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EuroBloodNet

Hereditary Stomatocytosis

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CEINGE, advanced biothecnologies
ERN-EuroBloodNet subnetwork: Red blood cell defects
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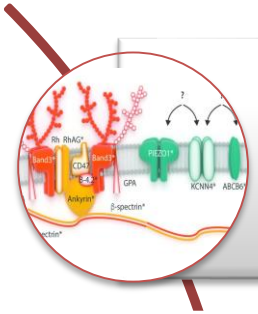
Conflicts of interest

I have nothing to disclose

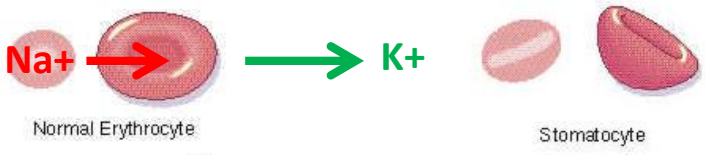
Learning objectives of the webinar



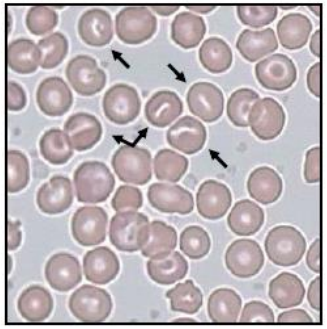
Classification of hereditary stomatocytosis (HSt): clinical and genetic aspects

Hereditary stomatocytosis (HSt)

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

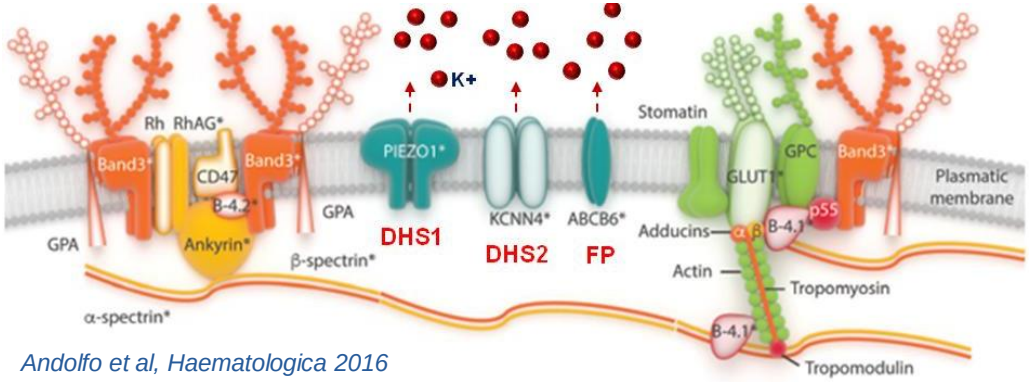


- The cation leak results in deregulation of cellular volume, which leads to morphological abnormality of RBCs (stomatocytes, RBCs with a stoma across the center, at peripheral blood smear)



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

- Genetic and allelic heterogeneity



Andolfo et al, Haematologica 2016

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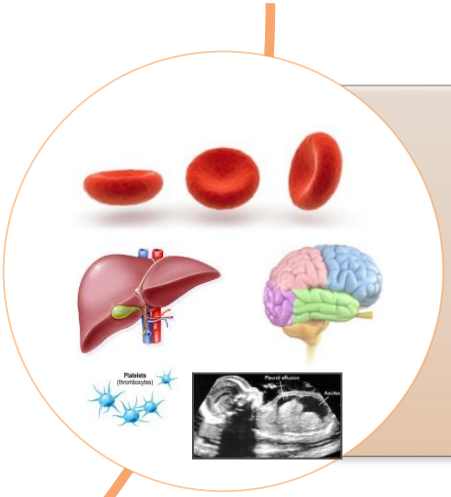
Hereditary stomatocytosis (HSt): classification



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)***

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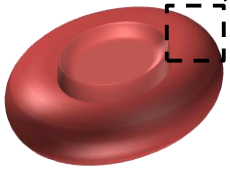
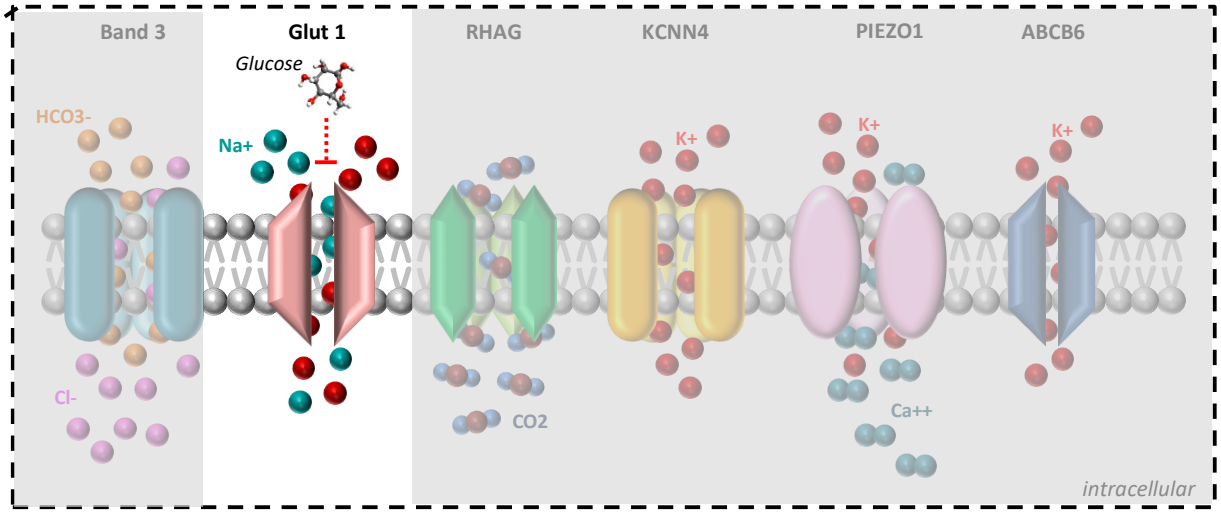
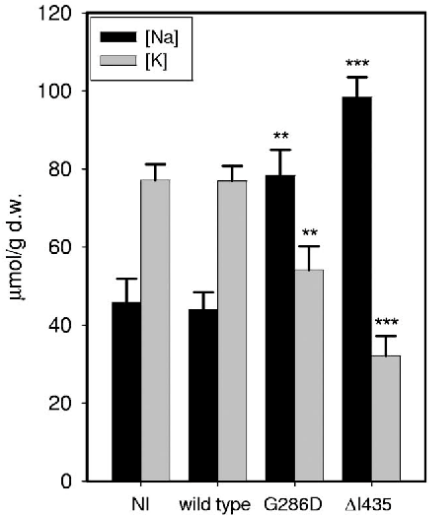
Andolfo et al. AJH 2017

Syndromic HSt: Stomatin deficient cryohydrocytosis



Red blood cell





- ✓ It is a rare form of stomatocytosis associated with a **cold-induced cation leak**, **hemolytic anemia**, **hepatosplenomegaly**, **cataracts**, **seizures**, **mental retardation**, and **movement disorder.**
- ✓ It is caused by mutations in ***SLC2A1 gene***, that codifies for the **GLUT1** transporter (associated with both loss of glucose transport and a cation leak). Autosomal recessive inheritance.

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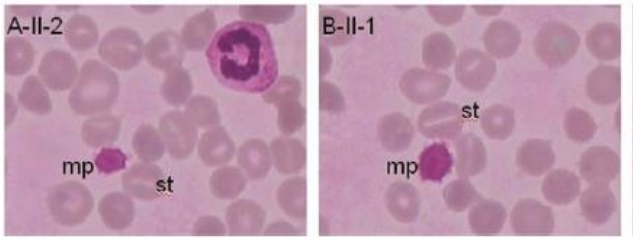
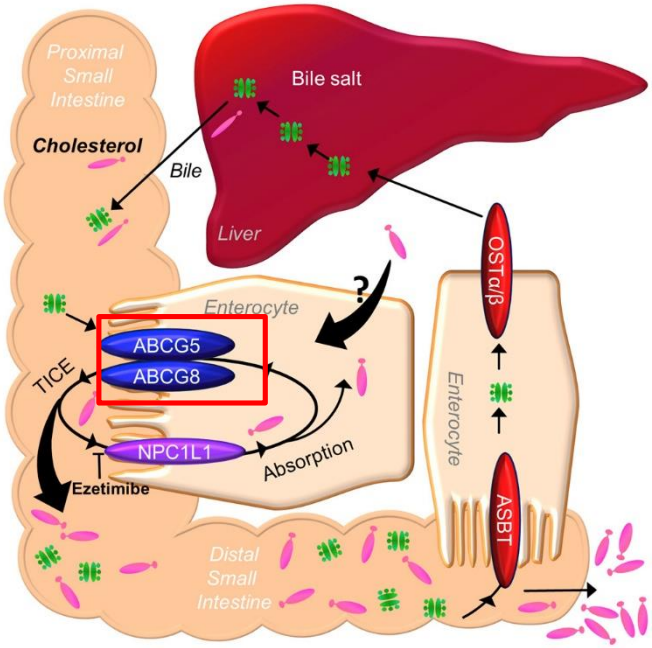
Syndromic HSt: Phytosterolemia

- ✓ It is characterized by **lipid metabolic disorder, stomatocytic hemolysis, and macrothrombocytopenia**.



- ✓ They showed normal erythrocyte cation content.

- ✓ The causative genes are: ***ABCG5*** and ***ABCG8***, that codify for two ATP-cassette transporters that mediate efflux of dietary sterols from the **small intestine**. Autosomal recessive inheritance (ABCG5/ABCG5; ABCG8/ABCG8; ABCG5/ABCG8).



- ✓ Incorporation of sterols into RBCs and platelets results in abnormal morphology and function.

Table 2. Serum Sterols Levels of Phytosterolemia Patients in 3 Families

Patient	Sex	Age, years	Serum, μmol/L		
			Stigmasterol	Cholestanol	Sitosterol
A-II-2	F	34	409.0	99.8	1103.4
B-II-1	F	43	209.8	102.9	716.9
C-II-1	M	62	118.5	94.9	348.4
C-II-2	M	60	344.3	329.0	776.9
C-II-3	M	57	273.1	138.6	725.8
C-II-4	F	55	449.5	246.7	1195.3
Controls (n = 15)[a]			13.0 ± 5.1[a]	25.3 ± 8.8[a]	28.3 ± 8.2[a]

[a] For serum plant sterol measurements, the data are expressed as mean ± standard deviation. The serum sterol levels of all patients were increased in comparison with those of normal controls.

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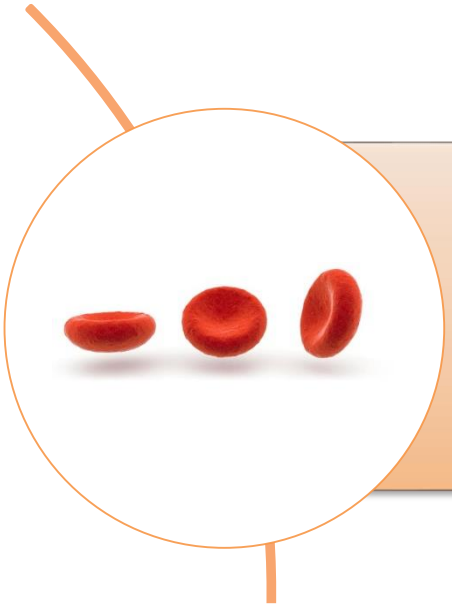
Hereditary stomatocytosis (HSt): classification

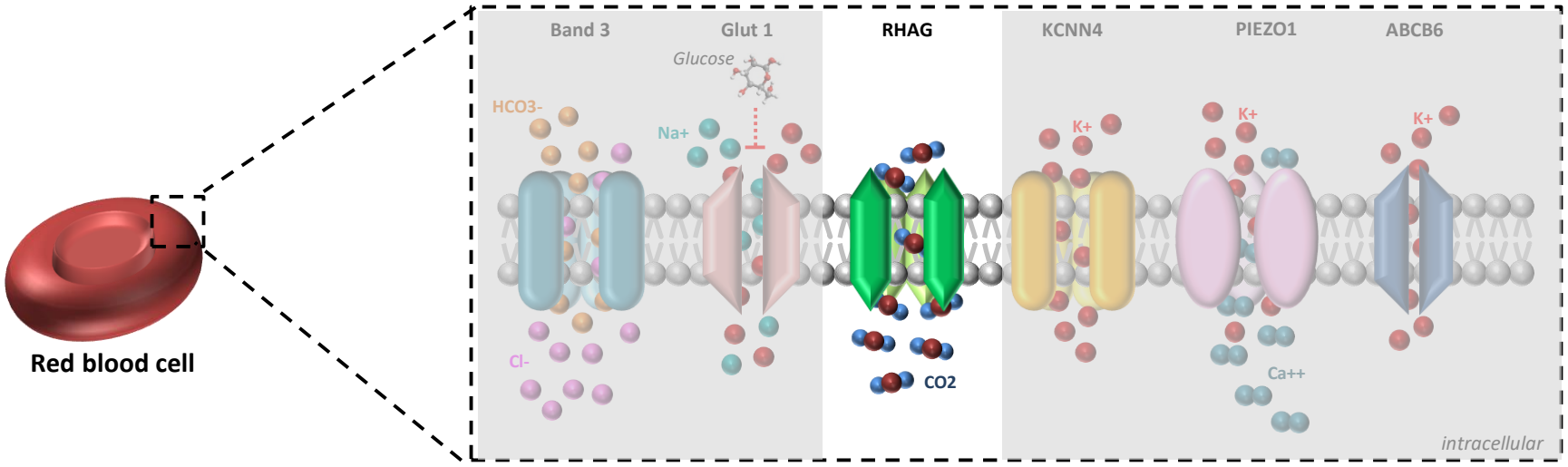


Non-syndromic

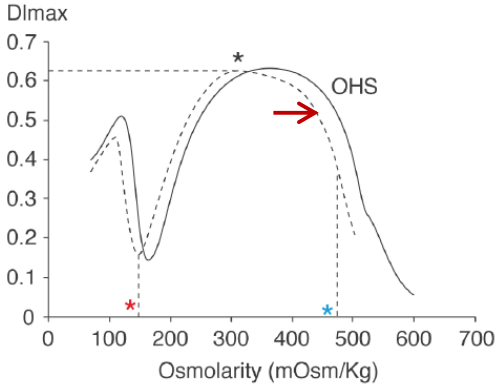
- Overhydrated Hereditary Stomatocytosis (OHS) ***(RHAG)***
  Cryohydrocytosis ***(Band 3)***
  Familial Pseudohyperkalemia (FP) ***(ABCB6)***
  Dehydrated Hereditary Stomatocytosis (DHS1/DHS2) ***(PIEZO1; KCNN4)***

Andolfo et al. AJH 2017

Non-Syndromic HSt: Overhydrated Hereditary Stomatocytosis



- ✓ **OHS** is characterized by anemia of a variable degree with macrocytosis, low MCHC, and a right shift of the osmolarity curve at ektacytometric analysis
- ✓ It is characterized by an increase in the **monovalent cation leak** also associated with the absence of stomatin
- ✓ It is caused by mutations in the ammonium transporter **RHAG** (**autosomal dominant** inheritance)



- ✓ At peripheral blood smear we can observe more than 20% of stomatocytes

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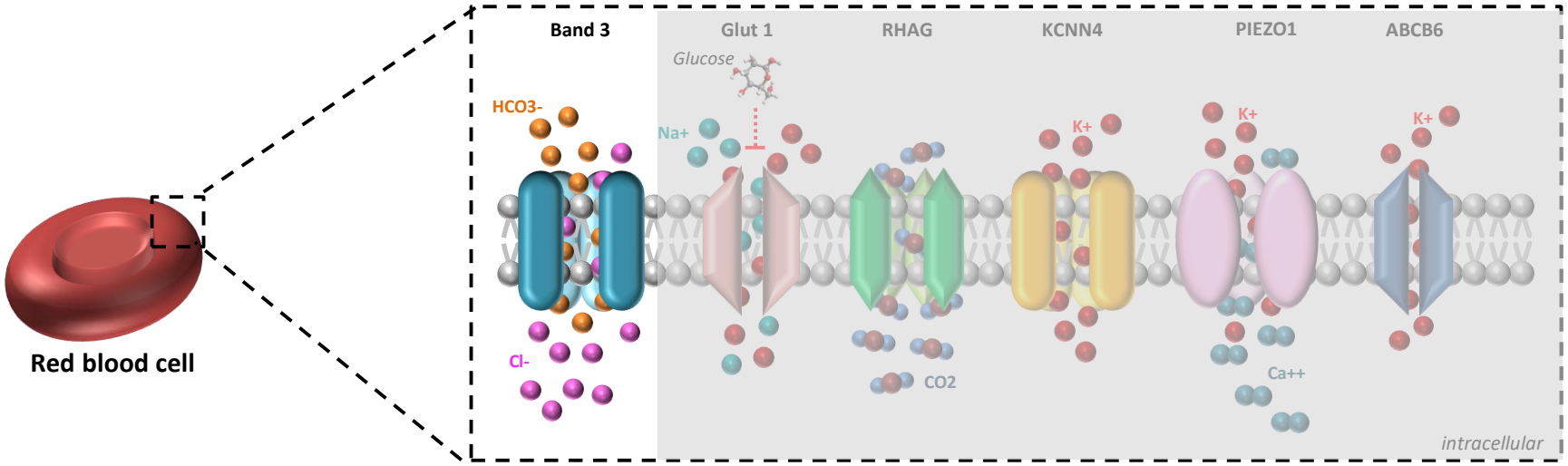
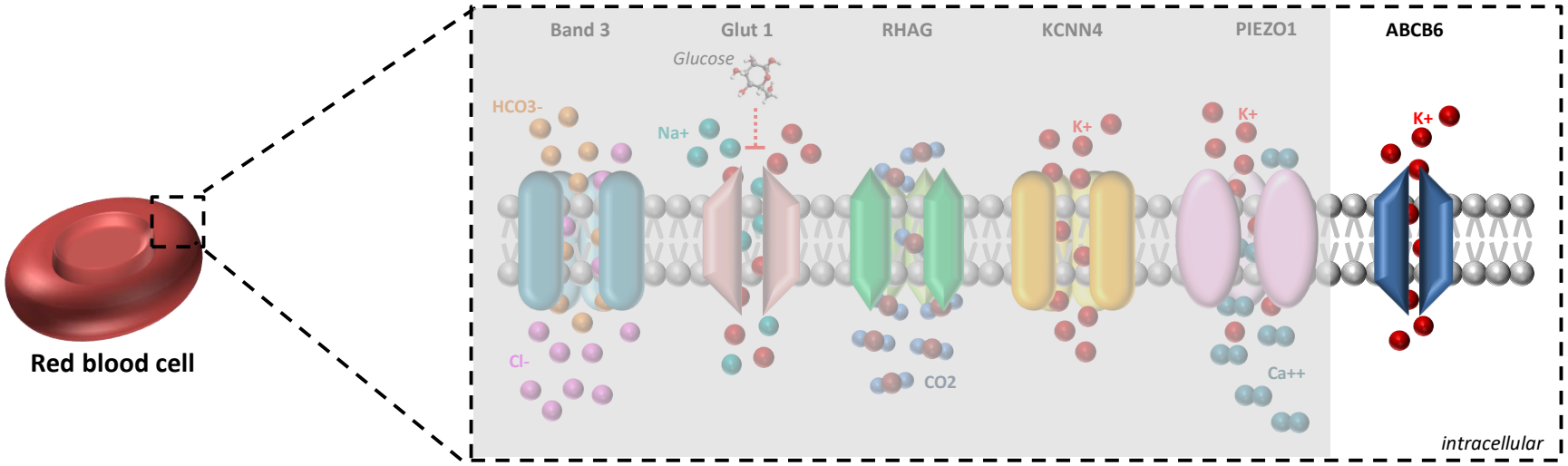
Non-Syndromic HSt: Cryohydrocytosis



- ✓ **Cryohydrocytosis** is characterized by increased permeability to Na+/K+ cations at low temperatures (0–4°C).
- ✓ It is a mild hemolytic anemia due to a minimal cation leak.
- ✓ Its pathophysiology has been linked to **missense mutations** in the ***SLC4A1*** gene that encodes the **band 3** protein.
- ✓ These substitutions convert band 3 from an **anion exchanger** into a **cation channel**, which is a pathogenic mechanism entirely different from the loss-of-function mechanism that causes hereditary spherocytosis.

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

Non-Syndromic HSt: Familial Pseudohyperkalemia



- ✓ Dominantly inherited genetic trait
- ✓ Characterized by a 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
- ✓ The patients show alterations in MCV
- ✓ Missense mutations in ***ABCB6*** gene were identified in FP

		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)

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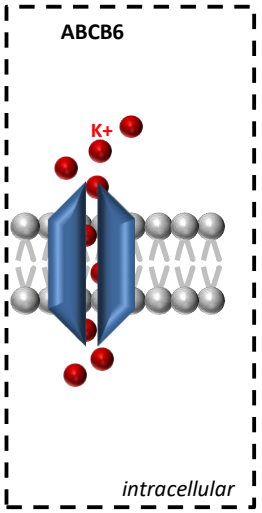
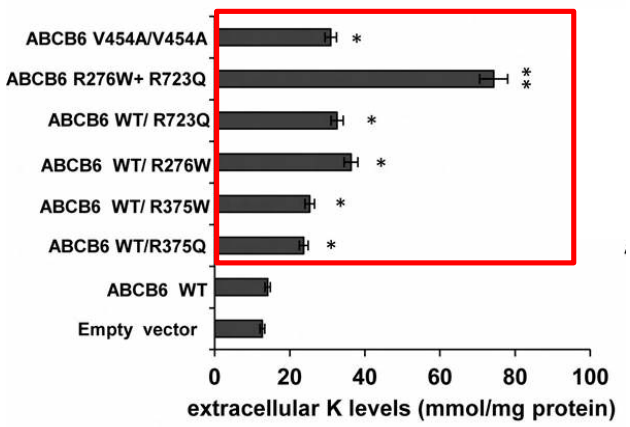
ABCB6 variants screening in blood donors population

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

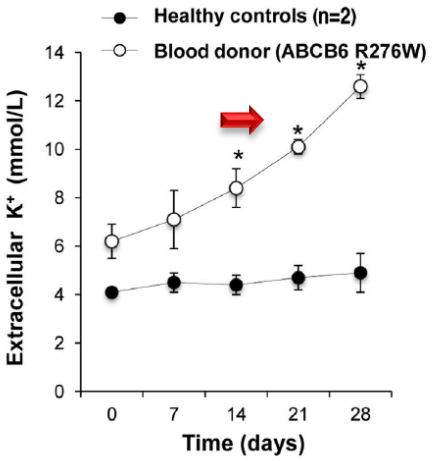
- ✓ Storage of FP blood causes a significant increase in blood K+ levels causing problems mostly in **pediatric/neonatal care**, indeed several cases of whole blood transfusion in infants leading to cardiac arrest and death have been described

- ✓ Genetic test for FP could be used to **screen potential donors of blood**





Cell lines



Blood samples

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Andolfo et al, Haematologica 2016

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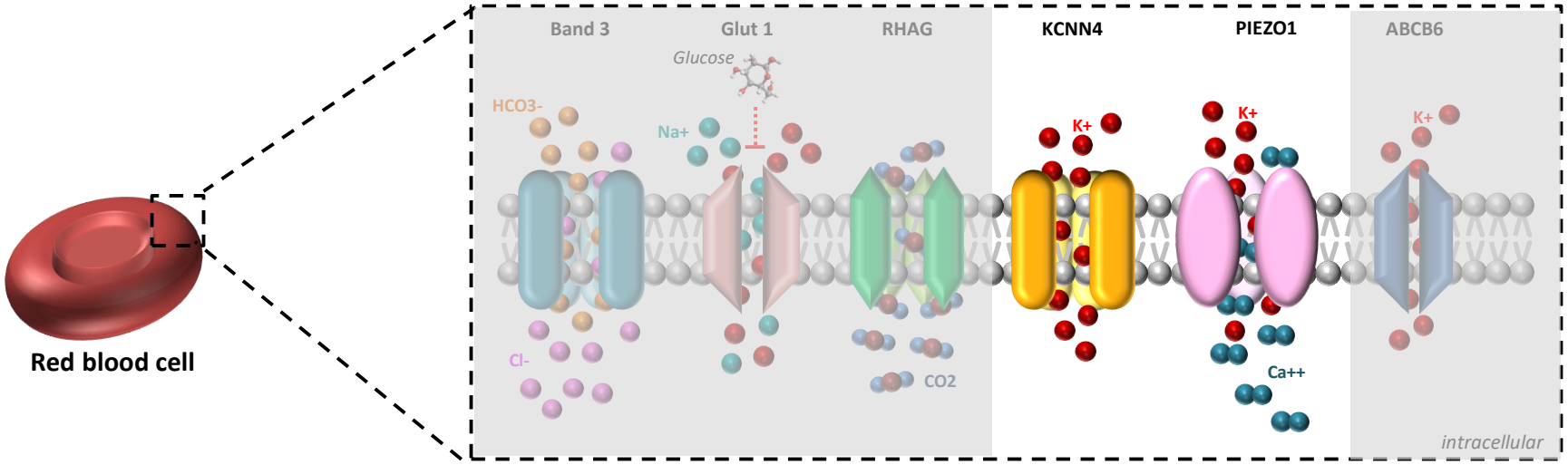
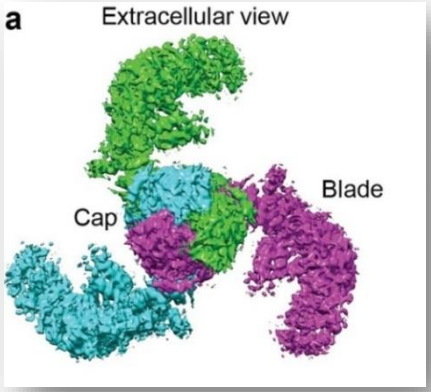
Syndromic/Non-Syndromic HSt: Dehydrated Hereditary Stomatocytosis



- ✓ 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 estimated an incidence of 1 case in 8000 adults.



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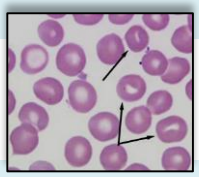
Dehydrated Hereditary Stomatocytosis (DHS)

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 ↑



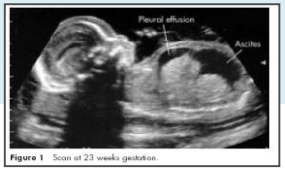


Figure 1 Scan at 23 weeks gestation.

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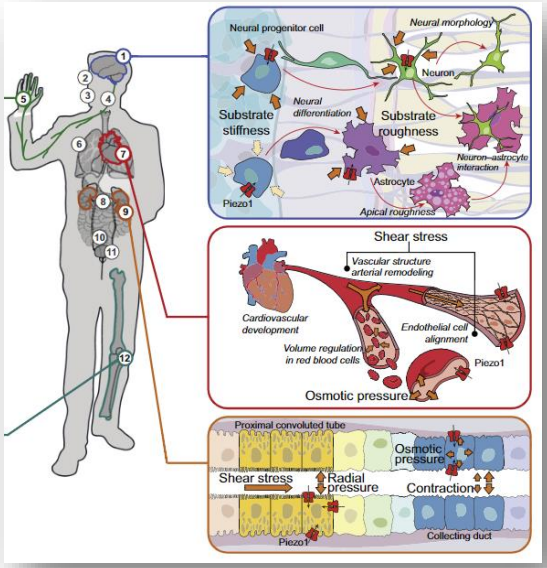
Andolfo et al. AJH 2018; Picard et al., Haem. 2019;
Andolfo et al. Haematologica 2016;
Andolfo et al. Blood 2013

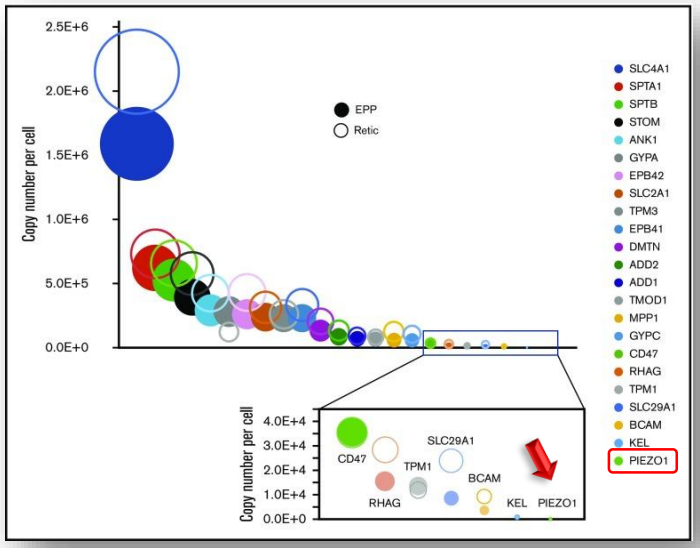
PIEZO1: physiological functions

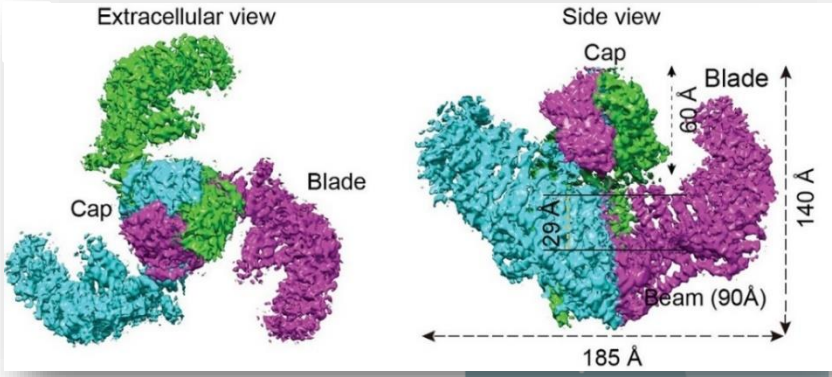
- ✓ PIEZO1 is a **mechanoreceptor** (non-selective cation channel activated by several mechanical stimuli) that forms a **trimeric propeller-like structure** of about 900 kDa in the plasma membrane

- ✓ It plays an important **physiological** role in **several biological processes** such as regulation of urinary osmolarity, control of blood pressure, regulation of hydration and volume of erythrocytes, sensor of epithelial cell crowding and stretching, formation and development of blood and lymphatic vessels

- ✓ It is present only at a **few hundred copies** per RBC but functions as major determinant of the RBC hydration status







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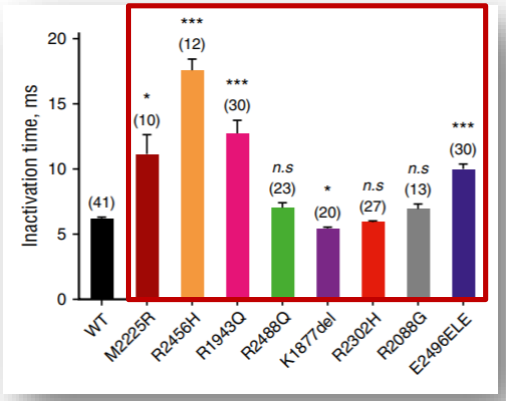
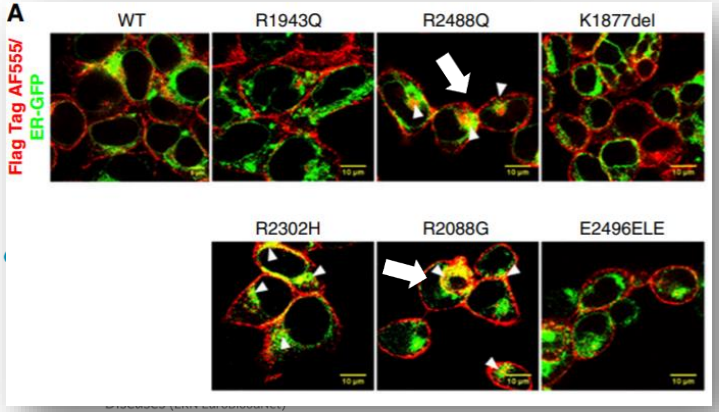
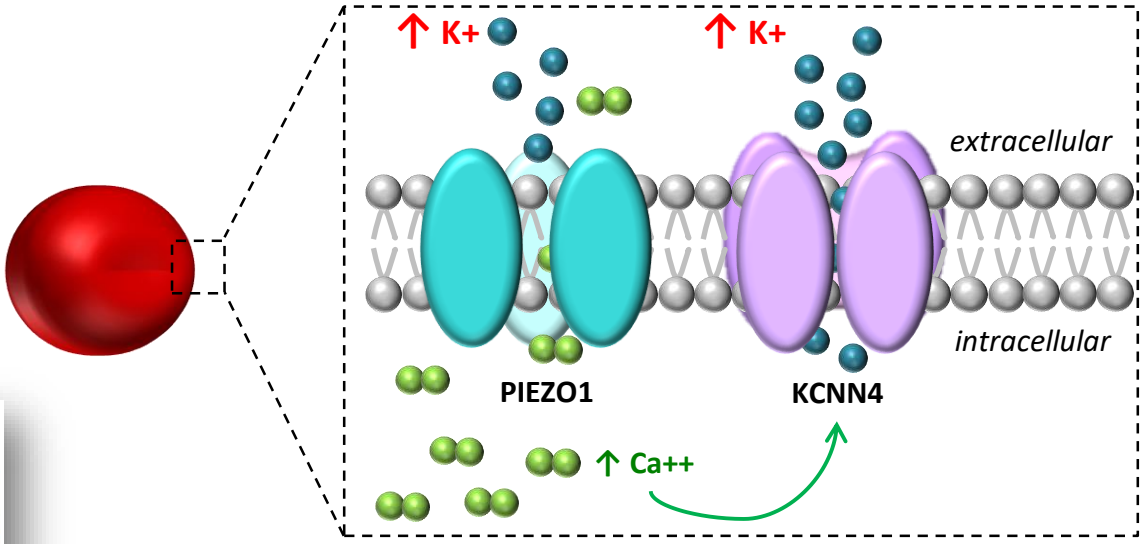
Wu J et al Trends in Bioc Sci 2017; Alper SL. Curr Top Membr. 2017; Martins JR, et al. Pflugers Arch. 2016; Wang S, et al. J Clin Invest. 2016; Gudipaty SA, et al. Nature. 2017; Li J, et al. Nature. 2014; Ranade SS, et al. 2014; Andolfo et al. Blood. 2013.

Gain-of-function (GOF) mutations in PIEZO1

- ✓ Several electrophysiology studies demonstrated that the pathogenic variants cause a **gain-of-function phenotype** with **delayed inactivation** of the channel
- ✓ RBCs dehydration is due to an **excessive potassium efflux** and **calcium influx,** accompanied by further potassium efflux through the **Gardos channel** and osmotic efflux of water
- ✓ Other mechanisms of PIEZO1 dysfunction include **altered response to osmotic stress** and **membrane trafficking** (phenotype heterogeneity of the disease)



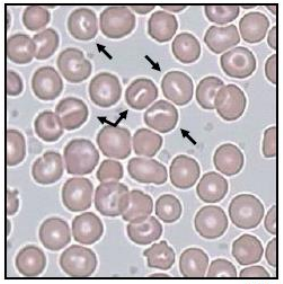


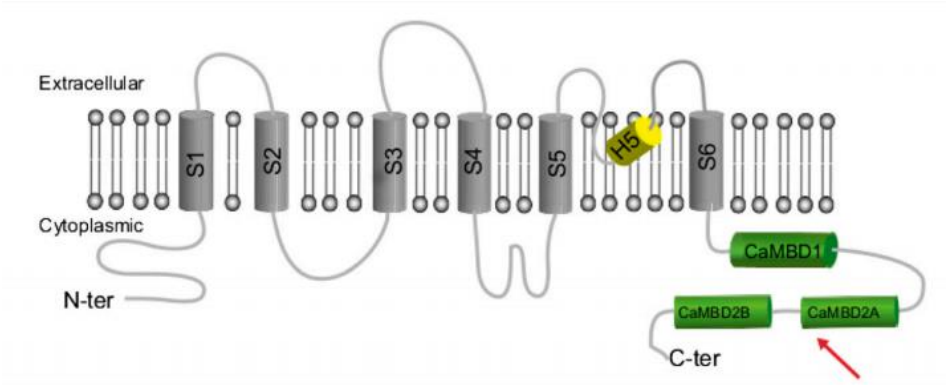


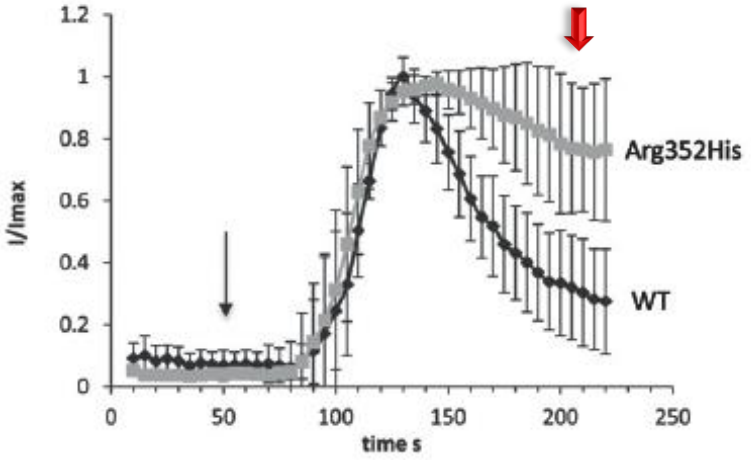
Andolfo et al., Haematologica, 2017; Rapetti-Mauss R. et al. Haematologica. 2017; Glogowska E., et al. Blood. 2017; Archer NM, et al. Am J Hematol. 2014; Shmukler BE. Et al. Blood Cells Mol Dis. 2014; Albuisson J. et al. Nat Commun. 2013; Bae C. et al. Proc Natl Acad Sci USA. 2013; Andolfo et al. Blood. 2013

KCNN4: second causative gene of DHS

- ***KCNN4*** gene encodes for the **Gardos channel** (KCa3.1), the erythroid Ca^{2+}-sensitive K^+ channel
- The families described until now are few (recurrent mutations R356H, V282M and V282R)
- The mutated channel showed a **higher activity** when compared to the wild type channel demonstrating that the mutations are **gain-of-function**
- Is it the same disease? "**Gardos channelopathy**". There are differences in cellular pathophysiology and clinical presentation







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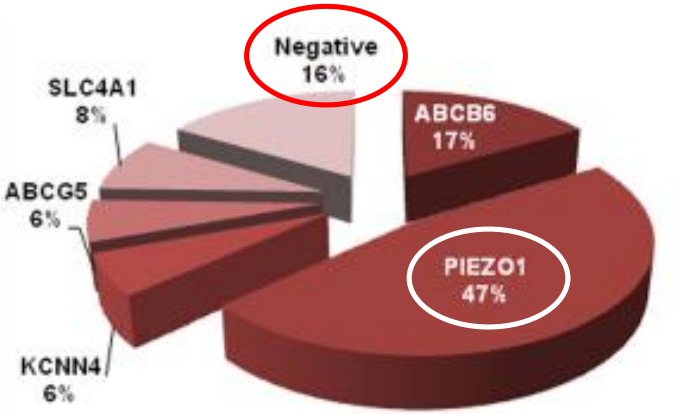
Rapetti Mauss et al, Blood 2015; Andolfo et al, AJH 2015; Glogowska et al, 2015; Fermo et al, 2017 Andolfo et al, AJH 2018

DHS phenotypes: PIEZO1 vs "Gardos channelopathy"

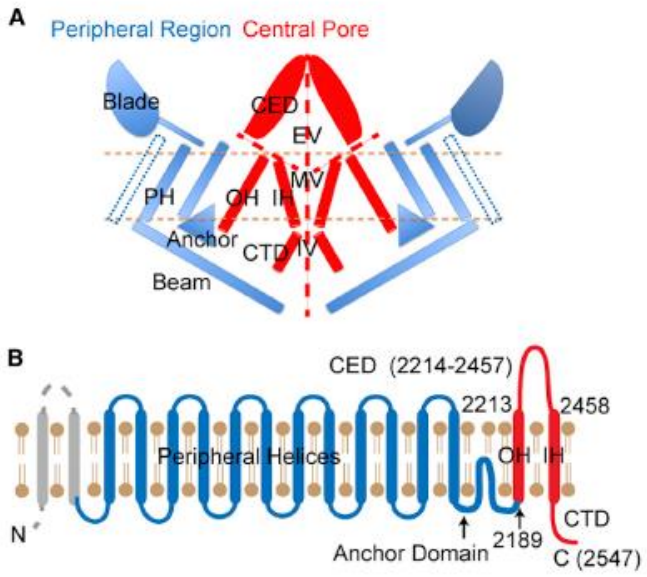
DHS1 - *PIEZO1*

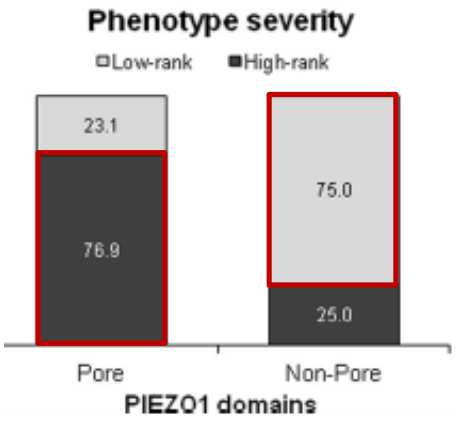
DHS2 - *KCNN4- Gardos*

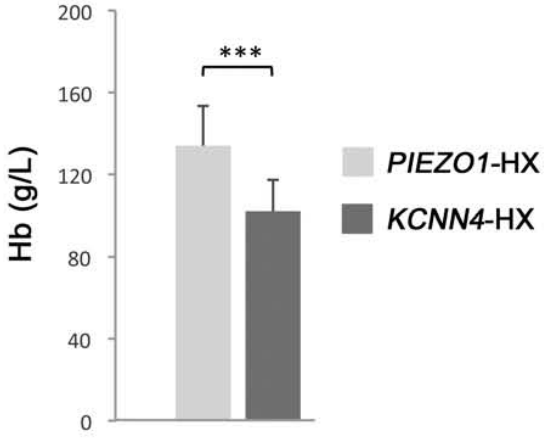
Two large cohort studies: 123 and 126 patients with HSt



Patients with PIEZO1 mutations	High-rank (*n = 14*)	Low-rank (*n = 15*)	P§
Age at diagnosis (years)	17.4 ± 3.3 (17.5; 14)	24.9 ± 6.5 (20.0; 11)	0.39
Gender (Female/Male)	4 (28.6)/10 (71.4)	9 (60.0)/6 (40.0)	0.09
Hematological data			
Hb (g/dL)	11.4 ± 0.8 (11.3; 14)	12.6 ± 0.4 (12.2; 15)	0.30
MCH (pg)	35.0 ± 1.5 (36.0; 13)	36.5 ± 1.5 (36.0; 15)	0.84
MCHC (g/dL)	36.7 ± 1.7 (34.8; 14)	33.9 ± 0.3 (33.7; 15)	0.12
Retics abs count (x10^3/µL)	181.3 ± 34.4 (165.6; 13)	153.5 ± 26.4 (139.3; 13)	0.57
Laboratory data, iron balance, and transfusion regimen			
Total bilirubin (mg/dL)	4.4 ± 0.7 (4.3; 14)	2.5 ± 0.7 (1.5; 8)	0.06
LDH (U/L)	333.8 ± 51.0 (315.0; 11)	232.6 ± 18.2 (242.5; 8)	0.17
Ferritin (ng/mL)	720.9 ± 129.3 (626.0; 14)	196.7 ± 57.1 (182.5; 6)	0.02
Ferritin level/dosage age‡	47.2 ± 8.3 (38.4; 14)	17.4 ± 3.7 (16.3; 6)	0.01

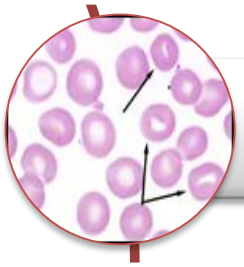






Andolfo et al, AJH 2018; *Picard et al., Haem. 2019*

Learning objectives of the webinar



Diagnosis and therapy of HSt

Diagnostic workflow of HSt

First-line investigations:
1. Hb, MCV, MHCH, Ret
2. peripheral blood (PB) smear
3. family history and transmission pattern

MCV, MCHC, Ret, hemolytic markers

PB smear: stomatocytes (variable degree: 5-20% DHS; >20%OHS)

AD transmission

Second-line investigations:
1. Osmotic fragility (OF), AGLT50, Pink, EMA tests
2. Ektacytometry

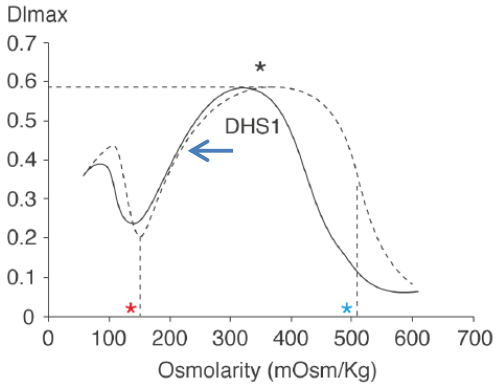
Osmotic resistance: increased
EMA test: normal

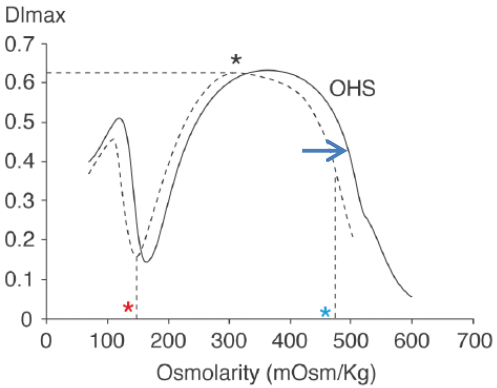
Ektacytometry: Left DHS/right shift OHS

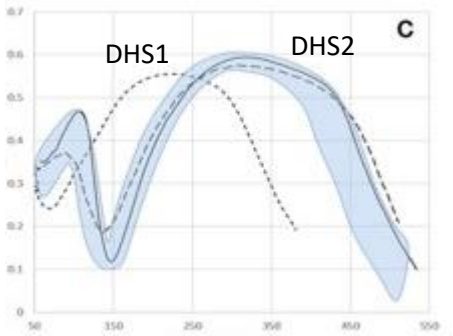
Third-line investigations:
1. Direct sequencing of causative genes
2. NGS custom panels

Molecular analysis: *single gene*

t-NGS panel or WES (RedPlex: 86 genes of HA)







Ektacytometry: Laser diffraction viscometer that measures **red cell deformability**

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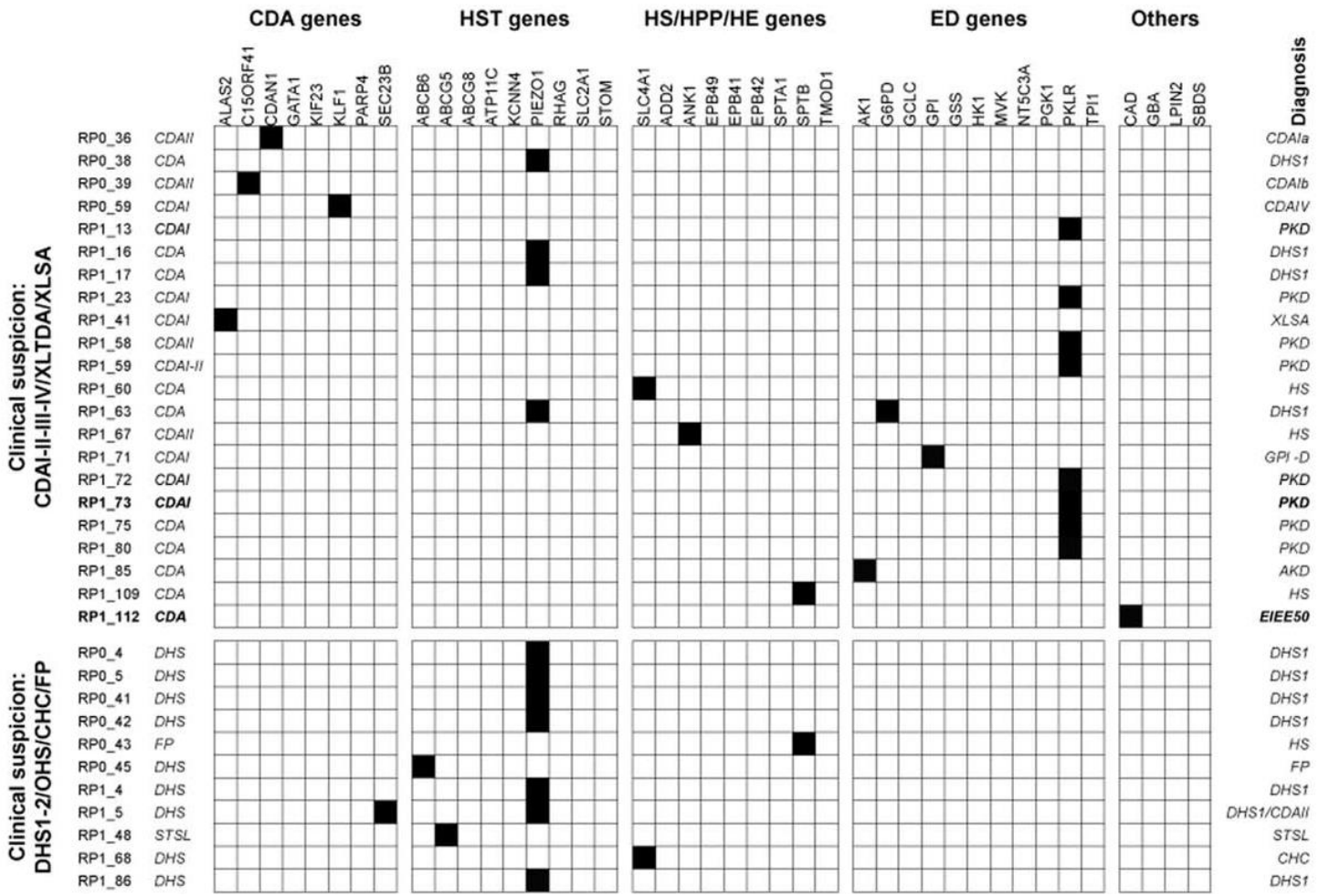
Russo et al 2018; Andolfo et al, 2018; Zaninoni et al, 2018

Genetic testing of HSt in the NGS era



We obtained an overall diagnostic yield of 65% using our t-NGS panel

- ✓ *18% of patients with clinical suspicion of congenital dyserythropoietic anemias (CDAs), **mainly CDAI and II**, carried mutations in PIEZO1 gene.*
- ✓ *9% of patients with clinical suspicion of **hereditary spherocytosis** (HS) carried mutations in PIEZO1 gene*

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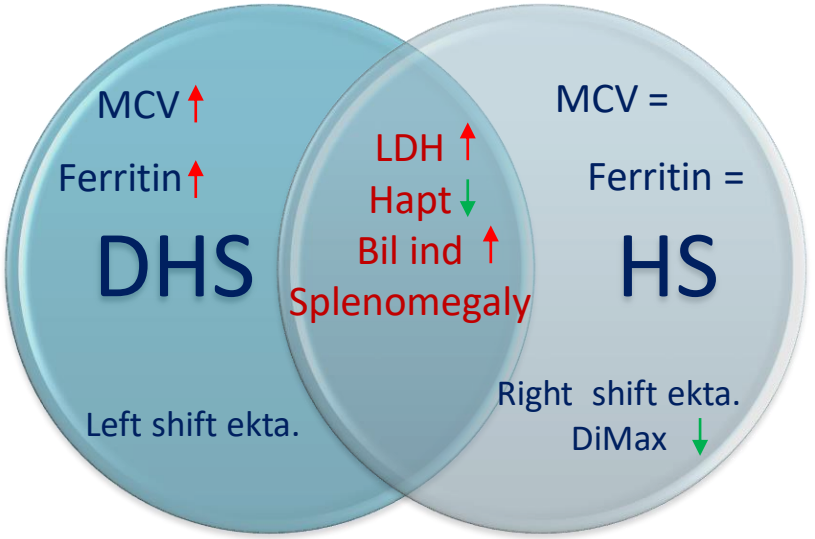
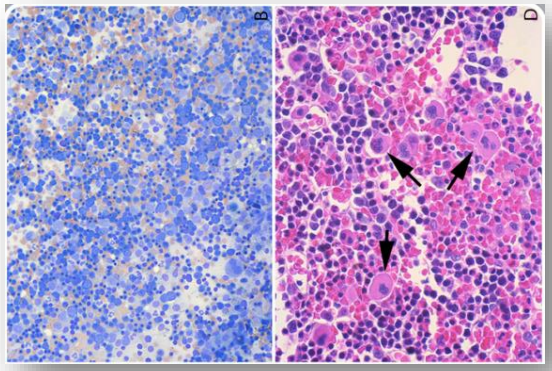
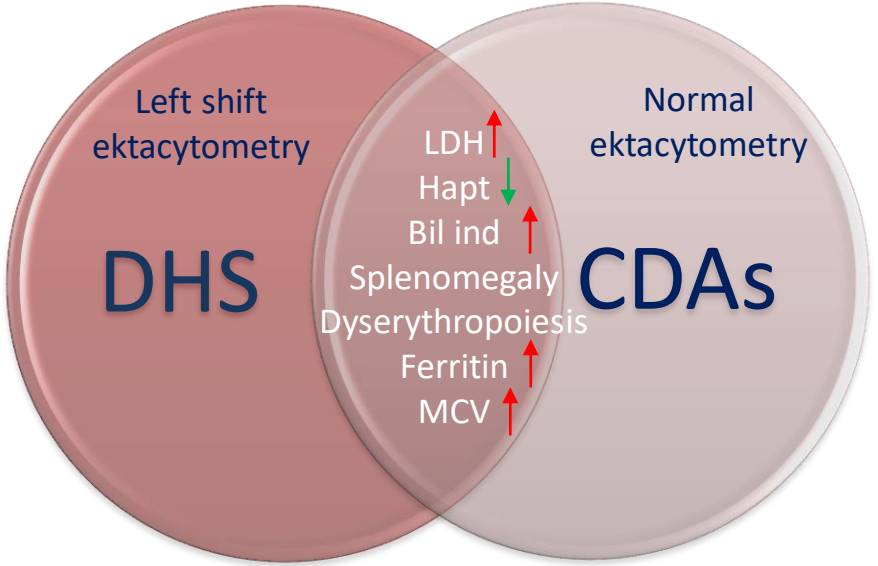
Differential diagnosis

- *DHS is often **misdiagnosed**, at clinical level, as **hereditary spherocytosis** (HS) or **congenital dyserythropoietic anemias** (CDAI/II)*
- *In several cases DHS can also be misdiagnosed as **hereditary hemochromatosis***
- *The **genetic analysis** is crucial also to avoid not useful treatments as for example splenectomy*
- *It is important to evaluate the possible **co-inheritance of other genetic traits** that could account for variability of the phenotype observed*





Hypercellular bone marrow with erythroid hyperplasia (mimicking myelodysplastic syndrome) in a patient with DHS
Paessler M, Hartung H. Blood. 2015



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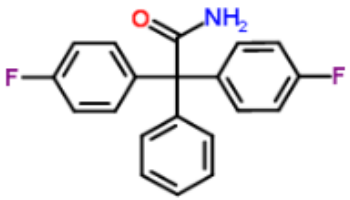
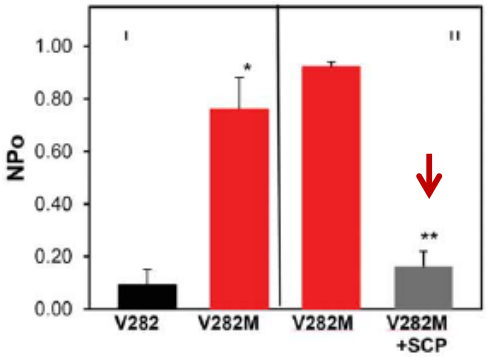
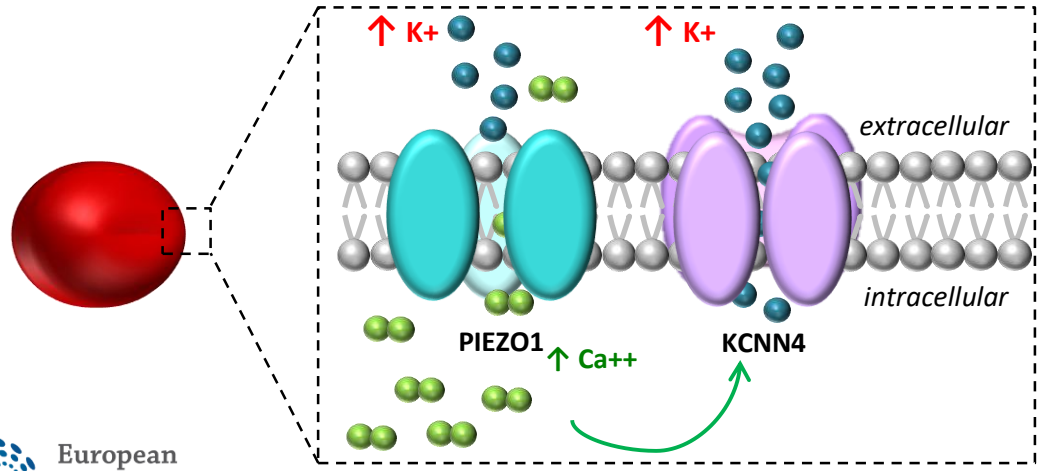
Standard treatment and possible future therapy

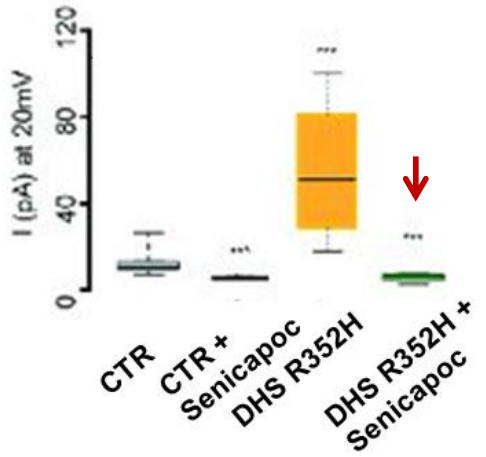
- ✓ The first-line treatment is based only on supportive care: folates, Vit.B12, transfusions, iron chelation.
- ✓ Splenectomy is <u>contraindicated</u> (increased risk of thrombosis).
- ✓ **SENICAPOC** (ICA -17043) is a **Gardos channel antagonist** , previously proposed for use in sickle cell anemia, tested in phase 3 study
- ✓ SENICAPOC is efficient in preventing RBC K+ loss and dehydration in both *PIEZO1* and *KCNN4* mutated cells.
- ✓ Other possible treatments are the **inhibitors** of PIEZO1



SENICAPOC







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Rapetti-Mauss R. et al., Haematologica 2016; Rivera A. et al., AJH 2017

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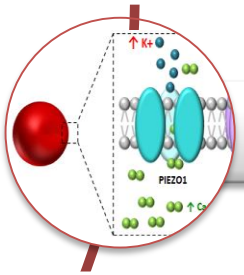
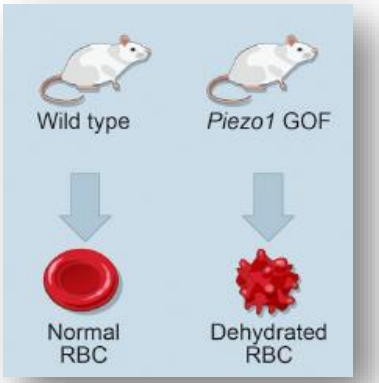


Dehydrated hereditary stomatocytosis: role of *PIEZO1 in RBCs*

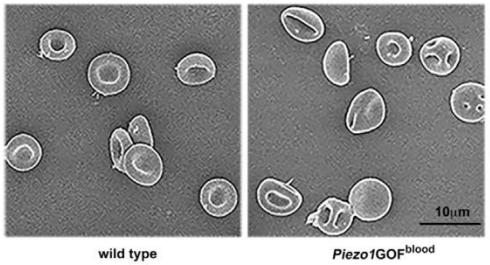
Piezo1 Gain-of-Function Mice

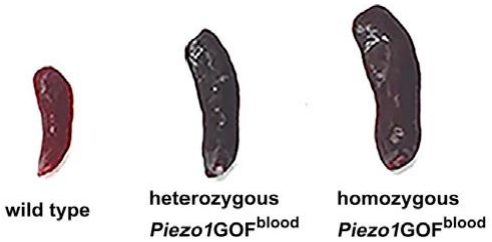
Constitutive Piezo1 GOF and blood-cell-specific PiezIo1 GOF transgenic mice (R2456H) showed:

- ✓ Stomatocytes at PB, reduced osmotic fragility, and splenomegaly
- ✓ Mild anemia, with lower Hb level and increased ret. Number/MCV



Gain-of-function Piezo1 mice display **hallmark clinical features observed in human DHS patients**, including RBC dehydration, mild anemia, and splenomegaly.





	wild type (n = 6)	Heterozygous Piezo1GOF^{blood} (n = 5)	Homozygous Piezo1GOF^{blood} (n = 7)
RBC (M/uL)	9.82 ± 0.35	9.98 ± 0.39	9.50 ± 0.37
HGB (g/dL)	14.90 ± 0.22	14.02 ± 0.16**	12.19 ± 0.34****
HCT (%)	56.27 ± 0.57	51.22 ± 1.09**	42.06 ± 1.25****
MCV (fL)	49.43 ± 0.12	51.08 ± 0.56*	54.64 ± 0.37****
MCH (pg)	14.12 ± 0.05	14.34 ± 0.02**	14.56 ± 0.07***
MCHC (g/dL)	27.35 ± 0.10	29.14 ± 0.15****	27.00 ± 0.59
RET # (k/ul)	375.68 ± 13.54	450.06 ± 7.03**	541.29 ± 11.79****

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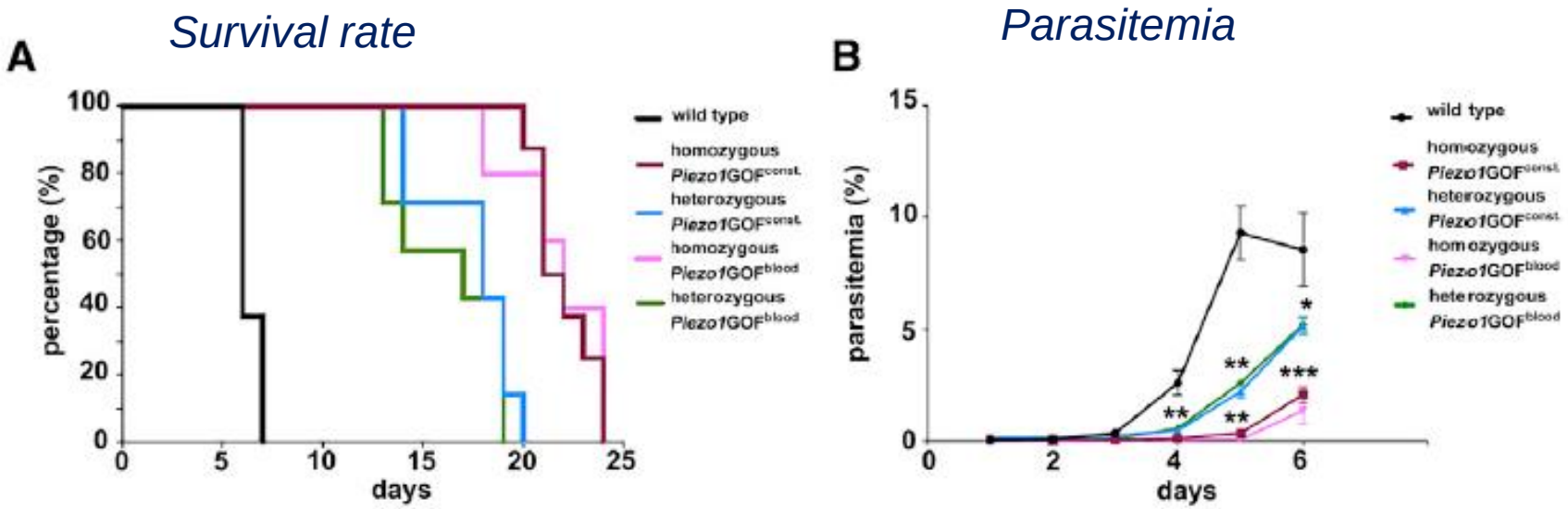
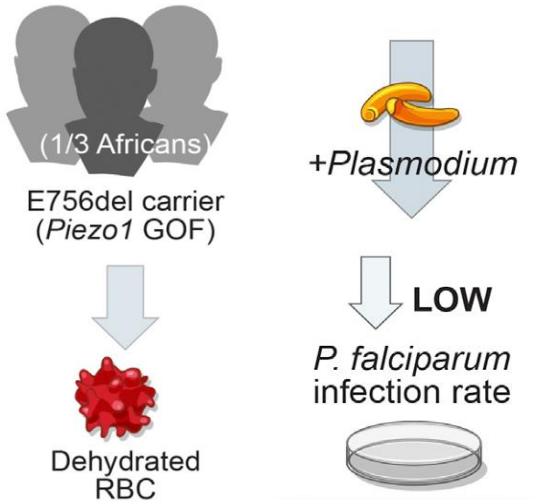
Shang Ma et al, Cell 2018

Piezo1 GOF mutations attenuate Plasmodium infection

GOF *PIEZO1* mice



- ✓ GOF *PIEZO1* mice showed increased survival rate after infection and decreased parasitemia.
- ✓ A novel human GOF *PIEZO1* allele, E756del, is present in a third of the African population.
- ✓ RBCs from individuals carrying this allele are dehydrated and resistant to malaria.



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Shang Ma et al, Cell 2018

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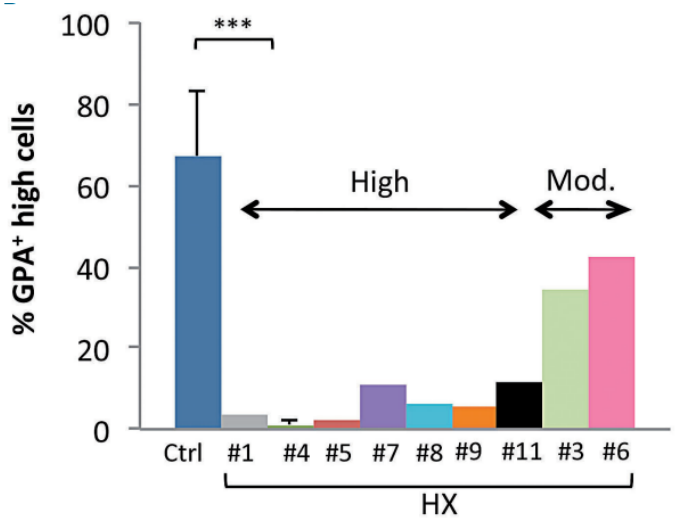
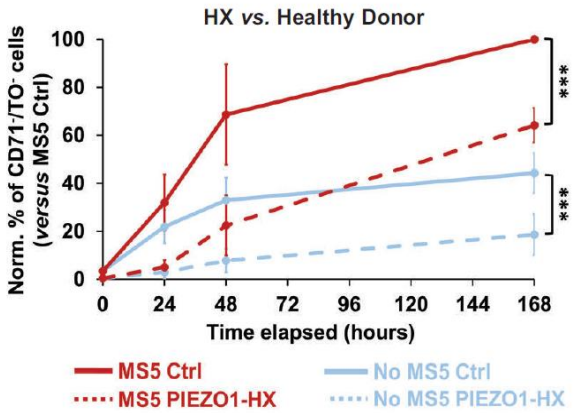
PIEZO1 activation delays erythroid differentiation and reticulocyte maturation in DHS1





- ✓ **PIEZO1**-patients showed **reduced reticulocyte count** compared to other patients with anemias due to membrane defects. This suggested that PIEZO1-patients might suffer from **delayed erythrocyte maturation**.
- ✓ In vitro culture assay showed **delay in erythroid differentiation of progenitor cells** obtained from patients with PIEZO1 mutations through transcriptional regulation (STAT5-ERK1/2-NFAT-EPO). It is mutations dependent.
- ✓ Characterization of reticulocytes and erythrocytes from 10 DHS1 patients revealing **alterations in deformability** and **vesicle content** that implicate a maturational defect in DHS1.
- ✓ DHS1 patients show differences in the **extent and rate of loss of CD71 and RNA content** over time. So, overactivation of PIEZO1 impacts **reticulocyte maturation.**

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Network Hematological Diseases (ERN EuroBloodNet)

Andolfo et al, AJH 2018; Pedro L. Moura et al., 2020; Caulier et al., 2020

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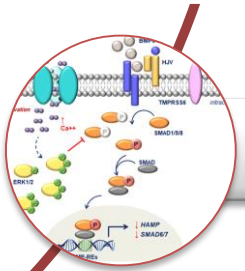
Learning objectives of the webinar



Dehydrated hereditary stomatocytosis: role of *PIEZO1 in hepatic cells*

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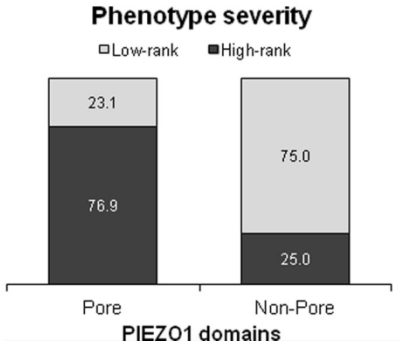
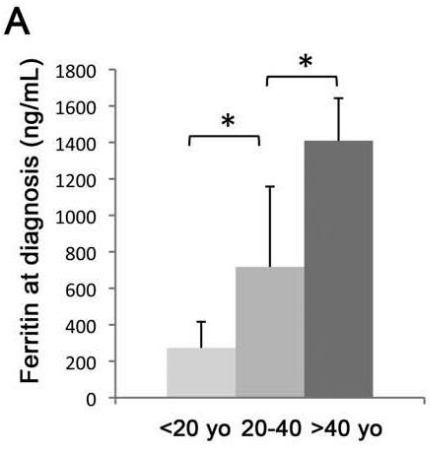
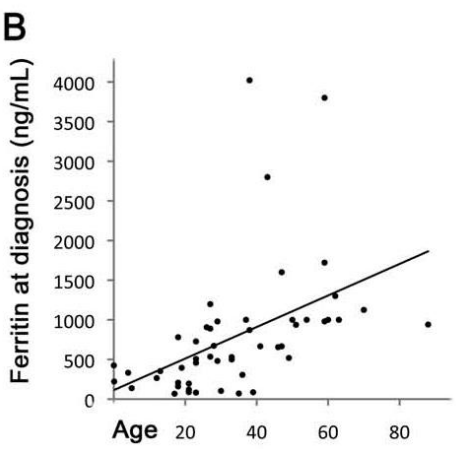
Hepatic iron overload in DHS1

- ✓ **Severe iron overload** with several cases of hepatosiderosis has been described for *PIEZO1* patients.
- ✓ Hepatic iron overload is **independent from the degree of anemia, the transfusion regimen, and the splenectomy**
- ✓ **Ferritin and ferritin/age** ratio is very high in DHS1. There is a poor correlation between ferritin levels and liver iron content.
- ✓ Most of the patients with a severe phenotype (mostly with impaired iron balance) carried mutations in the **pore domain**, while most of the patients with mild phenotype exhibited variations in the **non-pore domain**







		PIEZO1 patients[a] *DHS1*	KCNN4 patients[a] *DHS2*
Number of patients (%)		36 (49.3)	5 (6.8)
Gender (female/male)		16 (44.4)/20 (55.6)	2 (40.0)/3 (60.0)
Onset of symptoms (years)		7.7 ± 2.0 (1.5; 18)	9.4 ± 6.2 (7.0; 3)
Age of diagnosis (years)		21.5 ± 3.2 (20.0; 27)	29.3 ± 11.8 (27.5; 4)
Ferritin (ng/mL)	22.0-275.0	563.7 ± 106.3 (425.5; 20)	302.0 ± 127.0 (291.0; 4)
Ferritin level/ dosage age[b]	-	40.1 ± 6.7 (30.3; 19)	11.4 ± 4.5 (11.3; 4)

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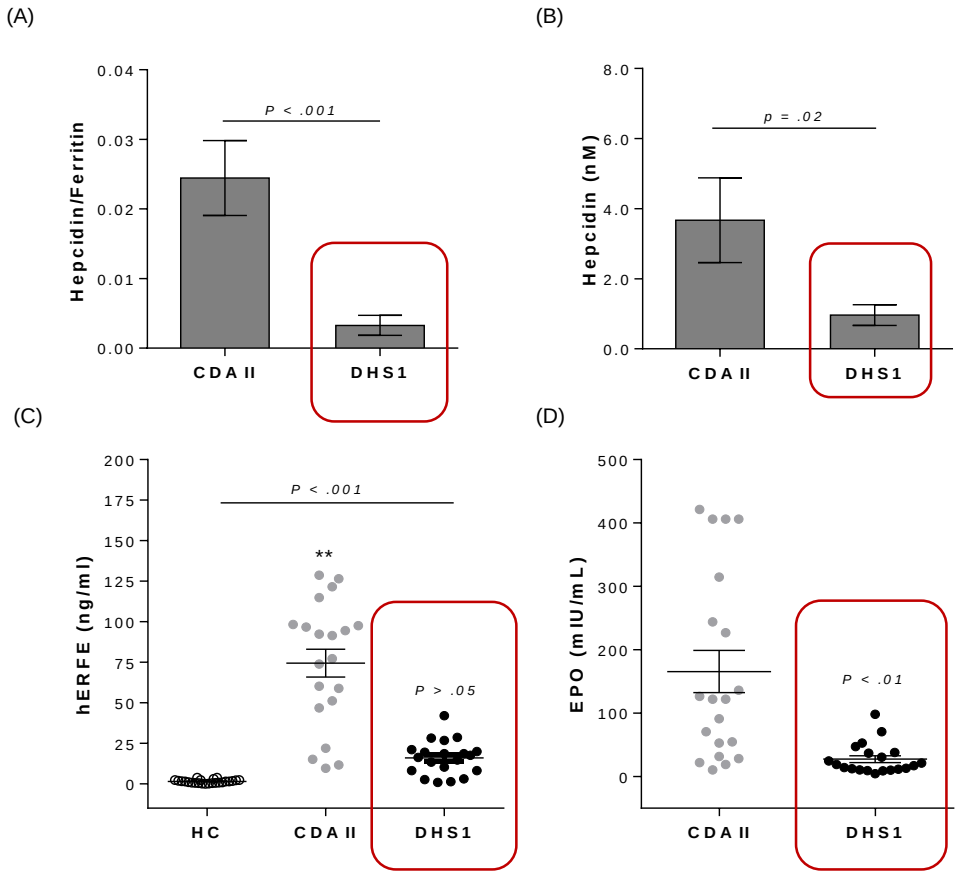
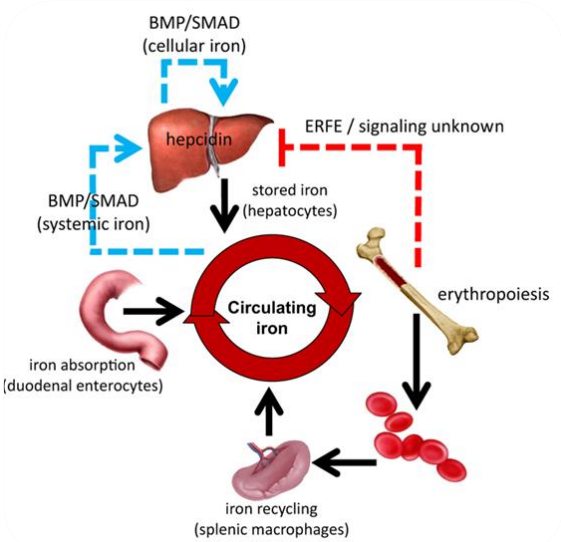
Picard et al., Haematologica 2019
Andolfo et al., AJH, 2018
Andolfo et al., Haematologica, 2017

Hepcidin and ERFE dosage in DHS1 patients



- ✓ Hepcidin resulted highly reduced in DHS1 patients compared to HC and CDAII patients.
- ✓ ERFE showed a slight, but not significant, increased levels in DHS1 compared to HC.



Hepcidin expression is impaired in DHS1 patients by a mechanism only partial regulated by ERFE

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Andolfo et al., AJH 2020

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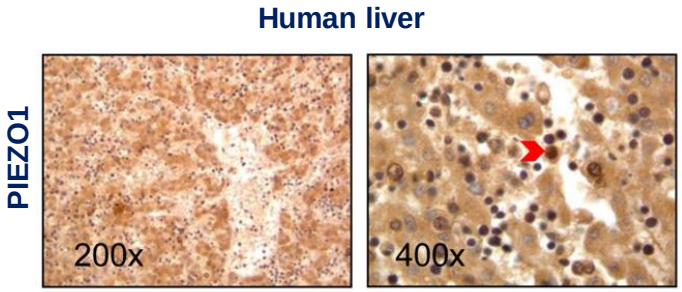
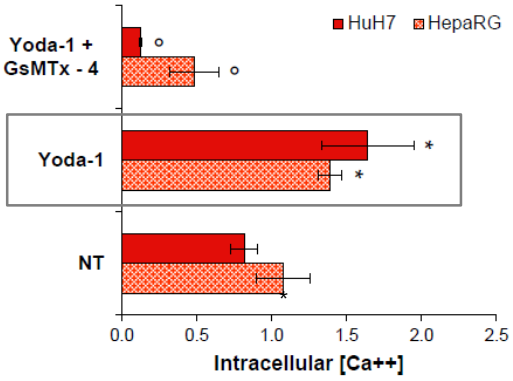
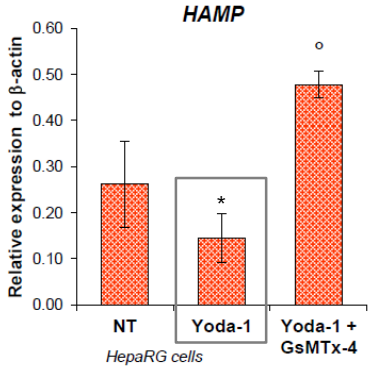
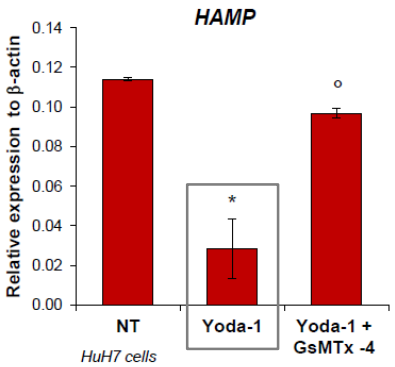
PIEZO1 in liver: physiological role

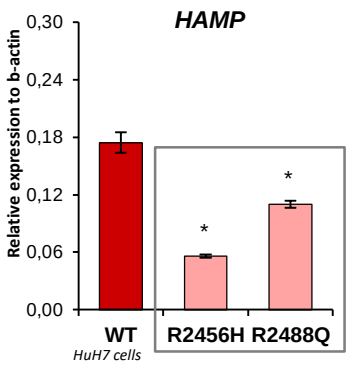


✓ PIEZO1 is expressed in the liver









✓ Intracellular calcium concentration increases after PIEZO1 activation by Yoda-1 in **primary hepatocytes**

✓ Activation of PIEZO1 by both Yoda-1 and GoF mutations cause *Hamp* suppression in hepatic cells

✓ Inhibition of PIEZO1 by GsMTx-4 leads to the rescue of the phenotype

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Andolfo et al. AJH 2020

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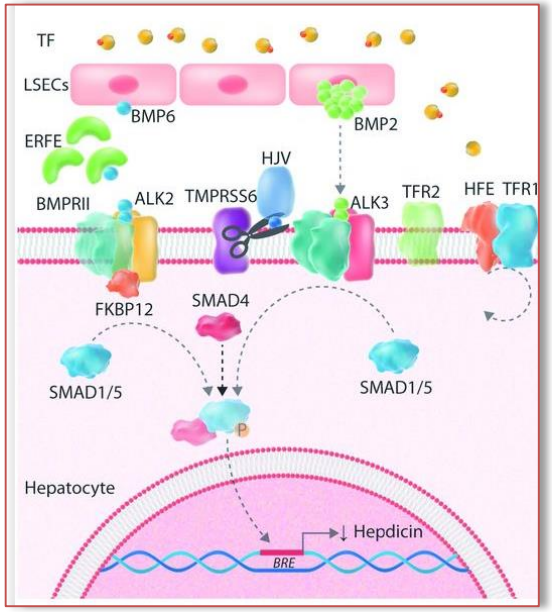
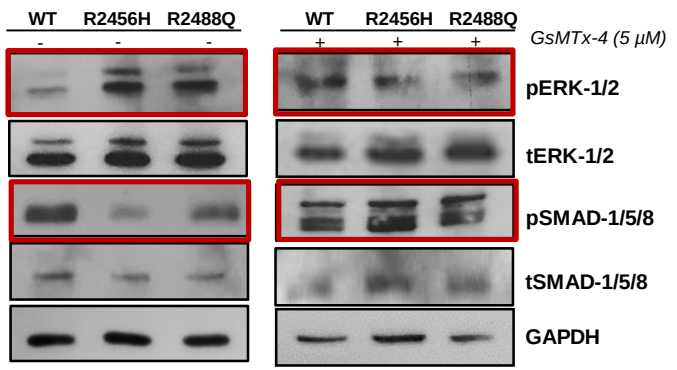
Impaired BMP-SMADs pathway in PIEZO1-GOF mutants

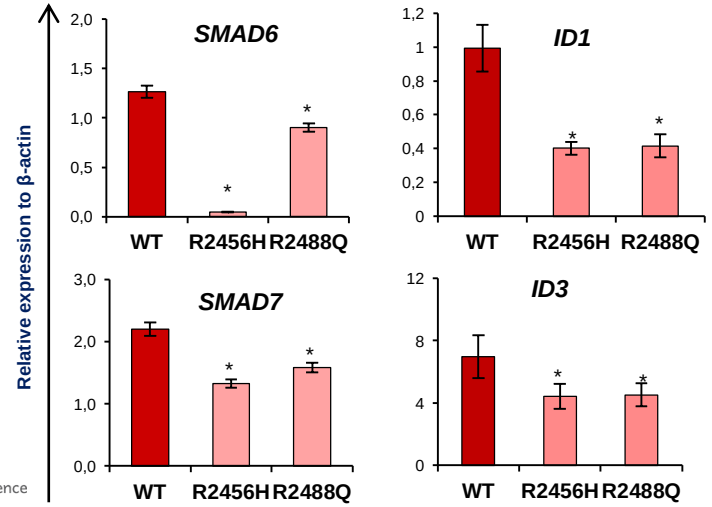


Camaschella C. et al., Haematologica 2020

- ✓ *HAMP gene expression is regulated by the BMP/SMADs pathway*
- ✓ *PIEZO1 activations leads to ERK1/2 phosphorylation in other cells*



PIEZO1 was activated by Yoda-1 (1.5μM)



PIEZO1 was activated by Yoda-1 (1.5μM)

- ✓ *PIEZO1 GOF mutants showed increased phosphorylation of ERK1/2 in hepatic cells and inhibition of BMP-SMADs pathway*
- ✓ *The inhibition of BMP/SMADs signaling was confirmed by the downregulation of the target genes: SMAD6/SMAD7/ID1/ID3*

Andolfo et al., AJH 2020

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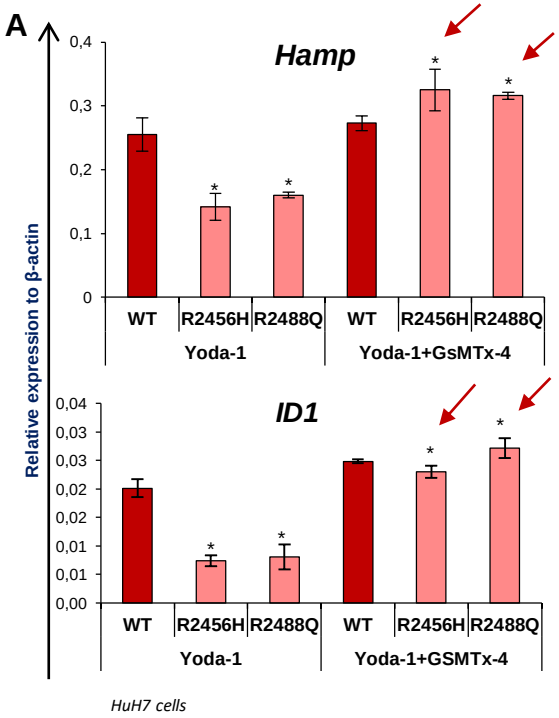
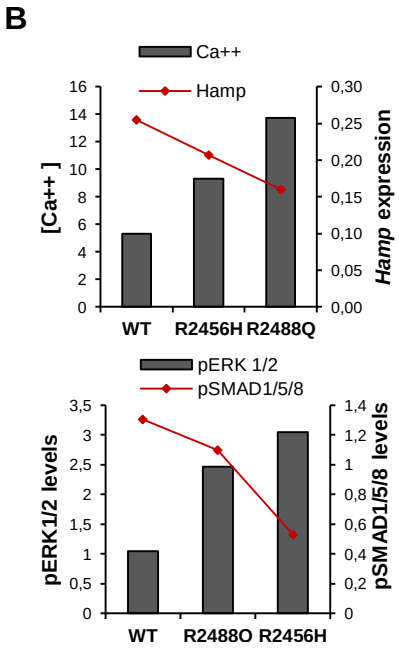
Phenotype rescue after GsMTx-4 treatment



✓ The inhibition of PIEZO1 by GsMTx-4 **rescued** the *Hamp* and *ID1* gene expression.



✓ There is an inverse correlation between intracellular [Ca++] and *Hamp* expression and between pERK1/2 and pSMAD1/5/8.

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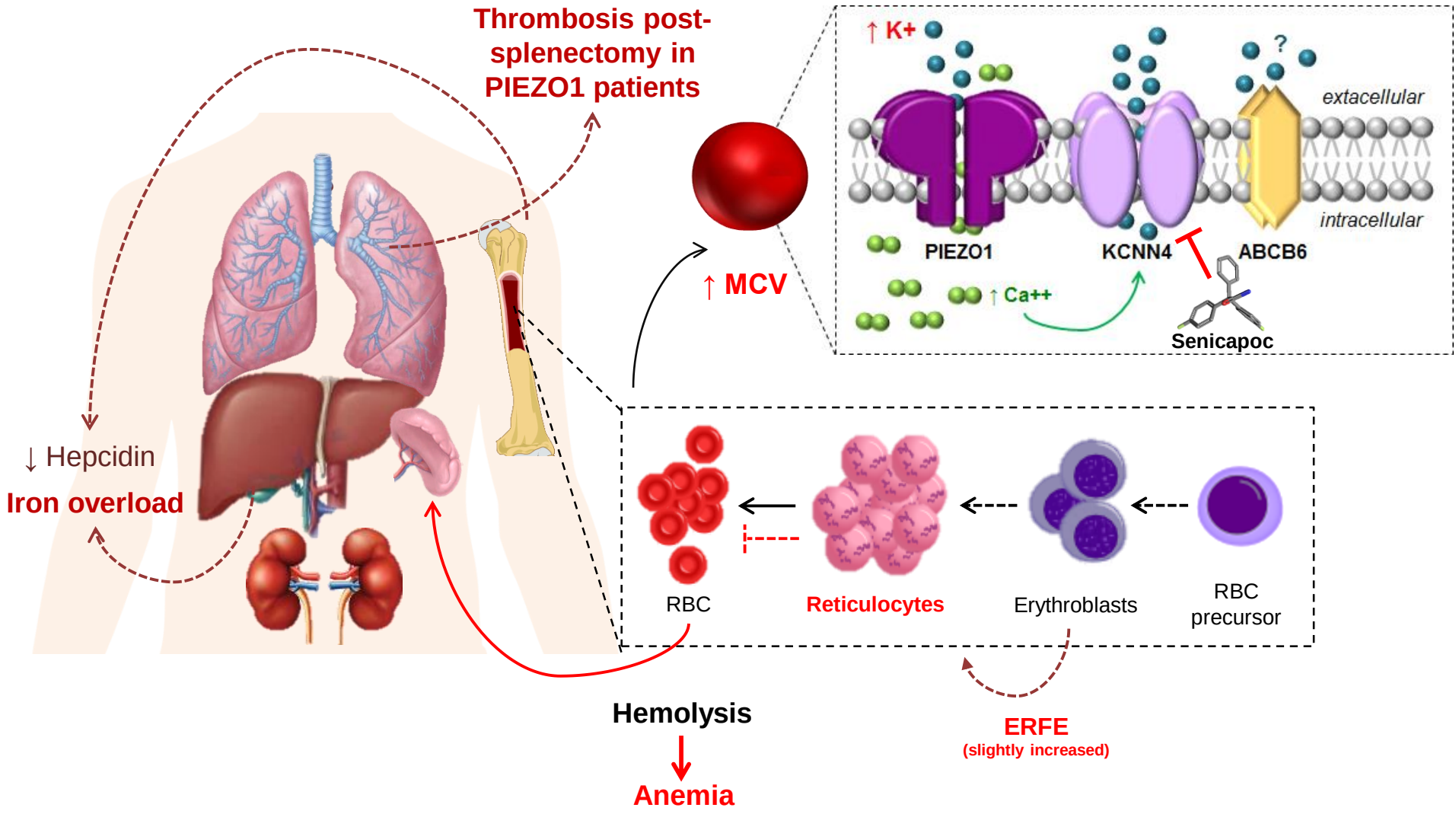
Model of pathogenic mechanism of DHS



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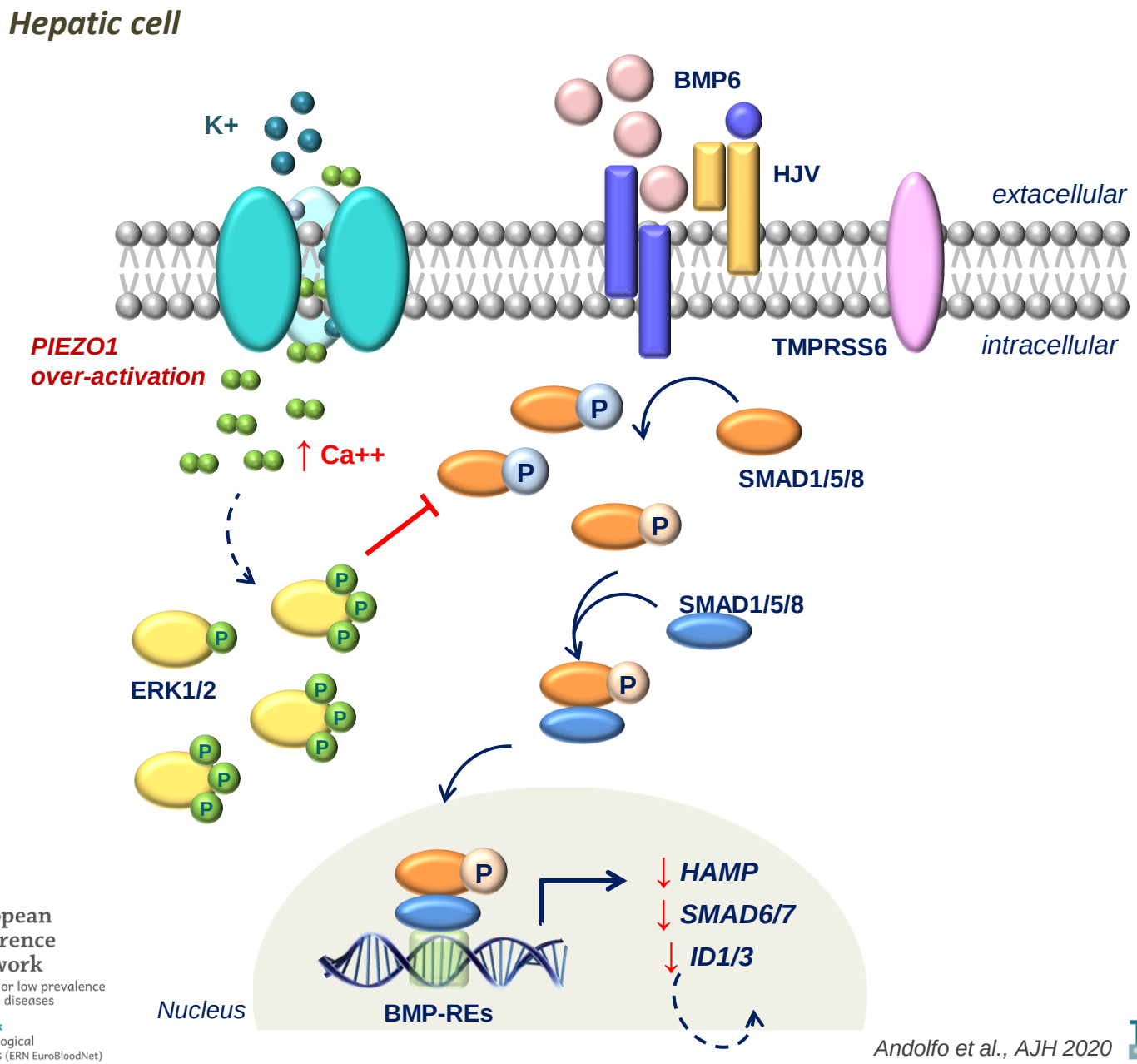
Proposed model: PIEZO1 regulation of hepatic iron metabolism



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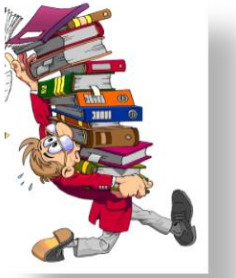
Take home messages



- ✓ HSt are a wide spectrum of inherited hemolytic disorders in which the RBC membrane cation permeability is increased.
- ✓ DHS is the most frequent condition within this class of anemias. It is an autosomal dominant hemolytic anemia caused by GOF mutations in both *PIEZO1* and *KCNN4* genes.
- ✓ The diagnosis of Hst is very challenging because of the presence of overlapping phenotypes, variable expressivity, allelic and genetic heterogeneity. DHS is in differential diagnosis with HS and CDAs.
- ✓ GOF mutations in *PIEZO1* caused impaired erythroid differentiation and reticulocytes maturation.
- ✓ GOF mutations in *PIEZO1* cause decreased *Plasmodium* infection.
- ✓ Iron overload in DHS1 is directly caused by GOF mutations of *PIEZO1* at hepatic level by impairing of *Hamp* gene expression.
- ✓ This finding opens a new field of study on *PIEZO1* and iron metabolism.

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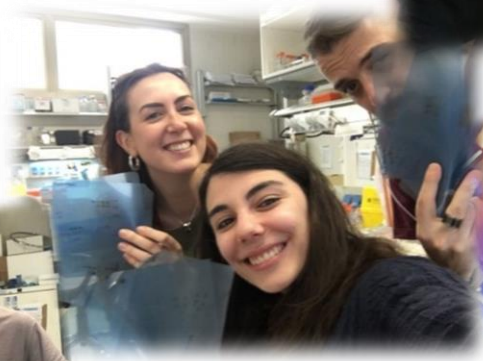

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