Thursdays Webinars

EuroBloodNet

Diagnosis and clinical management of hereditary stomatocytosis

Pr Loic Garçon, Hematology department, CHU Amiens
Dr Véronique Picard, Red cell reference Lab, CHU Bicêtre

ERN-EuroBloodNet Hematological Diseases
Amiens / le Kremlin Bicêtre – France
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Co-funded by the Health Programme of the European Union

European Reference Network for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

When should you think of stomatocytosis ?

Mr X., 37 y.o

- originates from North of France
- Follow up since age 20 for chronic hemolysis , hepatosplenomegaly
- **History**
  - jaundice, asthenia
  - splenomegaly (3 travers de doigt)
  - hepatomegaly moderate
  - no arthralgia, no portal hypertension
  - no other medical, chirurgical history
- **Family history**
  - parents, one brother, one sister, one 3yo son are healthy
  - Wife : One medical interruption of pregnancy at 28 GW for anasarca (2nd child)
    - ascite 500 ml (décelé à 24SA), discret épanchement péricardique,
    - oedeme ss cutané (nuque)
      malformation (disproportion tronc/membres)
      hématome basal du placenta
      Absence de lésion macroscopique
      Pas de malformation
      Caryotype normal
      Bilan infectieux negatif
      Absence d'argument pour maladie de surcharge

Mr X.

Referred to hematology department

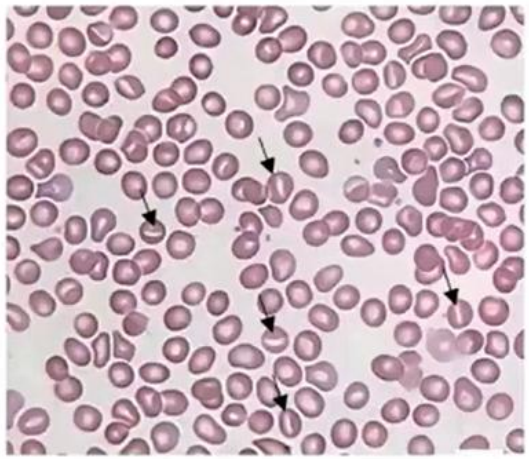
- Hb 13.8 g/dL
- MCV 106 fL
- MCHC 37%
- Reticulocytes 243 G/L

- Bilirubin 43 umol/L
- Haptoglobin 0.08 g/L,

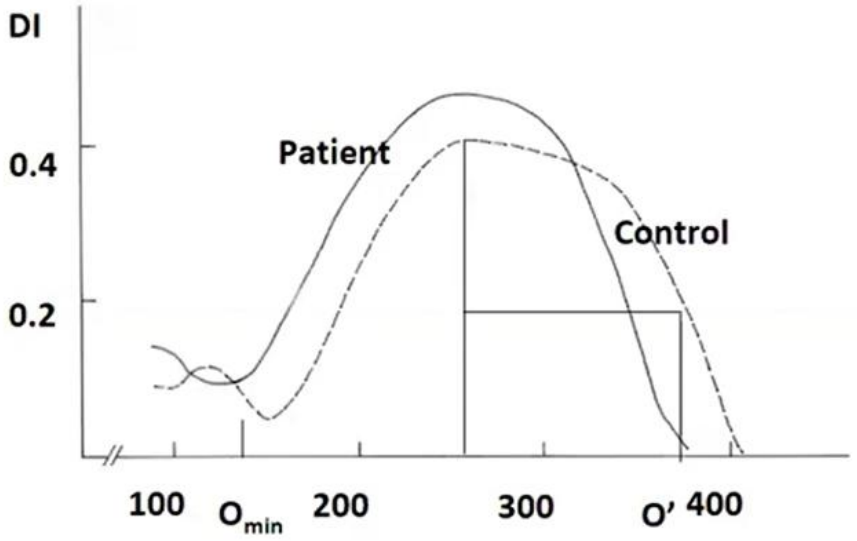
- K^+ 6.7 mmol/L
- controlled at 37°C 4.7mmol/L

- DAT neg.
- PK, G6PD, Hb study N[al]
- Ektacytometry :

→ diagnosis of dehydrated stomatocytosis



Few target cells and stomatocytes

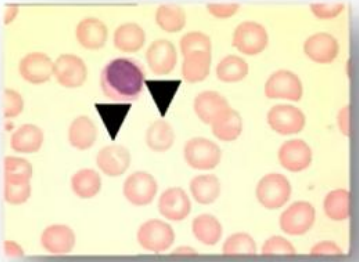


Hereditary Stomatocytosis : Definition

Heterogeneous group of hereditary red cell disorders characterized by
abnormal red cell membrane ion permeability as a primary defect

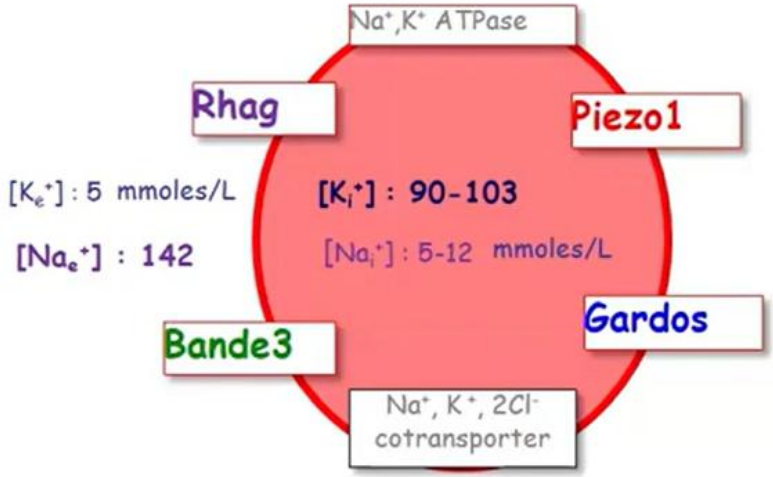
-> abnormal red cell morphology on blood film : stomatocytes
-> shortened red cell half life -> chronic hemolysis +/-
-> other symptoms according to the gene involved

NB : acquired stomatocytes are not treated here



Classification

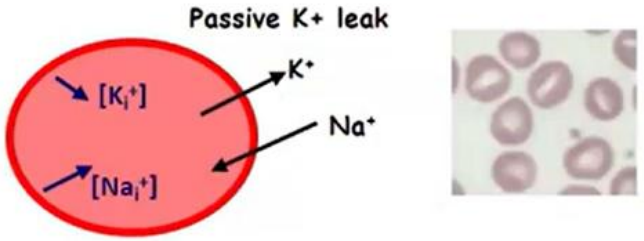
- **Dehydrated Stomatocytosis**
  - **DHS1 PIEZO1, most frequent = xerocytosis**
  - **DHS2 KCNN4 Gardos Channel**
  - SLC4A1 Bande 3 (rare)
- Overhydrated Stomatocytosis (OHS) very rare and severe
  - RHAG RH related antigen
- *Related but distinct disorders :*
  - *–South east Asian Ovalocytose (SAO)*
  - *–sitosterolemias*



Dehydrated Hereditary Stomatocytosis : history

60-70's : **Phenotype :**

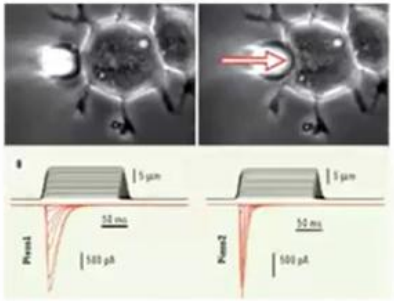
Description of **rare dominant hereditary hemolytic anemia with stomatocytes and unbalanced intracellular cations** suggesting altered membrane cations permeability



2012-13 : **Genotype**

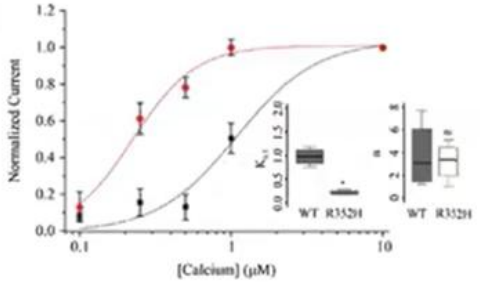
Gain of function mutations in the mecanotransducer cation channel ***PIEZO1*** cause DHS

(Zarychanski et al, Blood, 2012, Andolfo et al, Blood 2013, Albuisson et al, Nat Com, 2013)



2015 : Mutations in ***KCNN4*** encoding a potassium calcium-activated channel ' Gardos channel ' cause another form of DHS

(*Rapetti Mauss et al, Blood, 2015, Glogowska, Blood 2015)*



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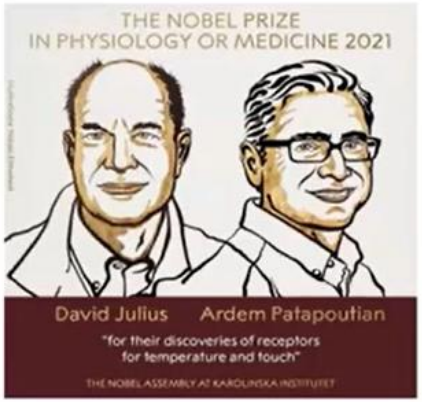
Discovery of Piezo 1

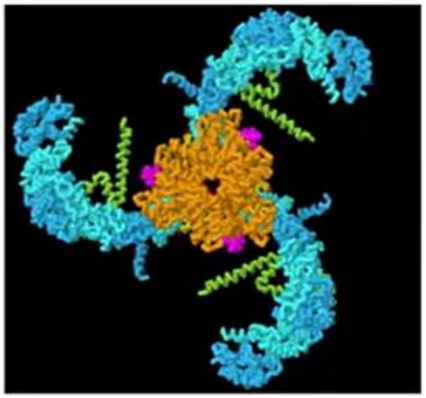
(Coste et al, Science, 2010, Nature 2012)

Discover the **first ion channel activated by mechanic pressure = mechanotransducer in mammals** (lignée neuroblastome de souris neuro 2A)

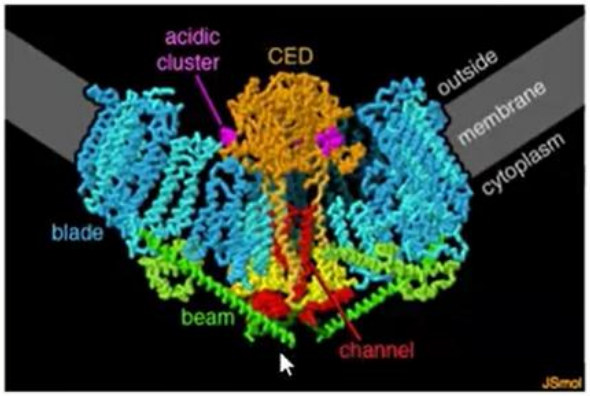
- no sequence or structural homology with other proteins
- highly conserved in eucaryotes (present in plants, invertebrates, protozoaires)
- Piezo 1 is a non specific **cation channel**
- low tissue specificity : in humans, RNA is expressed in lungs, colon, liver, bladder, bone marrow, weak in the nervous system



2021 Nobel Prize in Physiology or Medicine



trimer, 3 x 2521 aminoacids



pdb.101.rcsb.org

July 2018, David Goodsell

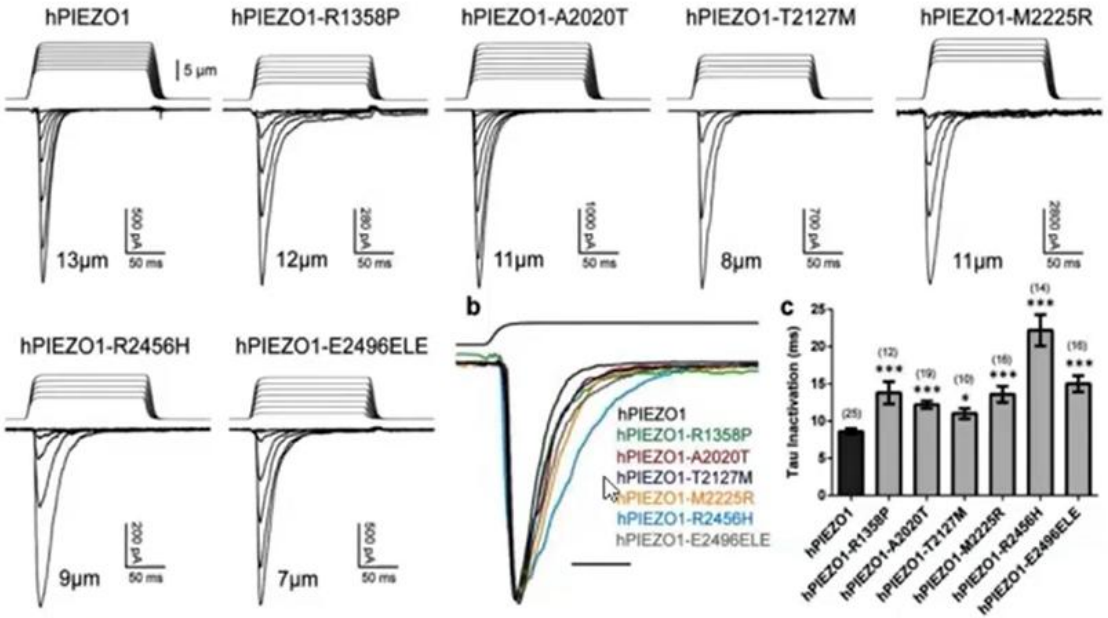
doi:10.2210/rcsb_pdb/mom_2018_7

DHS1 is caused by gain of function PIEZO1 mutations

Nat Commun. 2013; 4: 1884.
doi: 10.1038/ncomms2899
PMID: 23695678

Dehydrated Hereditary Stomatocytosislinked to gain-of-function mutations in mechanically activated PIEZO1 ion channels

Juliette Albuisson, Swetha E. Murthy, Michael Bandell, Bertrand Coste, Hélène Louis-dit-Picard, Jayanti Mathur, Madeleine Fénéant-Thibault, Gérard Tertian, Jean-Pierre de Jaureguiberry, Pierre-Yves Syfuss, Stuart Cahalan, Loic Garçon, Fabienne Toutain, Pierre Simon Rohrlich, Jean Delaunay, Véronique Picard, Xavier Jeunemaitre, and Ardem Patapoutian



Recombinant hPIEZO1 channels with DHS-associated mutations display slow inactivation kinetics

(a)Representative traces of mechanically activated inward currents recorded at −80 mV from HEK293T cells expressing either WT or indicated mutant hPIEZO1. Cells were stimulated by a series of mechanical steps (150 ms duration) in 1 µm

PIEZO1 Stomatocytosis

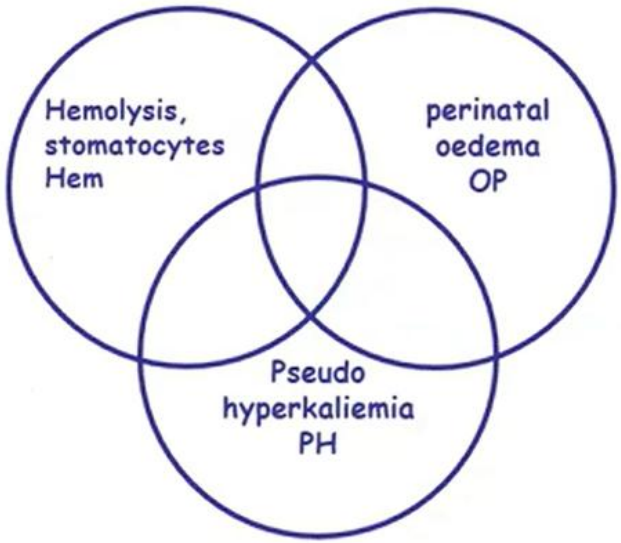
- Rare genetic disease : about 1/50 000 birth , mostly subjects from European descent
- Dehydrated form = xerocytosis = DHS1
- Transmission : dominant in most cases, *de novo* mutations not so rare
- **heterogeneous clinical expression even** in the same family :

1998: **pleiotropic syndrome** (**MIM #603528)** *a genetic syndrome associating*

hemolysis with stomatocytes,

pseudohyperkalaemia

perinatal oedema



- Hem
- Hem + OP
- Hem + OP + PH
- PH only (asymptomatic)
- OP seuls ???

Biological diagnosis : hematological features

- Retrospective study of **126 patients (56 index) with a *PIEZO1* (49 index) or *KCNN4* (7 index) DHS** diagnosed in the hematology lab of Bicêtre Hospital between 1993 and 2017

- Patients originated from all parts of France

- investigated for : undiagnosed chronic hemolysis / perinatal edema / iron overload
- DAT negative, no Hb or enzyme defect explaining their symptoms

adressed to search for red cell membrane disease

Diagnosis strategy

1. Red cell count on ADVIA 2120 ® analyser
2. Red cell morphology after MGG staining
3. EMA test (spherocytosis screening) always **normal**
4. Osmolar gradient ektacytometry
5. Gene analysis (50 genes) including most membrane red cell genes (NGS)

} diagnosis

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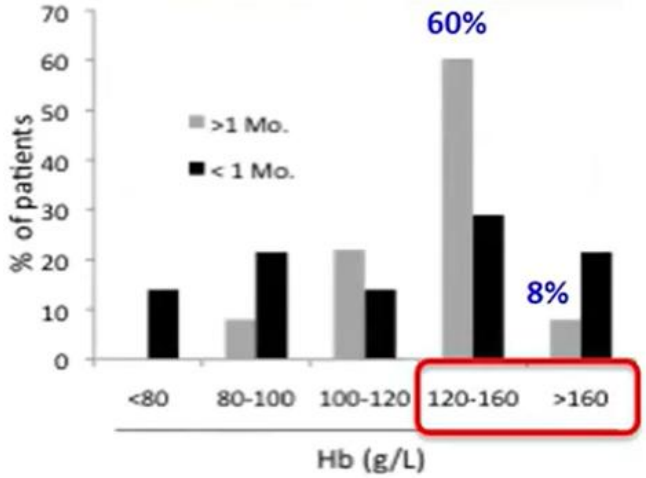
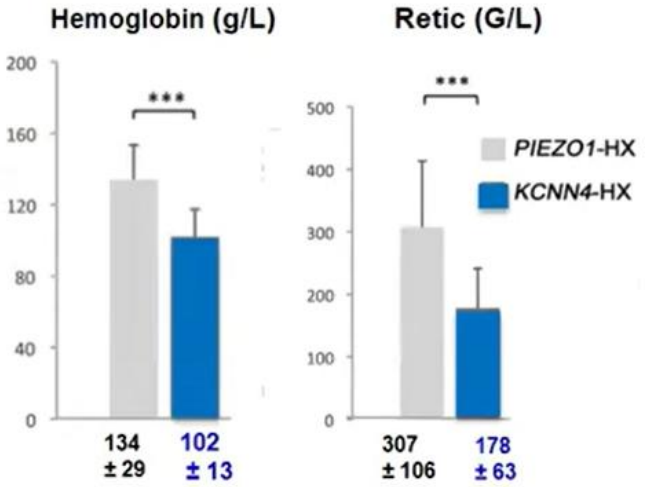
V Picard et al, Haematologica, 2019

Hemoglobin level in DHS

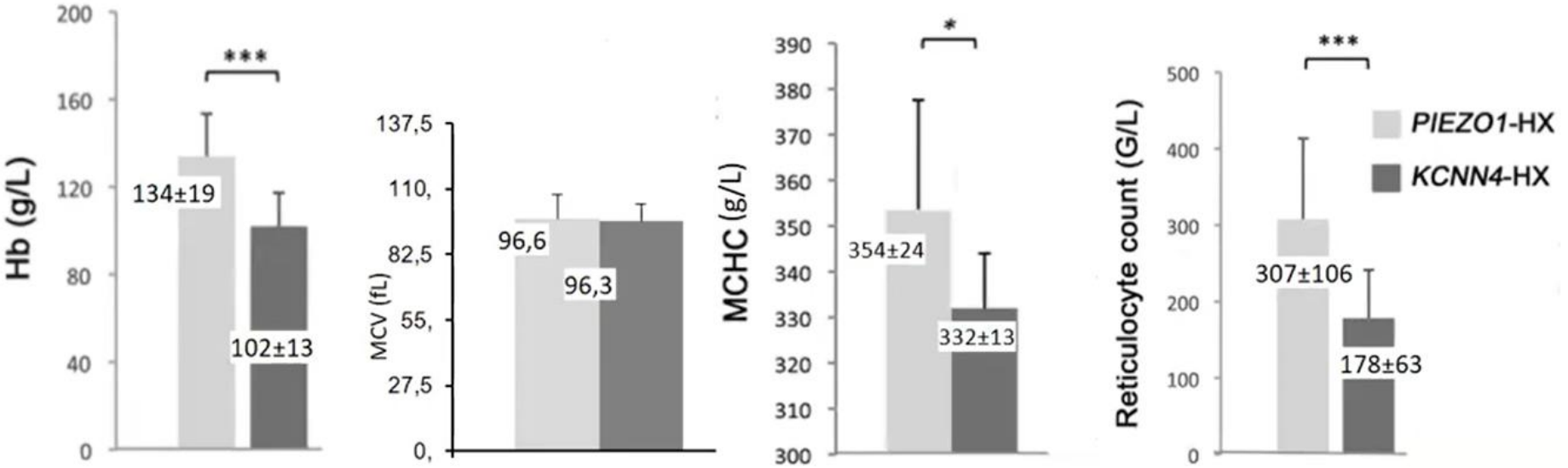


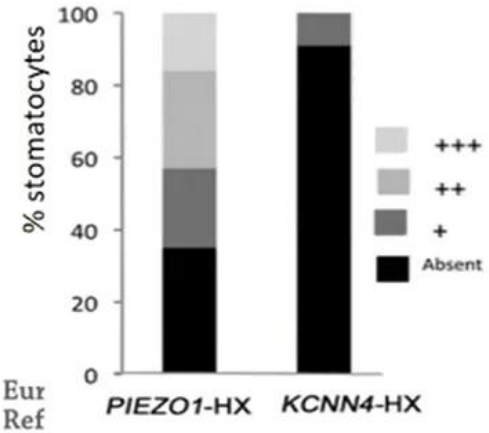


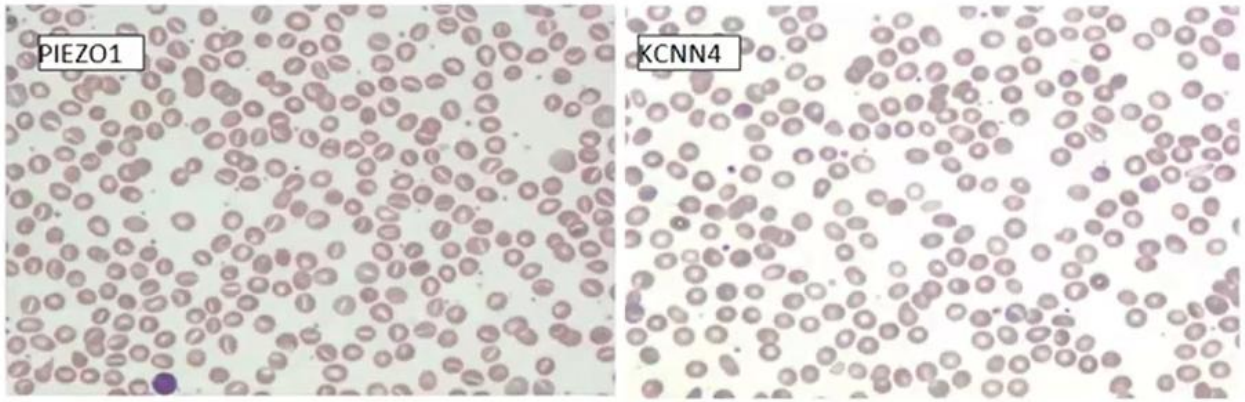
- **In most subjects DHS presents a compensated hemolysis**
- Elevated Hb level in 8% : relative 'polycythemia'
- Occasional transfusion in 7 patients
- Only 27% patients were anemic
- Anemia most frequent in Gardos-DHS

Haematologica. 2019 Aug;104(8):1554-1564.

Hematological parameters in PIEZO1 vs KCNN4 subjects







Red cell morphology MGG, x50 x10

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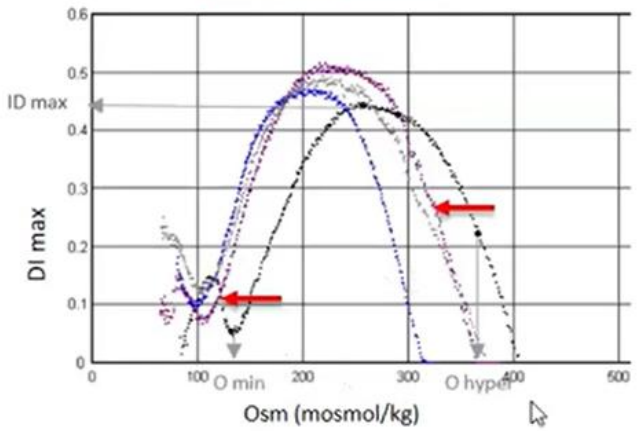
Osmolar gradient ektacytometry

49 probands (87%)

89 subjects (86%)

Typical DHS profiles



	Moy	ET
ID Max	0,50	0,1
O min	114,3	12,9
O hyper	302,6	25,7

Normal range

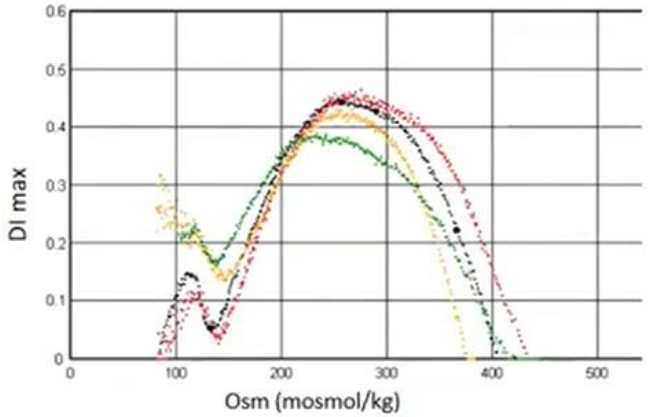
Hypo	135-155 mosm/kg
ID max	0,35-0,45
Hyper	325-360 mosm/kg

***PIEZO1* mutation(s) -> dehydrated profile**

7 probands (12%)

13 subjects (12%)

Normal or atypical profile



	Moy	ET
ID Max	0,4	0,1
O min	141,4	9,5
O hyper	354,6	22,0

***KCNN4* mutation -> normal profile**

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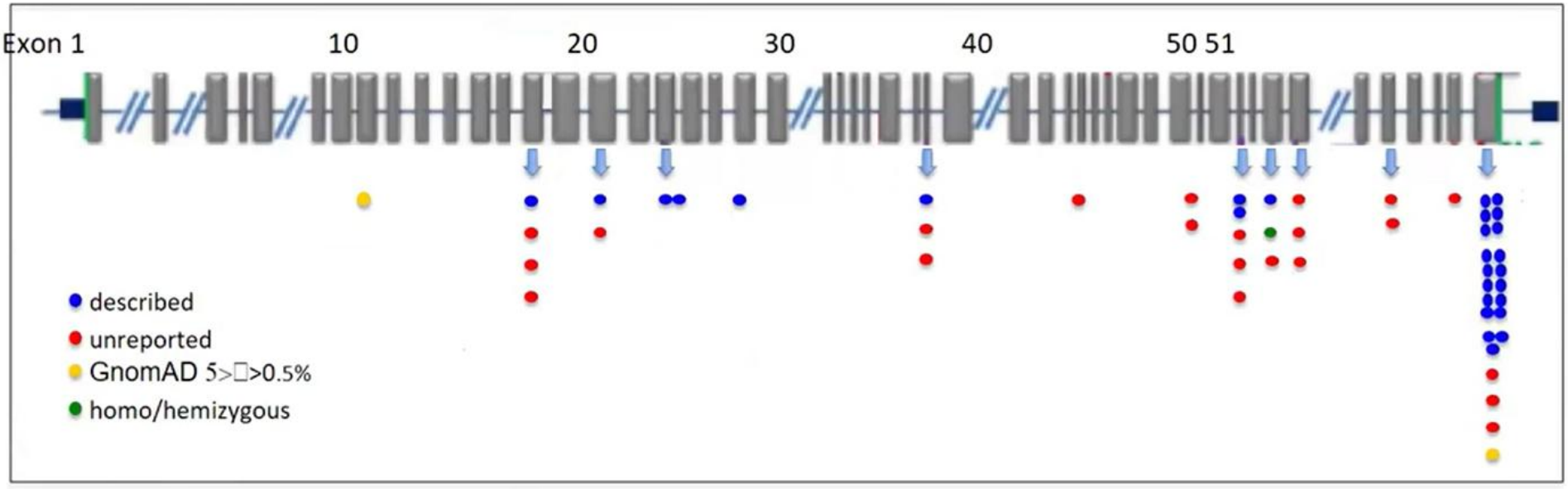
PIEZO1 mutations



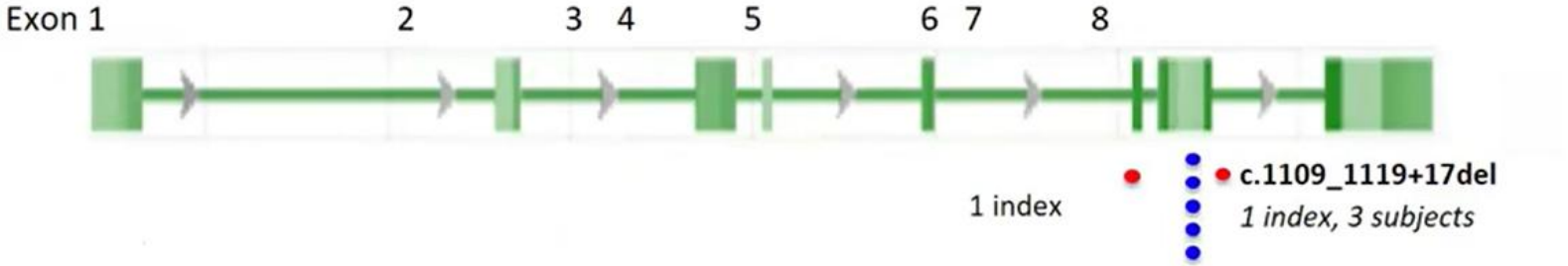
private missense mutations
30/49 index (61%)

- 6 described pathogenic mutations (functional tests)
- 15 carried a new unique missense mutation
  - probably pathogenic n=11
  - unknown significance n=4
- 7 index with 2 mutations
- 1 homo/hemizygous mutation
- 1 polymorphism

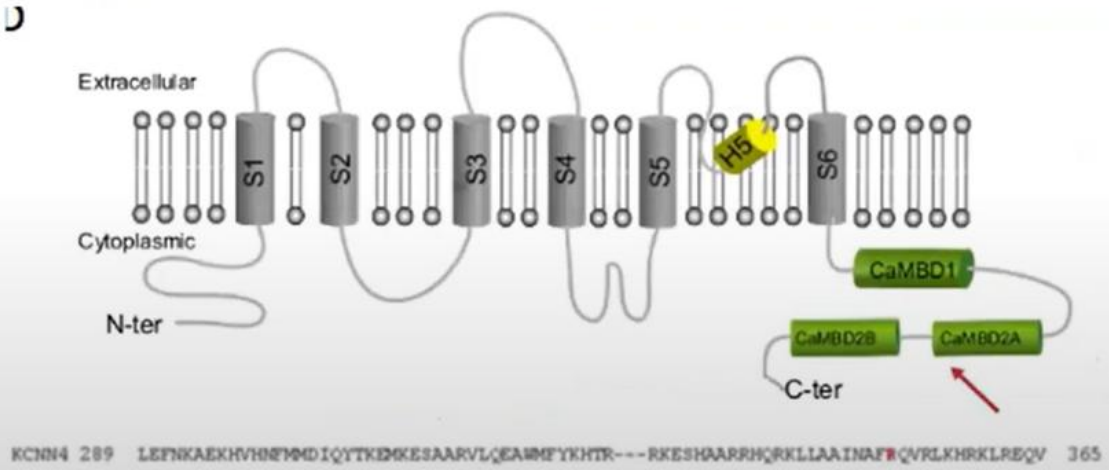
recurrent mutation in exon 51
19/49 index (39%)

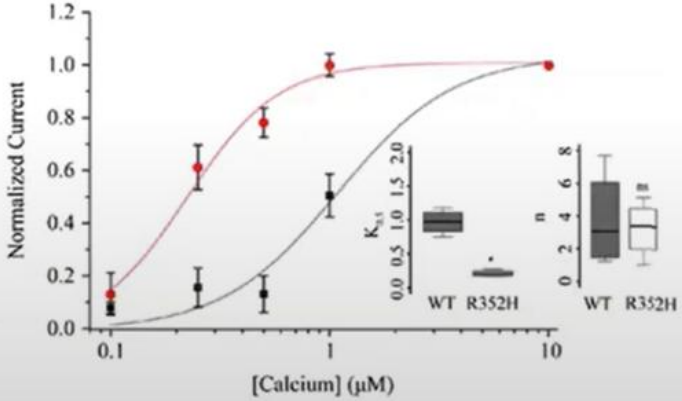
- p.Arg 2456 His 6 index 15 subjects
- p.Arg 2488 Gln 3 index 6 subjects
- p.Leu2495_Glu2496dup 10 index 20 subjects

KCNN4 mutations



c. 1055 G>A
p.Arg 352 His
5 index, 9 subjects





Rapetti Mauss et al, Blood, 2015

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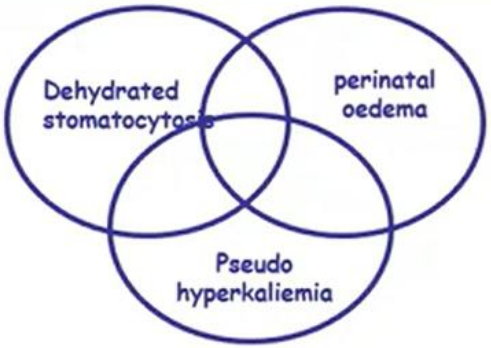
CLINICAL AND BIOLOGICAL FEATURES IN HEREDITARY XEROCYTOSIS



PLEIOTROPIC SYNDROM OMIM #603528

Genotype-phenotype correlation and risk stratification in a cohort of 123 hereditary stomatocytosis patients

Andolfo Immacolata[1,2*], Russo Roberta[1,2*], Rosato Eleni Barbara[1,2], Manna Francesco[1,2], Gambale Antonella[1,2], Carlo Brugnara,[3] Iolascon Achille[1,2].

Clinical and biological features in *PIEZO1*-hereditary xerocytosis and Gardos channelopathy: a retrospective series of 126 patients

Véronique Picard,[1,2] Corinne Guitton,[3] Isabelle Thuret,[4] Christian Rose,[5] Laurence Bendelac,[1] Kaldoun Ghazal,[6] Patricia Aguilar-Martinez,[7] Catherine Badens,[8] Claire Barro,[9] Claire Bénéteau,[10] Claire Berger,[11] Pascal Cathébras,[12] Eric Deconinck,[13] Jacques Delaunay,[14] Jean-Marc Durand,[15] Nadia Firah,[16] Frédéric Galactéros,[17] Bertrand Godeau,[18] Xavier Jaïs,[19] Jean-Pierre de Jaureguiberry,[20] Camille Le Stradic,[21] François Lifermann,[22] Robert Maffre,[1] Gilles Morin,[23] Julien Perrin,[24] Valérie Proulle,[1] Marc Ruivard,[25] Fabienne Toutain,[26] Agnès Lahary[27] and Loïc Garçon[1,28]

Protocole National de Diagnostic et de Soins (PNDS)

Sphérocytose héréditaire et autres anémies hémolytiques par anomalies de la membrane érythrocytaire

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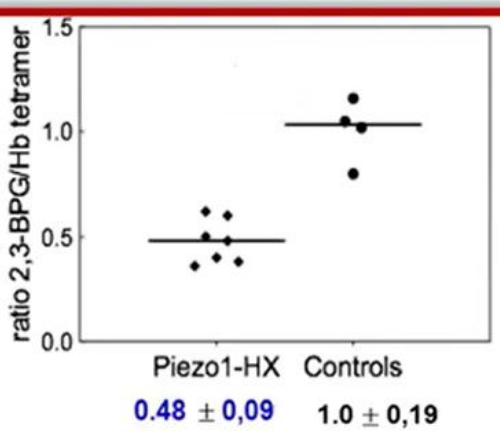
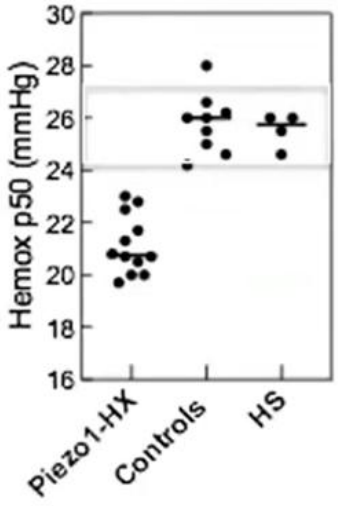
Grootenboer S et al, Blood, 1996
Andolfo I et al, Am J Hematol, 2018
Picard V et al, Haematologica, 2019

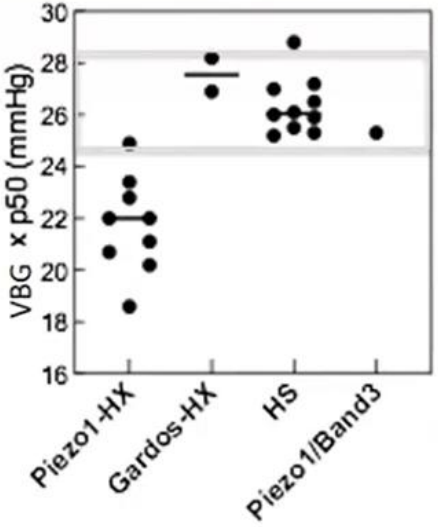
Mr K., 35 y.o

- Referred to Hematology for « polycythemia » in 2016
- Mild splenomegaly, jaundice
- Blood tests:
  - Hb: 18.5 g/dL
  - MCV=88fL
  - MCHH 36.2%
  - Reticulocytes 199 G/L
  - Bilirubin 79 umom/L
  - Haptoglobin, LDH in the normal range
  - « Rare spherocytes »
  - OGE: HX
  - Mutation heterozygous PIEZO1 (exon 51 c.7391A>C:p.H2464P)



2,3-BPG/Hb ratio is decreased in subjets presenting with Piezo1 HX





- p50 is decreased in subjets presenting with Piezo1 HX
- In 2 gardos cases and one Band 3 case, p50 was normal

Mr X., 37 y.o

- Referred to Hematology for hemolysis in 2012
- Moderate Jaundice, splenomegaly

- Hb: 13.8 g/dL
- MCV=106 fL
- MCHC: 37%
- Reticulocytes 243 G/L
- DAT neg.
- PK, G6PD, Hb study N^{al}
- $K^{+:}$ 5.7 mmol/L
- Bilirubin : 43 umol/L
- Haptoglobin: 0.08 g/L

- Ferritin 1100 ng/mL
- RMI liver: 198 umol/g
- HFE H63D HTZ

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- Haptoglobin: 0.08 g/L
- Ferritin 1100 ng/mL
- RMI liver: 198 umol/g
- HFE H63D HTZ

His wife was followed at CHU Amiens hospital during her second pregancy: severe fetal anasarque (at 28 SA)

Perinatal edema

	KCNN4	PIEZO1	
	1 subject	18 subjects (20%) /10 families	
Anemia	Y, in utero, severe	Y , In utero	n=2
		N	**n=16**
Edema	no	**Y**	**n=18 (100%)**
		hydrops	n= 7
		ascite	n=12
		pleural effusions	n= 5
Outcome	favorable after 3 transfusions	death	n=2
		medical termination	n=1
		favorable	**n=15 (83%)**
Mutations	p.Arg352His	**exon 51 recurrent mutation**	**n=8**
		p.Val598Leu	n=1
		p.Val598Met	n=1
		p.Gly782Ser+Arg808Gln	n=5 (1 fam)
		p.Gln2308Glu	n=1
		p.Arg2456His	**n=5 (3 fam)**
		p.Glu2489Asp	n=2 (1 fam)
		p.Leu2495_Glu2496dup	**n=3 (3 fam)**

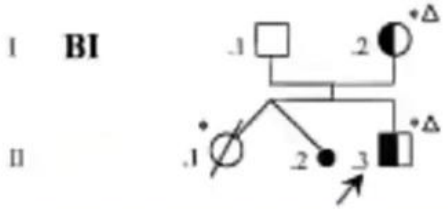
Perinatal edema

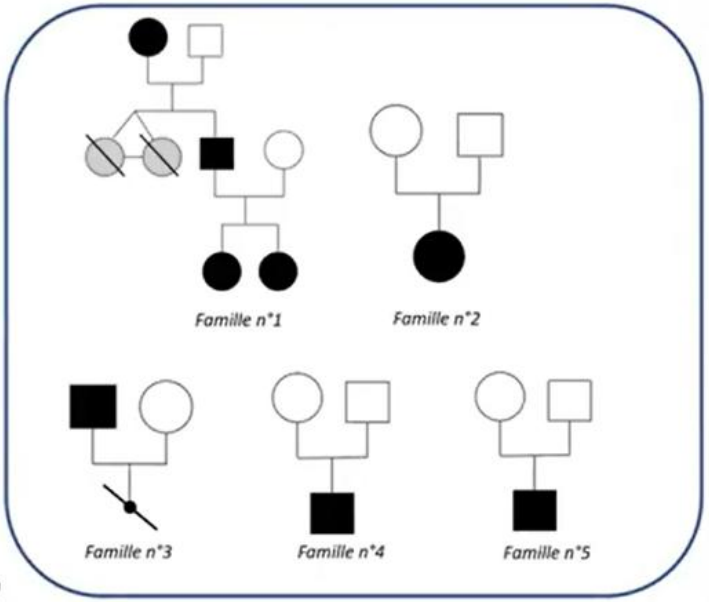
Received 4 Mar 2015 | Accepted 15 Jul 2015 | Published 3 Sep 2015 DOI: 10.1038/ncomms9085 OPEN

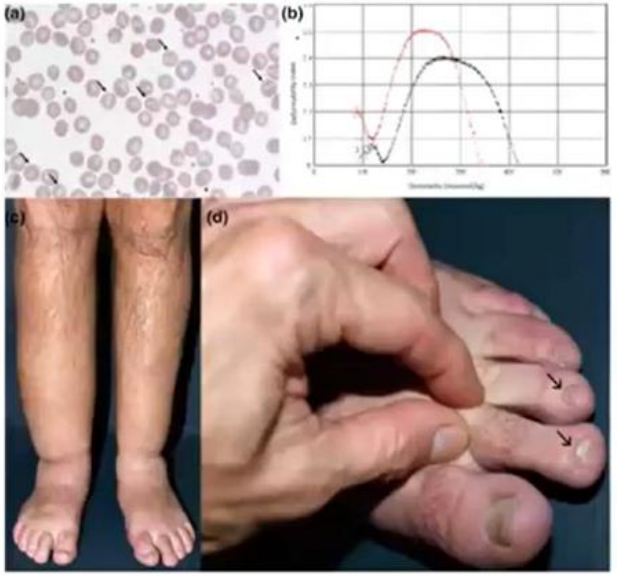
Novel mutations in *PIEZO1* cause an autosomal recessive generalized lymphatic dysplasia with non-immune hydrops fetalis

Elisavet Fotiou[1,*], Silvia Martin-Almedina[1,*], Michael A. Simpson[2], Shin Lin[3,4], Kristiana Gordon[5], Glen Brice[6], Giles Atton[6], Iona Jeffery[7], David C. Rees[8], Cyril Mignot[9], Julie Vogt[10], Tessa Homfray[6], Michael P. Snyder[4], Stanley G. Rockson[3], Steve Jeffery[1], Peter S. Mortimer[1], Sahar Mansour[6] & Pia Ostergaard[1]

PIEZO1
p.Gly782Ser p.Arg808Gln
pVal598Leu







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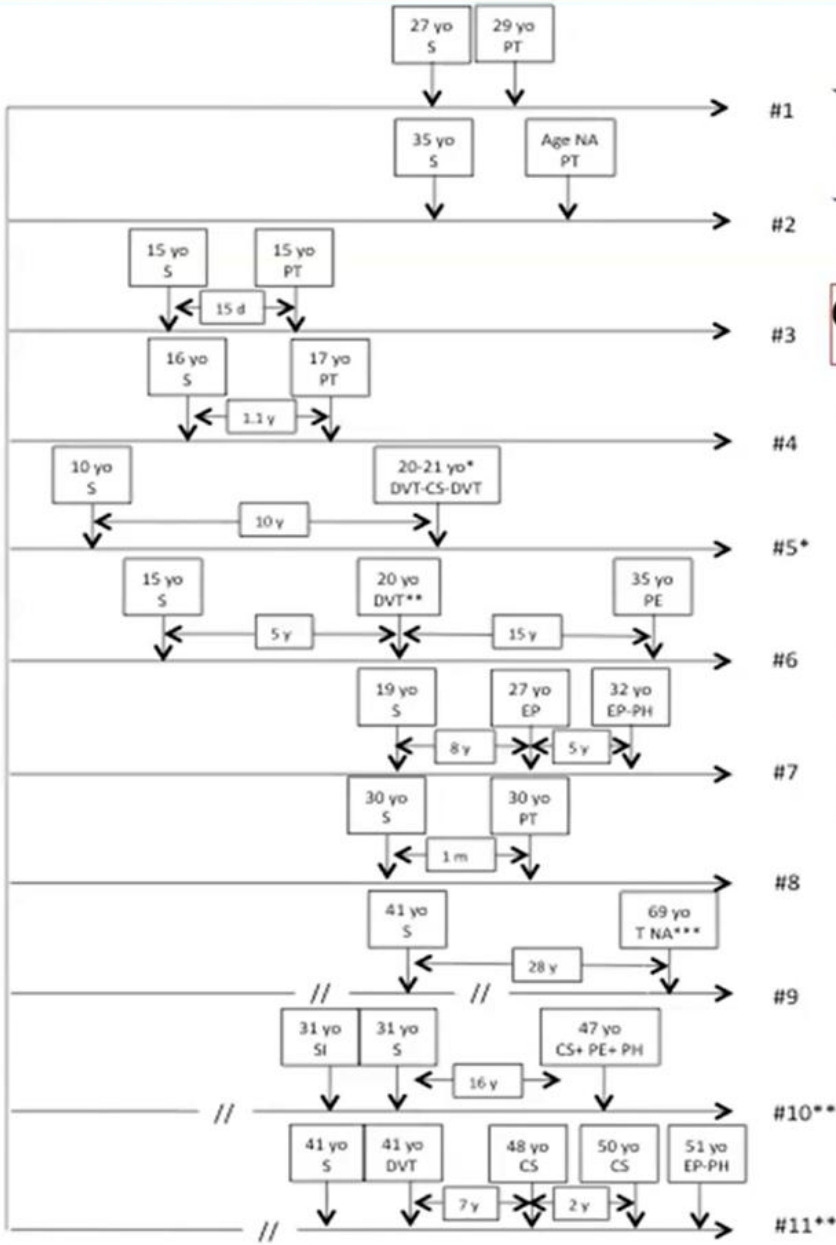
Network Hematological Diseases (ERN EuroBloodNet)

Thursdays Webinars



#1: *PIEZO1* 7479-84 dup 6bp

#2: *PIEZO1* c.7467C>T

OGE

#4: *PIEZO1* c.7367G>A

OGE atypique (pas de DesH2O)
VGMr<VGMm CHCMr>CHCHm

KCNN4
(Mansour-Hendili L)

#6:*PIEZO1* 2152 G>A, 7463 G>A

OGE

#8:*PIEZO1* 6479 C>T

#9: *PIEZO1* 7479-84 dup 6bp

OGE

#11:*PIEZO1*4556A>C, 5728 G>A

Trait AS

Béta Thal

Conclusion

- Rare causes of constitutional hemolysis / hyperferritinemia
- Have to be eliminated before any decision of splenectomy
- No correlation between red cell dehydration and severity

‘ Gardos channelopathy ‘ vs ‘ PIEZO1 DHS or xerocytosis ‘

	KCNN4	PIEZO1
Anemia	+	-/+
Hemolysis	++	+/-
Red cell dehydration	-	+
Simple genetics	+	+/-
Iron overload	++	+/-
Pre/post natal anemia	+/-	+/-
Pre/post natal edema	-	+
Post thrombotic splenectomy	-?	++

Take home messages

1. Most frequent presentation of hereditary stomatocytosis is compensated hemolysis, iron overload and risk of perinatal edema are complications to be aware of

2. Most frequently due to PIEZO1 gain of function mutation, or KCNN4 mutation (Gardos channelopathy, rare). Biological diagnosis is not easy, made by specialized labs, using genetic analysis (NGS) and ektacytometry if available

3. splenectomy in DHS patients should be avoided , because of a high risk of thrombosis, anticoagulation for splenectomized patients

Hôpitaux universitaires Paris-Sud
Antoine-Béclère Bicêtre Paul-Brousse

Dr Guitton
Hématologie Pédiatrique,
Hôpital Bicêtre

Pr Garçon
Hématologie Adulte,
CHU Amiens,
Hôpital Bicêtre

Hematology / Biochemistry / Genetic Team, Bicêtre Hospital

Pr Delaunay	Pr Da Costa
Dr Feneant-Thibault	Dr Lebigot
Dr V Proulle	A Proust
Dr K. Ghazal	L Bendelac
Dr R Maffre	D David Ponn
Dr Tertian	Dr Kiger
Dr Barreau	

And many collaborators

CLUB DU GLOBULE ROUGE ET DU FER

MCGRE
FILIÈRE SANTÉ MALADIES RARES

sfh
Société Française d'Hématologie

European Reference Network
for rare or low prevalence complex diseases
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