45° Convegno Nazionale di Studi di Medicina Trasfusionale
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SIMTI

Difetti enzimatici e membranopatie nell'orizzonte trasfusionale

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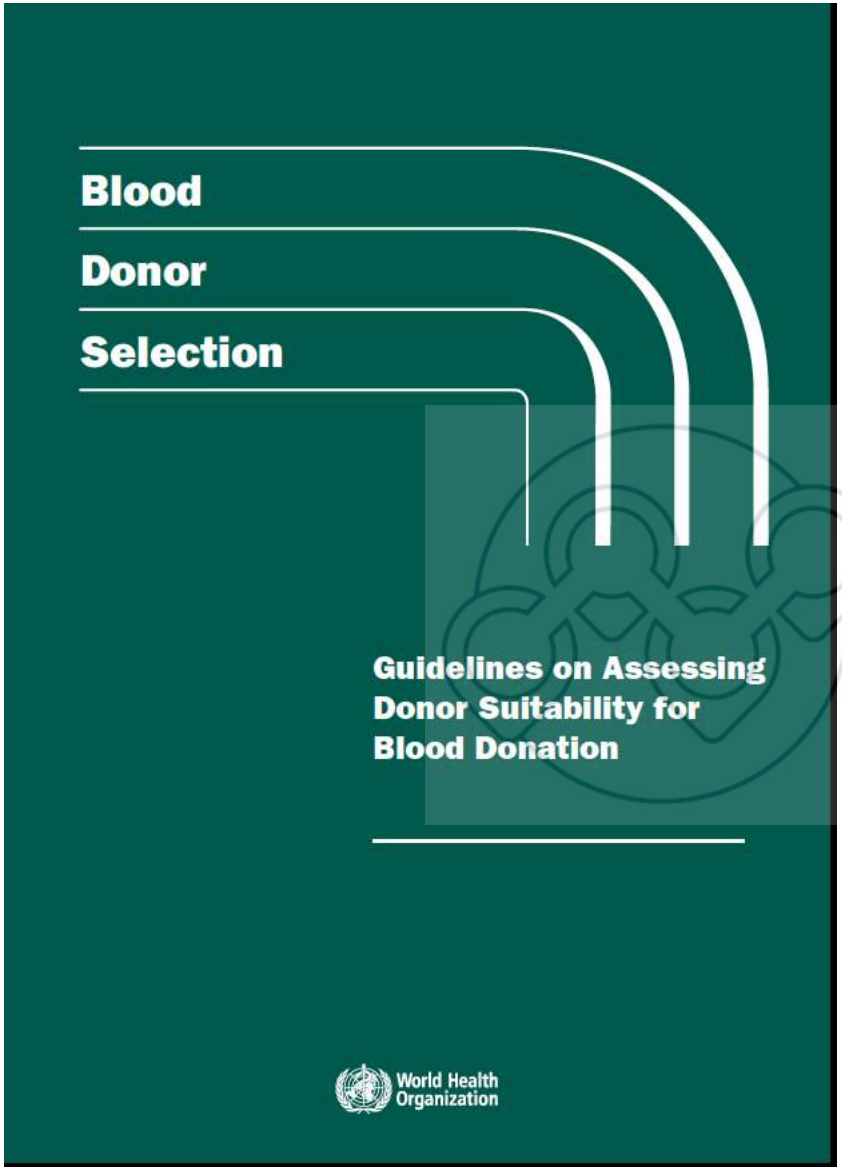
La sottoscritta, in qualità di Relatrice
dichiara che

negli ultimi due anni ha avuto i seguenti rapporti anche di finanziamento con i soggetti portatori di interessi commerciali in campo sanitario:

- Research Grant: Agios, Bristol, Afimmune

- Advisory Board: Vertex, Roche, NovoNordisk, Vifor



Recommendations

- Policies for the assessment of prospective donors should be developed by BTS in regions where there is a high incidence of enzymopathies and inherited red cell membrane defects

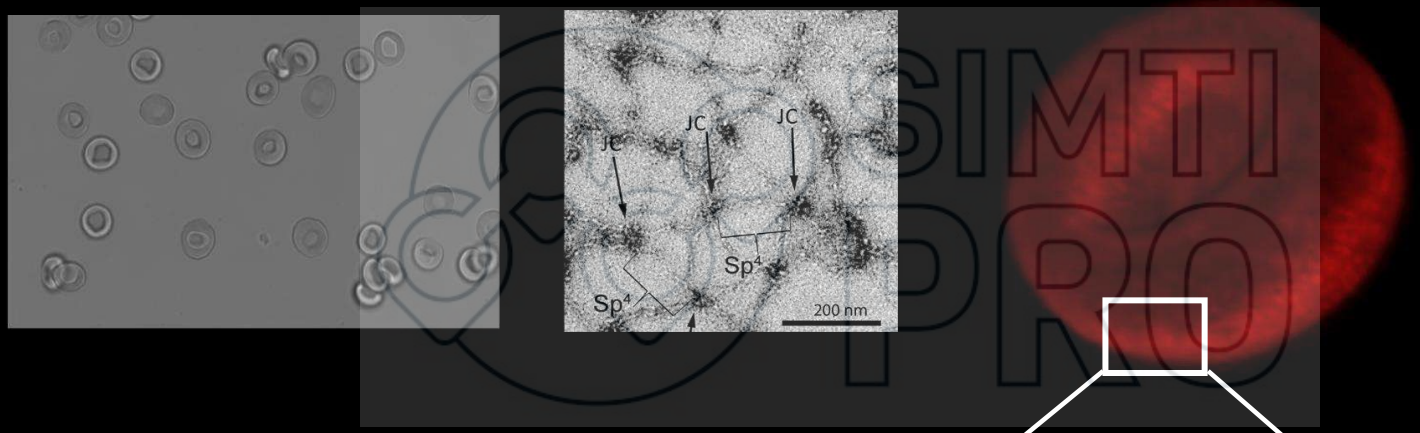
Accept

- Individuals with G6PD deficiency or other inherited red cell membrane defects, without a history of haemolysis; however, their blood is not suitable for intrauterine transfusion, neonatal exchange transfusion or for patients with G6PD deficiency

Defer permanently

- Individuals with G6PD deficiency or inherited red cell membrane defects, with a history of haemolysis

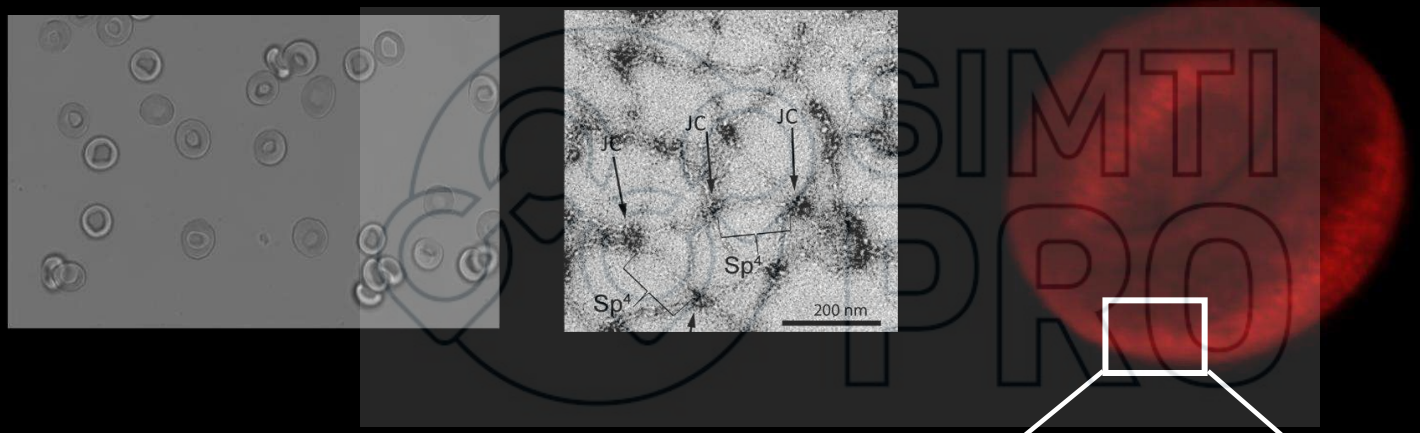
Blood donors and membranopathies or enzymopathies



Membranopathies:
- HS
- HO
- Stomatocytosis (HSt)

Enzymopathies:
- **PKD**
- **G6PD**

Blood donors and membranopathies or enzymopathies



Membranopathies:

- ~~HS~~
- ~~HO~~
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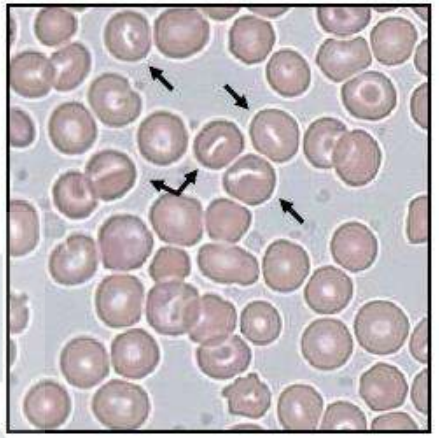
Enzymopathies:

- ~~PKD~~
- **G6PD**

Hereditary stomatocytosis (HSt)

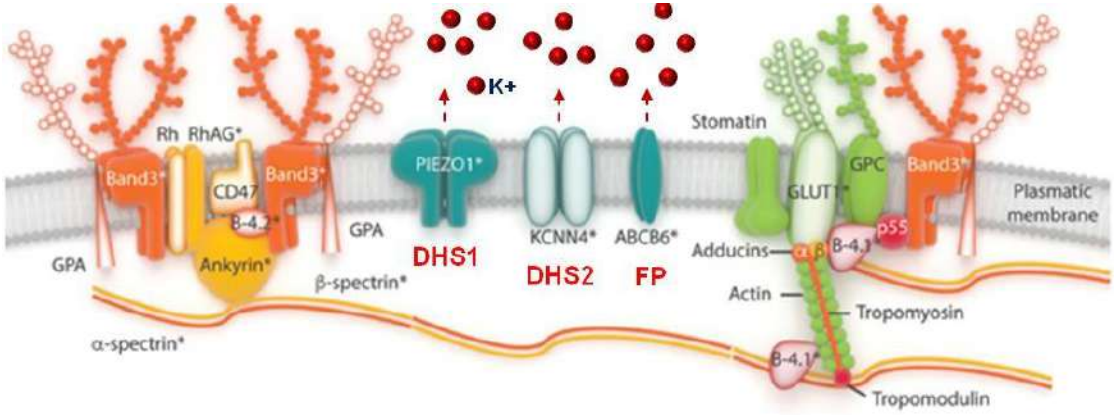
- Wide spectrum of inherited hemolytic disorders in which the red cell membrane cation permeability is increased (cation leak)



- The cation leak results in deregulation of cellular volume, which leads to morphological abnormality of RBCs (stomatocytes, RBCs with a stoma across the center, at peripheral blood smear)

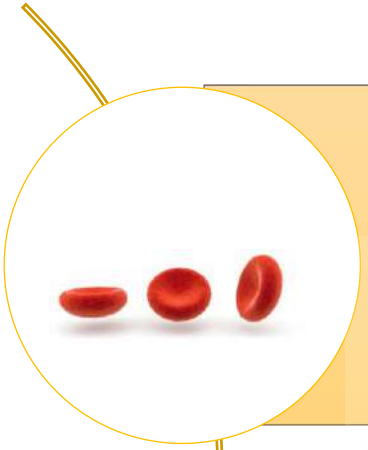
- The clinical presentation of HSt is highly variable: variable expressivity

- Genetic and allelic heterogeneity



Andolfo et al, Haematologica 2016

Hereditary stomatocytosis (HSt): classification



Non-syndromic

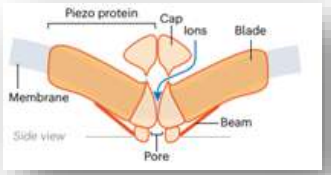
- Dehydrated Hereditary Stomatocytosis (DHS1/DHS2) ***(PIEZO1; KCNN4)***
- Overhydrated Hereditary Stomatocytosis (OHS) ***(RHAG)***
- Cryohydrocytosis ***(Band 3)***
- Familial Pseudohyperkalemia (FP) ***(ABCB6)***

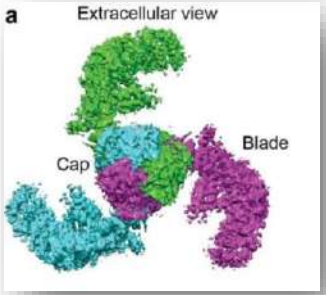
Syndromic

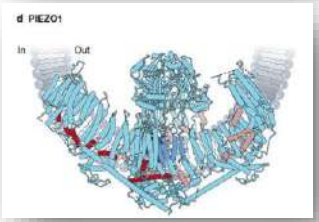
- Stomatin deficient cryohydrocytosis with mental retardation, seizures, hepatosplenomegaly **(*GLUT1*)**
- Phytosterolemia non-leaky stomatocytosis with macrothrombocytopenia **(*ABCG5; ABCG8*)**
- Dehydrated Hereditary Stomatocytosis (DHS1) with perinatal edema and/or pseudohyperkalemia ***(PIEZO1)***

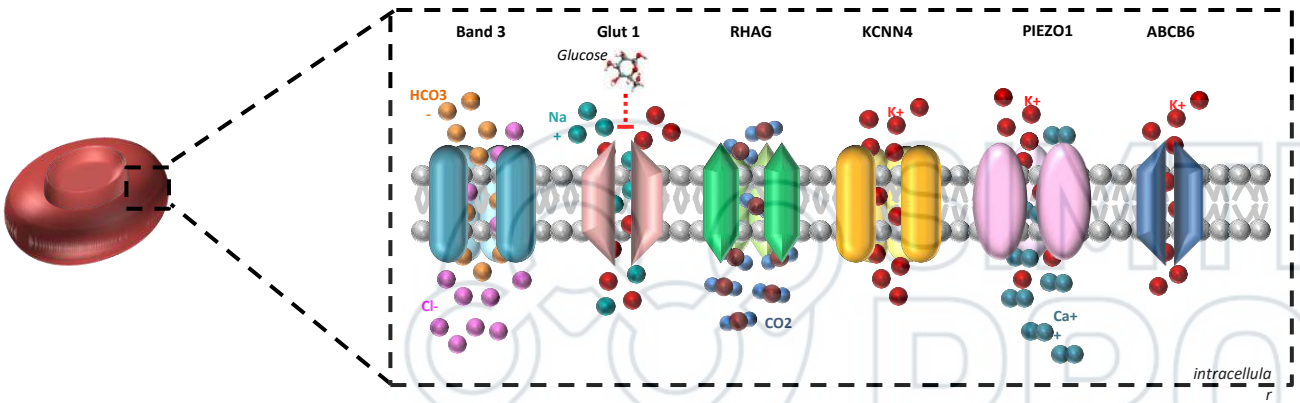
Andolfo et al. AJH 2017

PIEZO1 as causative of syndromic/non-syndromic Dehydrated Hereditary Stomatocytosis (DHS)









- Autosomal dominant hemolytic anemia associated with cation leak
- The two causative genes identified until now are ***PIEZO1*** and ***KCNN4***
- It is a rare condition, but rather underdiagnosed. A recent study estimate an incidence of 1 case in 8000 adults -> linked to malaria resistance.

Iolascon A, Andolfo I, Russo R. BJH 2019; Andolfo et a. AJH 2017, Andolfo et al, AJH 2018

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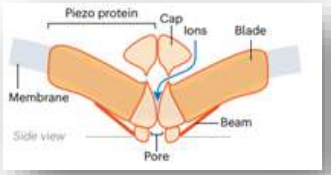

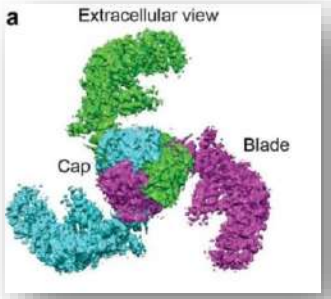

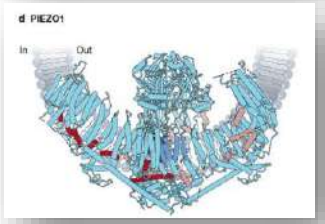

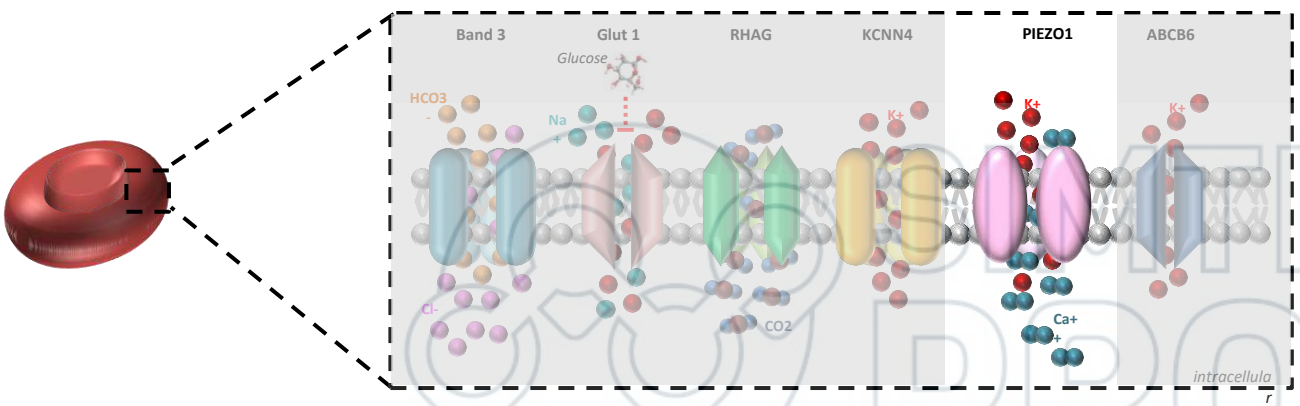

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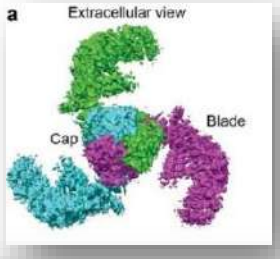
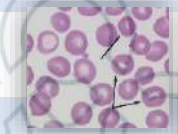
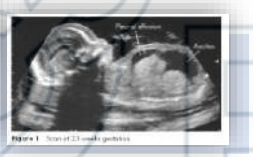
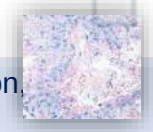
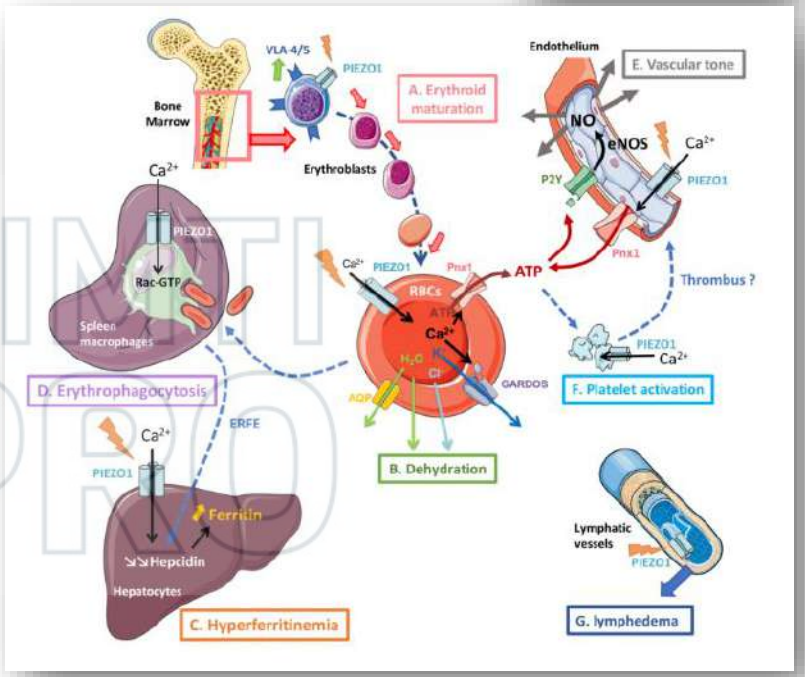
Dehydrated Hereditary Stomatocytosis (DHS)



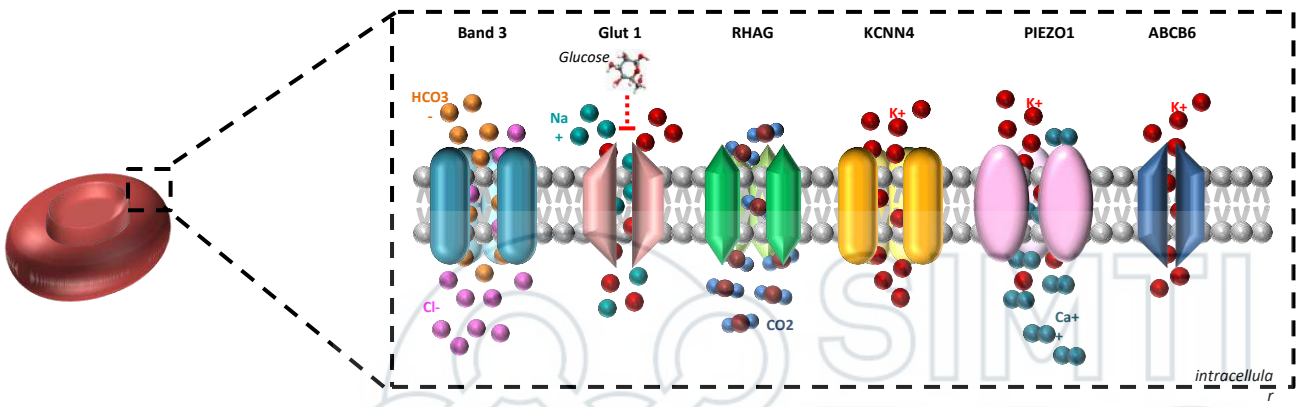
Main characteristics	
Macrocytic anemia	Hb ↓ MCV ↑ MCHC ↑
Hemolysis	Ret count ↑ LDH ↑ Hap ↓ Bil (tot, ind) ↑
Splenomegaly and gallstones	Splenectomy is contraindicated due to increased risk of severe thromboembolic complications
Variable numbers of stomatocytes at PB smear	<20%  
Pre-and/or perinatal edema (syndromic form). Pregnancy should be monitored	
Pseudohyperkalemia (syndromic form)	Kalemia ↑
Severe iron overload (hepatosiderosis)	Ferritin, transferrin saturation, and liver iron concentration ↑ 



- DHS is a pleotropic syndrome characterized by variable phenotypes.
- Different tissues/cell types that express PIEZO1 may be involved in the pathophysiology of DHS.

Jankovsky N, et al AJH 2021; Andolfo et al. AJH 2018; Picard et al., Haem. 2019; Andolfo et al. Haematologica 2016; Andolfo et al. Blood 2013

Non-Syndromic HSt: Familial Pseudohyperkalemia



		ABCB6 patients FP
Number of patients (%)		11 (15.1)
Gender (female/male)		10 (90.9)/1 (9.1)
Onset of symptoms (years)		42.5 ± 6.6 (40.5; 8)
Age of diagnosis (years)		47.1 ± 5.6 (43.5; 8)
Blood count		
	Ref range[c]	
RBC (10^6/μL)	*3.9-5.6*	3.6 ± 0.4 (3.8; 11)
Hb (g/dL)	*11.0-16.0*	13.5 ± 0.4 (13.1; 11)
Hct (%)	*33.0-45.0*	42.6 ± 1.3 (42.0; 11)
MCV (fL)	*70.0-91.0*	101.3 ± 2.3 (100.2; 11)
MCH (pg)	*23.0-33.0*	31.1 ± 0.6 (31.4; 11)
MCHC (g/dL)	*23.0–33.0*	33.2 ± 0.9 (32.5; 11)
Retics count ($x10^3$/μL)	-	140.3 ± 35.7 (140.3; 2)
Retics %	*0.5-2.0*	2.9 ± 1.2 (2.9; 2)

- Dominantly inherited genetic trait
- temperature-dependent, *in vitro*, loss of K^+ cation from red cells
- Plasma [K+] was increased when measured in blood stored at or below body temperature
- HSt patients show alterations in MCV
- Missense mutations in ***ABCB6*** gene were identified in FP

Iolascon A, Andolfo I, Russo R. BJH 2019; Andolfo et a. AJH 2017; Andolfo et al, AJH 2013

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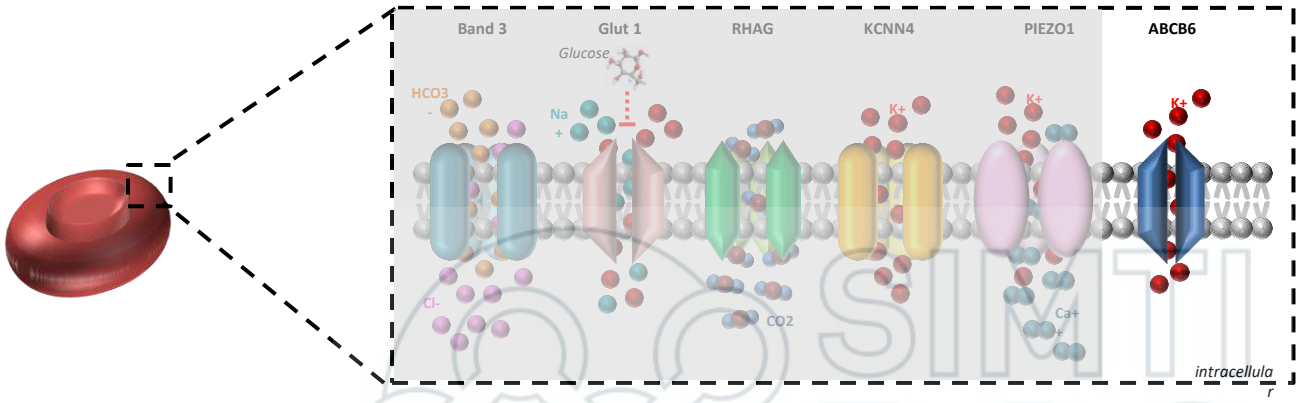

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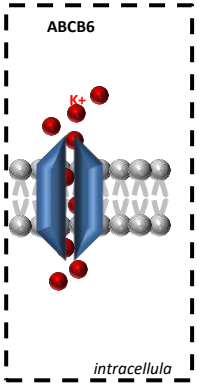
ABCB6 variants screening in blood donors' population

- Variants in ***ABCB6*** gene are present in **healthy subjects** and in **blood donor population**

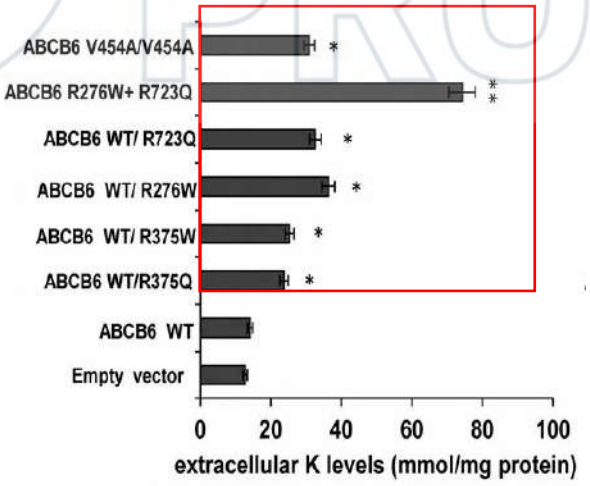


- Storage of FP blood causes a significant increase in blood K+ levels

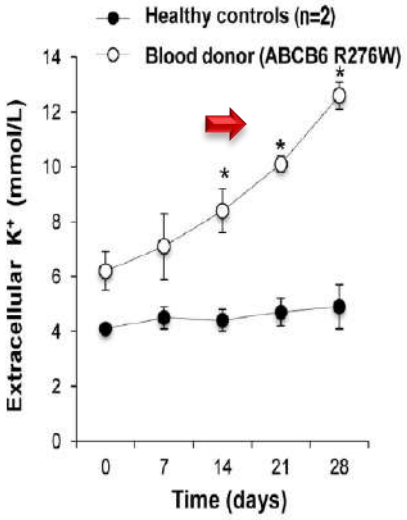
  ->**pediatric/neonatal care**-> several cases of whole blood transfusion in infants leading to cardiac arrest and death have been described

- Genetic test for FP could be used

to **screen potential donors of blood**



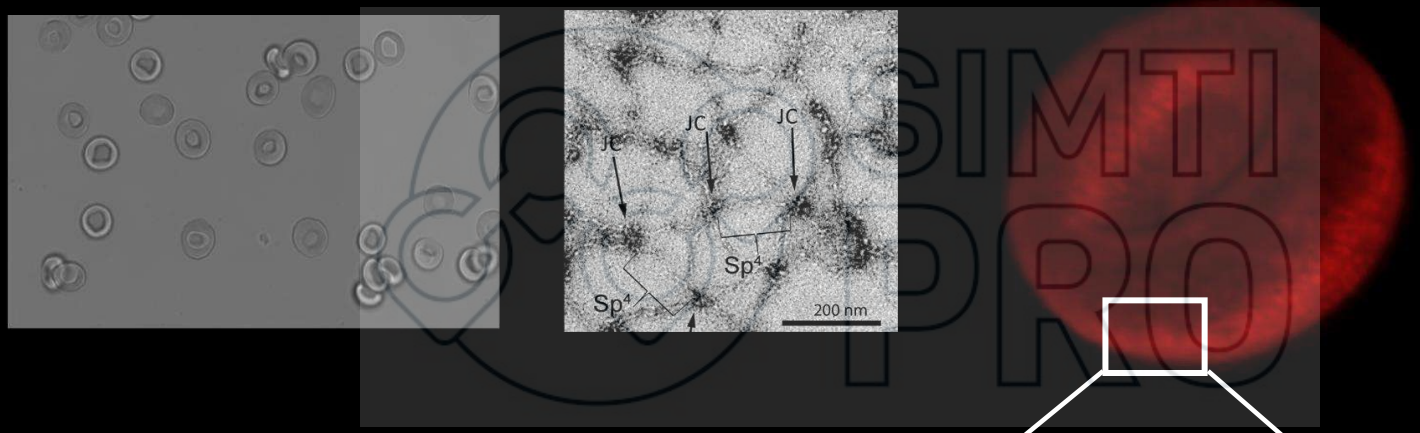
Cell lines



Blood samples

Andolfo et al, Haematologica 2016

Blood donors and membranopathies or enzymopathies



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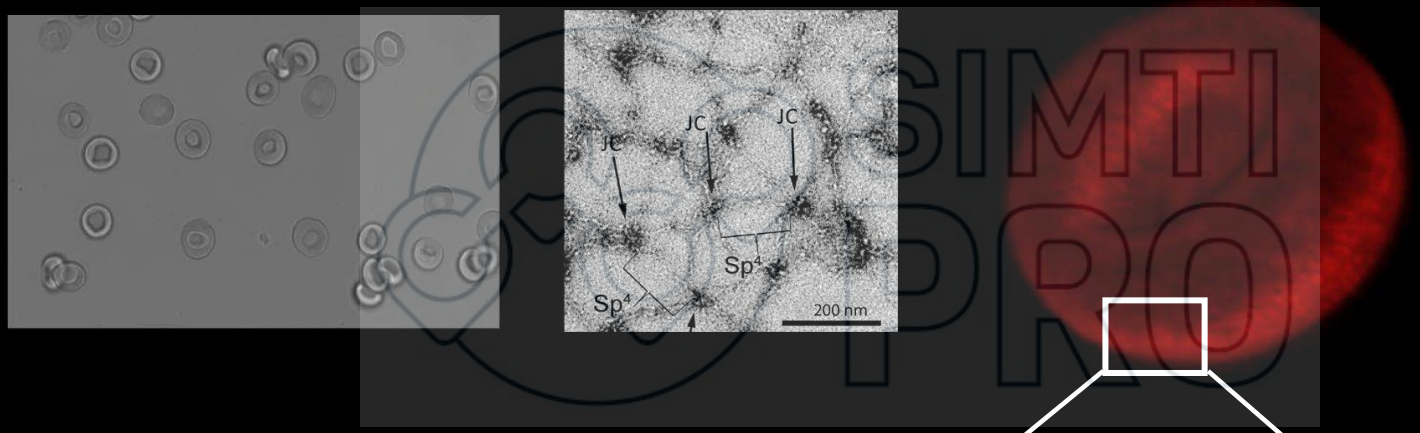

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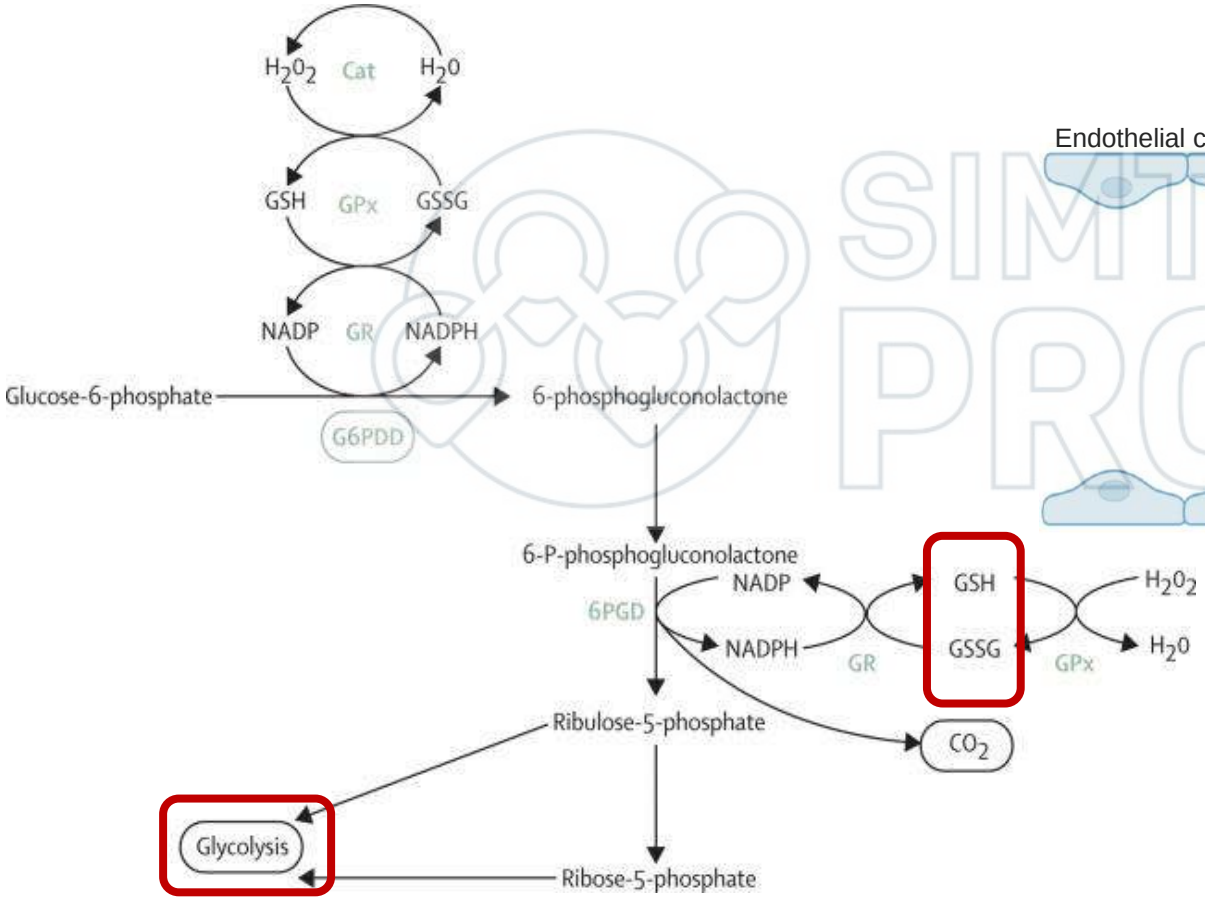
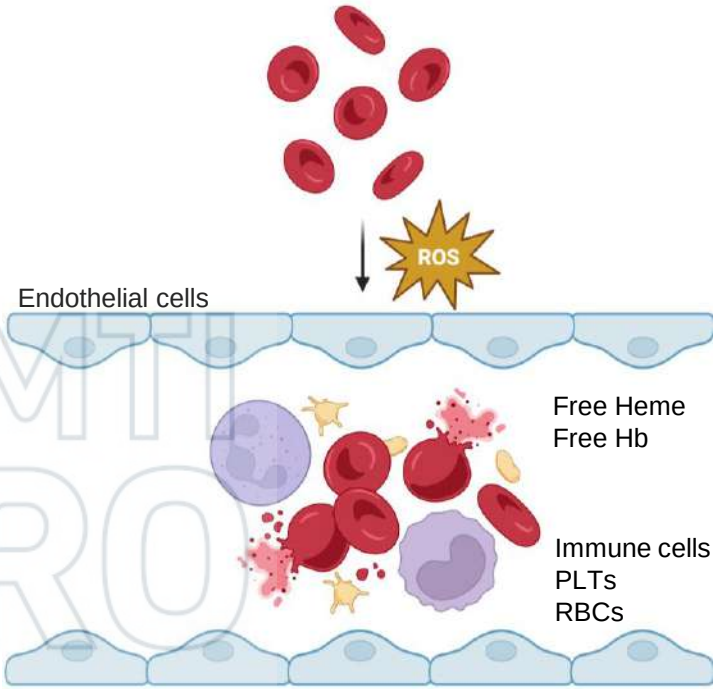
Enzymopathies:

- ~~PKD~~
- **G6PD**

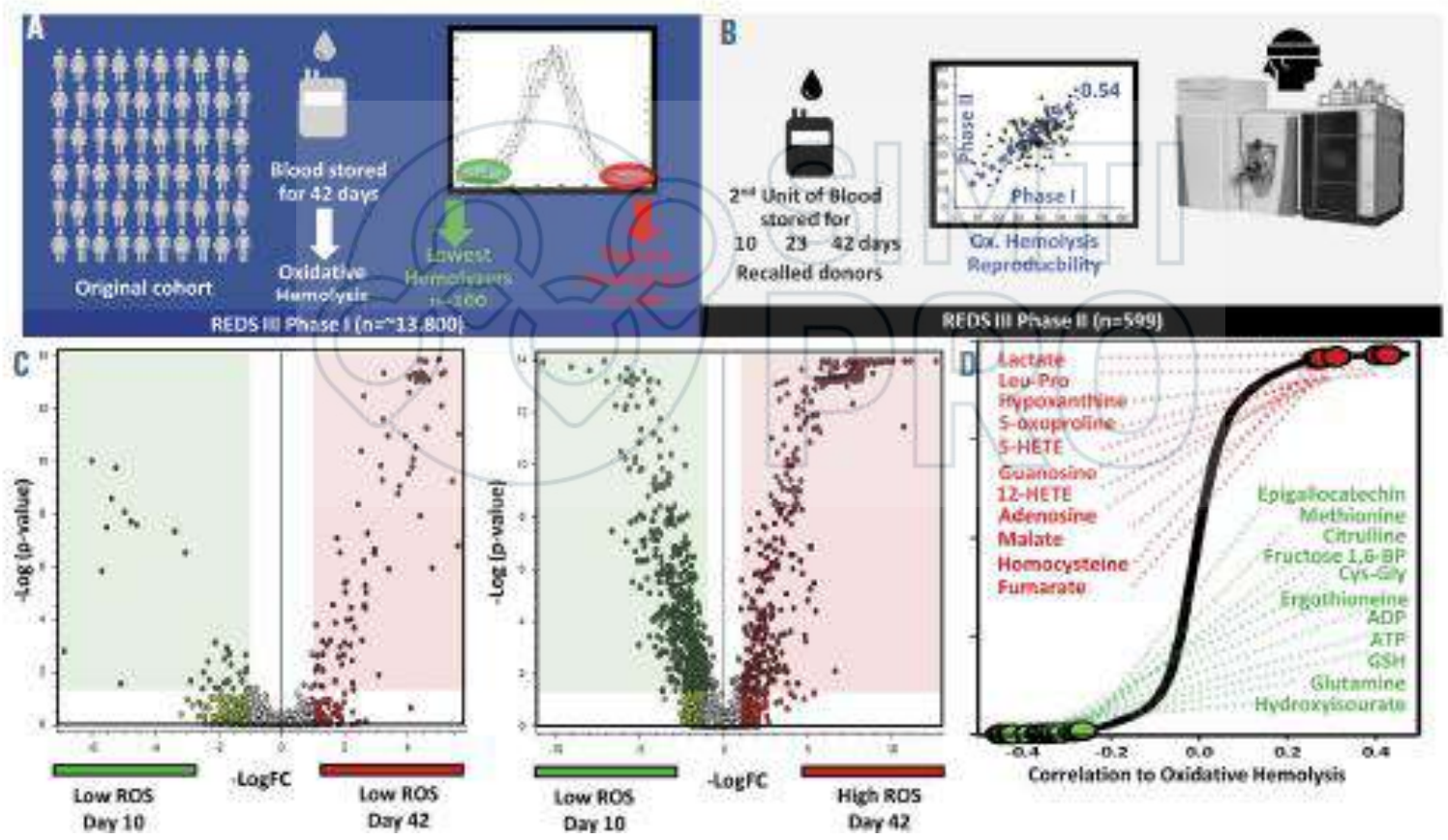
G6PD is the most common enzymatic defect, being present in most than 400 million of people





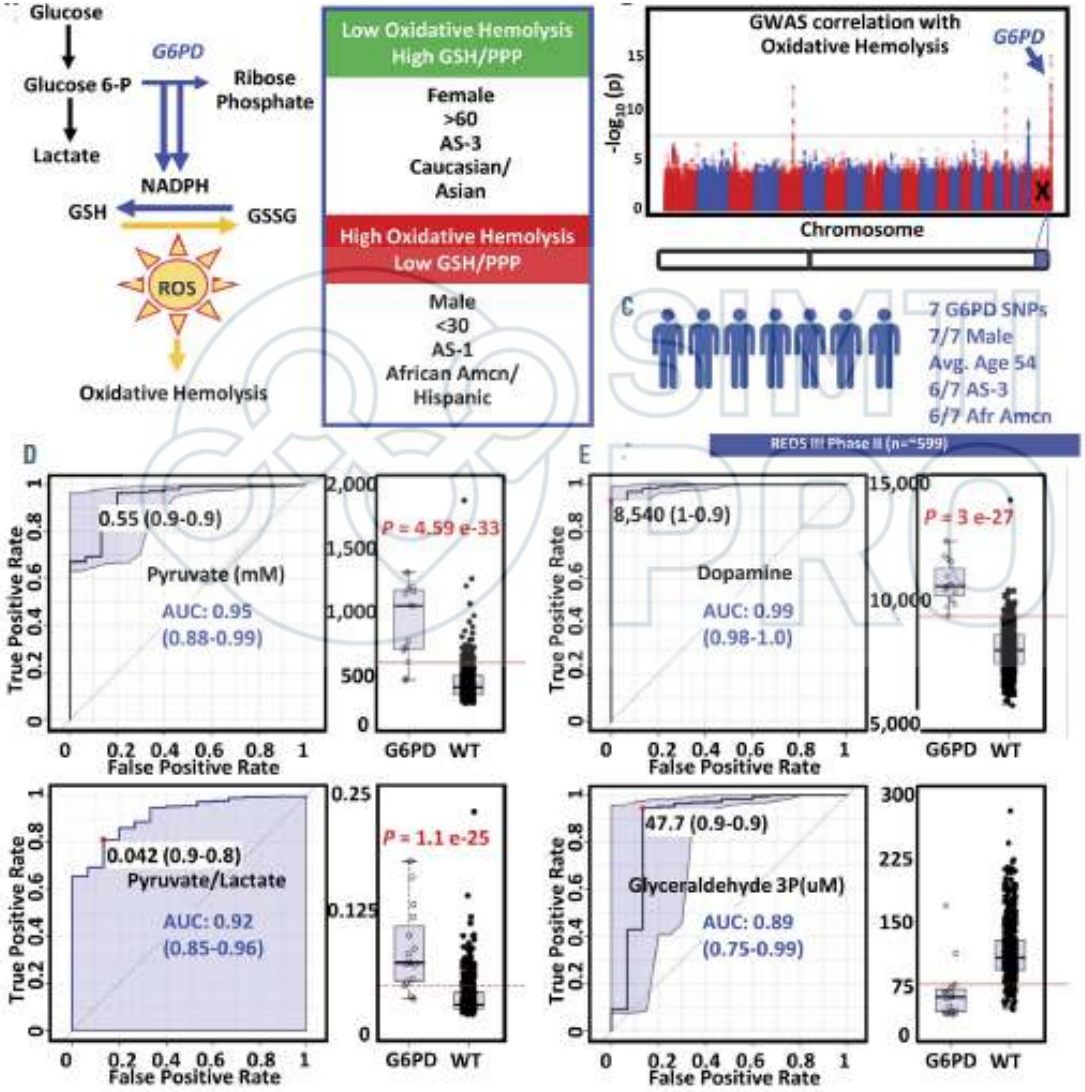
Cappellini MD & Fiorelli G The Lancet Hematology 371: 64, 2008

Omics study in Blood donors: REDS-III



Lantieri MC et al Transfusion 59: 79, 2019; D'Alessandro A et al Haematologica 106: 1290, 2021

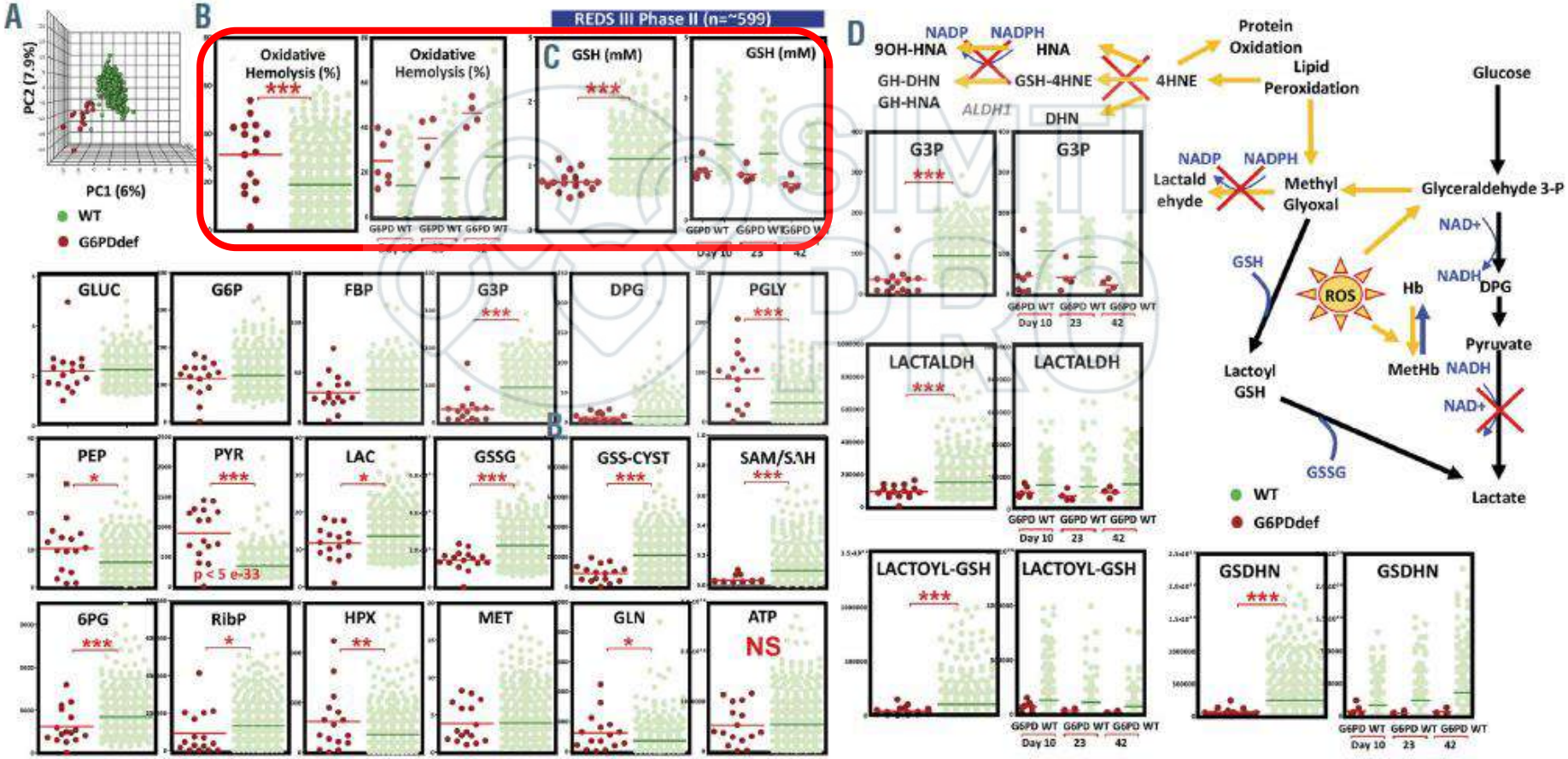
Metabolic analysis of blood donors identified risk factors for extreme hemolyzer(s)



G6PD deficient

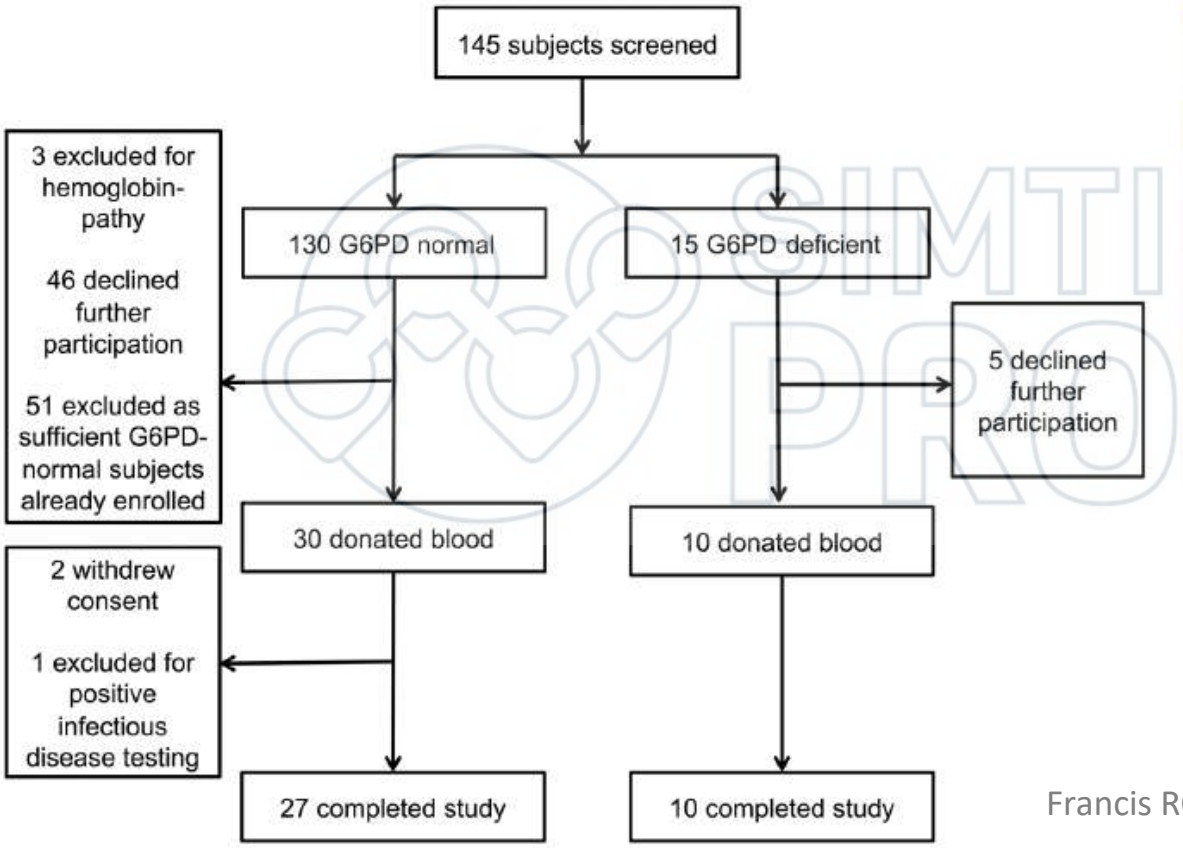
D'Alessandro A et al Haematologica 106: 1290, 2021

G6PD deficient blood donors display higher oxidative hemolysis, lower glutathione and PPP activation, resulting in reduced GSH and increased lipid-peroxidation



D'Alessandro A et al Haematologica 106: 1290, 2021

Exploring red cells from blood donors with G6PD deficiency

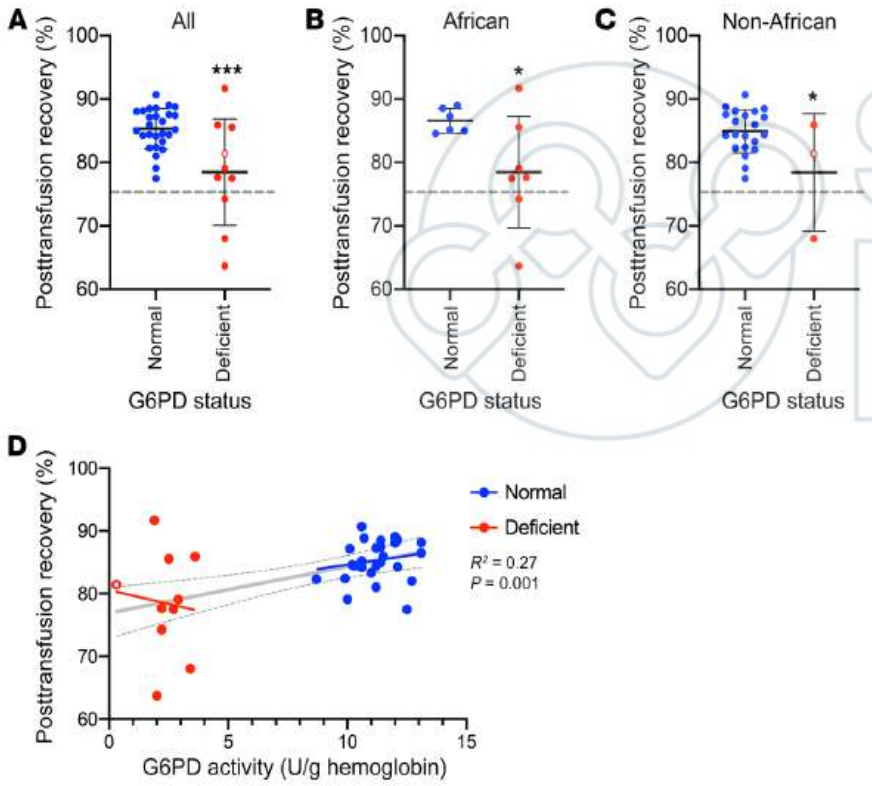


No difference in storage hemolysis

No difference in RBC ATP content

Francis RO et al JCI 130: 2270, 2020

Leukoreduced RBCs from blood donor with G6PD deficiency display 24 hrs PTR and impaired antioxidant machinery



- **Decrease 24 hrs post transfusion recovery (PTR) compared to healthy subjects (AS-3 preservation)**
- Reduced activation of PPP in fresh RBCs vs healthy erythrocytes
- Increased biliverdin, consistent with the reduction in NADPH driven conversion of bilirubin
- **Lower GSH levels during storage compared to healthy RBCs, which positively correlate with PTR**

Francis RO et al JCI 130: 2270, 2020

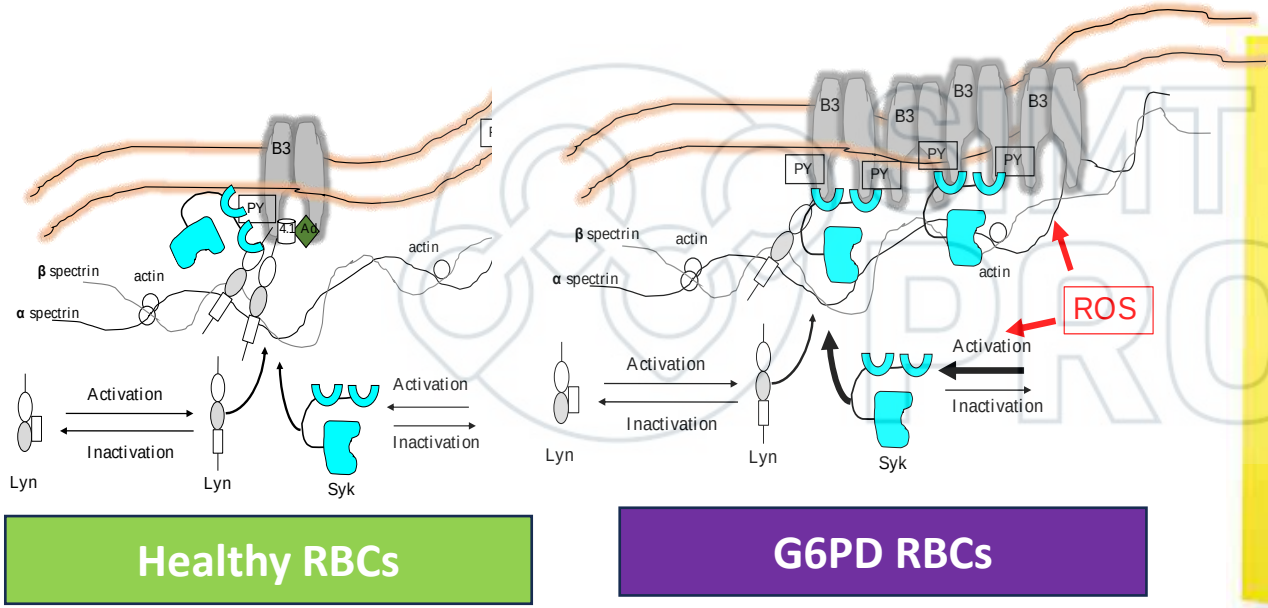
Blood donors with G6PD Mediterranean variant display normal storage hemolysis compared to healthy donors

Italian blood donors with G6PD Mediterranean variant show

- ⇧ MCV and ⇧ reticulocyte count compared to healthy subjects
- No differences in Hct and Hb compared to healthy controls
- No difference in storage hemolysis between G6PD deficient and healthy blood donors

Maffi D et al Vox Sang 106: 227, 2014; Piomelli S JCI 47: 940, 1968; Piomelli S BJH 16: 537, 1969

G6PD deficient RBCs display increased erythroid microparticle (MPs) formation and PS+ RBCs during blood processing and during storage compared to healthy subjects



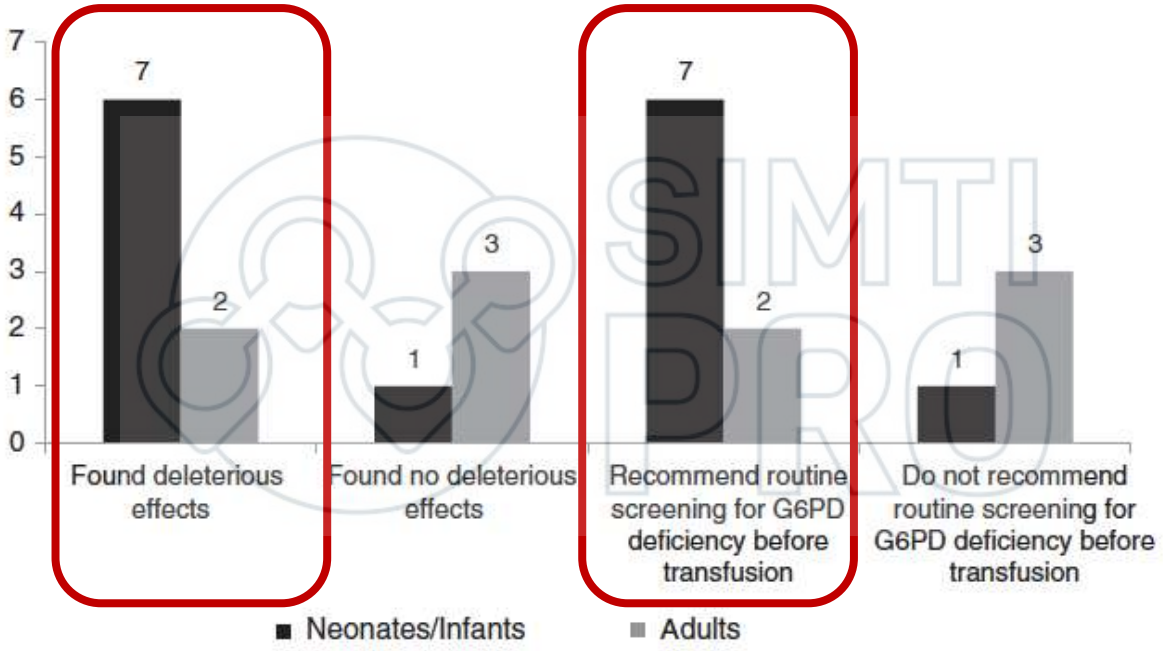
Healthy RBCs

G6PD RBCs

Blood transfusion product containing MPs are associated with **post-transfusion complications such as thrombosis or ALI**

Matte A et al Antioxidants 2021; Noomuna P et al. Br J Haematol doi: 10.1111/bjh.16671, 2020; Ferru E et al Blood 117: 5998, 2011; Pantaleo A et al Biochem J 418: 359, 2009; Noulsri E et al Lab Med 54: 6-12, 2023; Tzounakas VL et al Front Med 5: 16, 2018

Impact on patient outcomes of transfused RBCs from blood donors with G6PD deficiency

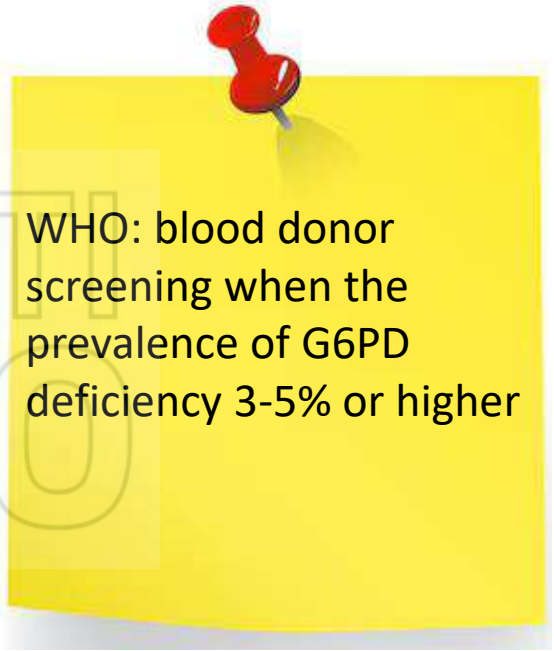


Increased total bilirubin and markers of hemolysis from 6 to 60 hours after transfusion of G6PD deficient RBCs

Renzato AMN et al Transfusion Med Review 28: 7, 2014; Francis O et al JCI 130: 2270, 2020

Are there special condition(s) to be take into consider when G6PD deficient RBCs are transfused in adult patients?

Poor capacity to circulate upon transfusion of G6PD RBCs in <u>patients with SCD and acute clinical manifestations such as VOCs</u>

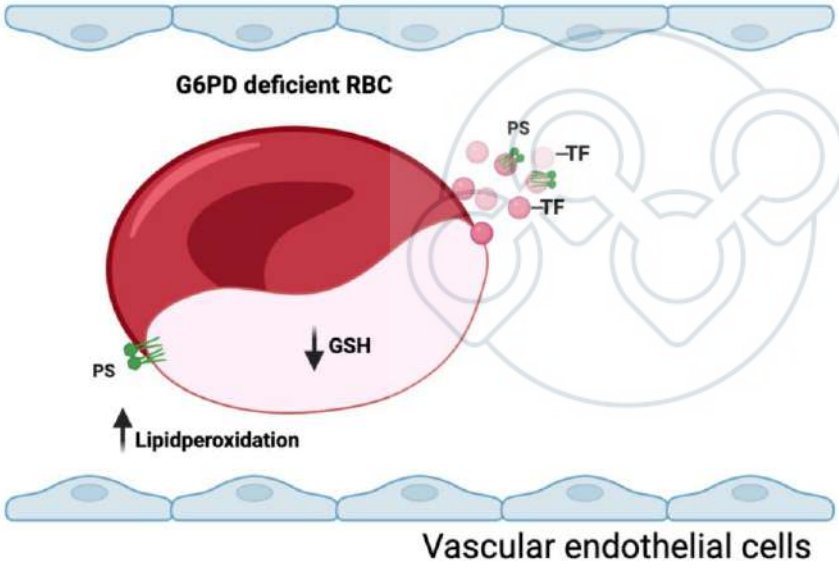


Karafin MS et al Curr Opin Hematol 25: 494, 2018; Sagiv E et al AJH 93: 630, 2018; Francis O et al JCI 130: 2270, 2020

Limitations of the analyzed studies

- **In transfusion studies:**
  - Old studies
  - Poor quality (e.g. different preservation solutions) and poor design
  - Lack of standardization in identification of RBCs as G6PD deficient
- **In the metabolomic studies:**
  - the analysis of post transfusion recovery was only at 24 hours
  - G6PD deficiency was sustained by variant(s) different from the Mediterranean one.
  - Lack of knowledge whether oxidative hemolysis might be predictive of clinical outcomes

RBCs from G6PD deficient blood donor: risk populations



- Intrauterine transfusion
- Premature neonates
- Neonatal exchange transfusion
- Patients with G6PD deficiency

New possible categories of patients:

- SCD patients during acute VOCs
- Extracorporeal circulation
- severely burned patients

Conclusions and Open Questions

- Consider screening for non syndromic hereditary stomatocytosis (ABCB6) to avoid possible severe complication in **pediatric/neonatal care**

- Growing evidence of decrease efficiency of RBCs from blood donors with G6PD deficiency (e.g. 24 hrs post transfusion recovery (PTR) and lower GSH levels compared to healthy subjects)

- New studies to evaluate the impact of G6PD Mediterranean variant on RBC metabolome under blood bank condition

- Identification of new risk population requiring G6PD screening based on the metabolomic data.

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