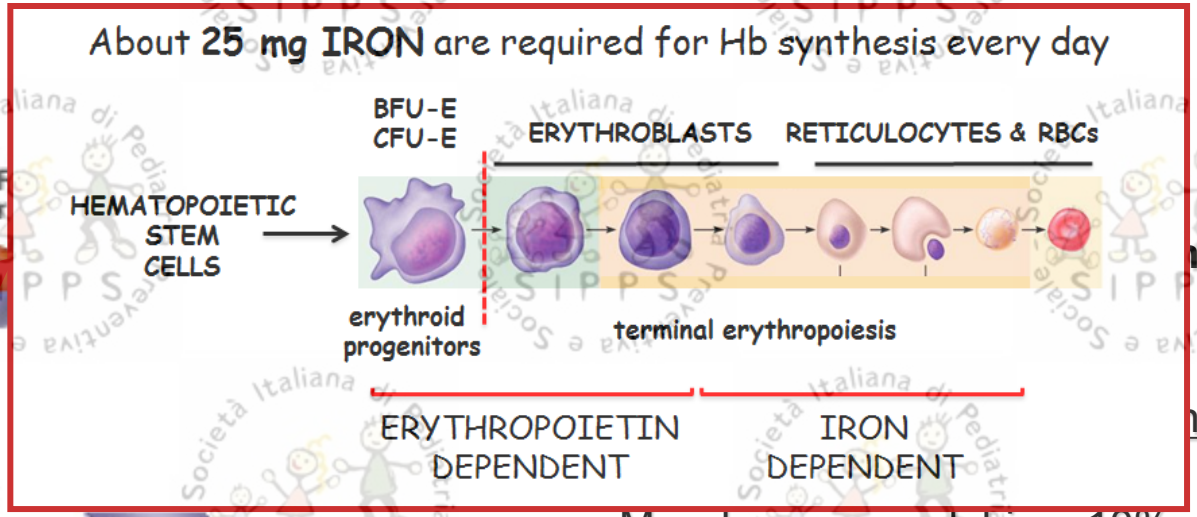
ceinge
BIOTECNOLOGIE AVANZATE s.c.a r.l.

Conflitto di interesse:
Nessuno

Iron metabolism



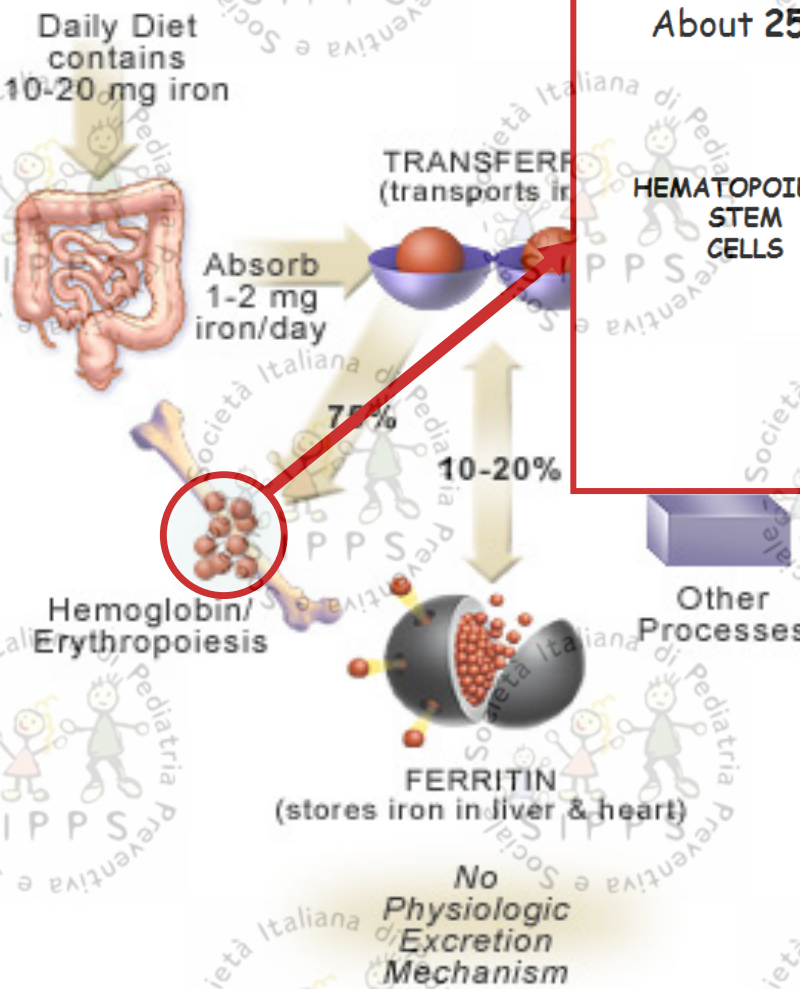


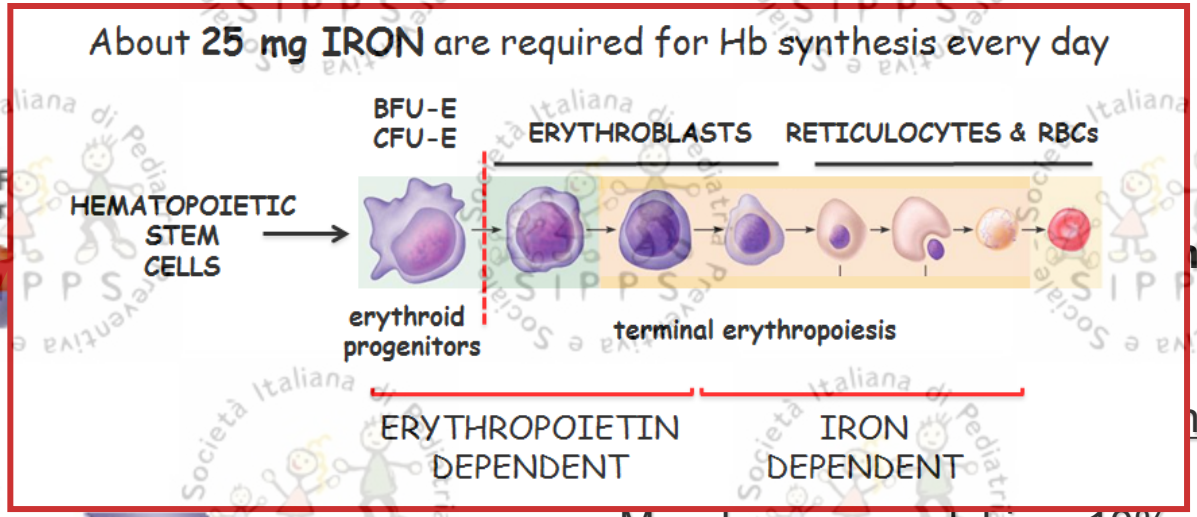
- Muscles as myoglobin – 10%
- Storage as ferritin - 10%
  - Bone marrow
  - Reticulo-endothelial cells
  - Liver (0.5-1 g)
- Other Haem proteins - 5%
  - Cytochromes, others
- In Serum - 0.1%

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)

Iron metabolism





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(Andrews, NEJM, 1999)

Body Iron Content during different ages

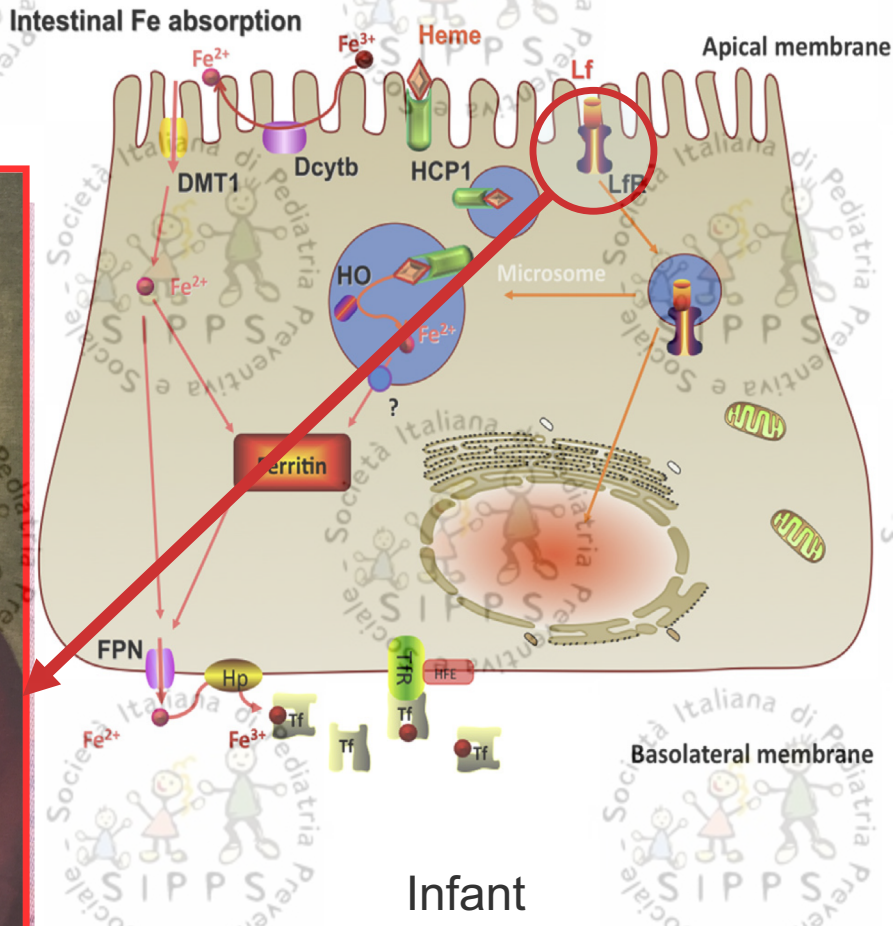
1kg body weight=
50 mg Fe

	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%)
Enzymes		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

Absorption and metabolism of iron

Adult

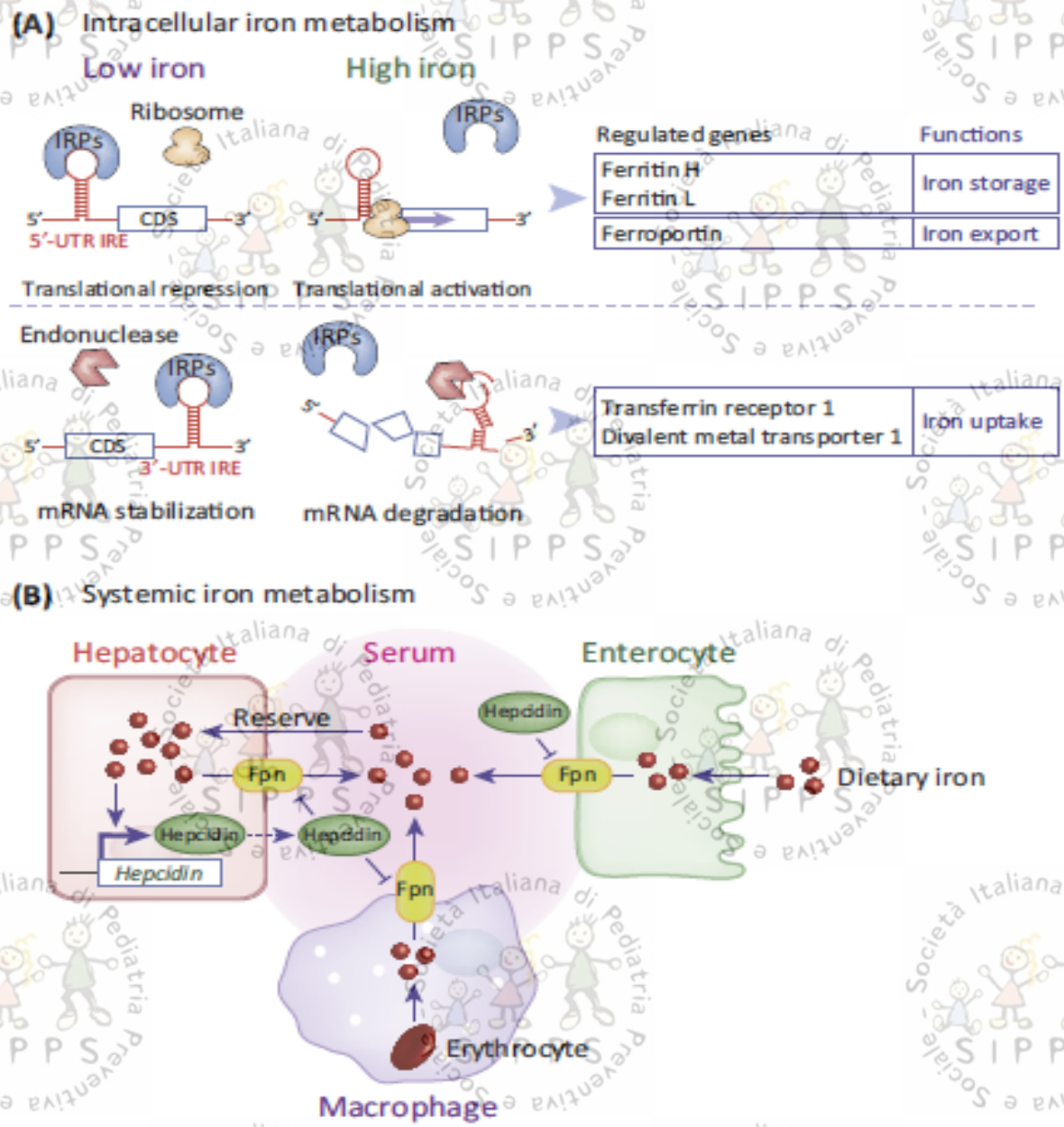


Infant

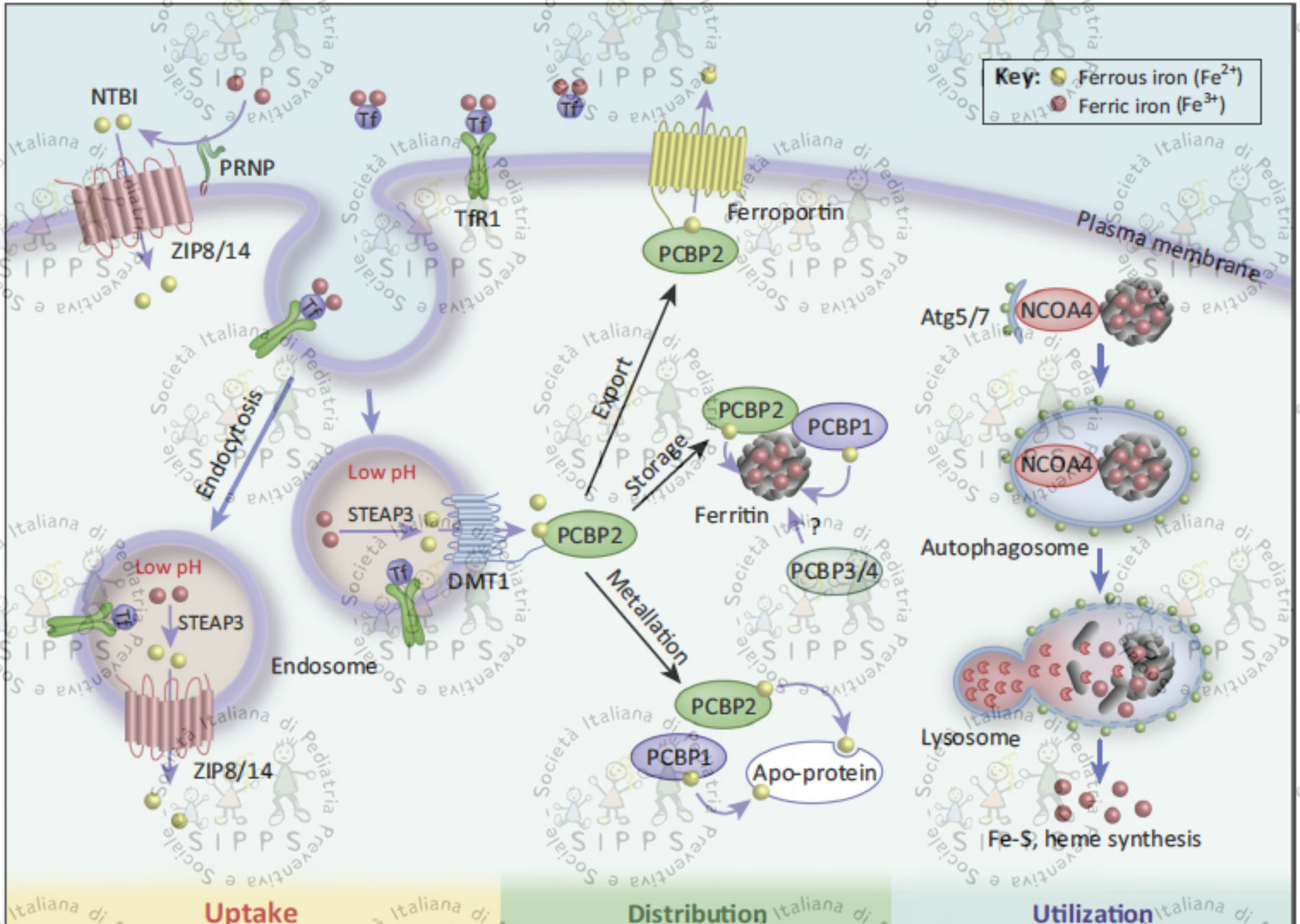
Iolascon A, De Falco L

Semin Hematol. 2009 Oct;46(4):358-70.

Mechanisms of systemic and intracellular iron homeostasis



New Mechanisms regulating Iron Intracellular Metabolism

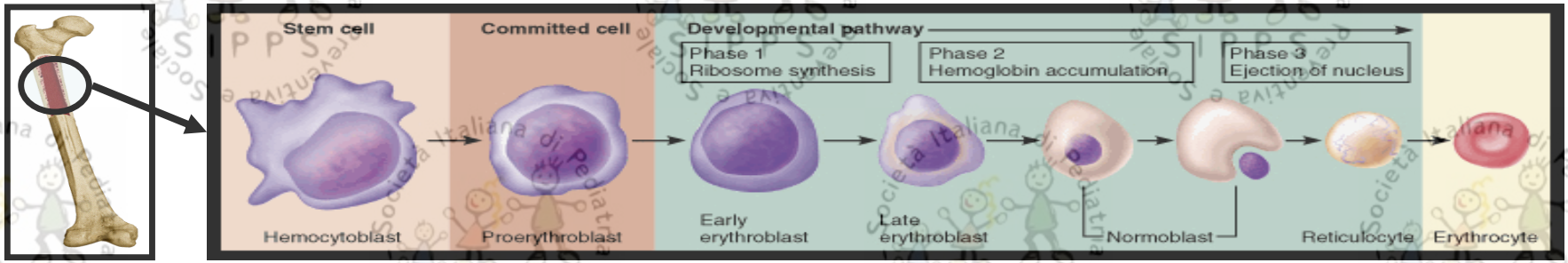


Iron deficiency and iron deficiency anemia

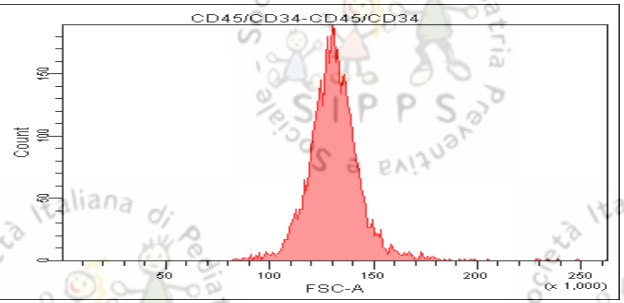
	Normal	Iron depletion	Iron deficient erythropoiesis	Iron deficiency anemia
Marrow iron	2-3+	0-1+	0	0
Serum Fe (µg/dl)	150	120	<100	<20
% saturation	40	30	<15	<10
HCT (%)	45	45	41	<40
RBC morphology	normal	normal	normal	microcytic hypochromic

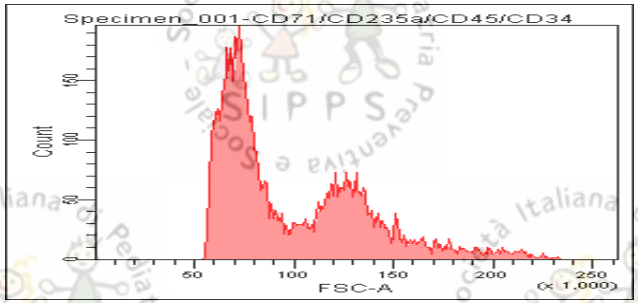
Iolascon A et al.,2013

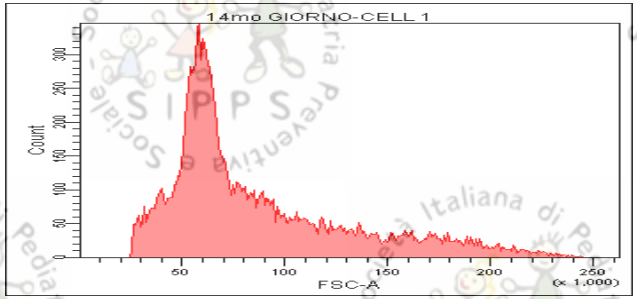
MCV During Differentiation



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

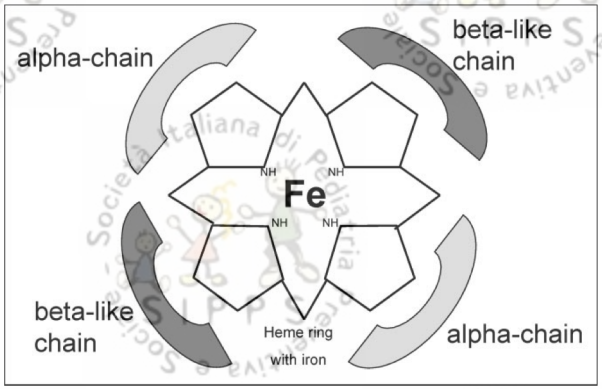






Mean MCV

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.

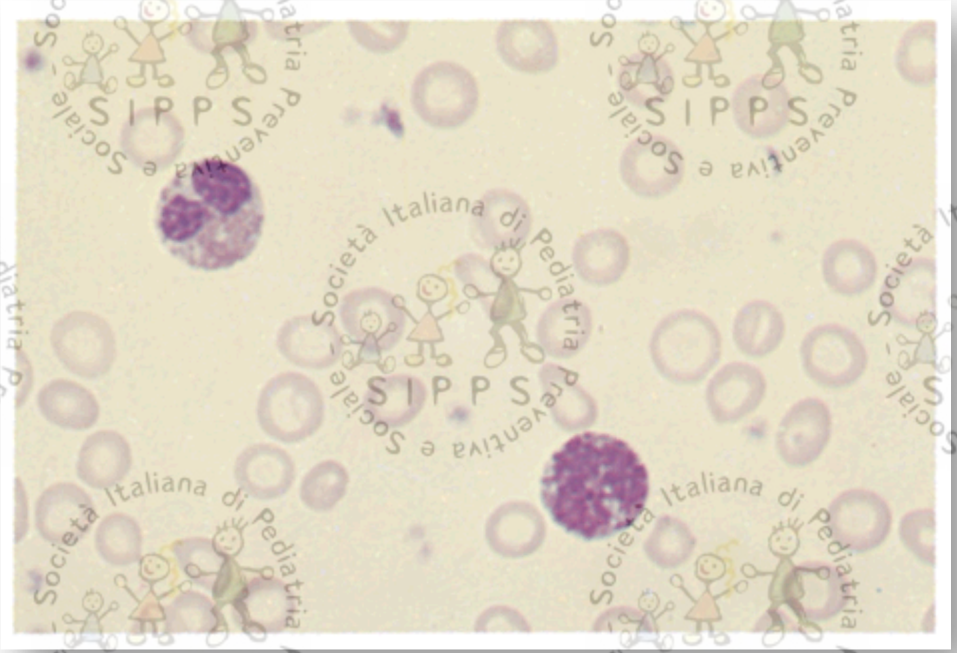
Characters of this story

✓**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

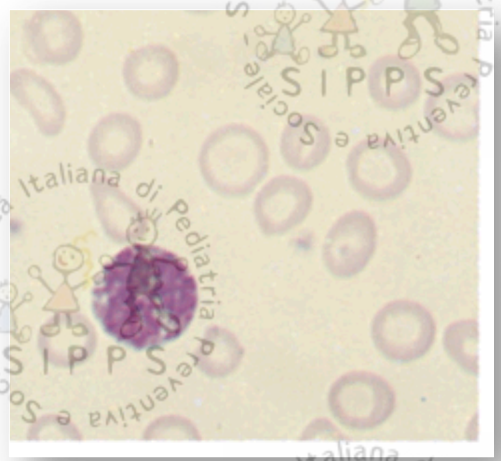
MCH: <26 pg (n.v 27-30)
MCHC: <30 g/dl (n.v.31-37)



Peripheral blood smear

Characters of this story

- ✓ **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 transferin receptor**
- ✓ **Hepcidin assay**



Diseases of Hepcidin Dysregulation

Iron deficiency anemia

Iron

Normal homeostasis

Hepcidin

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.

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

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

Defects of iron Metabolism

- **Defective iron transport or utilization**
  DMT1 deficiency, Hypo-transferrinemia

- **Defects of iron absorption**
  IRIDA (Iron-Refractory Iron Deficiency Anemia)

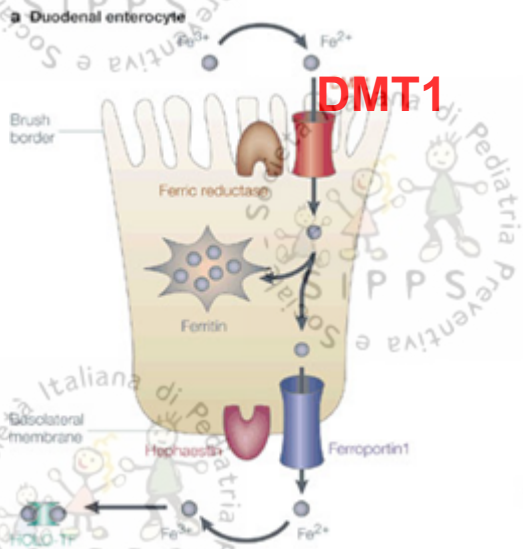
- **Defects of mitochondrial iron utilization**
  Inherited (and acquired) Sideroblastic Anemias

- **Defects of iron recycling**
  usually normocytic-normochromic anemias
  Aceruloplasmina, ACD (some cases)

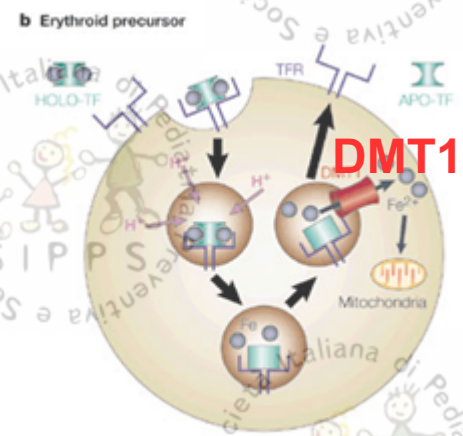
New rare disorders of iron entry and utilization: DMT1 deficiency

DMT1: Transporter of divalent metal cations
(Mn^{2+} Cu^{2+} Zn^{2+} Fe^{2+})

Duodenal cell: luminal non heme iron transporter
Erythroblasts: endosomal transferrin cycle



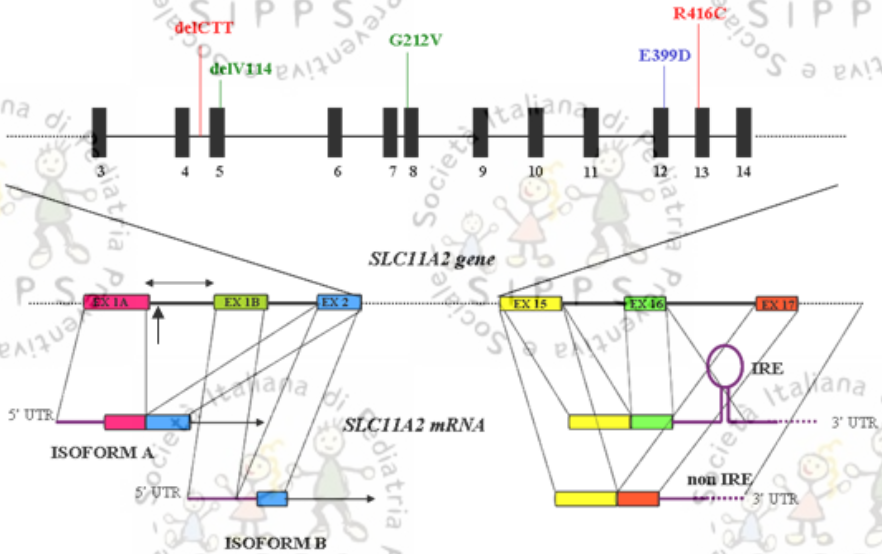
absorption

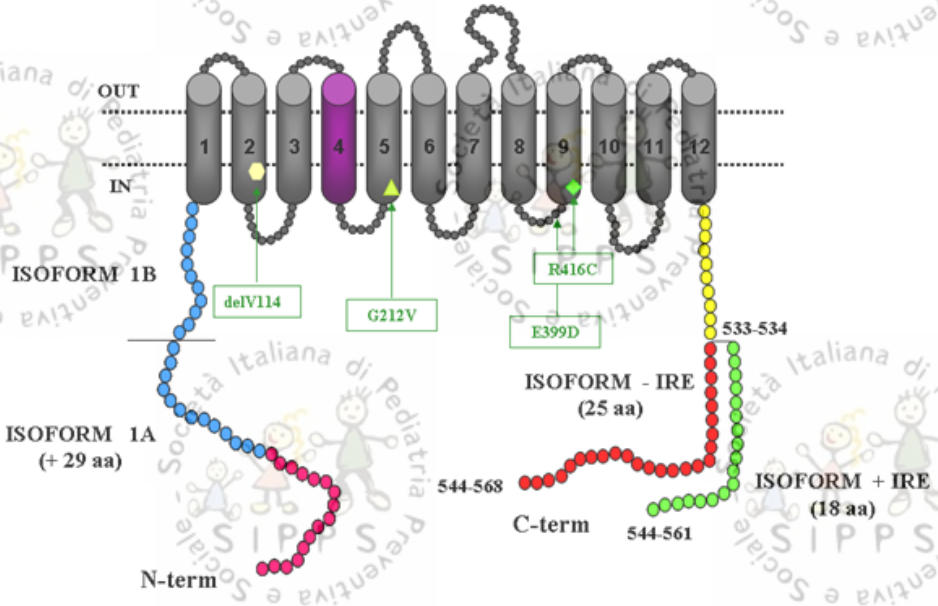


utilization

(Andrews, NEJM, 1999)

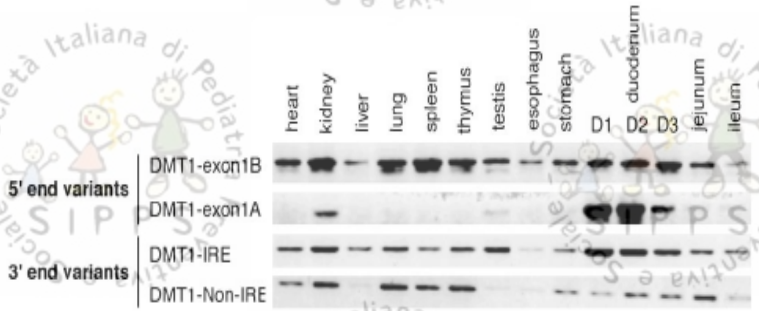
The iron transporter DMT1: 4 isoforms



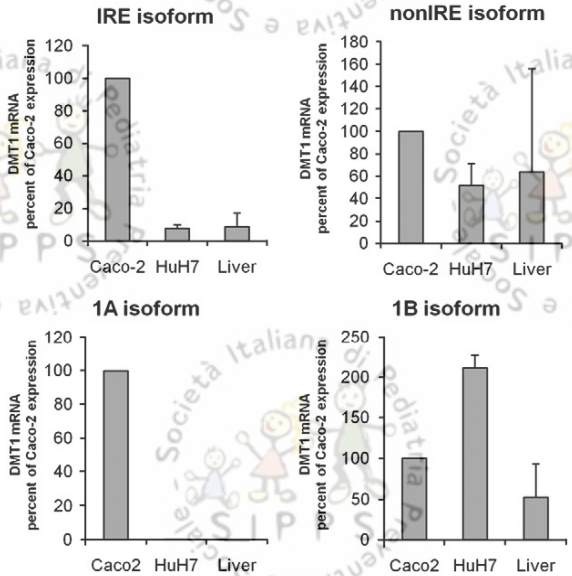


Iolascon A and De Falco L 2009 Semin Hematol 46:358–370

mouse



human

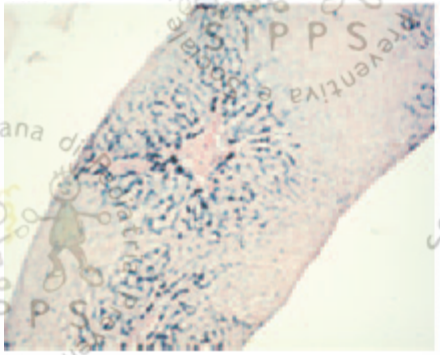


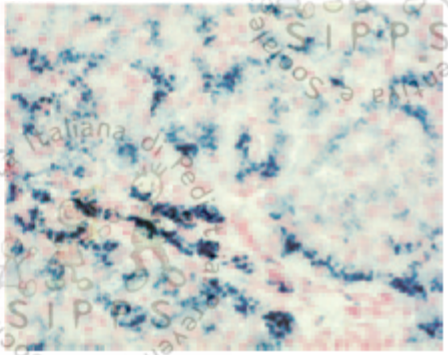
Bardou-Jacquet et al., 2011 Blood Cells Mol Dis 15;47(4):243-8

Microcytic anemia and hepatic iron overload in a child with compound heterozygous mutations in *DMT1 (SCL11A2)*

Achille Iolascon, Maria d'Apolito, Veronica Servedio, Flora Cimmino, Antonio Piga, and Clara Camaschella

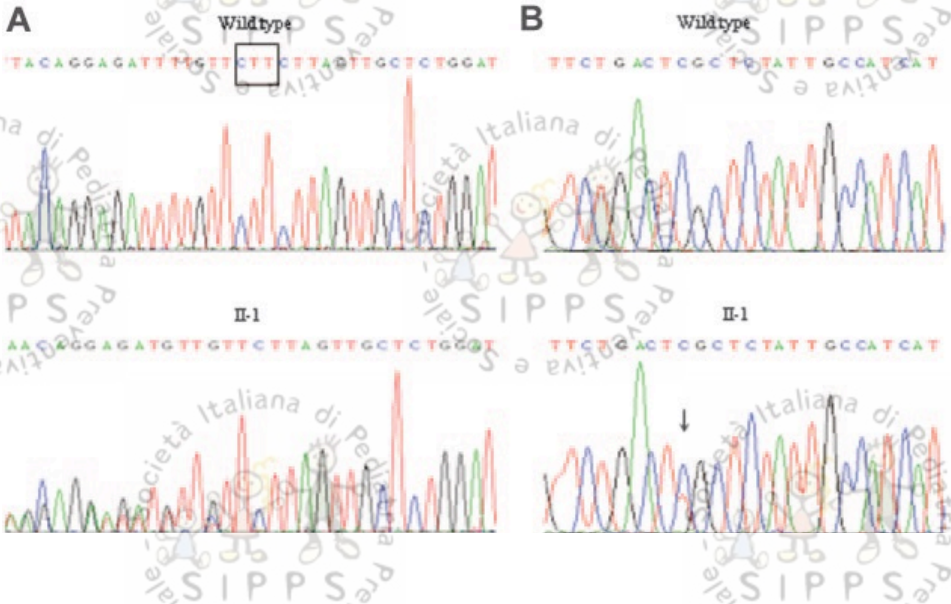
- Severe microcytic anemia with high transferrin saturation
- Severe hypochromia with liver iron overload and normal ferritin levels





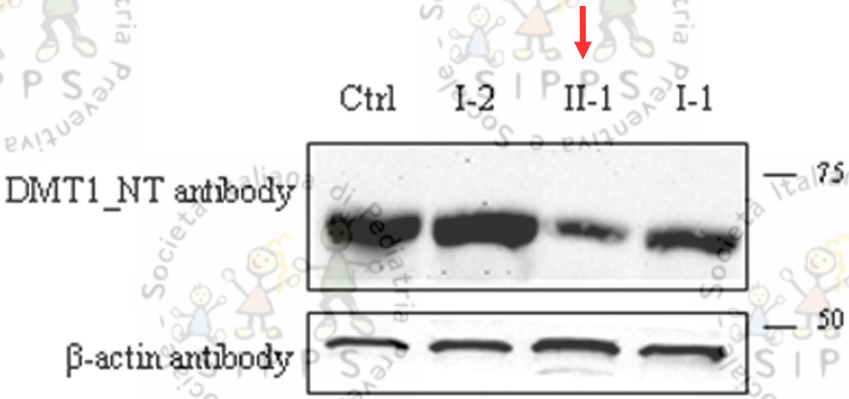
	Father, I-1	Mother, I-2	Proband, II-1							Normal values (range)
Age	35 y	32 y	Birth	2 mo	3 mo	6 mo	1 y	3 y	5 y	2-3 y
Body weight, percentile	NA	NA	< 3rd	3rd	5th	10th	15th	15th	25th	NA
Hb, g/L	149	128	40	74	76	82	98	90	85	130 (120-150)
MCV, fL	84	79.6	71	75	69	50	50	48	51	80
MCH, pg	28.8	27	14	14	15	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

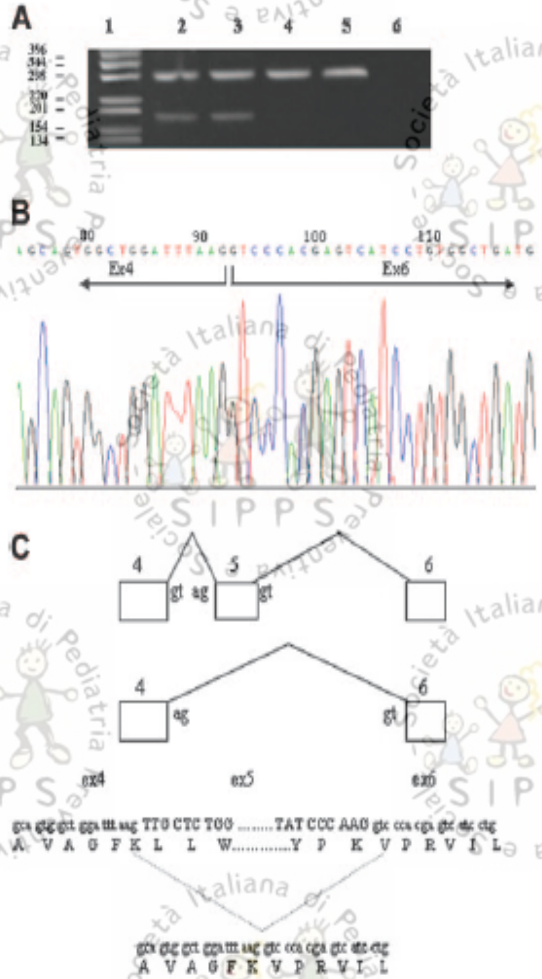
Analysis of *DMT1* mutations



delCTT

R416C





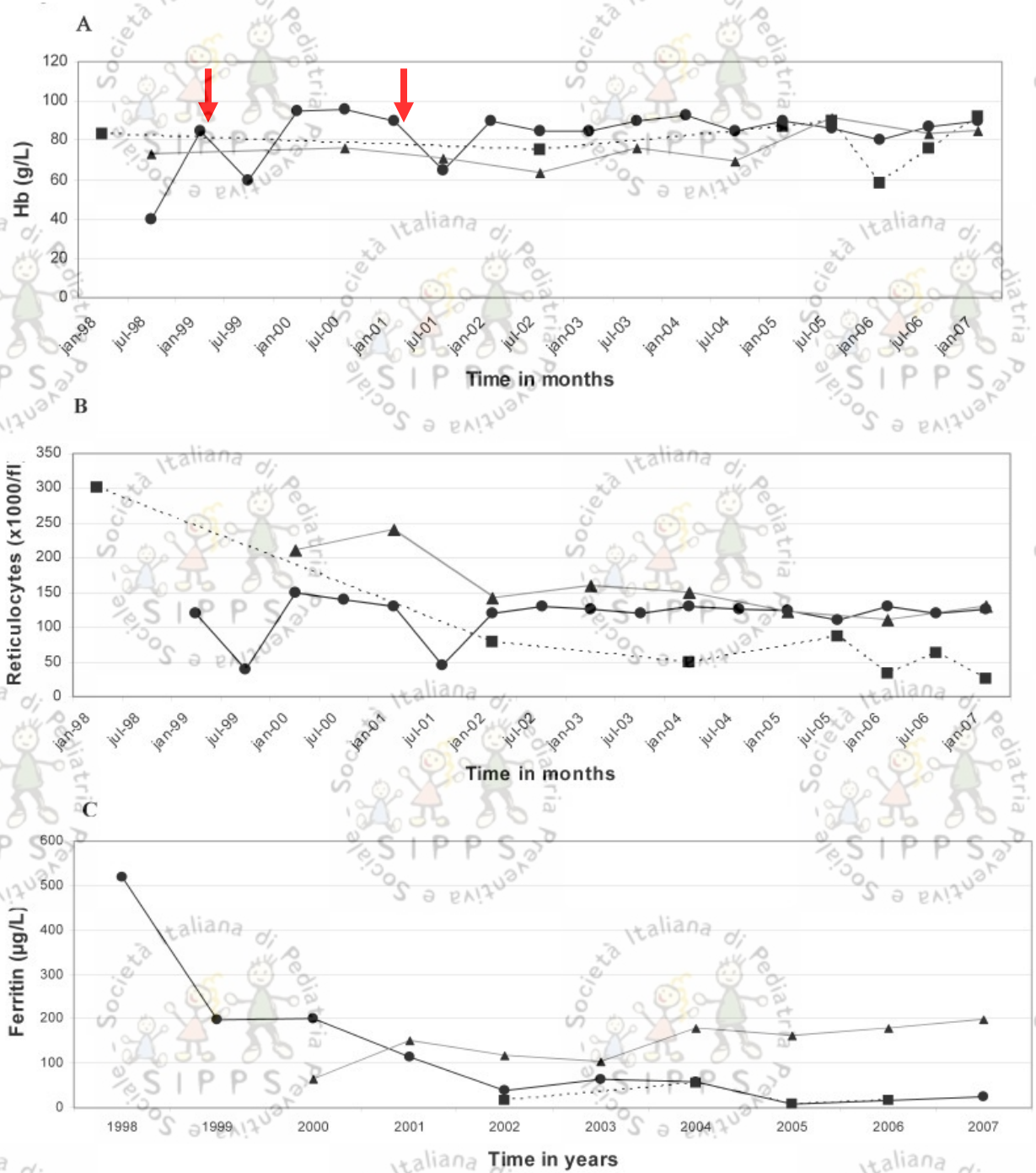
Iolascon, A. et al. Blood 2006;107:349-354

Mutations and Clinical features of DMT1 patients

Patient	Mutation	Hb at birth (g/L)	sTfR (mg/L)	Liver iron	Urinary hepcidin (ng/mg creatinin)	Functional Studies of The Mutation
Czech (homozygous)	G1285C, D399E (cytosolic loop) exon 12 skipping	74	38 (N,1.9–4.4)	+++ (age 19y)	1–2 (N, 10-200)	Reduced stability of del exon 13 mutation; Normal targeting and function of E399D mutation
Italian (compound heterozygous)	delCTT, intron 4 R416C, TM9	40	6.77 (N, 0.83–1.76)	2536 µg/g liver (N, 0–400)	98–102 (N, 45–115)	R416C, complete loss of function (defective processing and targeting, ER retention, loss of transport function)
French (compound heterozygous)	delVal 114, TM2, G212V, TM5	83	8.29 (N, 0.83–1.76)	250 +/- 50 µmol/g liver (age 9 y); 66 µmol/g (after 3 mo epo) (N,<36)	19–43 (on 2 separate occasions) (N, 45–115)	Not studied; G212V probably conservative mutation
Ecuadorian (homozygous)	G75R, TM1	51	6.16 (N, 0.8–2.3)	Absence of iron deposits	n.a.	Not studied
(compound heterozygous)	G212V, TM5 , N491S, TM11	86 (13 years old)	66 nmol/L (N<28 nmol/L)	300 µmol/g dry weight liver (N,<36)	n.a.	G212V probably affect irontransport function, N491S loss of function resulting from disturbed protein trafficking.

Haematological data from 3 patients affected with DMT1 deficiency

EPO TREATMENT



Iolascon et al, J. Pediat. 2008

Erythropoietin-driven signaling ameliorates the survival defect of DMT1-mutant erythroid progenitors and erythroblasts

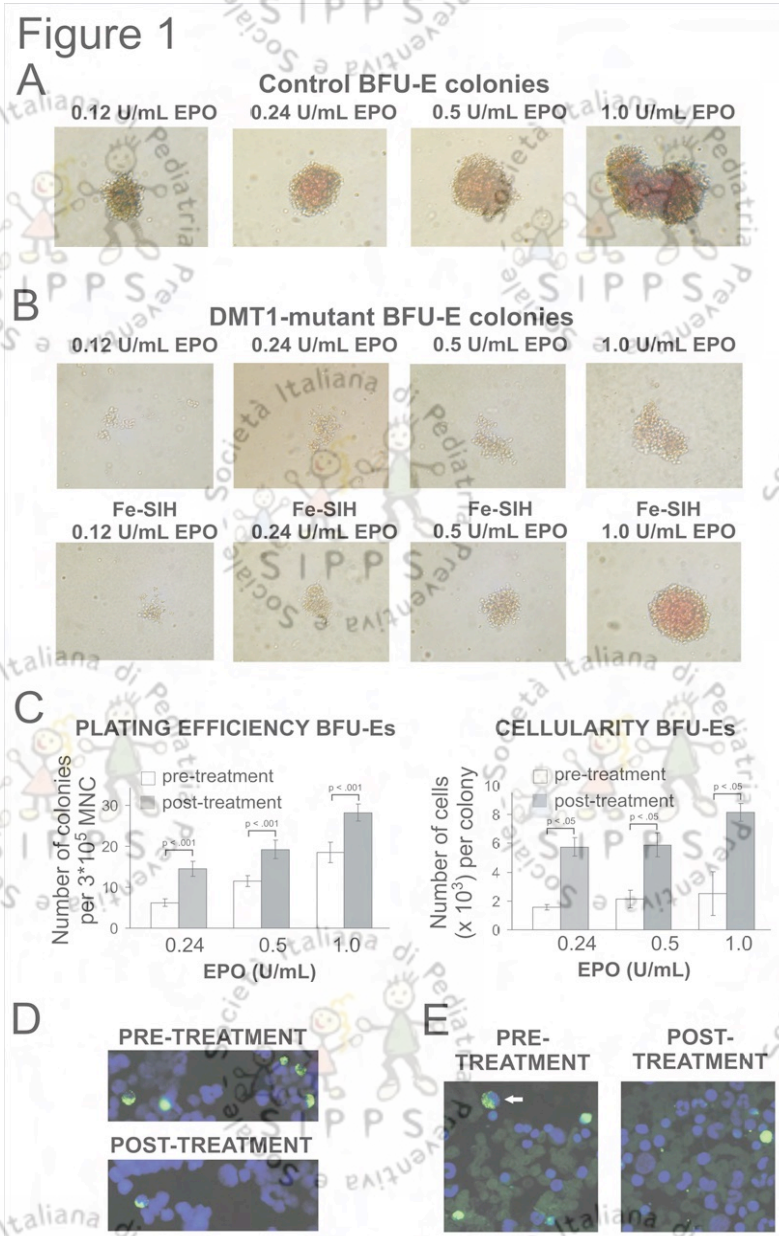
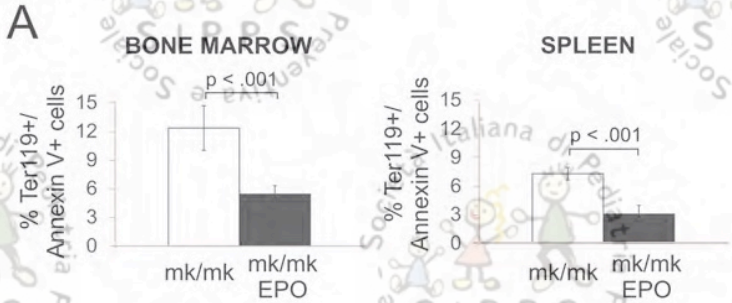


Table 1. Selected hematological values and iron status parameters in DMT1-mutant patient

	Patient		Normal values
	Pre-treatment	Post-treatment	
Red blood cell count ($x10^{12}$/L)	5.0 ± 0.4	6.1 ± 0.6	4.0-5.4
Hemoglobin level (g/dL)	7.5 ± 0.5	9.5 ± 0.5	12.0-15.6
Hematocrit (%)	29.0 ± 1.4	33.5 ± 0.7	36-45
Mean corpuscular volume (fL)	56.1 ± 1.0	57.0 ± 0.8	80-90
Mean corpuscular hemoglobin (pg)	15.2 ± 0.2	15.4 ± 0.2	27-34
Serum iron (µmol/L)	44.0 ± 1.4	43.5 ± 4.2	14.5-26.0
Total iron binding capacity (µmol/L)	50.7 ± 0.6	50.7 ± 1.2	44.8-71.6
Ferritin (ng/mL)	179 ± 26	175 ± 27	20-150
Serum transferrin receptor (mg/L)	24.1 ± 10.8	24.5 ± 3.1	1.9-4.4
Urinary hepcidin (ng/mg creatinine)	See Mims et al.[6]	55.3	126-986



Horvathova M et al, Haematologica. 2012

Table 1. Clinical and Laboratory Findings of *DMT1* Mutations

MCV	45-55
Serum iron	++
Tf saturation	++
sTfR	++
Bone marrow sideroblasts	–
FEP	+
Liver iron	+++
Neonatal appearance	Yes
Effect of oral Fe	No
Effect of intravenous Fe	No
Inheritance	Autosomal recessive
Therapy	Epo

Abbreviations: MCV, mean corpuscular volume; Tf, transferrin; sTfR, soluble transferrin receptor; FEP, free erythrocyte protoporphyrin; Fe, iron; Epo, erythropoietin.

- 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

Iolascon A, De Falco L Semin Hematol. 2009 Oct;46(4):358-70.

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
Prepublished online Jul 2, 2008;
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

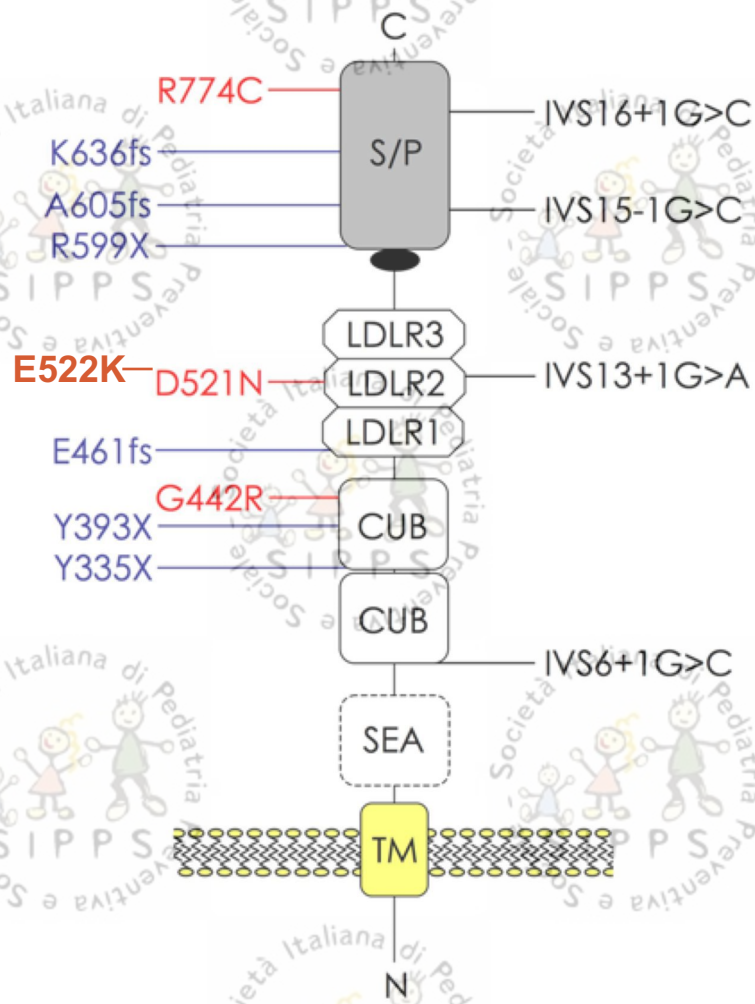
Maria Antonietta Melis,[1] Milena Cau,[1] Rita Congiu,[1] Gabriella Sole,[2] Susanna Barella,[2] Antonio Cao,[2] Mark Westerman,[4] Mario Cazzola[5] and Renzo Galanello[1,3]

blood

Prepublished online Apr 8, 2009;
doi:10.1182/blood-2008-12-195594

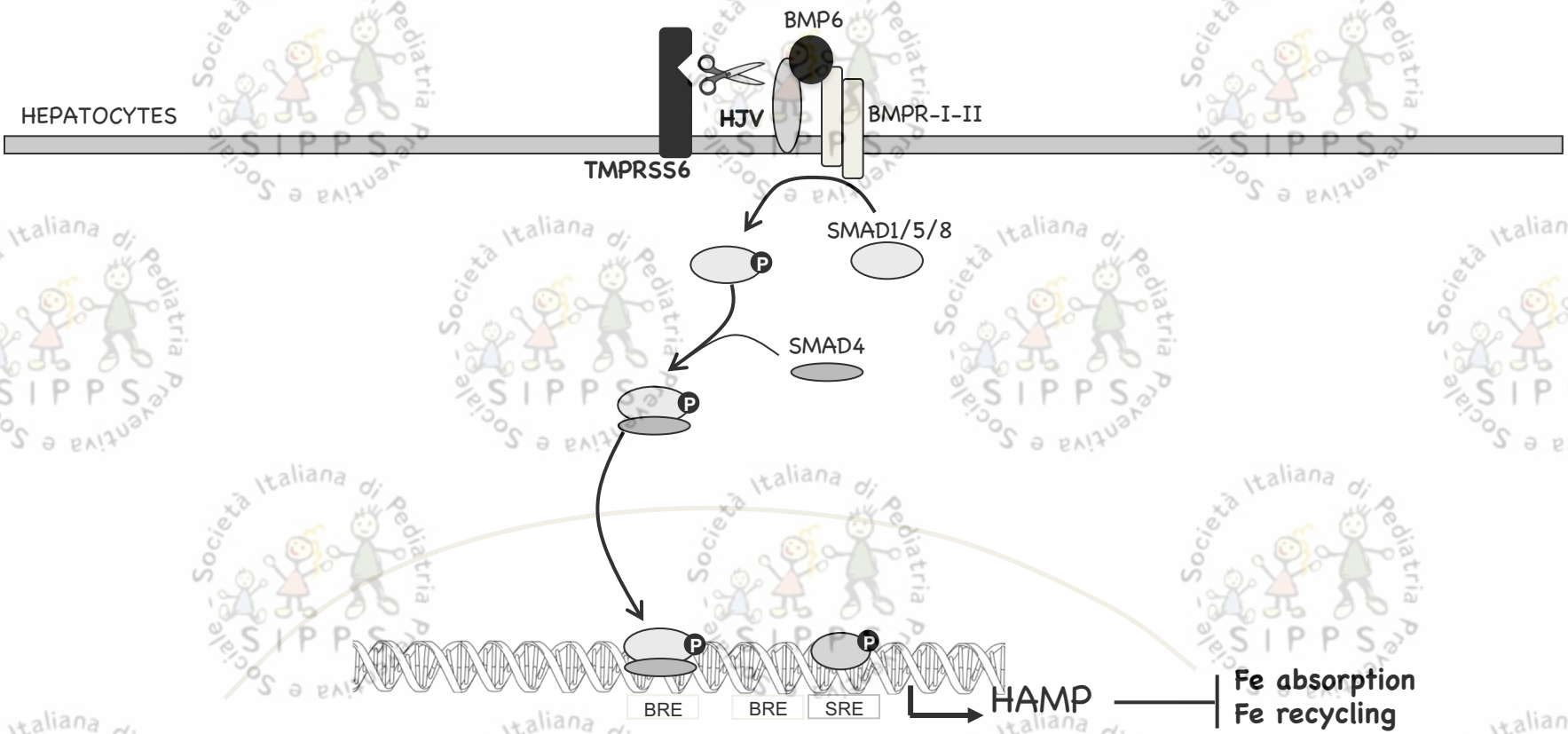
Molecular mechanisms of the defective hepcidin inhibition in TMPRSS6 mutations associated with iron-refractory iron deficiency anemia

Laura Silvestri, Flavia Guillem, Alessia Pagani, Antonella Nai, Claire Oudin, Muriel Silva, Fabienne Toutain, Caroline Kannengiesser, Carole Beaumont, Clara Camaschella and Bernard Grandchamp

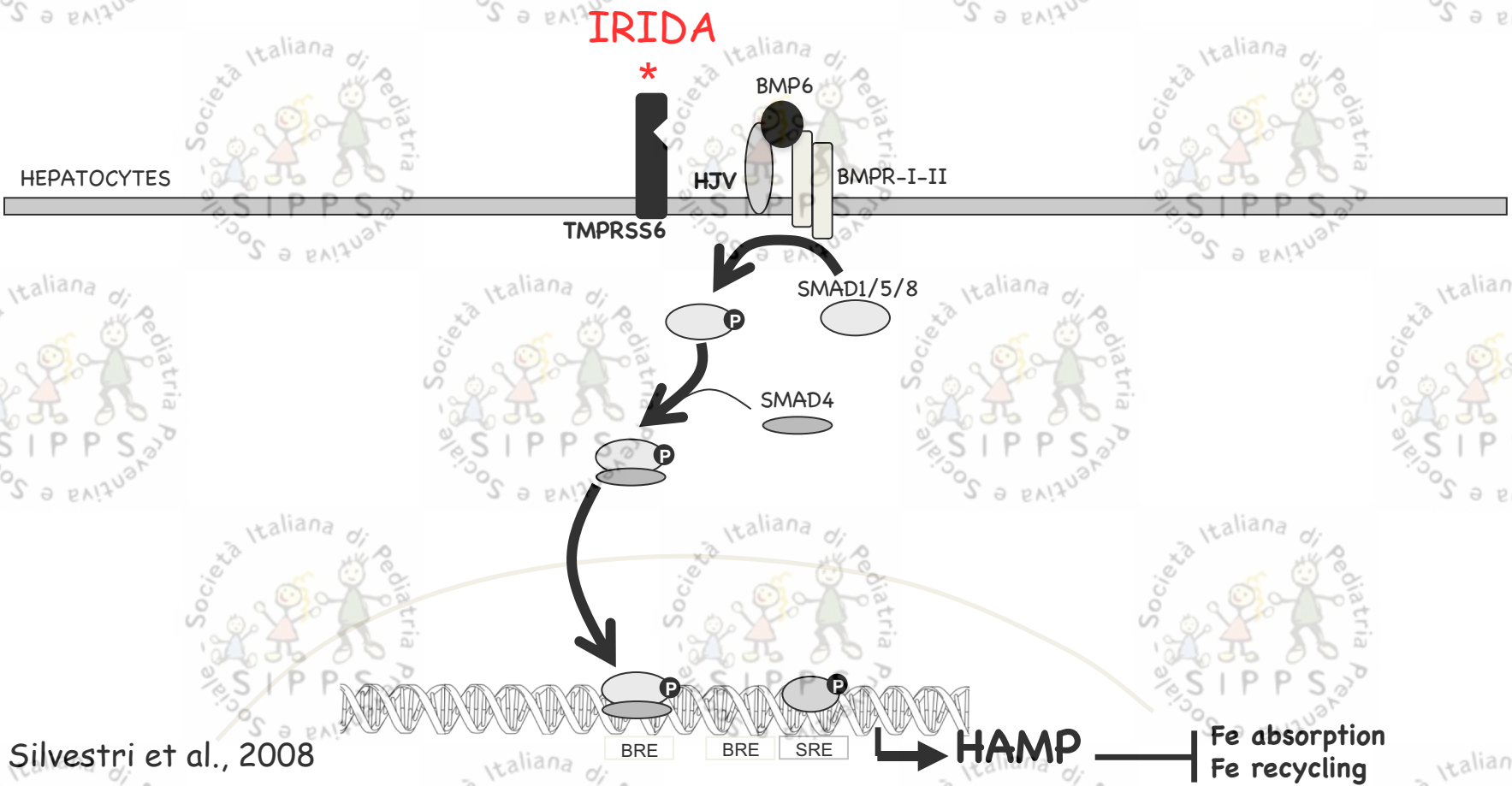


All patients have high hepcidin levels!

HEPCIDIN inhibition by LOW IRON

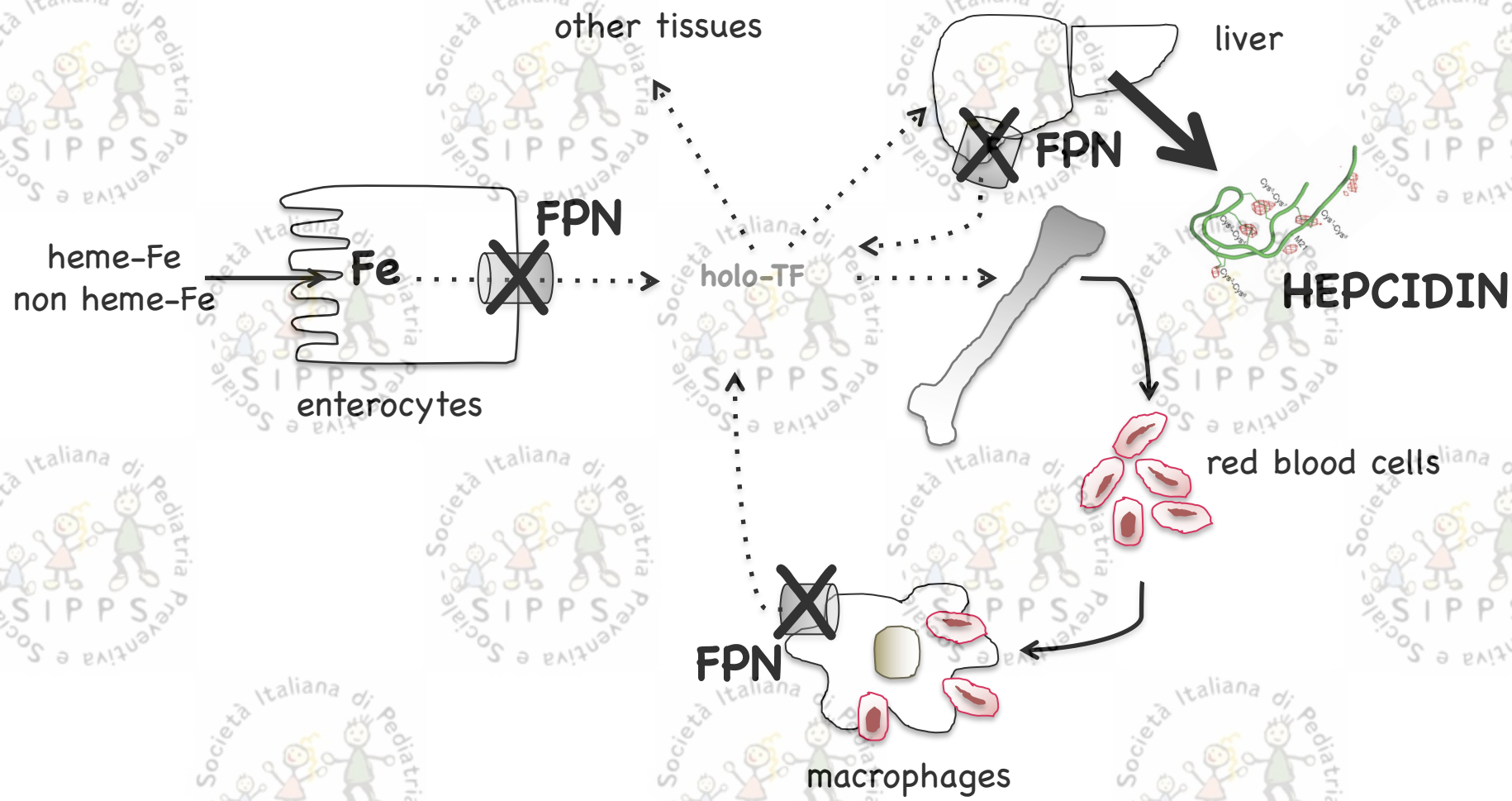


Mutations in TMPRSS6 cause IRIDA



Silvestri et al., 2008

IRON REFRACTORY IRON DEFICIENCY ANEMIA (IRIDA)



Clinical data of TMPRSS6 patients

	Normal values (range) m.	f.	A II-1	B II-1	C II-1	C II-2	C II-3	D II-1	E II-1	E II-2	F VI-1	F VI-2	F VI-3	G II-1
Age, years/sex			8/M	5/F	7/F	5/F	3/M	3/F	8/F	11/F	6/M	8/F	2/F	9/M
Hb, g/dL	12.0-17.5	12.0-16.0	9.1	9.5	10.6	10.4	9.8	6.6	6.8	8.9	8.01	8.83	7.93	10.4
WBC, x 10^3/µL	4.8-10.8			7.5		8.7	5.4	6.55	6.72	4.5	11.5	11	13.9	
RBC, x 10^6/µL	4.2- 5.6	4.0- 5.4	5.3	4.70		5.0	5.49	5.28	4.66					5.3
MCV, fL	80-97		60	62.8	62.8	68	65	47	58.8	59.8	46.3	53.3	49.3	63.5
MCH, pg	25-34		17	20.2	17.8	18	16.7	12	14.5	18.5	14.3	15.9	14.9	19.6
MCHC, g/dL	32-37		29	32.2		30.6	27	26	24.7	31	30.9	29.8	30.2	
RDW, %	11-16.5			16.4				17.5	19.2	17.3	25.4	19.6	22.5	17.5
Reticulocyte count, x 10^3/µL	20- 120		50	60		68	64	40	120					42.4
PLT, x $10*^3$/µL	130-400			420		383	410	740	647	406	778	526	592	
Serum Ferritin, µg/L	18-370	9-120	26	25	112	32	50	10	8	19	86	101	37.7	228
Serum iron, µg/dL	16-124		14	14	21	48	22	8	13	9	20	20	40	17
Transferrin, mg/dL	174-446		270	290	320	260	270	258	234					376
Transferrin saturation, %	15- 35		3.7	3.7	5	9.4	6.2	2.3	4.2		3.1	3.3		5
Soluble transferrin receptor, mg/L	0.83-1.15							24.8	6.22		4.15	5.59 - 19.6	22	
Serum Epcidin[a], nM	3-7		9.78	5.57	17.77	8.92	7.55	5.78	12.99	10.41				
Urinary Epcidin[b], 95% CI	0,34-0,96	0,2-0,41		2.03					1.33	3.68				

[a]Reference range: n=57 normal individuals (median 4.7)

[b]Reference range: normal individuals

Iolascon et al.2009

Answer to iron administration

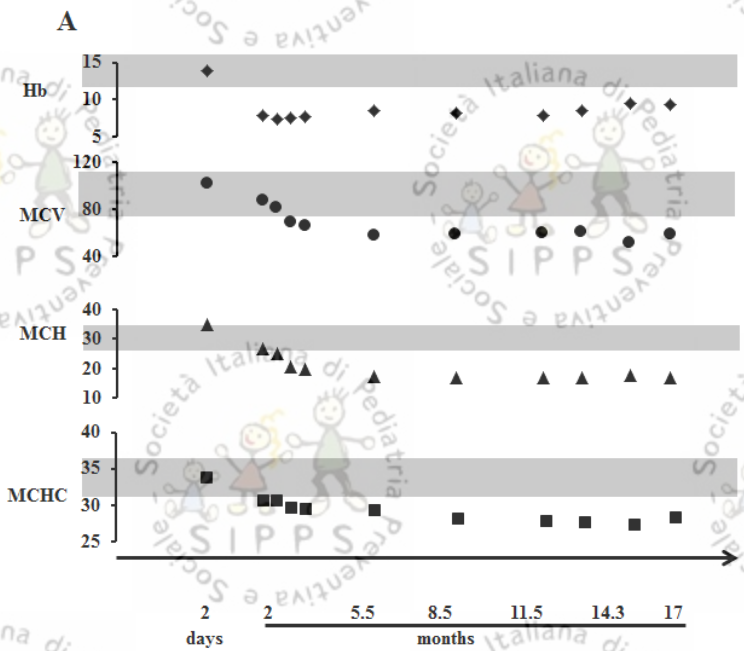
	Patient I-1 (age: 3 years) *Intravenous Iron*		**Patient II-1** (age: 3 years) *Intravenous Iron*		**Patient II-2** (age: 5 years) *Intravenous Iron*		**Patient III-1** (age: 3 years) *Intravenous Iron*	
	Before Treatment	*After Treatment*	*Before Treatment*	*After Treatment*	*Before Treatment*	*After 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

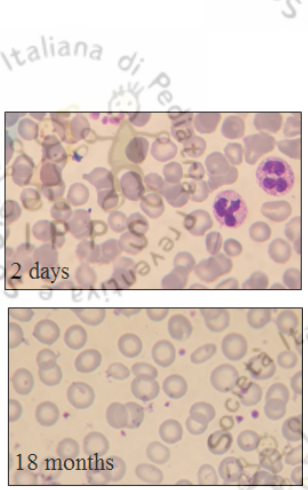
Iolascon et al.2010

The role of Matriptase-2 during the early postnatal development in humans

Table 1. Hematological parameters of IRIDA patients in the neonatal period.

							Reference values			
	2 days	2 weeks	1 day	1 week	1-3 days		1 week		2 weeks	
	A II3	B II3	C III1	D II1	mean	±SD	mean	±SD	mean	±SD
Hb, g/dL	13.8	12.5	17.3	13.8	17.3	1.9	16.7	2.2	15.9	1.9
MCV, fL	102	106.8	111.3	101.8	109.1	4.8	107.7	6.2	106.4	3.9
MCH, pg	34.8	33.3	36.1	34.3	34.1	1.3	33.8	1.9	33.7	1.5
MCHC, g/dL	33.8	31.2	32.4	33.7	31.3	0.9	31.6	1.3	31.7	0.9
RDW, %	14.9	15.6	20.4	17.4	16.2	1.1	15.6	1.1	15.5	0.9
RBC, $*10^6/\mu L$	3.9	3.3	4.8	4.02	5.1	0.6	4.9	0.7	4.7	0.6
WBC, $*10^3/\mu L$	15.7	14.5	16	6.7	14.9	17.1	10.8	2.5	11	2.4
PLT, $*10^3/\mu/L$	452	420	236	301	287	88	306	101	420	122





De Falco et al., Haematologica In Press

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

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MEDICAL GENETICS

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