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EuroBloodNet

Connection between PIEZO1, dehydrated hereditary stomatocytosis, and iron overload

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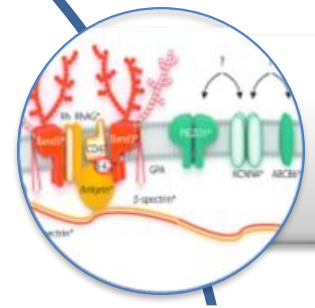
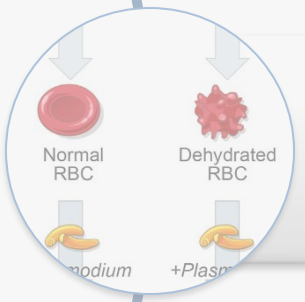
Network Hematological Diseases (ERN EuroBloodNet)

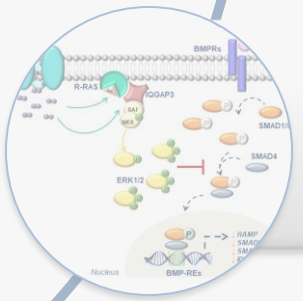
Outlines



***PIEZO1**: physiological roles and pathogenetic mechanism of dehydrated hereditary stomatocytosis*



***PIEZO1**: molecular genetics*



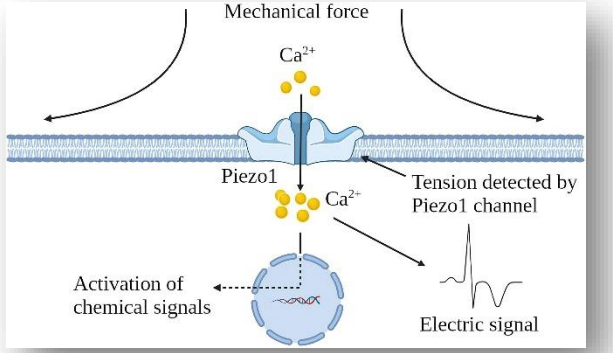
***PIEZO1:** iron metabolism*

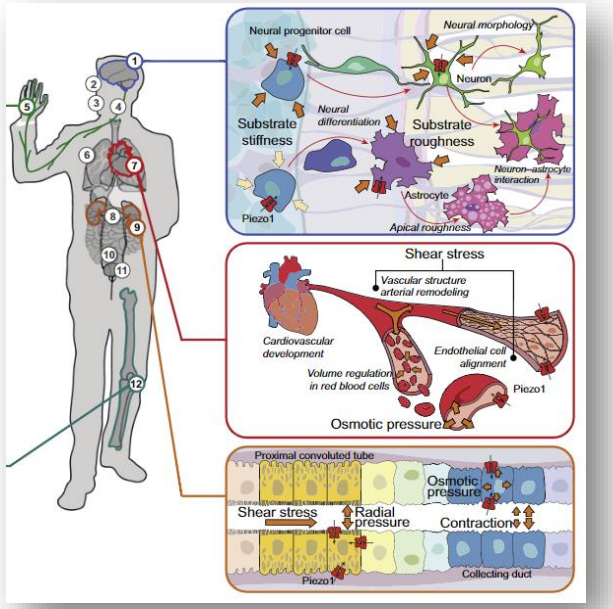
PIEZO1: physiological functions

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

- ✓ It can detect **mechanical stresses** such as **static pressure, shear stress**, and **membrane stretch**

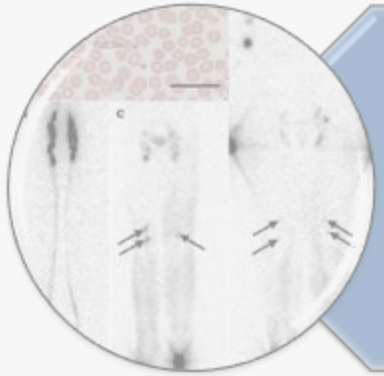
- ✓ PIEZO1 has wide-spread roles in **mechanotransduction processes**:
  - vascular and lymphatic system development
  - heart valve development
  - bone formation
  - regulation of blood pressure
  - endothelial cells response to shear stress
  - **control of the red blood cell volume and hydration**





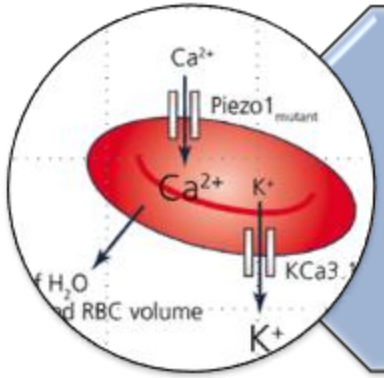
Wu J et al Trends in Bioc Sci 2017; Alper SL. Curr Top Membr. 2017; Martins JR, et al. Pflugers Arch. 2016; Wang S, et al. J Clin Invest. 2016; Gudipaty SA, et al. Nature. 2017; Li J, et al. Nature. 2014; Ranade SS, et al. 2014; Andolfo et al. Blood. 2013; A. Patapoutian et al Science 2010)

PIEZO1 mutations in human



Autosomal recessive generalized lymphatic dysplasia

Loss-of-function variants



Autosomal dominant dehydrated hereditary stomatocytosis

Gain-of-function variants

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

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Piezo1 Loss-of-Function Mice

- ✓ **Mice deficient in Piezo1** die in utero due to a diminished shear-induced alignment of endothelial cells and a **severe impairment of vascular development**
- ✓ **Mice with deletion of PIEZO1 in the hematopoietic system** showed RBCs:
  - with increased fragility
  - aberrantly retained within the spleen
  - overhydrated

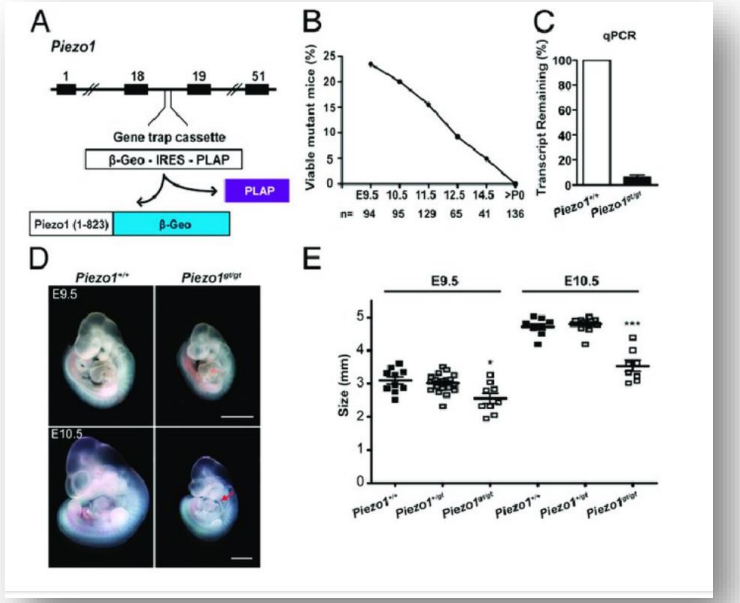
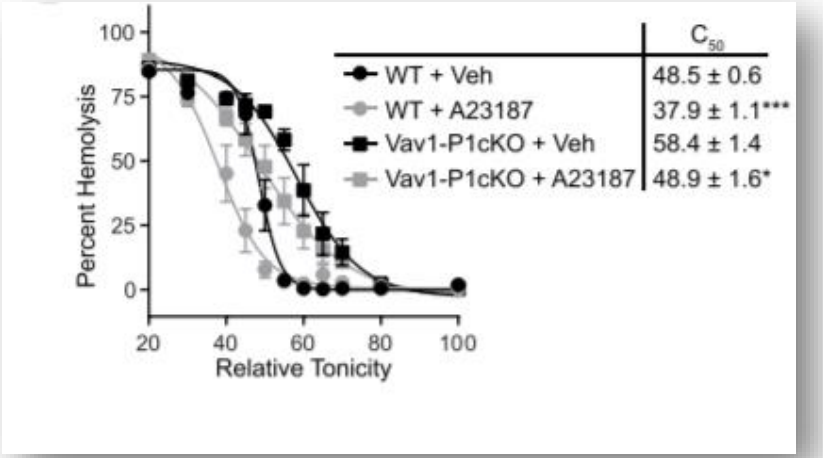


Table 1. Hematological indices from blood isolated from 8- to10-week-old WT and Vav1-P1cKO mice

	WT ± SEM (n = 19)	Vav1-P1cKO ± SEM (n = 18)
RBC	100 ± 0.58	96.60 ± 1.10*
HGB	100 ± 0.54	99.50 ± 1.10
HCT	100 ± 0.51	105.59 ± 1.13***
MCV	100 ± 0.23	109.51 ± 1.51***
MCH	100 ± 0.25	103.14 ± 0.48***
MCHC	100 ± 0.26	94.37 ± 1.08***
RDW	100 ± 0.92	114.49 ± 2.64***



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Ranade et al, PNAS 2014; Cahalan et al Elife 2015

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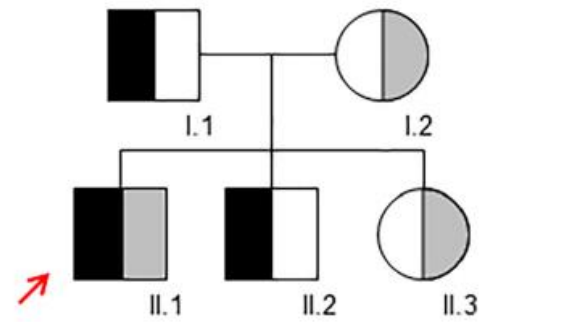
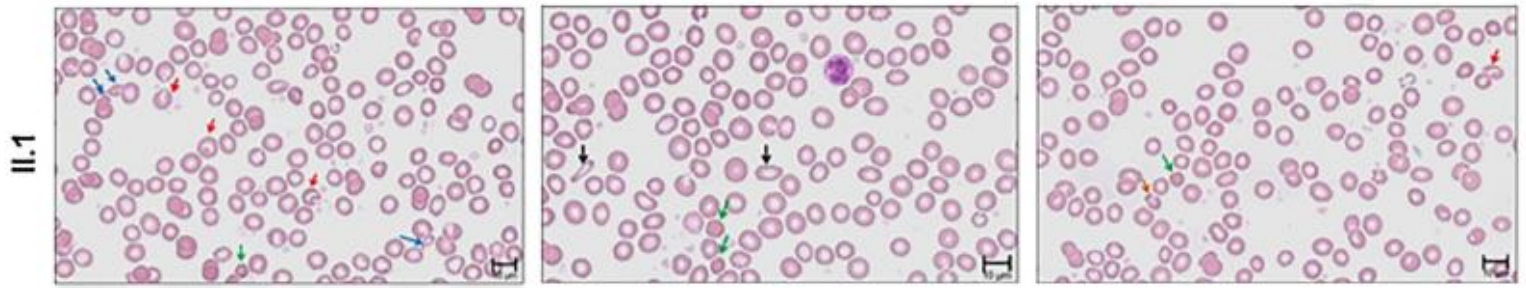
PIEZO1 hypomorphic variants cause alterations of RBCs hydration

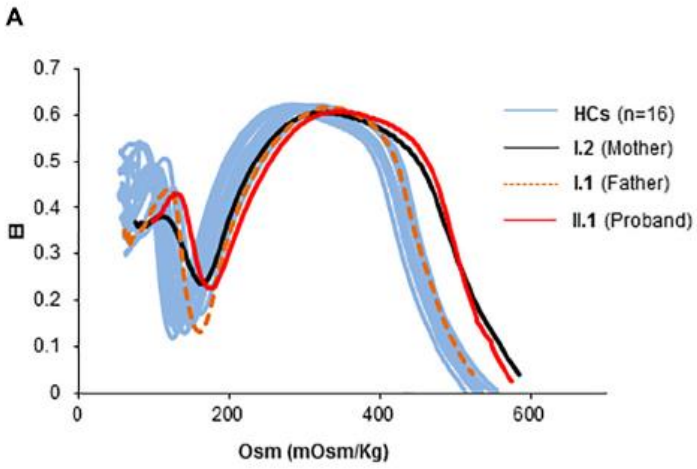


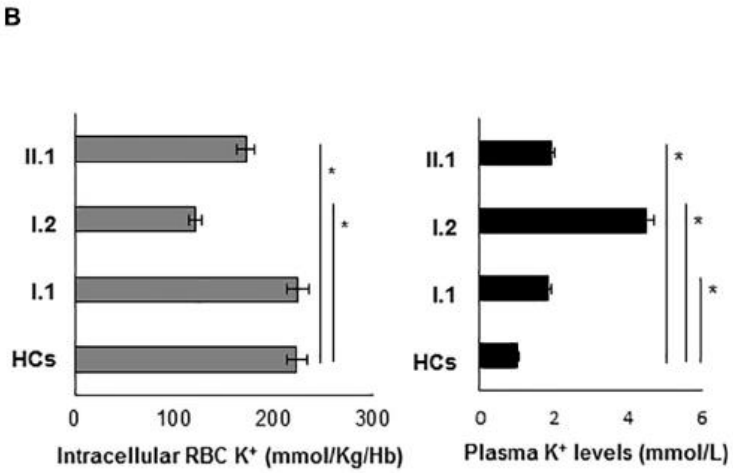
c.6165-7G>A (rs141011459; MAF = 0.0004)

c.5725delA; p.Arg1909Glufs*12



✓ The erythrocytes of the patient highlighted **altered hydration** with the intracellular loss of the potassium content and structural abnormalities with **anisopoikolocytosis** and presence of both **spherocytes** and **stomatocytes**.





This novel erythrocyte trait, sharing features with both hereditary spherocytosis and overhydrated hereditary stomatocytosis

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Andolfo et al Front in Phisiol. 2019

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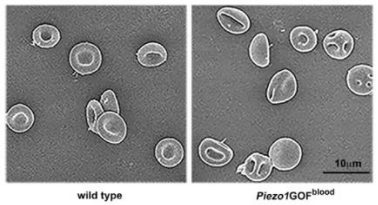
Piezo1 Gain-of-Function Mice

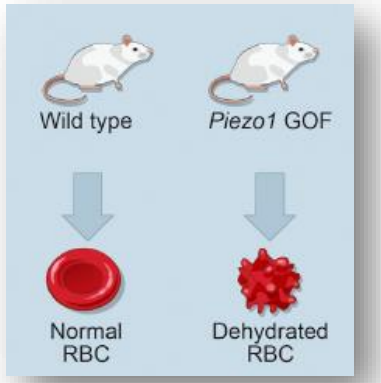
Constitutive Piezo1 GOF and blood-cell-specific Piezo1 GOF transgenic mice (R2456H) showed:

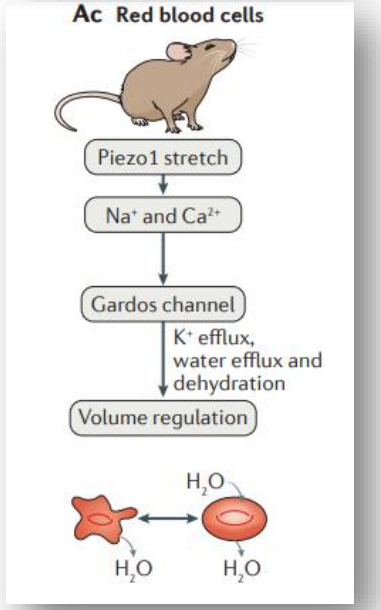
- ✓ Stomatocytes at PB, reduced osmotic fragility, and splenomegaly
- ✓ Mild anemia, with lower Hb level and increased reticulocytes count.

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 Piezo1 GOF^{blood} (n = 5)	Homozygous Piezo1 GOF^{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

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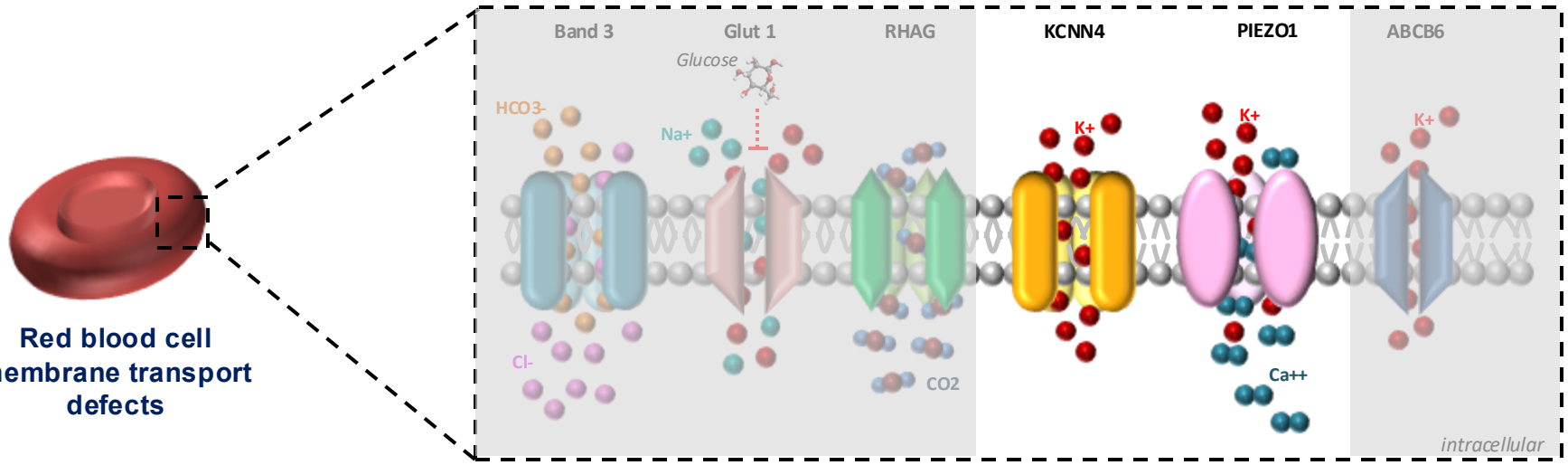
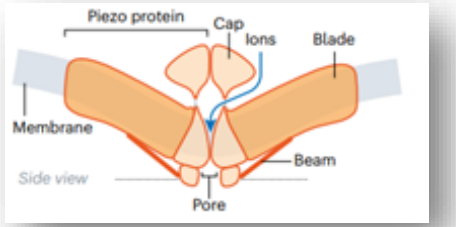
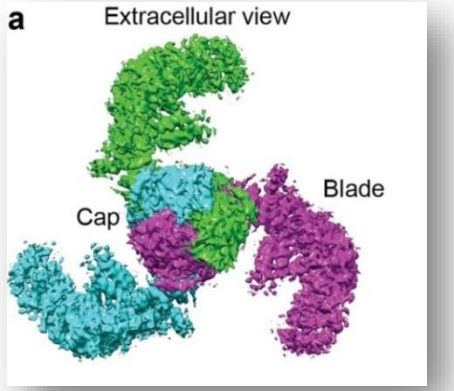
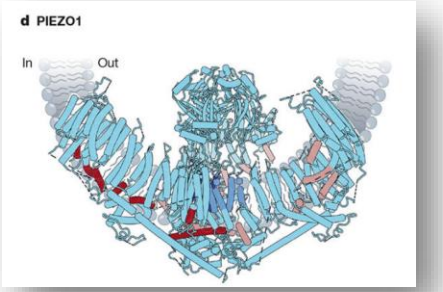
PIEZO1 and Dehydrated Hereditary Stomatocytosis (DHS)



Red blood cell membrane transport defects







- ✓ 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. It is linked to malaria resistance.

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

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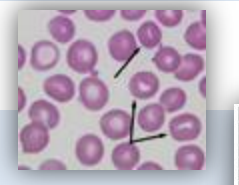
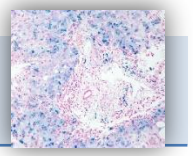
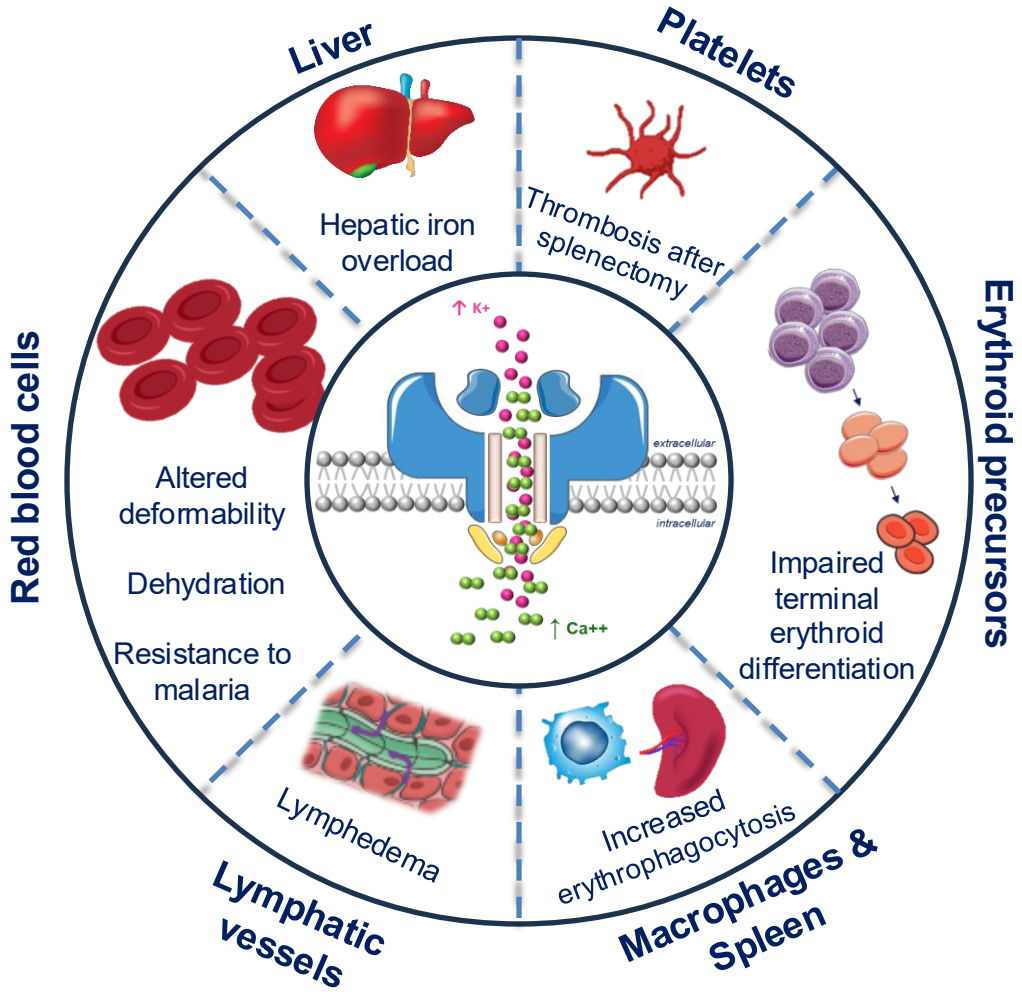
Dehydrated Hereditary Stomatocytosis (DHS)

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	 Figure 1 Scan at 23 weeks gestation.
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.

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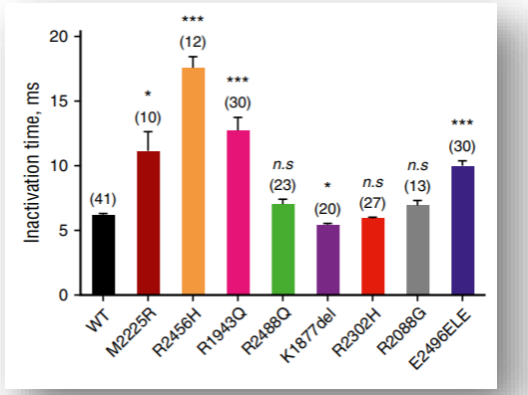
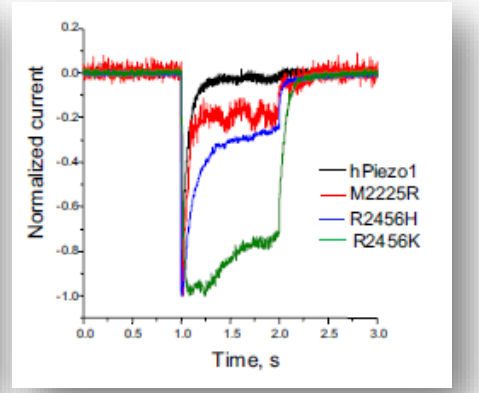
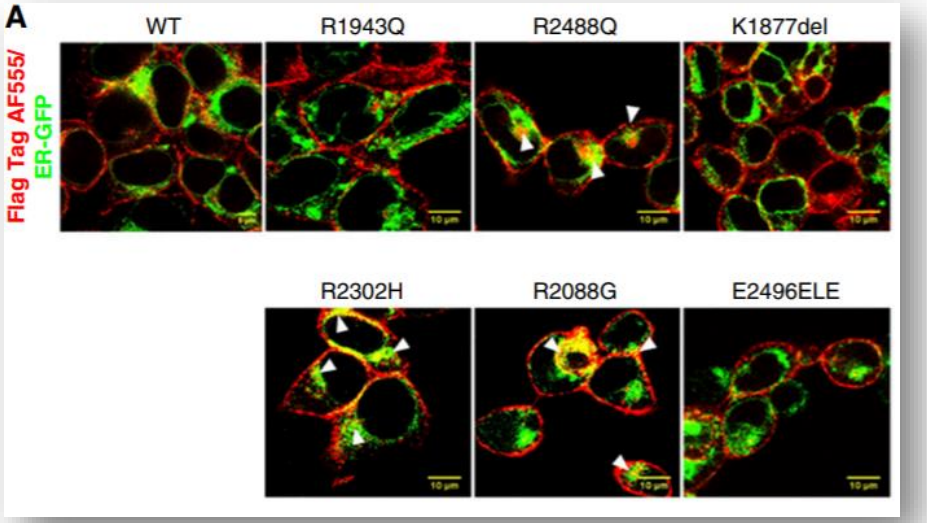
Jankovsky N, et al AJH 2021; Andolfo et al. AJH 2018; Picard et al., Haem. 2019; Andolfo et al. Haematologica 2016; Andolfo et al. Blood 2013

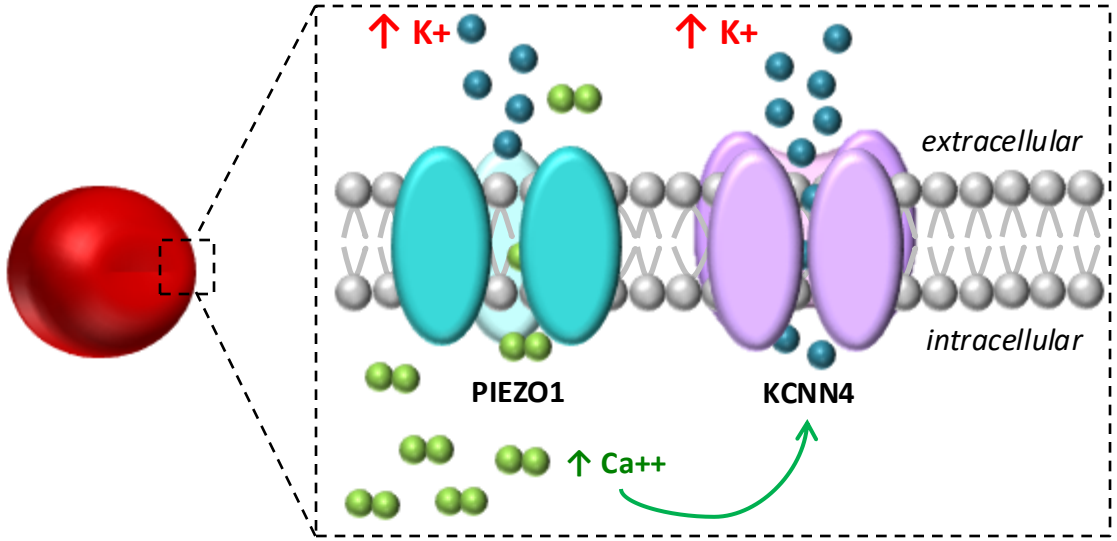
Gain-of-function (GoF) mutations in PIEZO1

- ✓ Several electrophysiology studies have 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)









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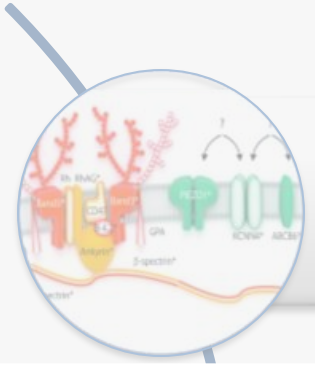
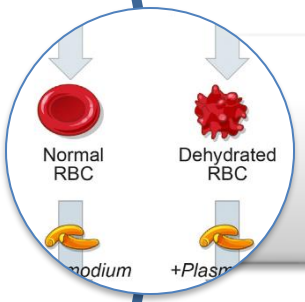
Andolfo et al., Haematologica, 2017; Rapetti-Mauss R. et al. Haematologica. 2017; Glogowska E., et al. Blood. 2017; Archer NM, et al. Am J Hematol. 2014; Shmukler BE. Et al. Blood Cells Mol Dis. 2014; Albuisson J. et al. Nat Commun. 2013; Bae C. et al. Proc Natl Acad Sci USA. 2013; Andolfo et al. Blood.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

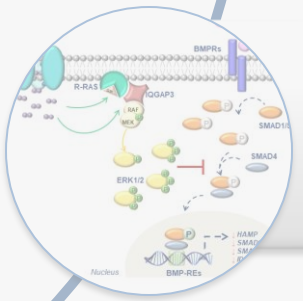
Outlines



***PIEZO1**: physiological role and pathogenetic mechanism of dehydrated hereditary stomatocytosis*

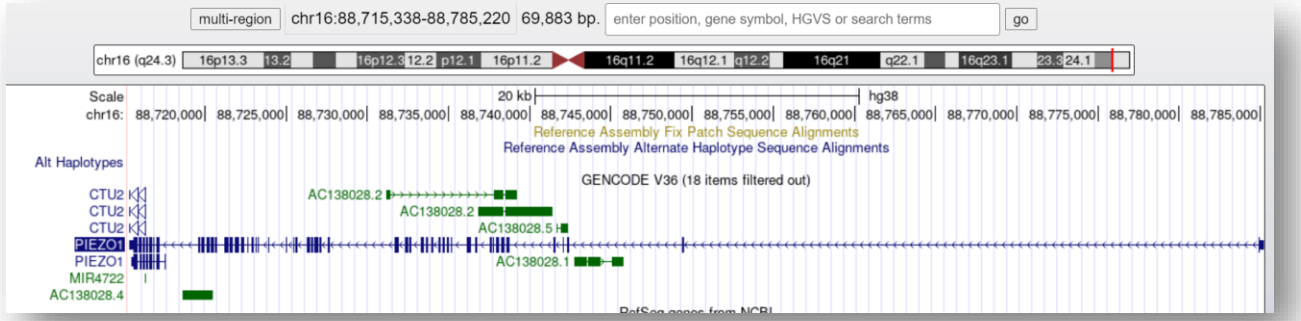


***PIEZO1**: molecular genetics*



***PIEZO1:** iron metabolism*

PIEZO1 gene



- ✓ Localized on Chr16
- ✓ 51 exons
- ✓ Transcript lenght: 8.089 bps
- ✓ Translation lenght: 2.521 residues
- ✓ **Two pseudogenes**
- ✓ Protein: 900KDa (trimer)

Constraint

Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	684.8	1086	Z = -12.05 o/e = 1.59 (1.51 - 1.67)
Missense	1527.4	1905	Z = -3.43 o/e = 1.25 (1.2 - 1.29)
pLoF	118.4	55	pLI = 0 o/e = 0.46 (0.37 - 0.58)

GnomAD v2.2.1: 76,156 genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies

- ✓ More missense and synonymouss variants than expetcted
- ✓ Among the 155 patients originally suspected of red blood cell defects ***PIEZO1*** is most the most mutated loci.
- ✓ The high frequencies of mutations in this gene is mainly related to its high genetic tolerance.

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Andolfo I, …, Iolascon A, Russo R. Genes 2021.

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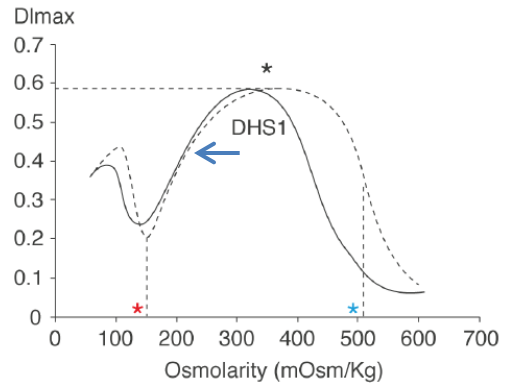
PIEZO1 VUS: reassessment of the pathogenicity

The American College of Medical Genetics and Genomics (ACMG)

Gene	HGVS Nomenclature		ACMG Rules †	Method	Class
PIEZO1	c.3935C>T	p.Ala1312Val		Automated	B
				Adjusted	LP
PIEZO1	c.4481A>C	p.Glu1494Ala		Automated	V
				Adjusted	LP
PIEZO1	c.5195C>T	p.Thr1732Met		Automated	B
				Adjusted	V
PIEZO1	c.5835C>G	p.Phe1945Leu		Automated	V
				Adjusted	LP
PIEZO1	c.5981C>G	p.Ser1994Cys		Automated	V
				Adjusted	LP
PIEZO1	c.6205G>A	p.Val2069Met		Automated	V
				Adjusted	LP

- ✓ The high frequencies of mutations in PIEZO1 is mainly related to its high **genic tolerance**
- ✓ Accordingly, most of the variants in PIEZO1 were originally predicted as variants of **uncertain significance (VUS) or likely benign**
- ✓ The reevaluation of **PIEZO1 pathogenic variants by ACMG rules** demonstrated that 26/35 (74%) and 17/35 (48.6%) PIEZO1 variants were predicted as VUS by InterVar and Varsome tools, respectively
- ✓ 72% (31/43) reclassified variants



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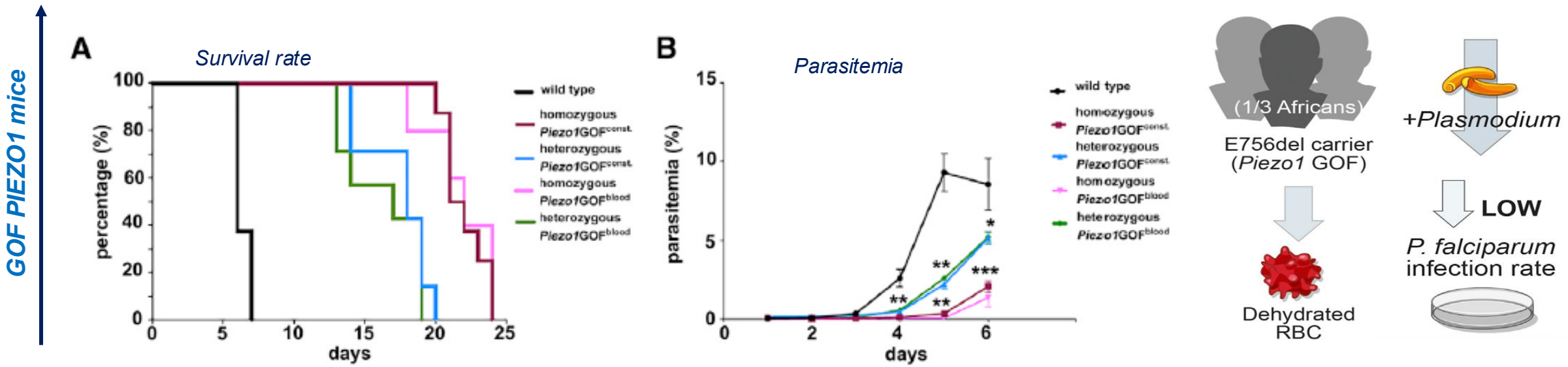
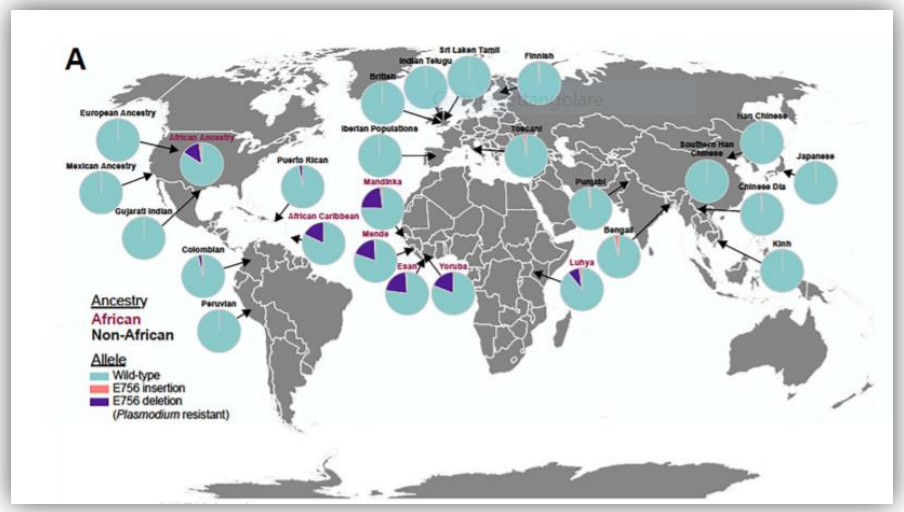
Piezo1 GOF mutations attenuate Plasmodium infection



- ✓ 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.



Shang Ma et al, Cell 2018

Diagnostic workflow of DHS

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

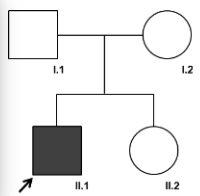
MCV, MCHC, Ret, hemolytic markers

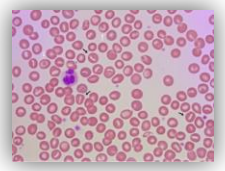
PB smear: stomatocytes (variable degree: 5-20% DHS)

AD transmission

Complete Blood Count

Analyte	Result	Normal range
Red cell count	5.5 x 10^{12}/L	4.5 – 5.7
White cell count	9.8 x 10^{9}/L	4.0 – 10.0
Hemoglobin	123g/L	133 – 167
Hematocrit	0.42	0.35 – 0.53
MCV	76fL	77 – 98
MCH	22.4pg	26 – 33
MCHC	293pg/L	330 – 370
RDW	14.5%	10.3 – 15.3

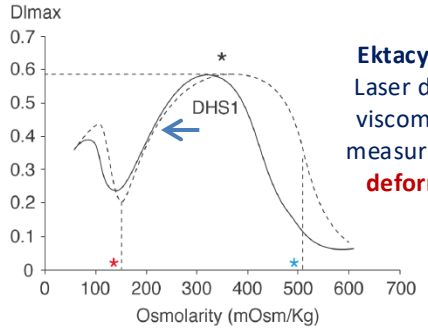




Second-line investigations:
1. Osmotic fragility (OF), AGLT50, Pink, EMA tests
2. Ektacytometry

Osmotic resistance: increased EMA test: normal

Ektacytometry: Left shift



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

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

Molecular analysis: *single gene*

t-NGS panel or WES (RedPanel: 125 genes)

RedPanel: 125-genes targeted-NGS panel for hereditary RBC defects:
(i) red blood cell membrane defects;
(ii) congenital dyserythropoietic anemias;
(iii) Diamond-Blackfan anemia;
(iv) enzymatic defects;
(v) iron deficiency anemias;
(vi) hemochromatosis;
(vii) sideroblastic anemias;
(viii) erythrocytosis

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Russo et al 2018; Andolfo et al, 2018 and unpublished data

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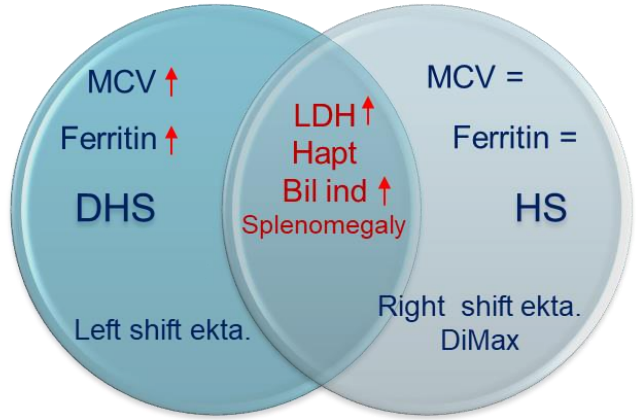
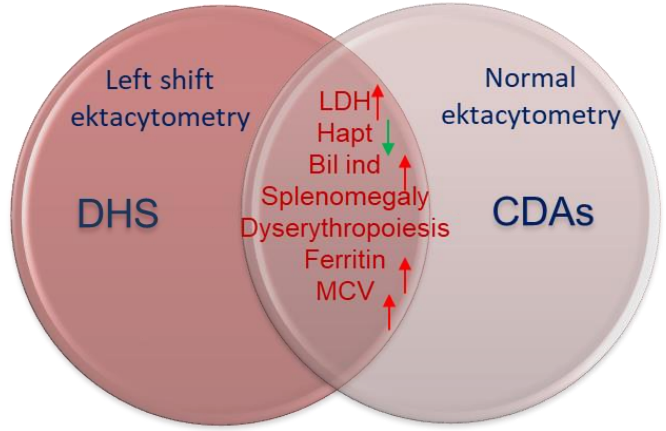
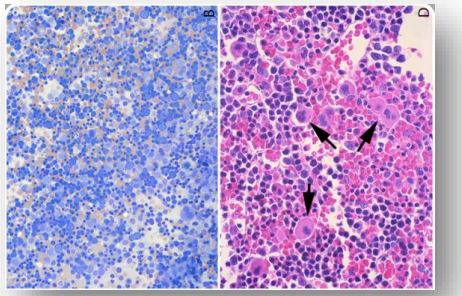
Differential diagnosis of DHS

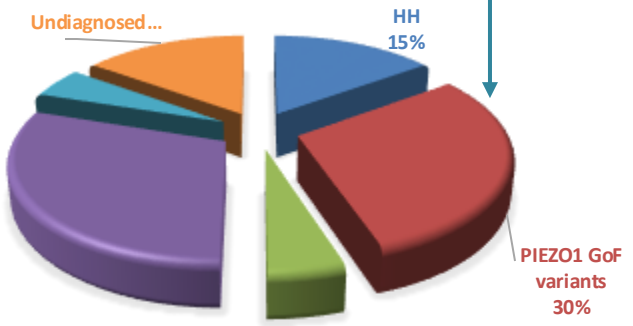
- ✓ Dehydrated Hereditary Stomatocytosis (DHS) is often **misdiagnosed** with Hereditary spherocytosis (HS), and Congenital Dyserythropoietic Anemias (CDAI/II)
- ✓ In several cases DHS can also be misdiagnosed as **hereditary hemochromatosis**
- ✓ It is important to evaluate the possible **co-inheritance of other genetic traits** that could account for variability of the phenotype observed or the presence of multi-locus inheritance
- ✓ The **genetic analysis** is crucial also to avoid not useful treatments as for example splenectomy





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





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Coinheritance of PIEZO1 mutations and beta-thalassemia trait

20 symptomatic BT

Heterozygous subjects for *HBB* gene mutations in absence of *HBA* pathogenic SNV/CNV with anemia, splenomegaly, and alteration of hemolytic indices

Evaluation of the clinical phenotype clear demonstrated the worsening of the phenotype in the **20 symptomatic BT compared to 53 asymptomatic BT**

Evaluation of family history, peripheral blood smear, ektacytometry curve, and t-NGS panel for hereditary RBC defects in the **20 symptomatic BT**

15/20 symptomatic BT resulted also affected by **RBC membrane defects** mainly DHS caused by ***PIEZO1*** alterations

The analysis of the present cohort of patients demonstrated that the clinical phenotype was more severe for patients with BT and DHS or multi-locus inheritance compared to asymptomatic BT in terms of RBCs, Hb, splenomegaly, iron balance, and hemolytic indices

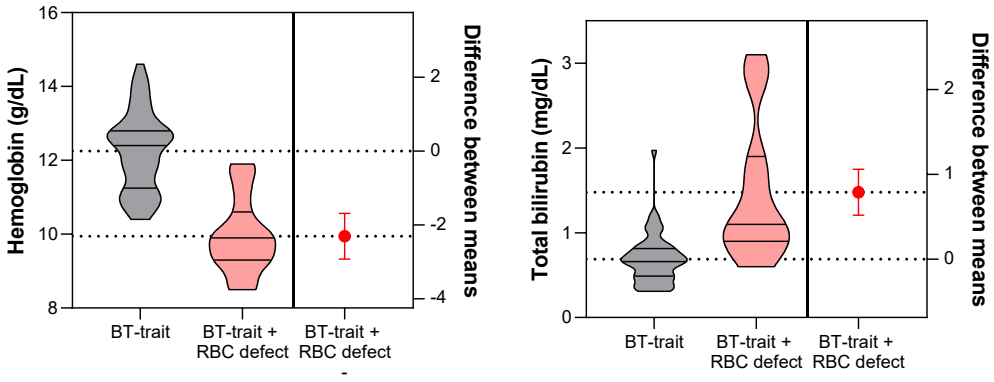

Table S2. Laboratory data of patients with asymptomatic BT and symptomatic BT with causative variants in RBC defects

	Unit	Asymptomatic BT n = 53	Symptomatic BT/RBC defects n = 15*	*P1*	*P2*
Gender	male/female	34 (0.64)/19 (0.36)	6 (0.46)/7 (0.54)	ns	-
Age	years	33.0; 37.1 ± 16.6	38.0; 38.9 ± 17.6	ns	ns
RBC	× 10^6/µL	6.1; 6.1 ± 0.6	5.2; 5.0 ± 0.9	<0.0001	<0.0001
Hb	g/dL	12.4; 12.3 ± 1.1	9.9; 9.9 ± 1.0	<0.0001	<0.0001
MCV	fL	63.5; 63.3 ± 3.2	61.7; 63.2 ± 8.0	ns	ns
MCH	pg	20.1; 20.2 ± 1.2	20.1; 20.4 ± 2.9	ns	ns
MCHC	g/dL	31.8; 31.9 ± 0.9	32.3; 32.3 ± 1.1	ns	ns
Retics	%	1.2; 1.5 ± 0.7 (n=11)	2.1; 3.0 ± 2.9 (n=14)	0.10	0.02
Tb	mg/dL	0.7; 0.7 ± 0.3	1.1; 1.5 ± 0.8	<0.0001	<0.0001
LDH	U/L	160.0; 172.5 ± 44.4 (n=52)	202.0; 199 ± 35.5 (n=13)	0.05	0.01
Haptoglobin	mg/dL	86.7; 92.2 ± 41.8 (n=52)	18.0; 21.1 ± 22.5 (n=14)	<0.0001	<0.0001
Ferritin	ng/mL	167.5; 193.4 ± 147.2 (n=52)	181.0; 310.0 ± 332.1	0.05	0.30
Ferritin/age	-	4.9; 5.5 ± 4.0 (n=52)	4.8; 9.7 ± 13.1	0.05	0.52

Data are median; average ± standard deviation
RBC, red blood cells; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, mean corpuscular hemoglobin concentration; Tb, total bilirubin; LDH, lactate dehydrogenase
P1, Unpaired t-test for quantitative variables; Chi-square test for quantitative variables.
P2, Mann Whitney test for quantitative variables.
*The remaining 5 symptomatic BT-trait patients negative for the t-NGS panel of RBC defects were excluded from this analysis.

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V.M.Pinto, R. Russo*, …, A. Iolascon, GL.Forni, I. Andolfo. AJH 2023*

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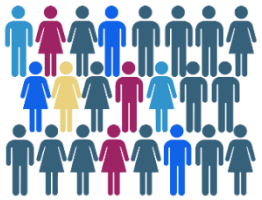
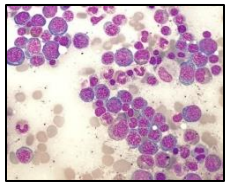
PIEZO1 mutations impact on early clinical manifestation of MDS

"young" patients with chronic anemia
< 60 years



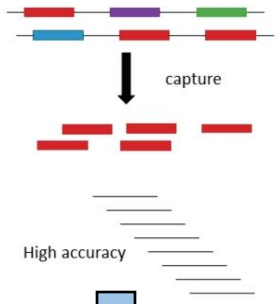
BM evaluation



21 MDS cases

DNA from PB and saliva

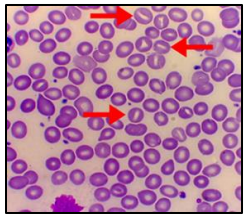
Whole exome sequencing



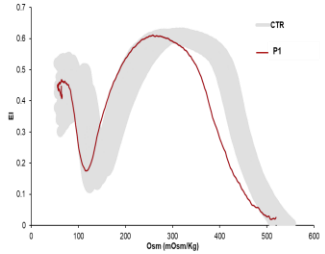
24% of MDS patients carried germline pathogenetic variants in *PIEZO1* gene

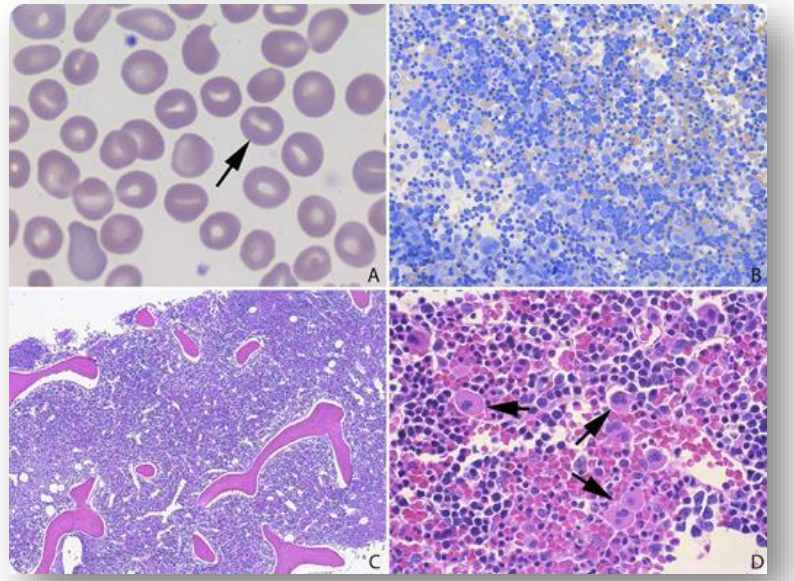
RBC study on patients and relatives

- **morphology by PB**



- **ektacytometry**





- ✓ Patient with diagnosis of **myelodysplastic syndrome (MDS)** based on a history of iron overload and bone marrow biopsy findings of a **hypercellular marrow with erythroid hyperplasia**
- ✓ Whole-exome sequencing revealed a pathogenic germ line mutation in ***PIEZO1***, **c.6239_6256dup18**, consistent with the diagnosis of dehydrated hereditary stomatocytosis

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E. Attardi, I. Andolfo*, R. Russo, ... Iolascon, and V. Santini. AJH 2023.*
Paessler M, Hartung H. Blood. 2015

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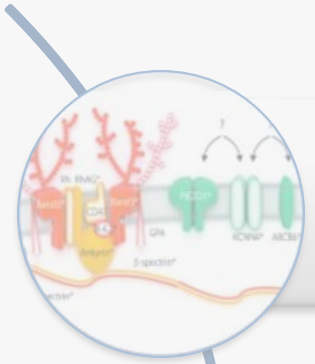
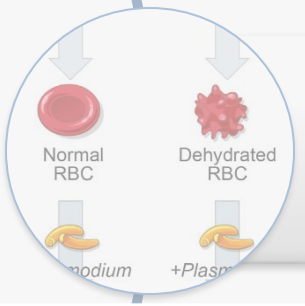
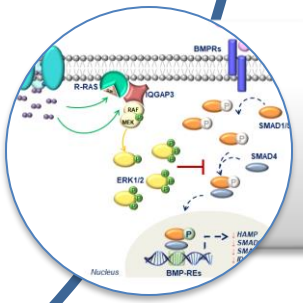
Outlines



***PIEZO1**: physiological role and pathogenetic mechanism of dehydrated hereditary stomatocytosis*



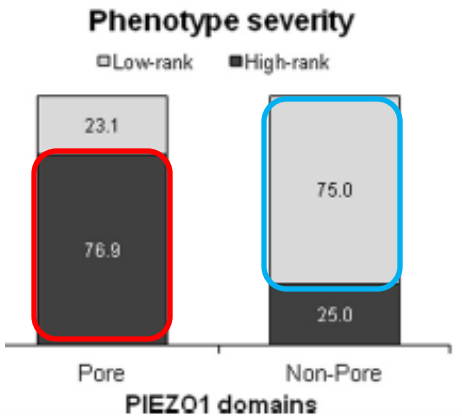
***PIEZO1**: molecular genetics*

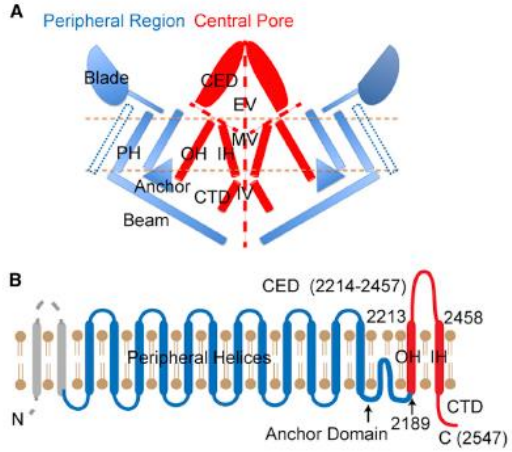


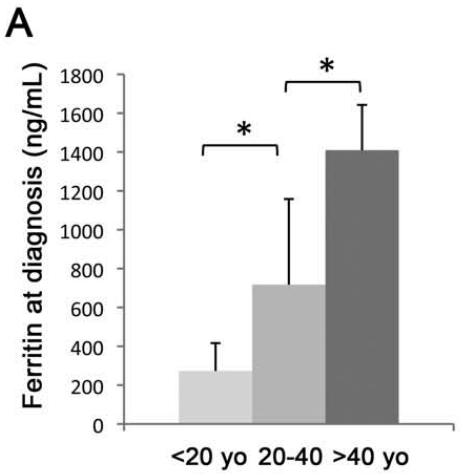
***PIEZO1:** iron metabolism*

Genotype-phenotype correlation and hepatic iron overload in DHS

DHS patients	High-rank (n = 54)	Low-rank (n = 65)	P§
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







- ✓ Hepatic iron overload is **independent from the degree of anemia, and the transfusion regimen**
- ✓ **Severe iron overload** with several cases of hepatosiderosis has been described for *PIEZO1* patients
- ✓ Genotype-phenotype correlation on **123** patients with DHS demonstrated that most of the patients with a **severe phenotype** (mostly with impaired iron balance) carry mutations in the **pore domain**, while most of the patients with **mild phenotype** exhibit variants in the **non-pore domain**

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

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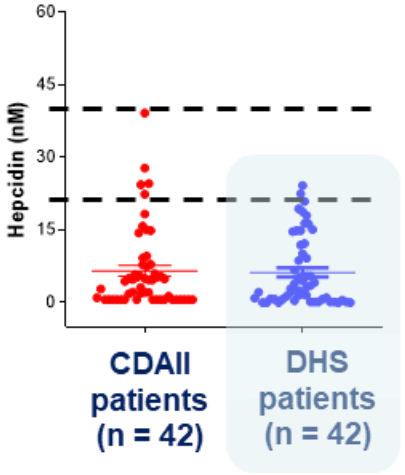
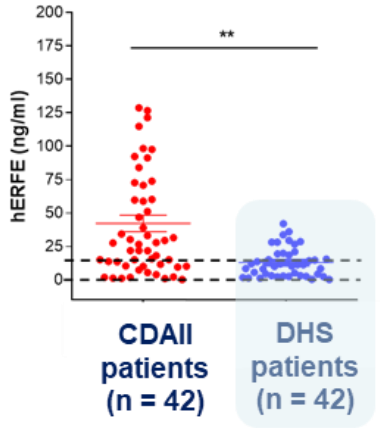
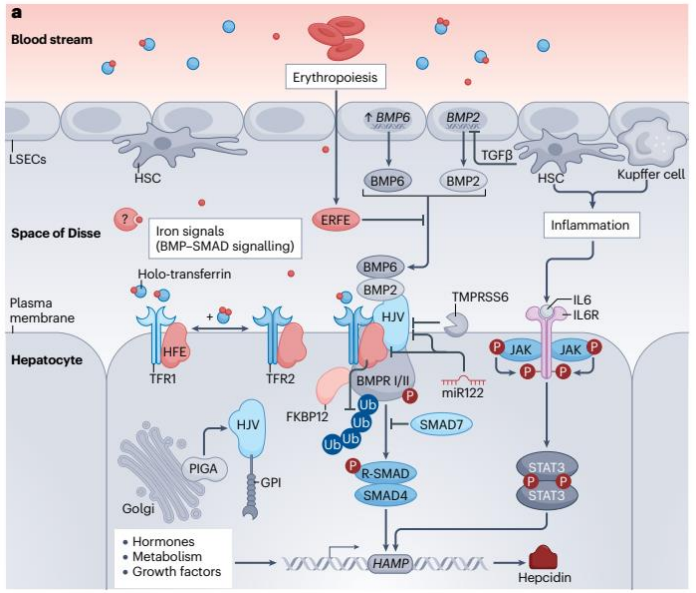
Hepcidin and ERFE dosage in DHS patients



✓ Hepcidin resulted highly reduced in DHS patients compared to controls.



✓ Erythroferrone (ERFE), the only known erythroid regulator of hepcidin suppression, showed a slight increased level in DHS compared to controls.

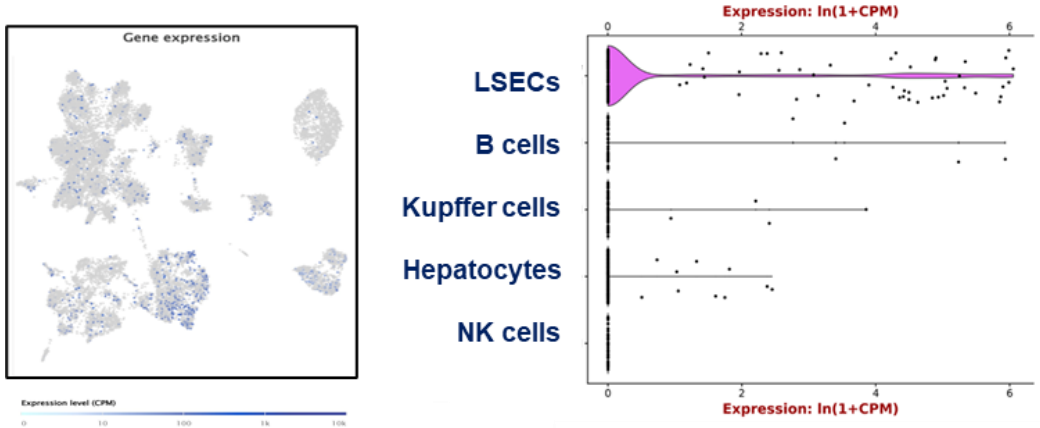


Galy B, Conrad M, Muckenthaler M. Nat Rev Mol Cell Biol. 2024

Could be a specific role of PIEZO1 at hepatic level?

Andolfo et al AJH 2020

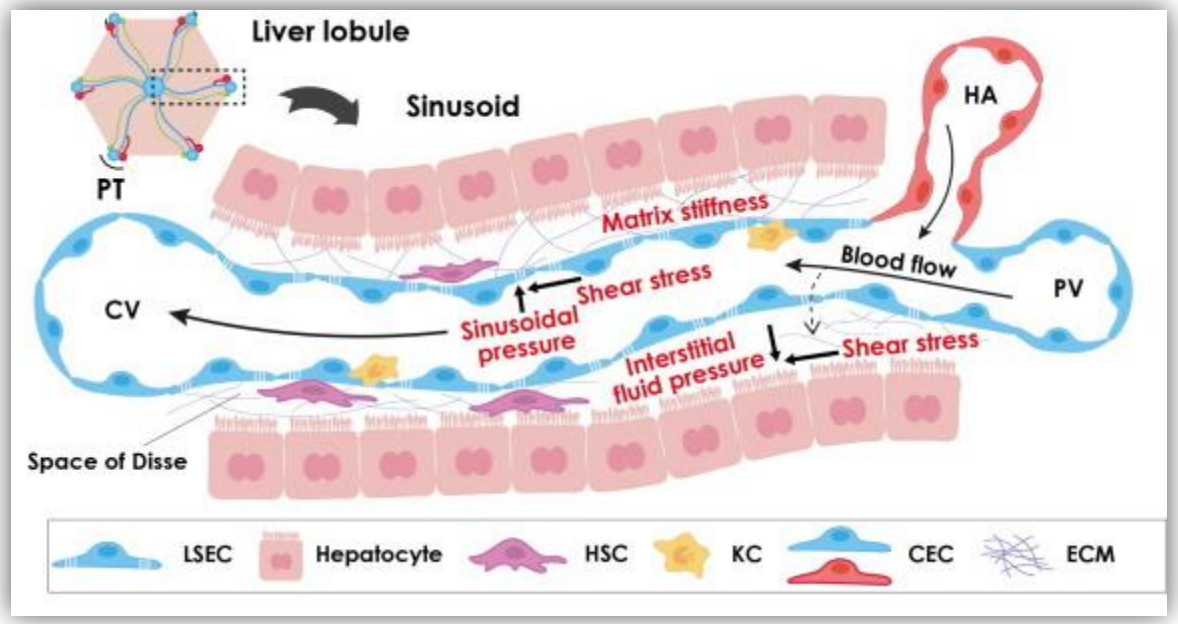
PIEZO1 and mechanotransduction in the liver



Human and murine livers

- ✓ The liver is located in a complicated **mechanical microenvironment** (tissue stiffness, shear flow and hydrostatic pressure) that is crucial for maintaining physiological homeostasis.
- ✓ Liver resident cells, especially **hepatocytes, liver sinusoidal endothelial cells (LSECs),** and **hepatic stellate cells (HSCs),** are all sensitive to mechanical forces, and able to alter their behaviors and functions through mechanotransduction pathways

- ✓ **Piezo1** is **highly expressed** in the different cell types of the liver in both human and mice



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Ning Li et al. Advanced Drug Delivery Reviews. 2022

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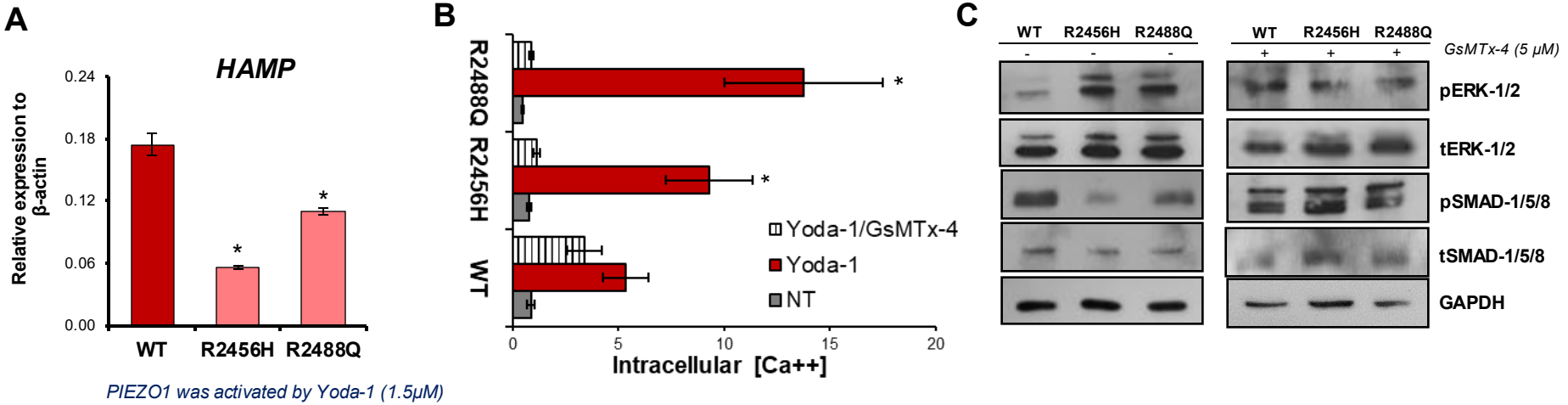
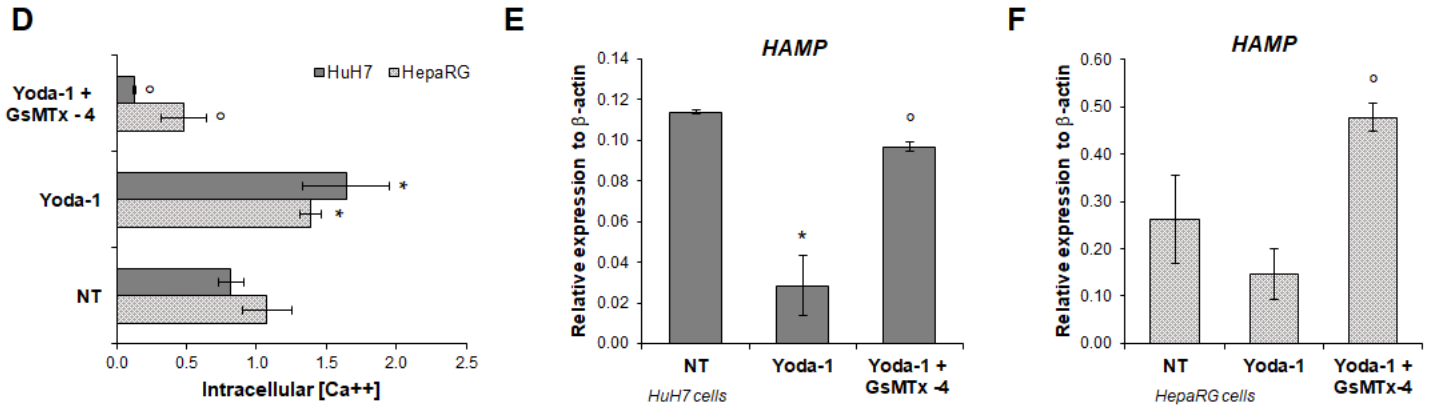
Impaired BMP-SMADs pathway in PIEZO1-GoF mutants



PIEZO1 was activated by Yoda-1 (1.5μM)

PIEZO1 GoF mutants showed:

- ✓ decreased *HAMP* gene expression.
- ✓ increased intracellular calcium concentration
- ✓ increased phosphorylation of ERK1/2 and inhibition of BMP-SMADs pathway



Primary hepatocytes

- ✓ PIEZO1 activation, at physiological level, increased calcium concentration and inhibit *HAMP* gene expression in primary hepatocytes.

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Andolfo et al., AJH 2020.

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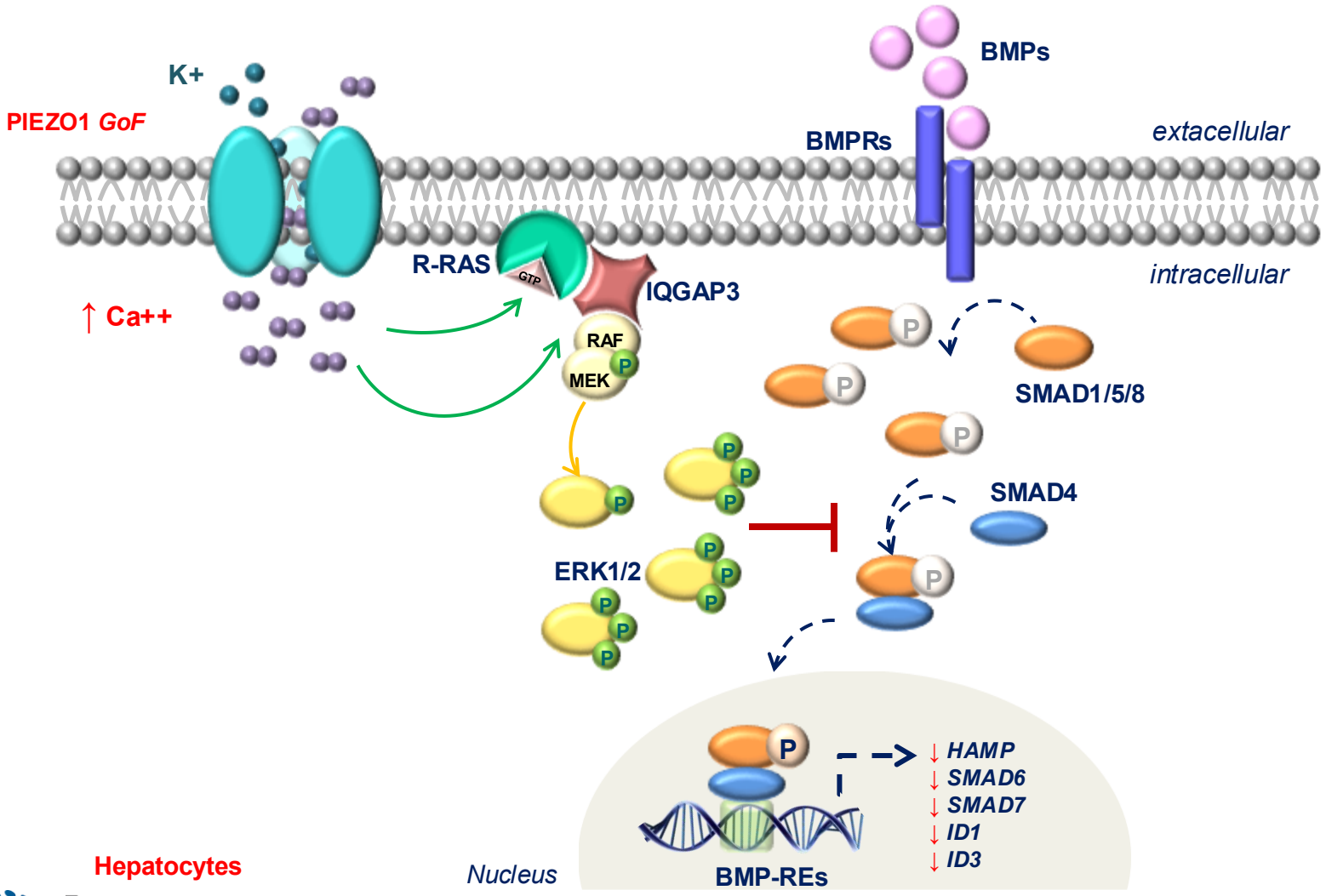
PIEZO1 and regulation of *HAMP* gene expression



- ✓ Engineered KI hepatic PIEZO1 cells showed alterations in several genes/proteins belonging to **MAPK** pathways, and revealed new genes/proteins linked to the increased **calcium** concentration and to the activation of the intracellular pathways as **TGF-beta** and **R-RAS**
- ✓ The activation of **ERK1/2** pathway leads to **BMP/SMADs** inhibition
- ✓ The inhibition of BMP/SMAD pathway impair ***HAMP*** gene transcription
- ✓ The new identified players of intracellular signaling pathways could be future druggable targets

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Rosato, Marra, D'Onofrio, Lasorsa, Capasso, Russo, Iolascon and Andolfo, in submission

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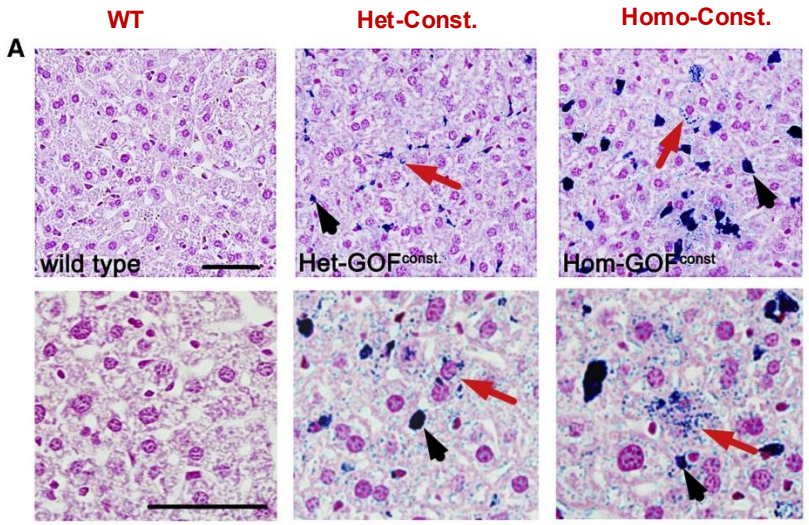
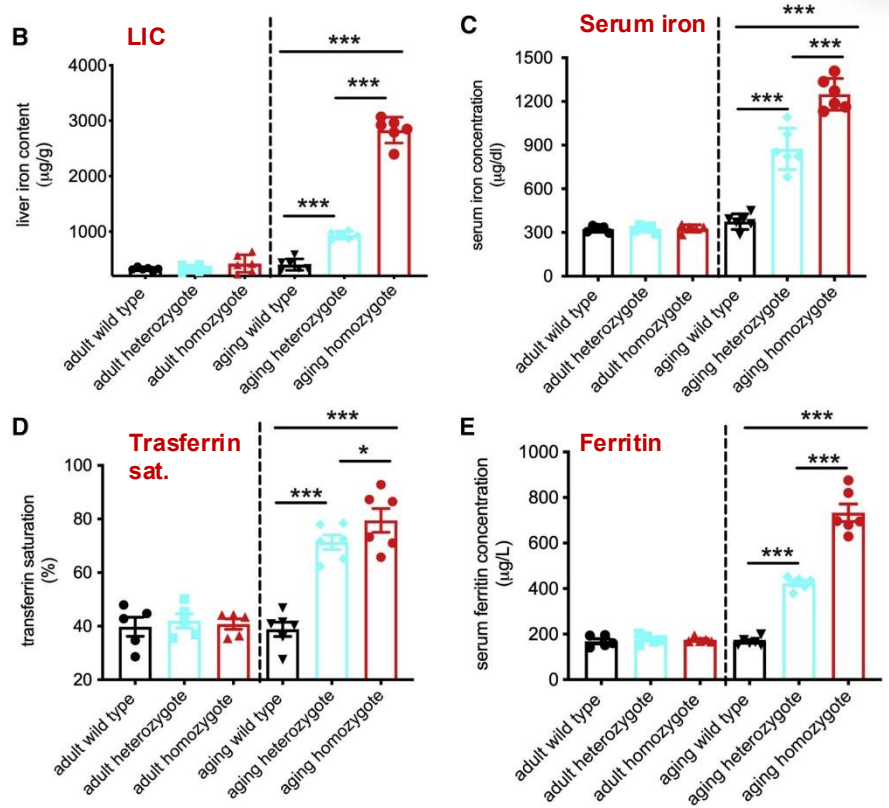
Constitutive GoF Piezo1 mice developed age-onset iron overload



Perls Prussian blue staining in 10-mm paraffin sections of livers. Blue color represents iron staining in hepatocytes (red arrow) and Kupffer cells (black arrowhead).

- ✓ **Constitutive heterozygous GOF Piezo1** aging mice (over 1 year old) developed **iron overload.**

Piezo1 GOF



- ✓ Iron deposition was **more severe in homozygous** GoF Piezo1 mice than in heterozygous mice.
- ✓ Both the heterozygous and homozygous mice show alterations of iron parameters such as liver iron concentration **(LIC), serum iron, transferrin saturation and ferritin levels.**

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S. Ma, A. E. Dubin, …, I. Andolfo, A. Patapoutian. Cell, 2021.

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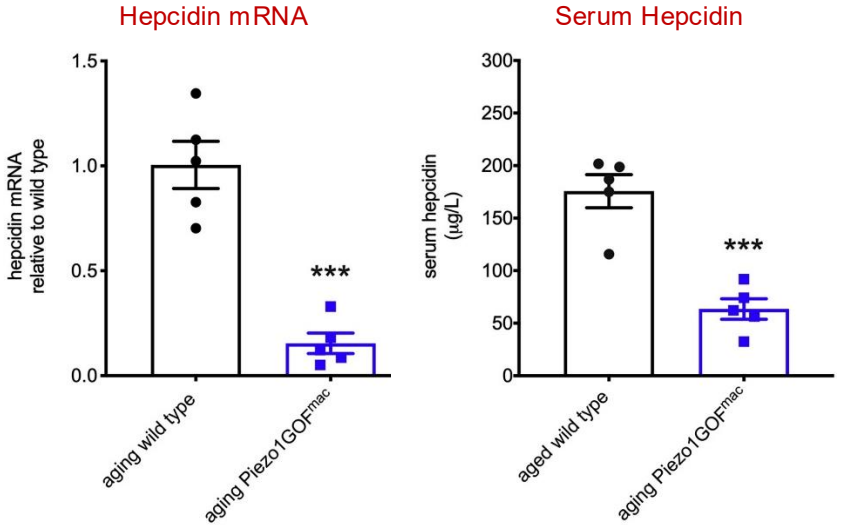
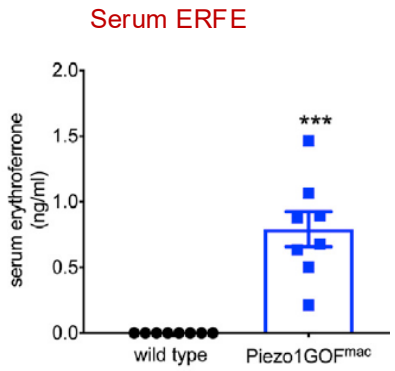
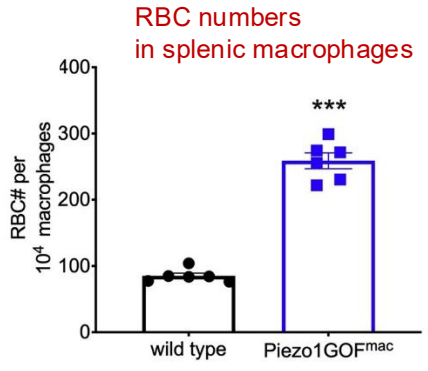
Hepcidin level was decreased in macrophage-specific GoF Piezo1 mice







- ✓ Macrophage-specific expression of a GoF Piezo1 allele showed dramatically reduced hepcidin mRNA and serum levels in aging mice compared to Wt ones.
- ✓ Erythroferrone (ERFE) levels were significantly increased in adult macrophage-specific GoF Piezo1 mice compared to wild-type mice.
- ✓ The *in vivo* RBC turnover analysis indicates that macrophages with overactive PIEZO1 recycle more RBCs over a given time period.
- ✓ Macrophages with overactive PIEZO1 enhance erythropoiesis and increase erythroferrone to reduce hepcidin expression.
- ✓ PIEZO1 is a key regulator of macrophage phagocytic activity and subsequent erythrocyte turnover.

Piezo1 GOF

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S. Ma, A. E. Dubin, …, I. Andolfo, A. Patapoutian. Cell, 2021.

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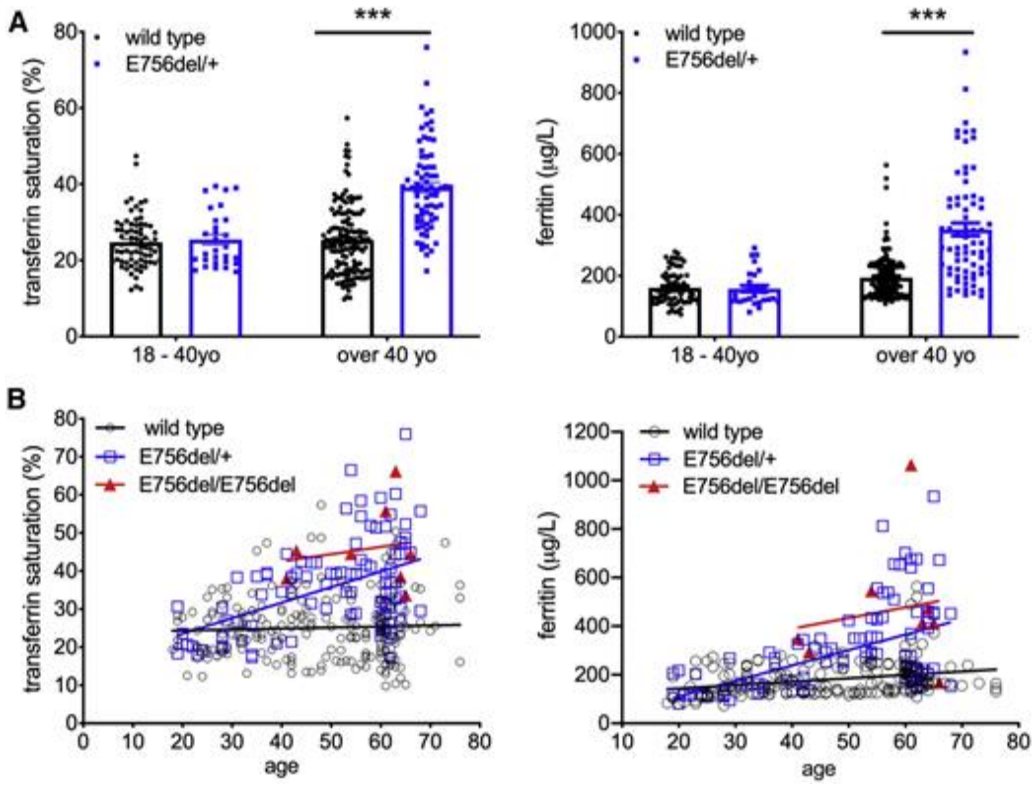
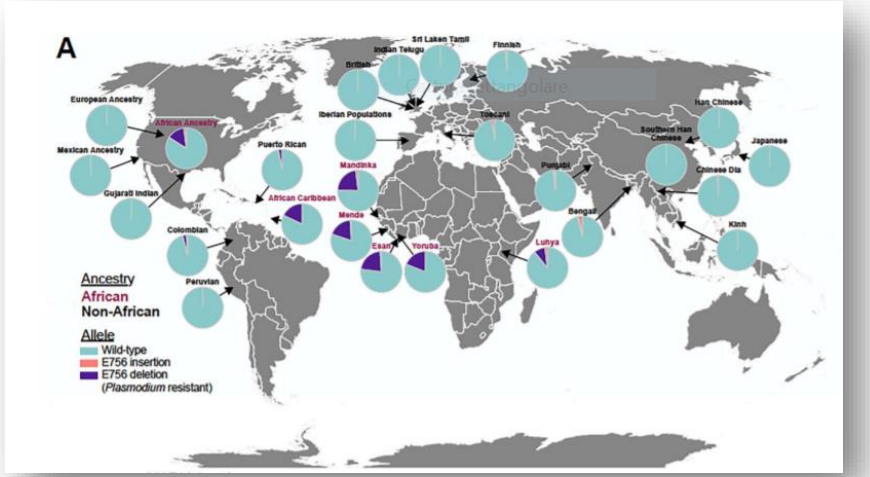
E756del GoF PIEZO1 allele causes iron overload



- ✓ **GOF PIEZO1 allele (E756del)** is a mild allele common in individuals of African descent and previously demonstrated to cause mild DHS. Thus, an estimated up to one-third of people of west African descent carry one or two copies of this allele.
- ✓ E756del heterozygous individuals over 40 years old had a statistically significant increase in transferrin saturation and ferritin concentrations compared to noncarriers within the same age group.
- ✓ We observed a clear positive correlation between age and transferrin saturation/ferritin concentration in heterozygous E756del carriers, but not in noncarriers.



S. Ma, A. E. Dubin, …, I. Andolfo, A. Patapoutian. Cell, 2021.

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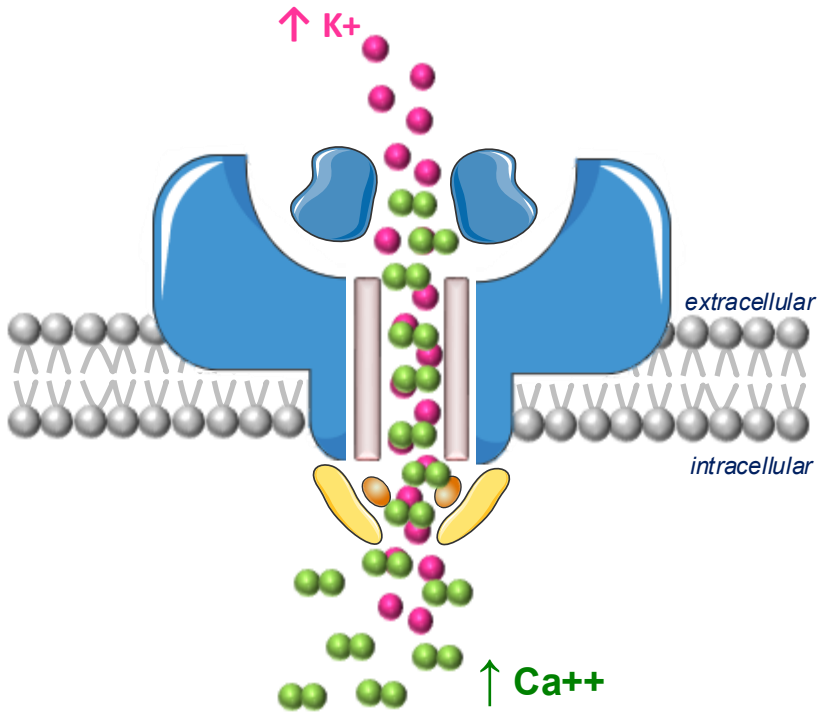
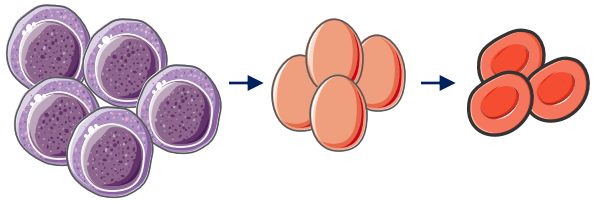
PIEZO1 and DHS pleiotropic syndrome

GoF variants in PIEZO1



Red blood cell



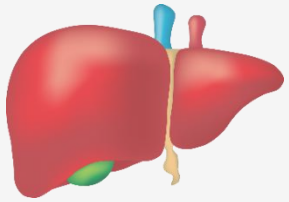
Erythroblasts Reticulocytes Erythrocytes

Platelet

Altered deformability
Dehydration
Resistance to malaria

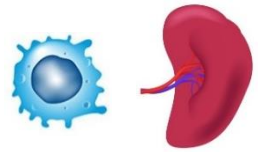
Impaired terminal erythroid differentiation

Increased thrombotic events after splenectomy



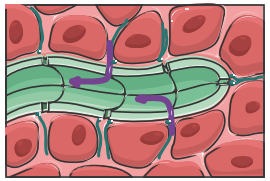
Liver

Hepatic iron overload



Macrophage Spleen

Increased erythrophagocytosis



Lymphatic vessels

Lymphedema

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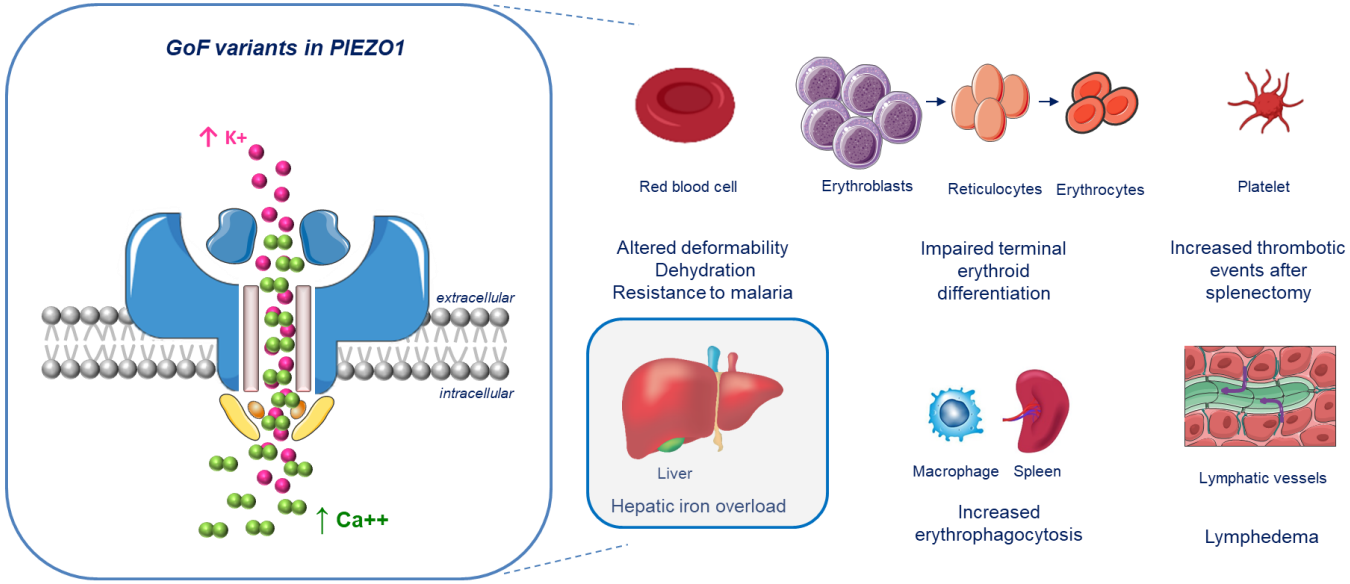
Take home messages

- ✓ PIEZO1 is a mechanoreceptor which plays an important physiological role in several biological processes.
- ✓ Gain-of-function mutations in *PIEZO1* are associated with dehydrated hereditary stomatocytosis, a hereditary hemolytic anemia characterized by severe hepatic iron overload, and several other phenotypes.
- ✓ Gain-of-function mutations in *PIEZO1* directly impair hepatic iron metabolism via the inhibition of the BMP/SMADs pathway.
- ✓ Constitutive and macrophages expression of a gain-of-function *Piezo1* variant in mice induces iron overload.
- ✓ E756del, a mild GoF PIEZO1 allele present in one-third of individuals of African descent, is strongly associated with alteration of iron parameters (relevance to larger population).
- ✓ **Our studies linked mechanotransduction to iron metabolism and identified in *PIEZO1* a genetic risk factor for increased iron levels.**



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