# LE ETA' DELL'ANEMIA

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XIV CONGRESSO NAZIONALE SITE

Roma, 11-13 Settembre 2025
Pontificia Università Urbaniana

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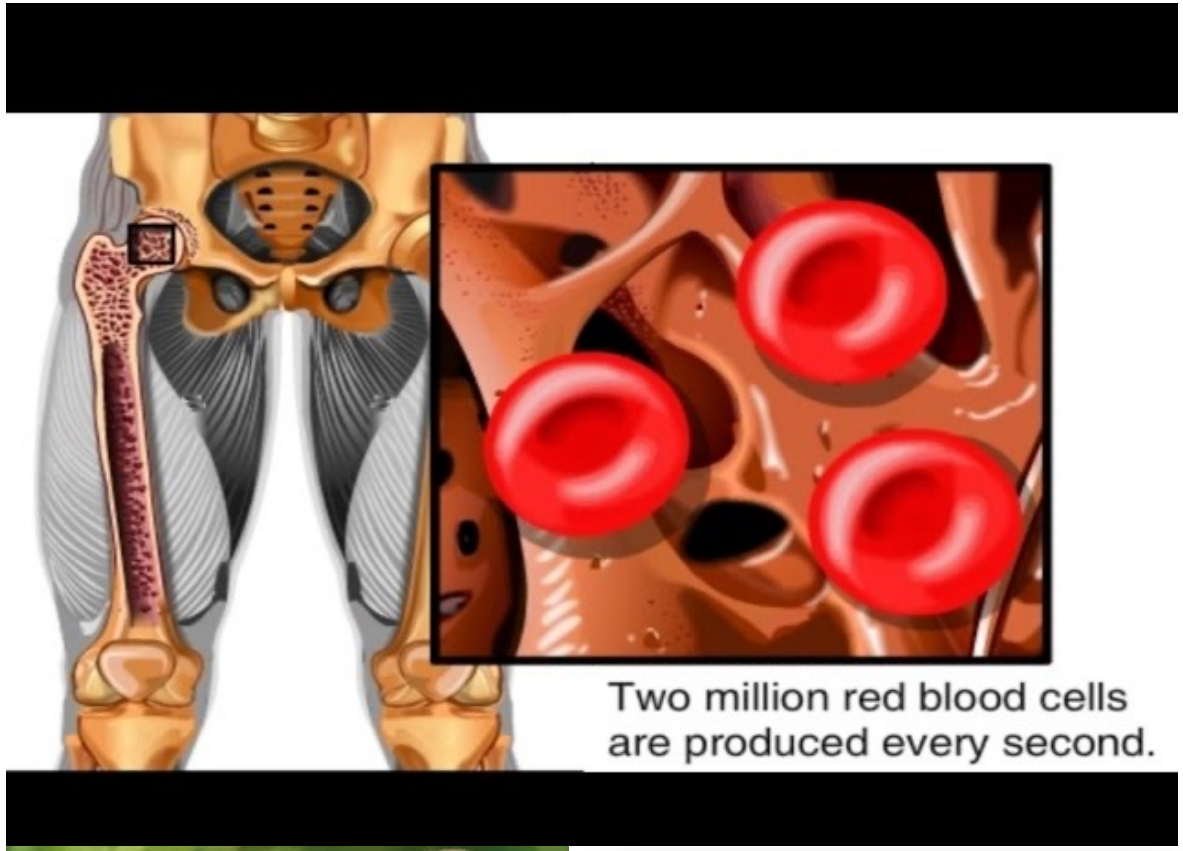
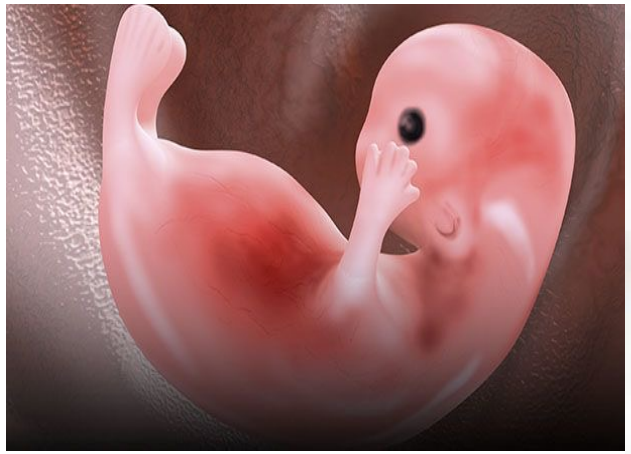
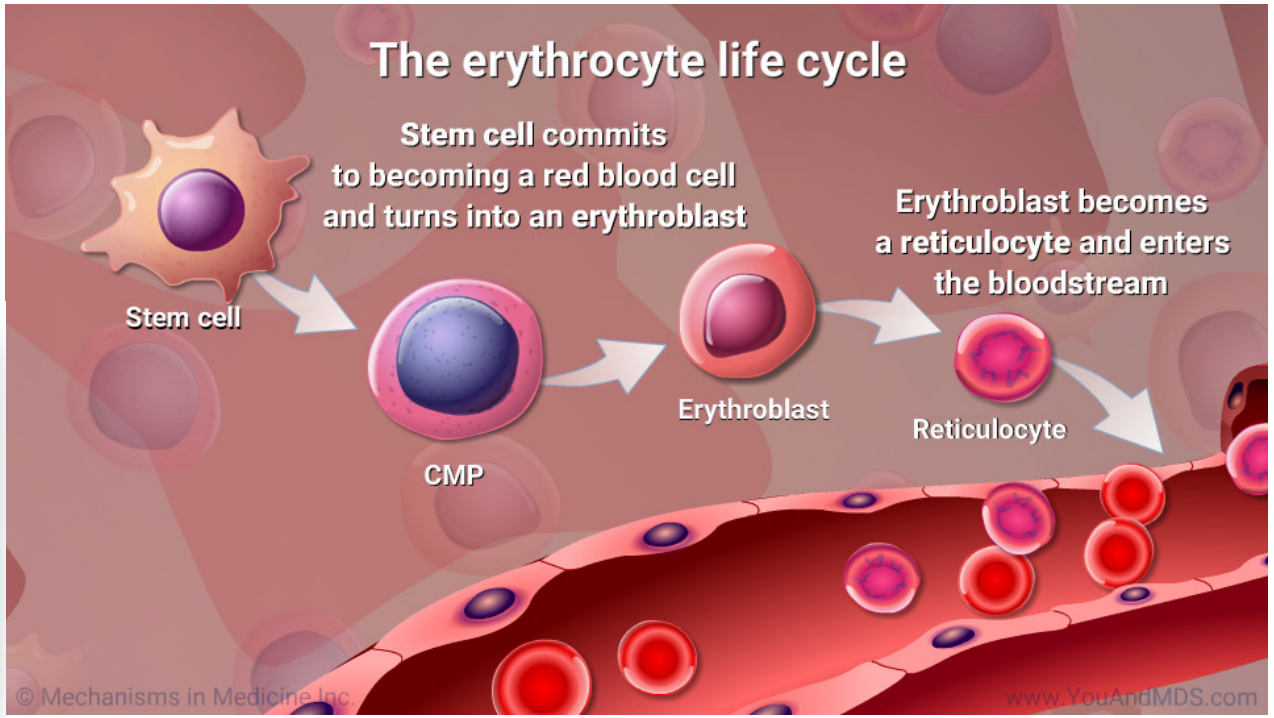
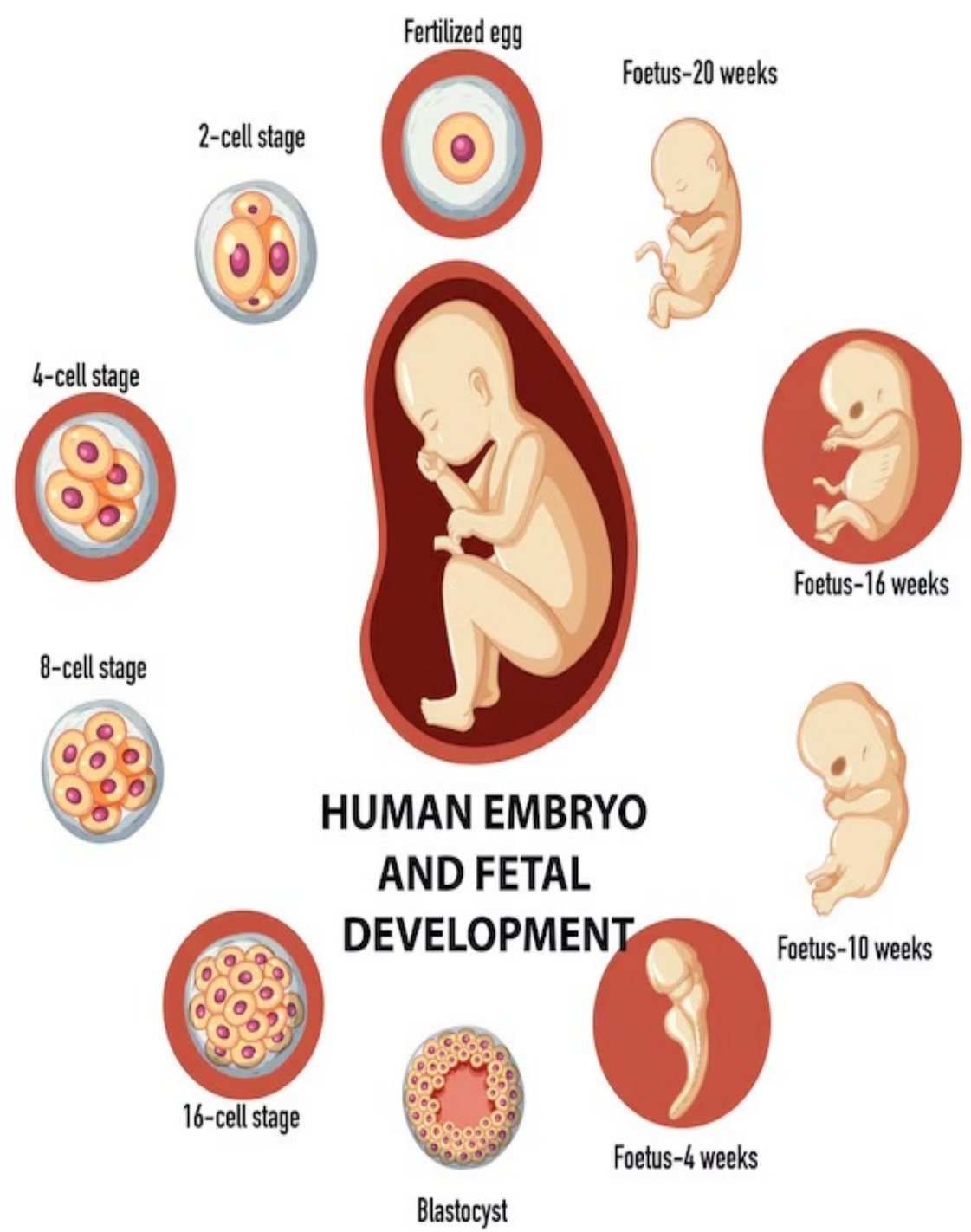
# Erythropoiesis



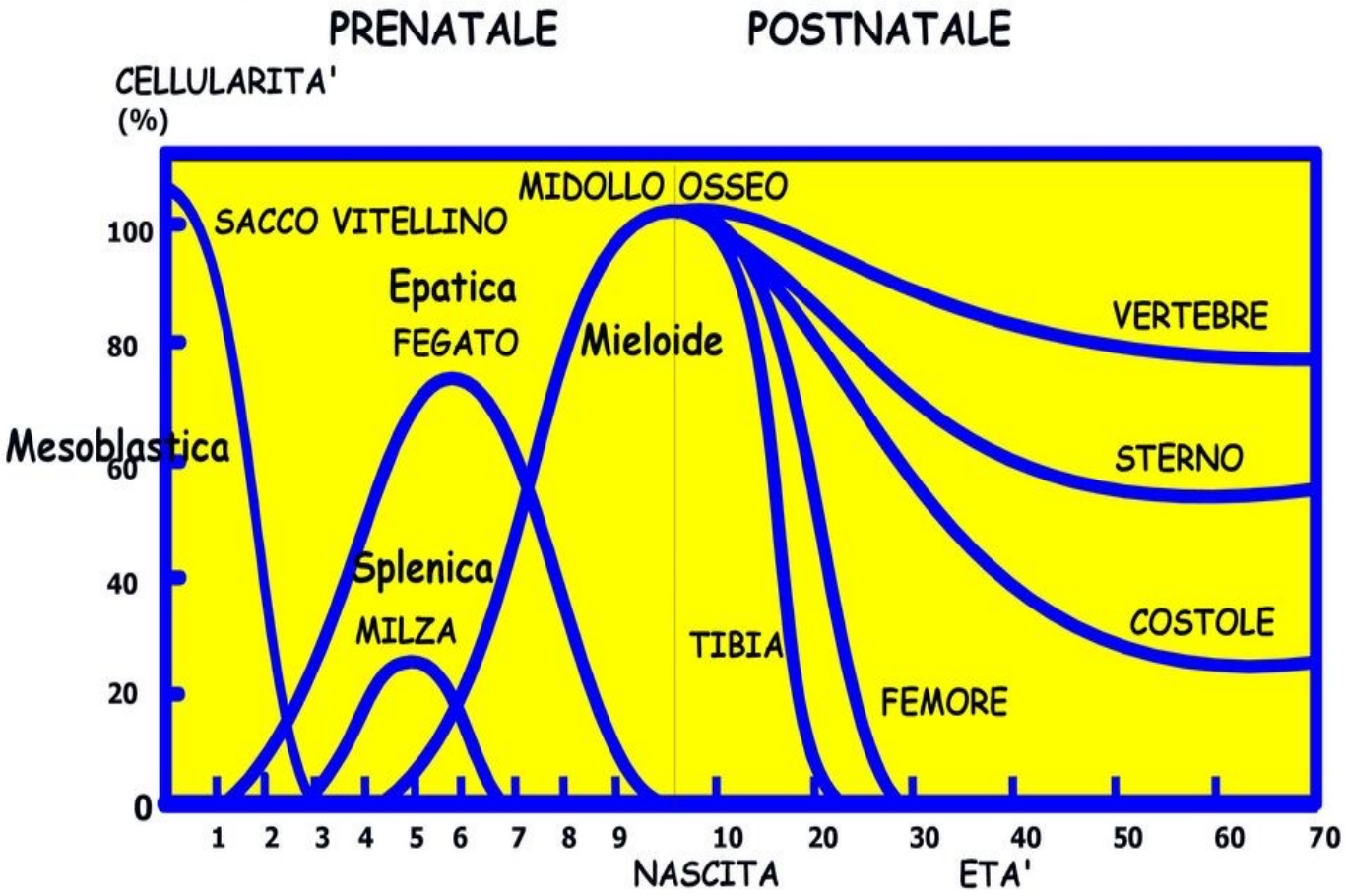



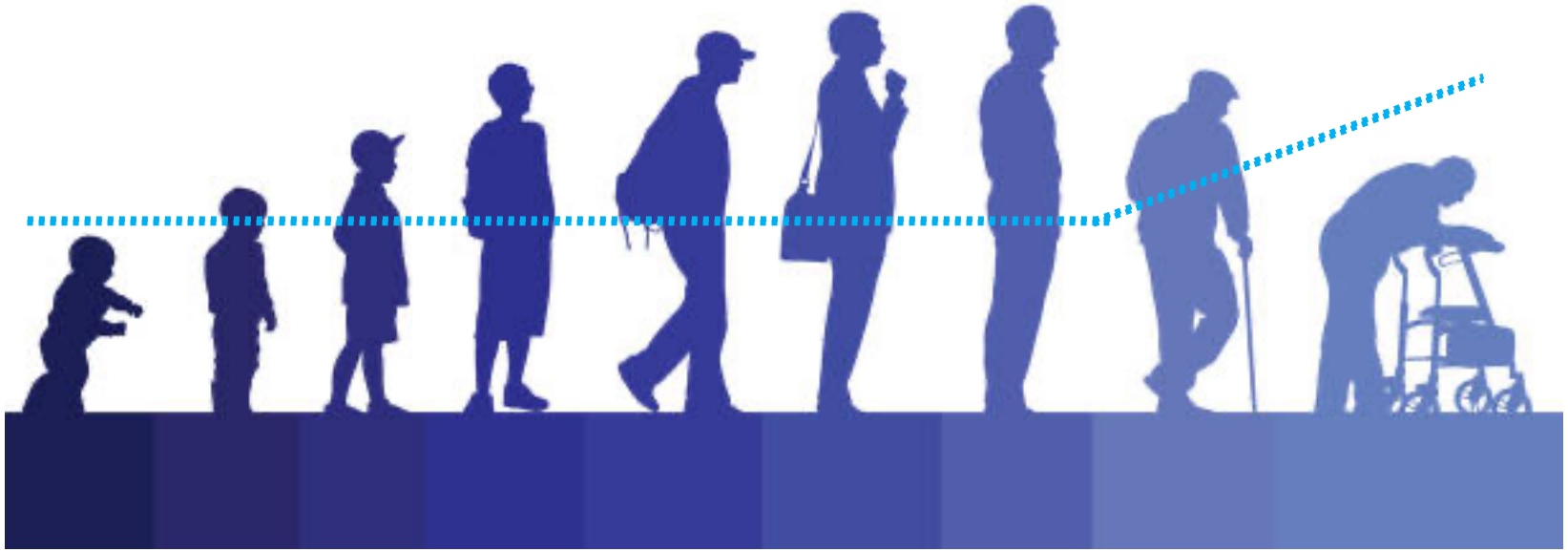


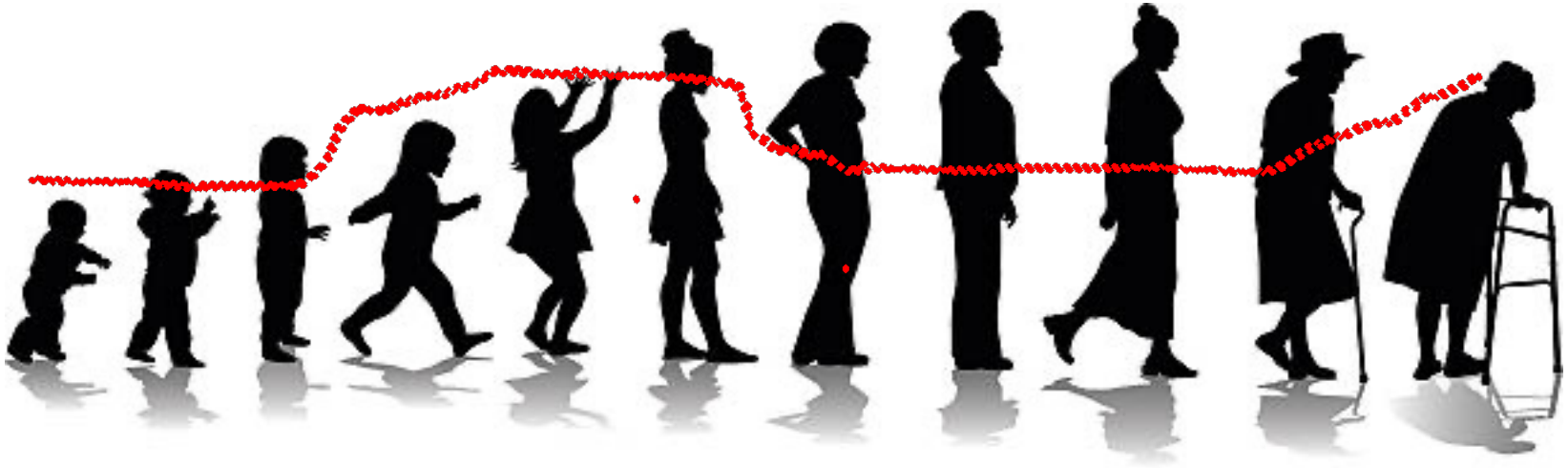



Nei vertebrati lo sviluppo delle cellule del sangue avviene in due fasi: una **fase embrionale transitoria** e una successiva **fase definitiva**. Queste fasi differiscono per i siti in cui gli elementi del sangue vengono prodotti, per la tipologia delle cellule prodotte e per i tempi necessari all'emopoiesi.

L'emopoiesi prenatale è, a sua volta, suddivisa in 4 fasi:







## Anemia in infants and children

| Age<br>Disorder | Newborn (0–30 days) | Infant (0–1 year) | Toddler (2–3 years) | Preschool (4–5 years) | Child (6–9 years) | Preteen (10–12 years) | Teenager (13–18 years) |
|---|---|---|---|---|---|---|---|
| Membrane defects | | | | | | | |
| Abnormalities of metabolism | | | | | | | |
| Unstable hemoglobins | | | | | | | |
| Sideroblastic anemia | | | | | | | |
| α-Thalassemia | | | | | | | |
| β-Thalassemia | | | | | | | |
| Sickle cell disease | | | | | | | |
| Congenital dyserythropoietic anemia | | | | | | | |
| Diamond blackfan anemia | | | | | | | |
| Fanconi anemia | | | | | | | |
| Hemolytic uremic syndrome | | | | | | | |
| Thrombotic thrombocytopenic purpura | | | | | | | |
| Disseminated intravascular coagulation | | | | | | | |
| Hemorrhage | | | | | | | |
| Chronic inflammation | | | | | | | |
| Malignancies | | | | | | | |
| Neonatal alloimmune hemolytic disease | | | | | | | |
| Primary autoimmune hemolytic anemia | | | | | | | |
| Secondary autoimmune hemolytic anemia | | | | | | | |
| Aplastic anemia | | | | | | | |
| Iron deficiency | | | | | | | |
| B12 deficiency | | | | | | | |
| Folate deficiency | | | | | | | |

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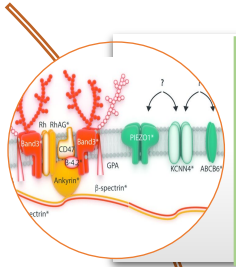
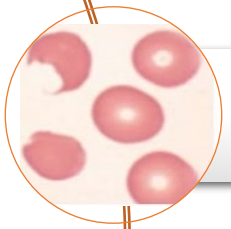
# Hereditary hemolytic anaemia (HHA)

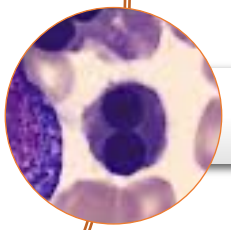
HHA are a heterogeneous group of conditions characterized by premature red blood cells (RBCs) destruction and anaemia due to intrinsic RBCs defects:



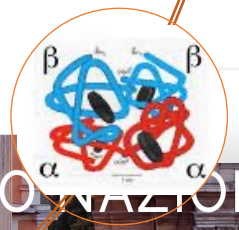

**Red cell membrane defects** (hereditary spherocytosis HS, hereditary elliptocytosis HE, hereditary pyropoikilocytosis HPP, southeast Asian ovalocytosis SAO, dehydrated hereditary stomatocytosis DHS, overhydrated hereditary stomatocytosis OHS, familial pseudohyperkalemia FP, and cryohydrocytosis CHC)



**Enzyme disorders** (the most frequent are glucose-6-phosphate dehydrogenase G6PD and pyruvate kinase PK deficiencies)



**Congenital dyserythropoietic anaemias** (CDAI and II)

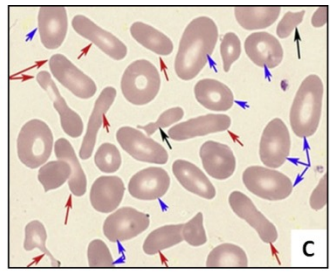



**Haemoglobinopathies** (thalassemia and sickle cell disease)

# Hereditary anemias associated with membrane defects

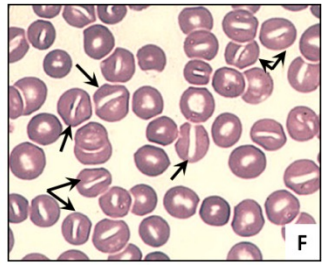
**altered membrane structural organization**

- Hereditary Spherocytosis
- Hereditary Elliptocytosis
- Hereditary Pyropoikilocytosis
- South East Asian Ovalocytosis



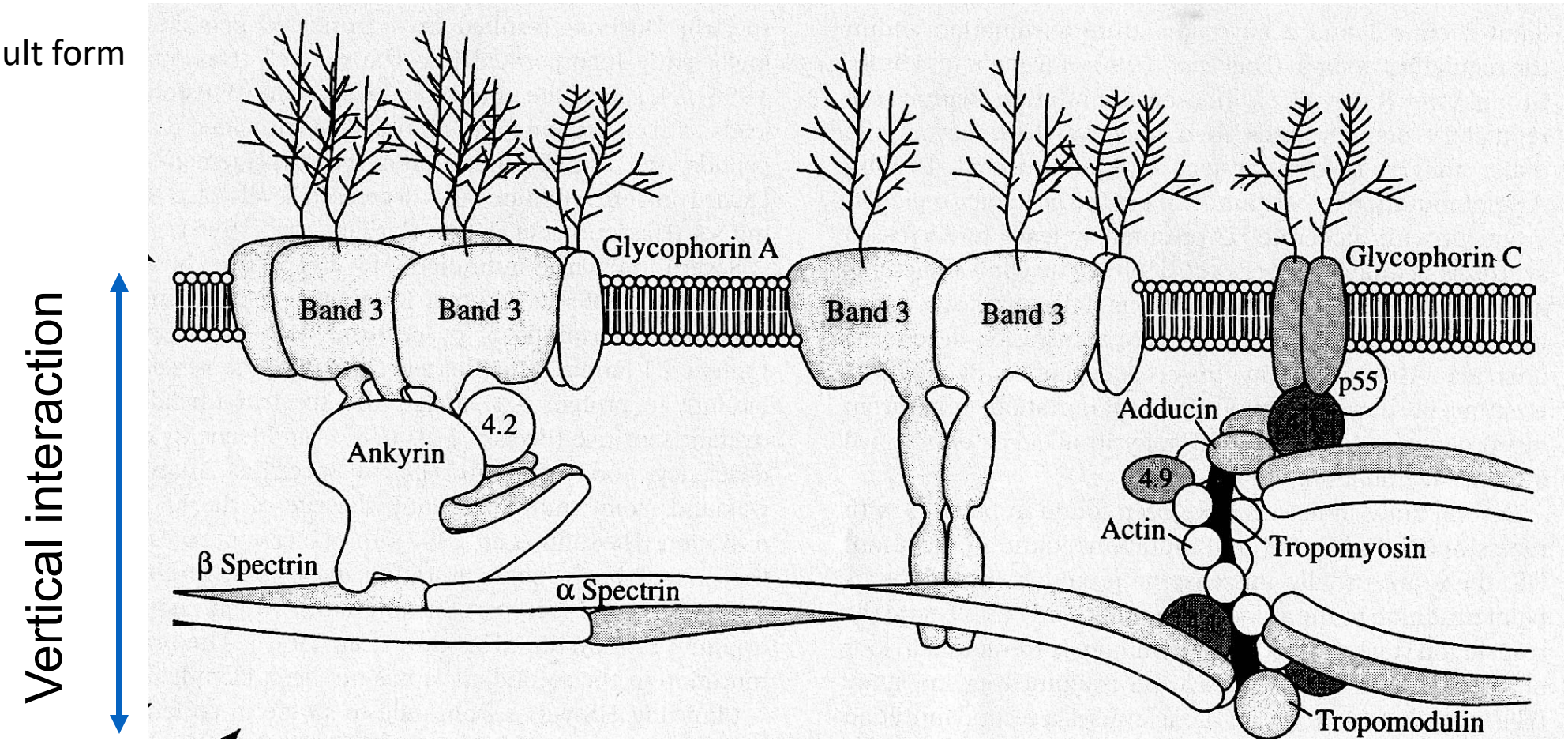

**altered membrane transport function**

- Dehydrated hereditary stomatocytosis
- Overhydrated Hereditary Stomatocytosis
- Familial Pseudohyperkalemia
- Cryohydrocytosis




# Schematic representation of red cell membrane

HS/HE
Neonatal vs adult form




Vertical interaction

Horizontal interaction

**Micronutrients** are needed in the body in tiny amounts. They do not provide energy, but are required for a number of important processes in the body.

There are two main groups of micronutrients:

- vitamins;
- minerals and trace elements.

**Iron** is an example of a mineral. Minerals are inorganic substances required by the body in small amounts for a variety of different functions.

## Iron is needed for:

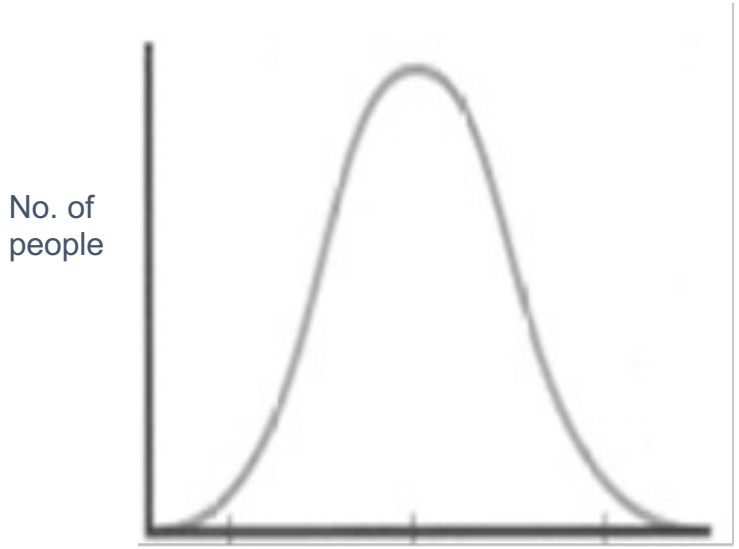
- the formation of haemoglobin in red blood cells;
- transport of oxygen in the body;
- production of energy;
- function of the immune system;
- reduction of tiredness and fatigue.

# Reference Nutrient Intakes (RNI) for Iron

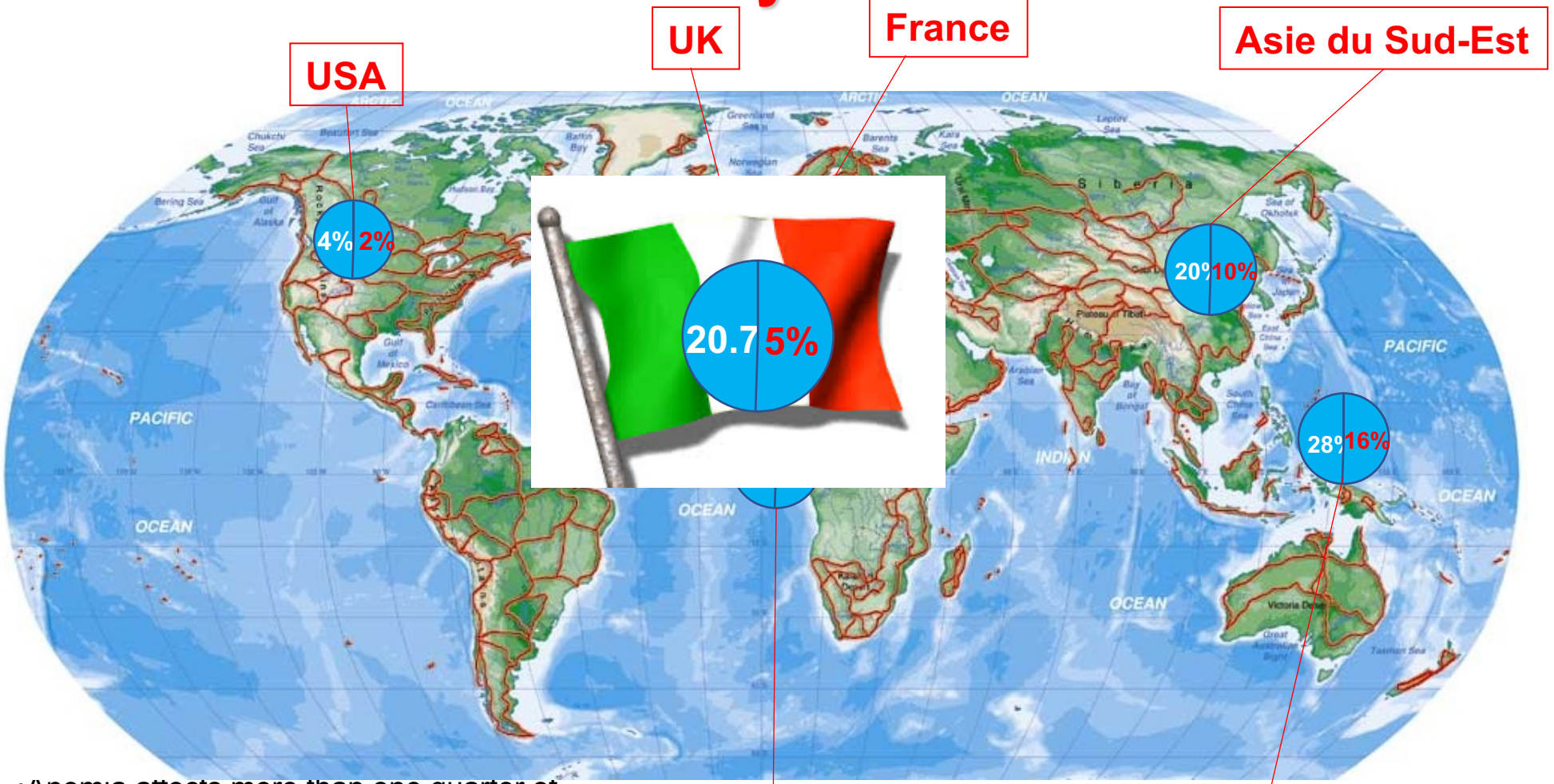
The RNI is the amount of a nutrient that is enough to ensure that the needs of nearly all the population (97.5%) are being met.

The RNIs for iron shown in the table below are in mg/day.

| Age | Males | Females |
|---|---|---|
| 1-3 years | 6.9 | 6.9 |
| 4-6 years | 8.1 | 8.1 |
| 7-10 years | 8.7 | 8.7 |
| 11-14 years | 11.3 | 14.6 |
| 15-18 years | 11.3 | 14.8 |
| 19-50 years | 8.7 | 14.8 |
| 50+ years | 8.7 | 8.7 |



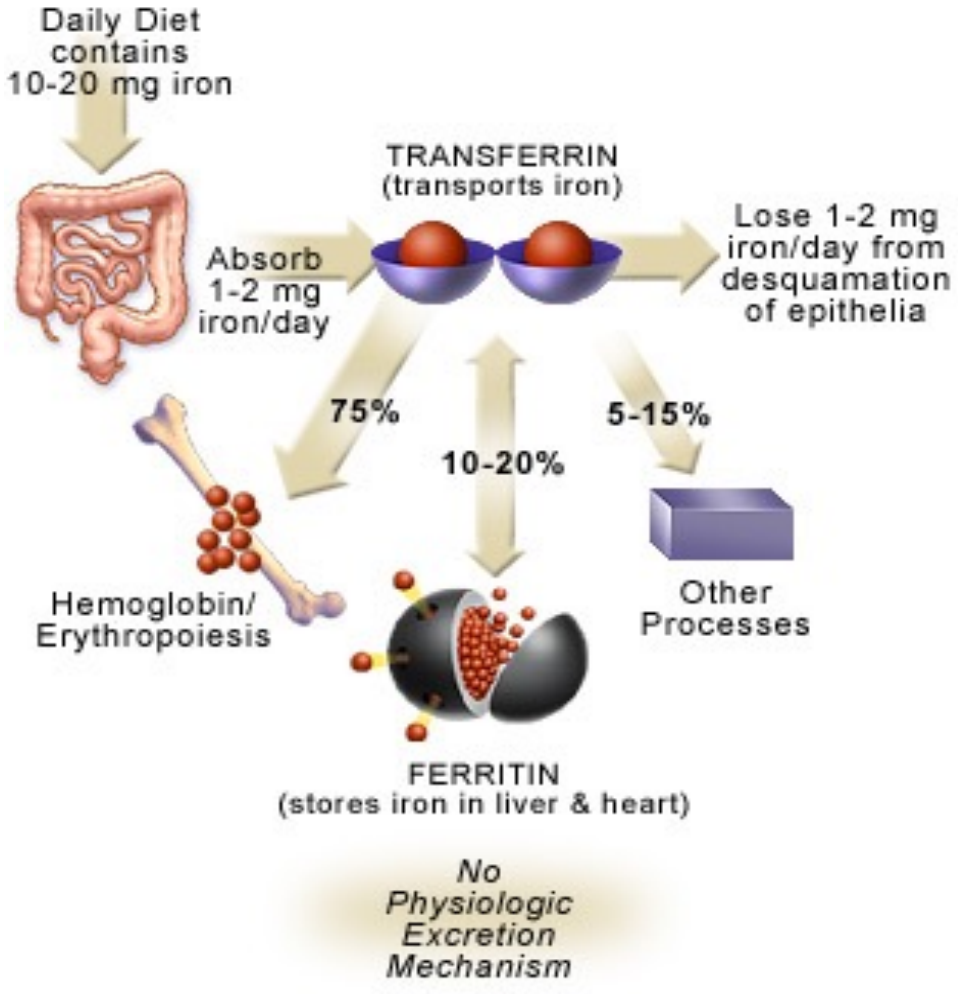
# Prevalence of Iron Deficiency and Iron Deficiency Anemia




- Anemia affects more than one quarter of the world's population
- About half have **iron deficiency anemia (IDA)**, prevalently children and pregnant women

(Kassebaum et al, Blood 2014)

# Iron metabolism



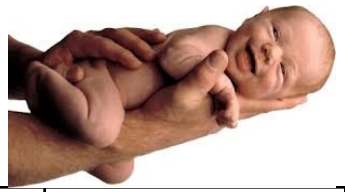

- **The total body iron content of an average male adult is about 4 g;**
- **Total iron:**
  - Red cell mass as haemoglobin – 65%-75%
  - Muscles as myoglobin – 10%
  - Storage as ferritin - 10%
    - Bone marrow
    - Reticulo-endothelial cells
    - Liver (0.5-1 g)
  - Other Haem proteins - 5%
    - 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)*

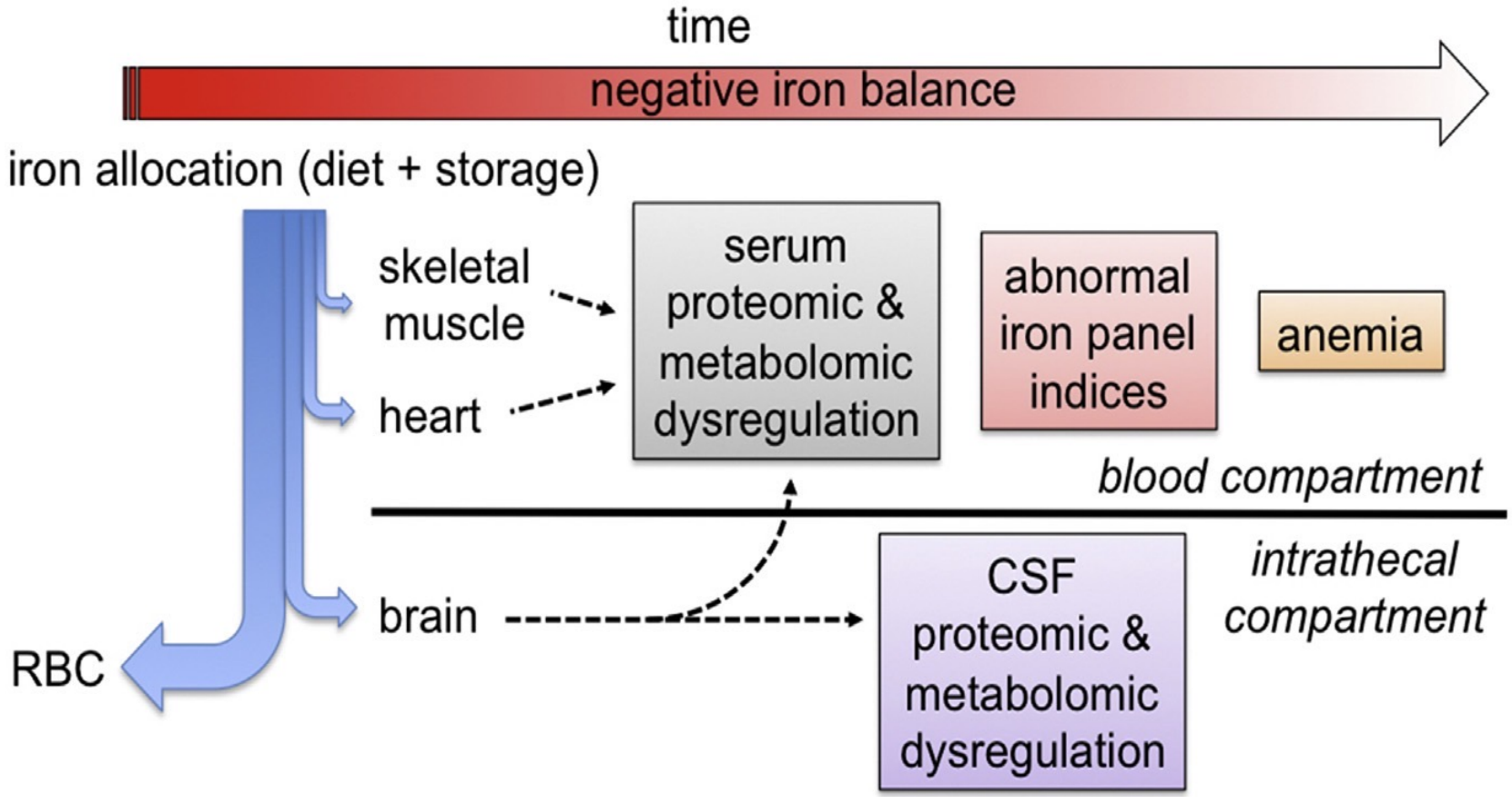
# Iron content in the body in different age

1kg body weight=
50 mg Fe

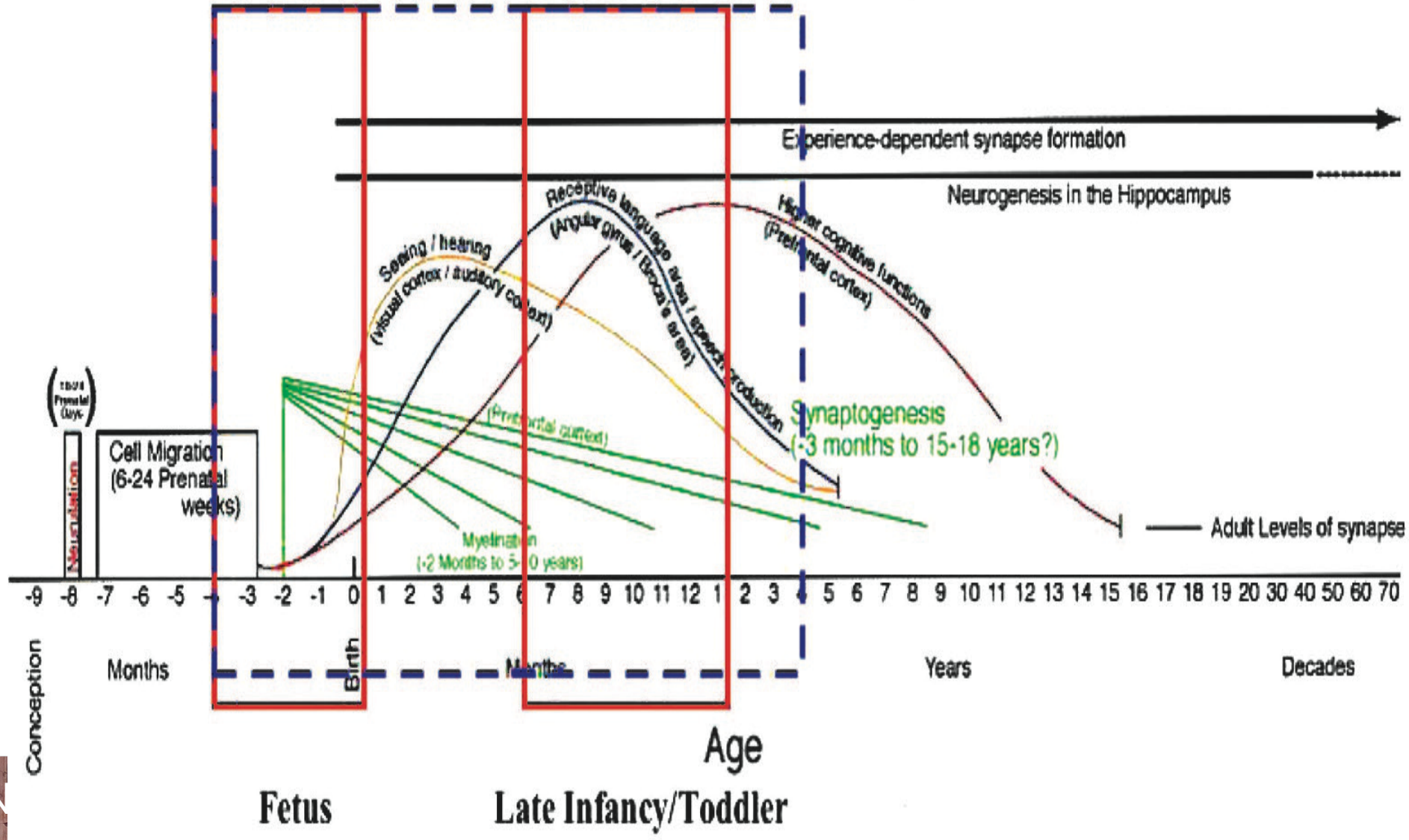


| | *Newborn (3,300 Kg)* | *Children(35 Kg)* | *Adult (75 Kg)* |
|---|---|---|---|
| **Total iron** | **240-250 mg** | **1,5 – 2 g** | **3 -4 g** |
| *HB* | 132 – 137,5 mg (55%) | 1 – 1,4 g (68%) | 2,04 – 2,72 g (68%) |
| *Ferritin* | 101 – 105 mg (42%) | 400 – 500 mg (27%) | 0,81 -1,08 g (27%) |
| *Myoglobin* | 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%) |

Idiascon A et al.,2013


time
negative iron balance
iron allocation (diet + storage)
skeletal muscle
heart
brain
RBC
serum proteomic & metabolomic dysregulation
abnormal iron panel indices
anemia
blood compartment
intrathecal compartment
CSF proteomic & metabolomic dysregulation

# Human Brain Development



# 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)

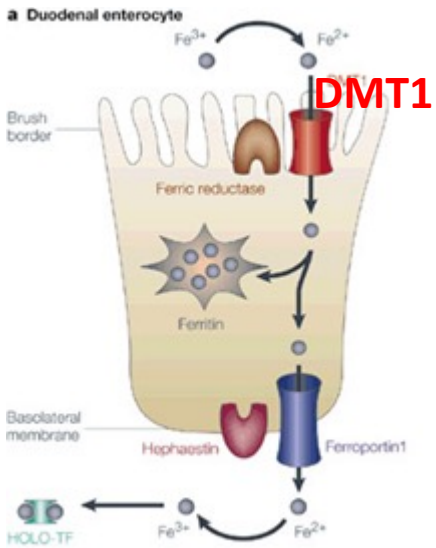
- **Defects of mitochondrial iron utilization**
  Inherited (and acquired) Sideroblastic Anemias

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

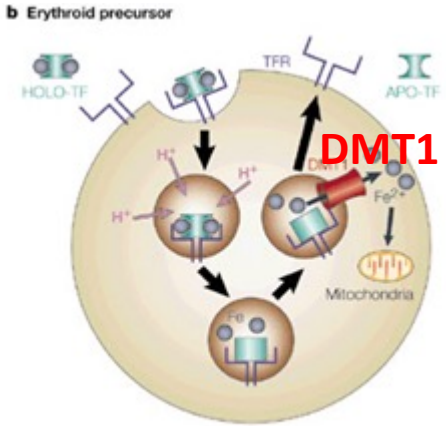
# New rare disorders of iron entry and utilization: DMT1 deficiency

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



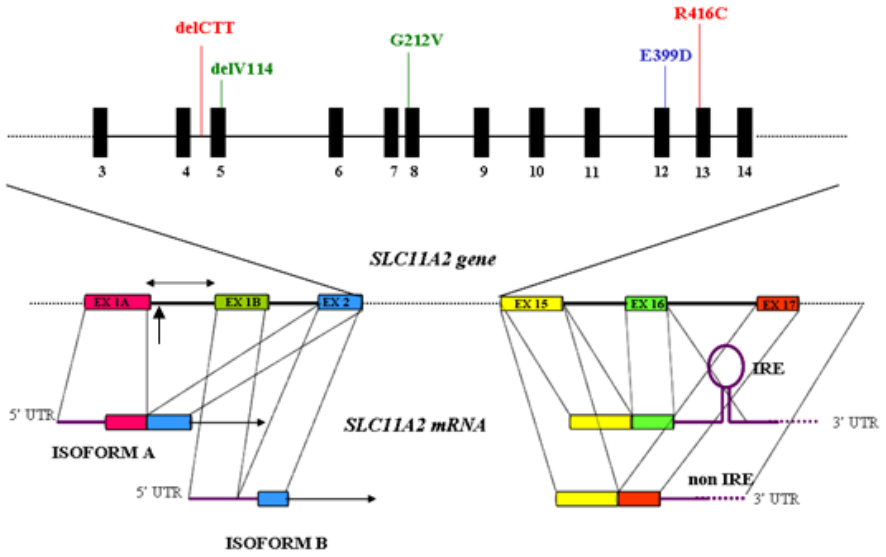

absorption



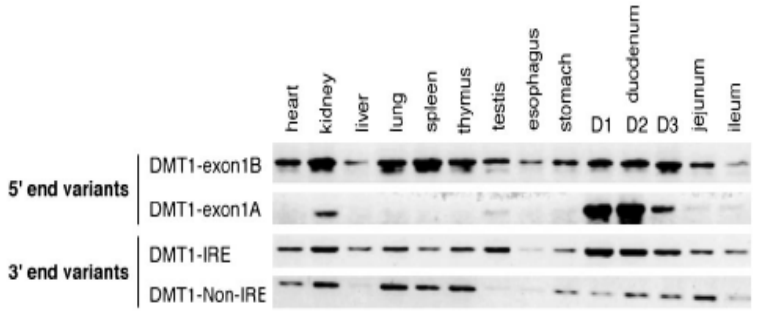

utilization

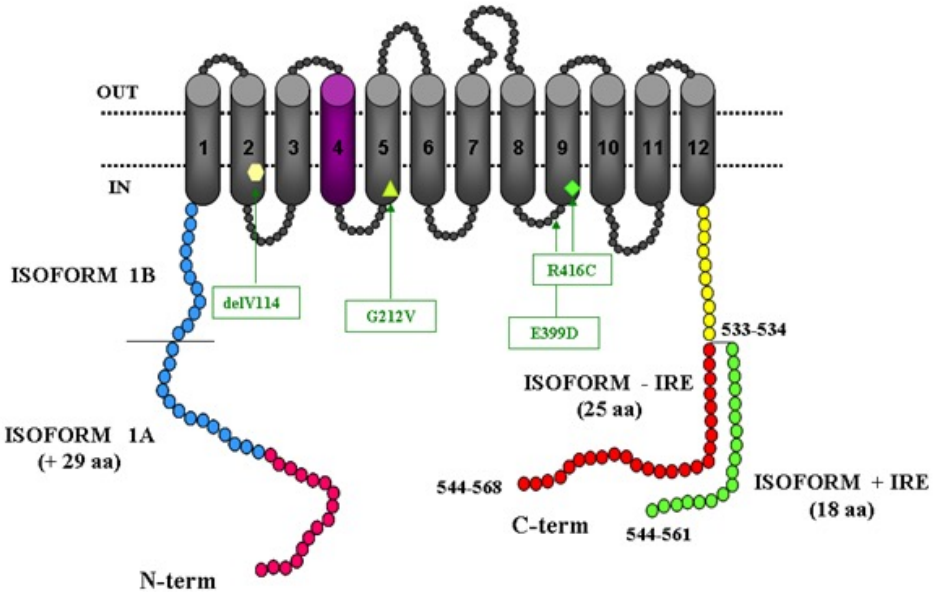
(Andrews, NEJM, 1999)

# The iron transporter DMT1: 4 isoforms

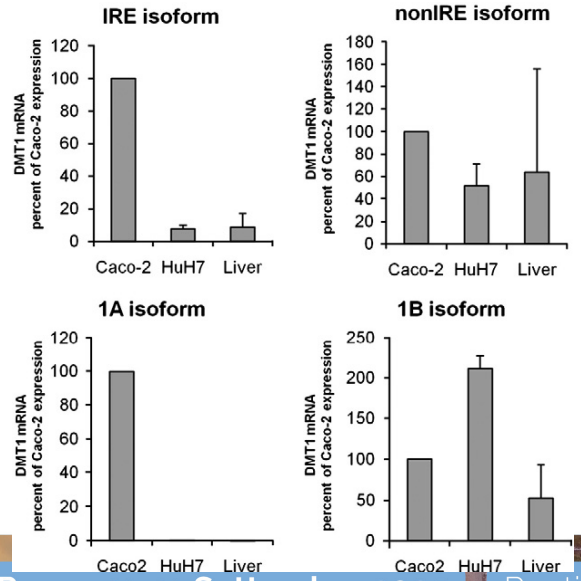



mouse




human





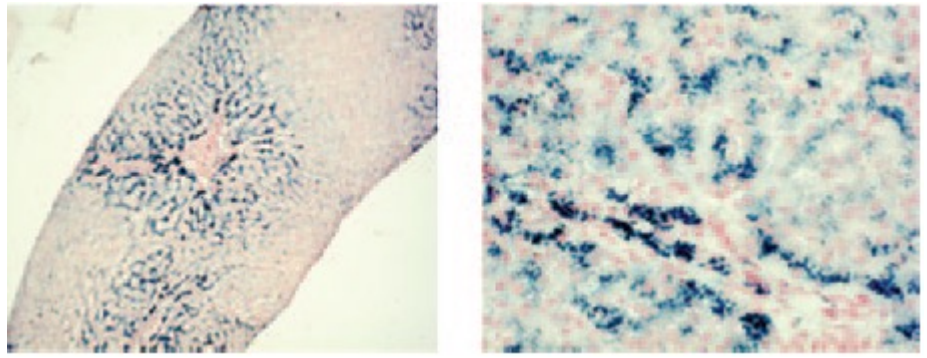

Iolascon A and De Falco L 2009 Semin Hematol 46:358-370

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



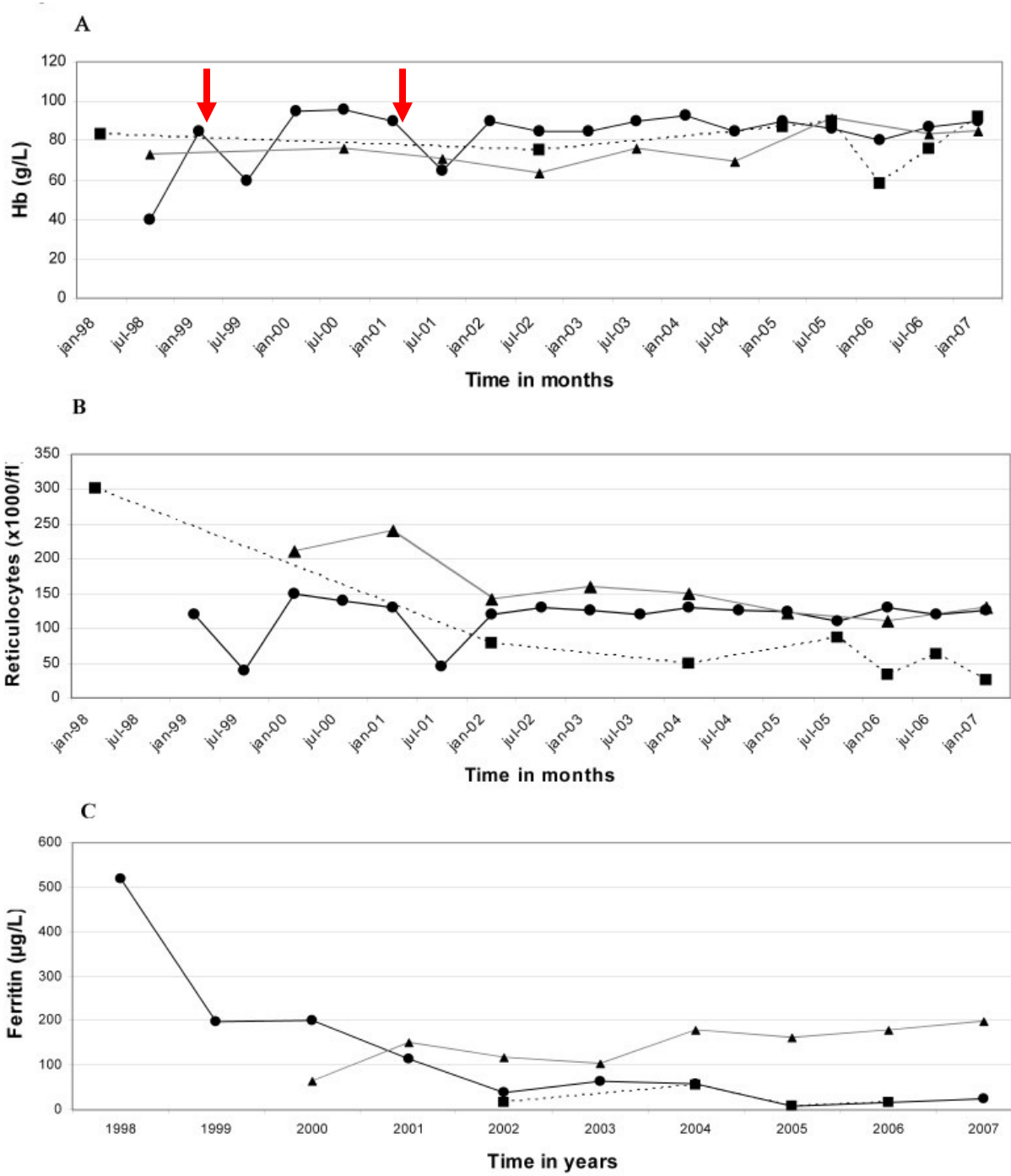
| | Father, I-1 | Mother, I-2 | Proband, II-1 | | | | | | | Normal values (range) |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | 35 y | 32 y | Birth | 2 mo | 3 mo | 6 mo | 1 y | 3 y | 5 y | 2-3 y |
| Body weight, percentile | NA | NA | < 3rd | 3rd | 5th | 10th | 15th | 15th | 25th | NA |
| Hb, 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 |

# 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 iron transport 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

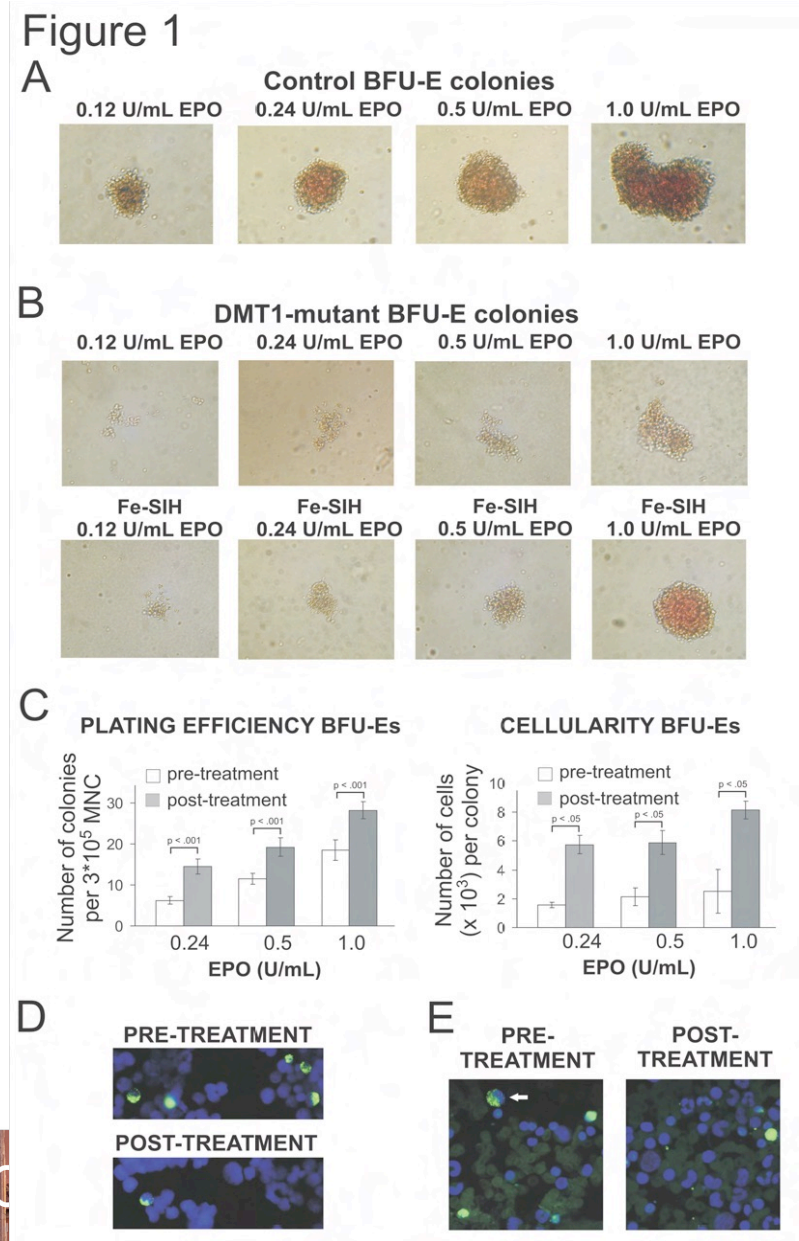
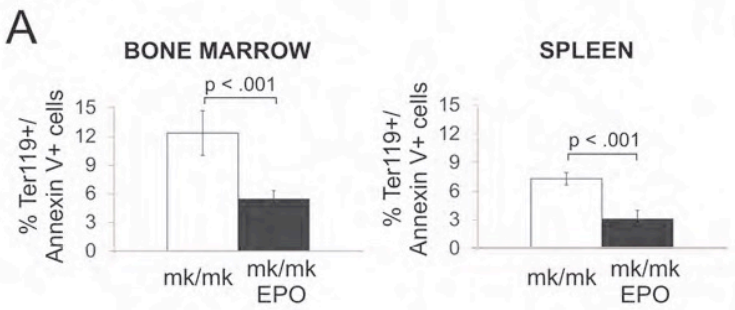



**Table 1. Selected hematological values and iron status parameters in DMT1-mutant patient**

| | Patient | | Normal values |
|---|---|---|---|
| | Pre-treatment | Post-treatment | |
| Red blood cell count (x$10^{12}$/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**

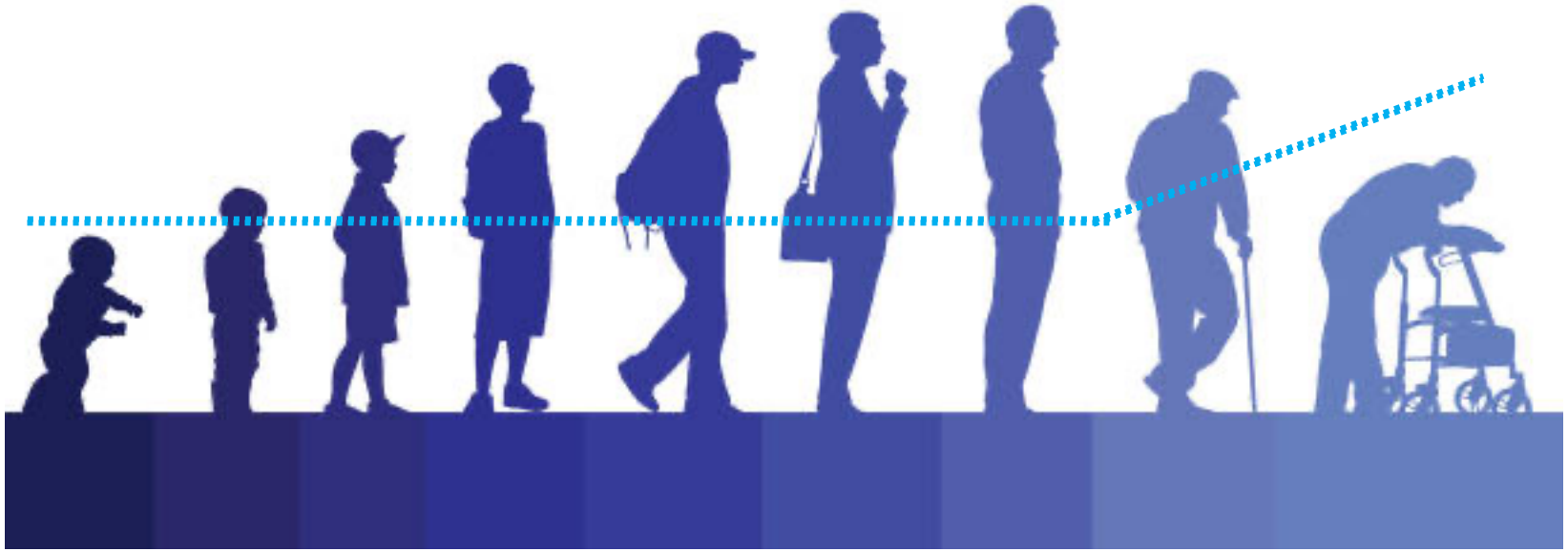
| | |
|---|---|
| MCV | 45-55 |
| Serum iron | ++ |
| Tf saturation | ++ |
| sTfR | ++ |
| Bone marrow sideroblasts | – |
| FEP | + |
| Liver iron | +++ |
| Neonatal appearance | Yes |
| Effect of oral Fe | No |
| Effect of intravenous Fe | No |
| Inheritance | Autosomal recessive |
| Therapy | Epo |

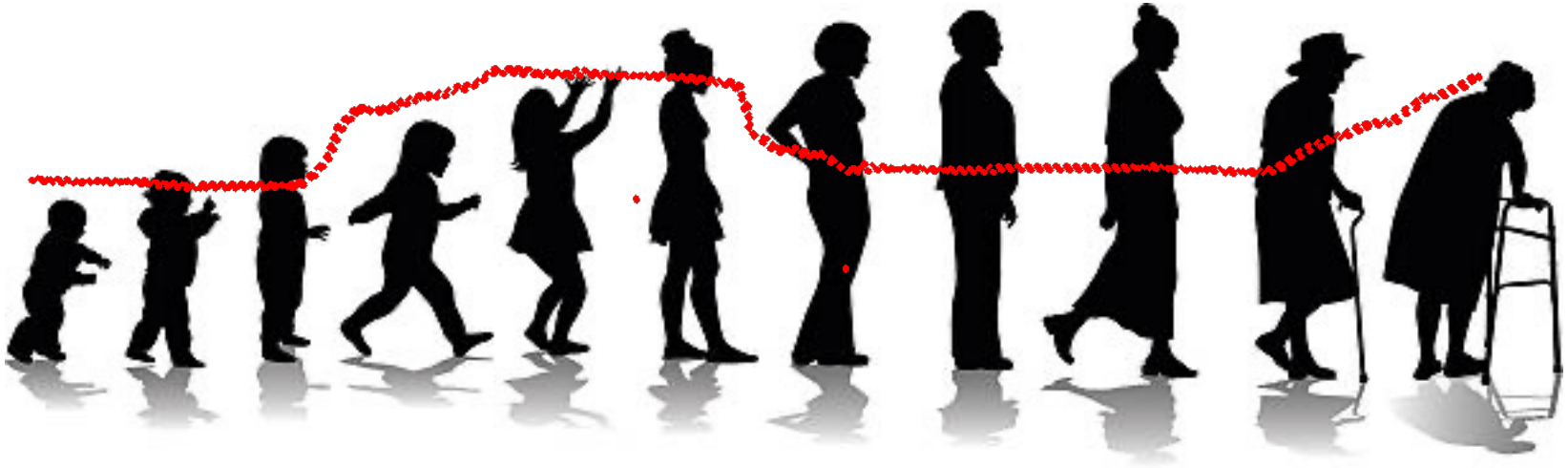
Abbreviations: MCV, mean corpuscular volume; Tf, transferrin; sTfR, soluble transferrin receptor; FEP, free erythrocyte protoporphyrin; Fe, iron; Epo, erythropoietin.

- 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.





# Acknowledgments …

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**Patients and their families**


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for rare or low prevalence complex diseases
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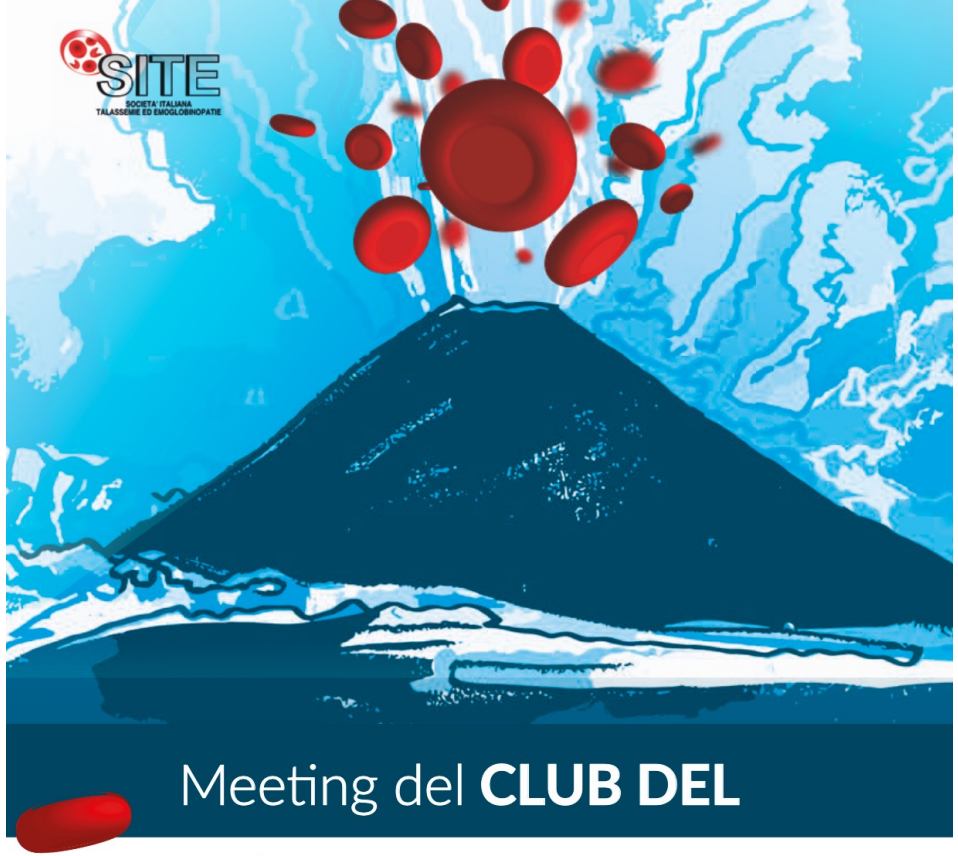


Meeting del **CLUB DEL**

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Complesso dei SS. Marcellino e Festo

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