Novità diagnostico-terapeutiche nelle Anemie Rare
Il punto di vista del biologo

Paola Bianchi

Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano

Bologna, 3 ottobre 2023

XLVIII
CONGRESSO NAZIONALE
AIEOP
Bologna
2-4 Ottobre 2023

La sottoscritta Paola Bianchi

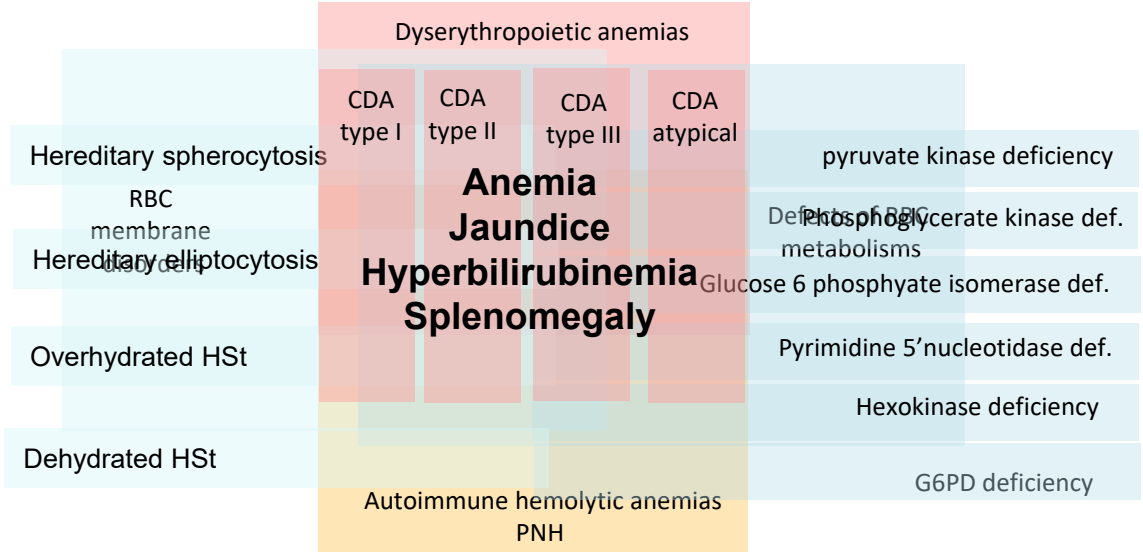
ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

dichiara

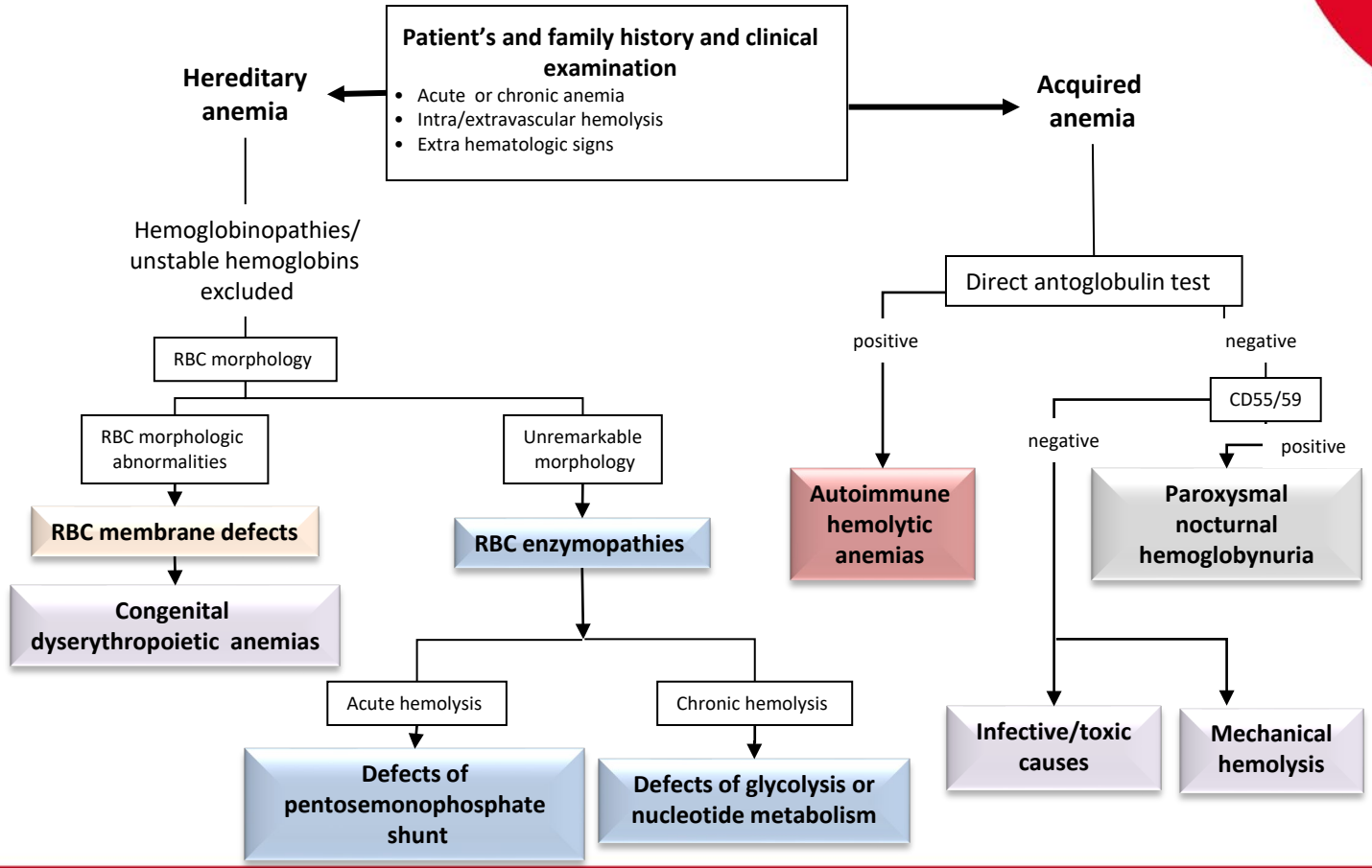
Che negli ultimi due anni ha avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario:

- *Agios Pharmaceutics (Consultancy - Advisory board)*

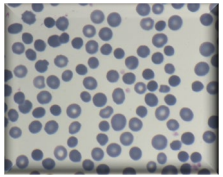
Hemolytic anemias:
Clinical presentation and differential diagnosis



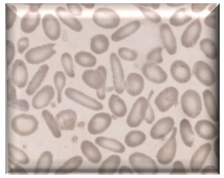
Hemolytic anemias: diagnostic flowchard



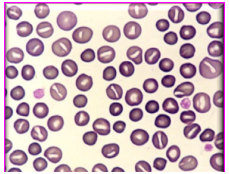
RED CELL MEMBRANE DISORDERS



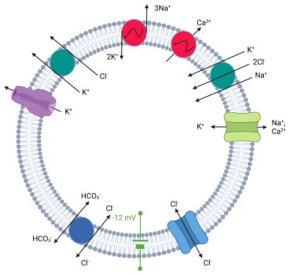
Hereditary Spherocytosis



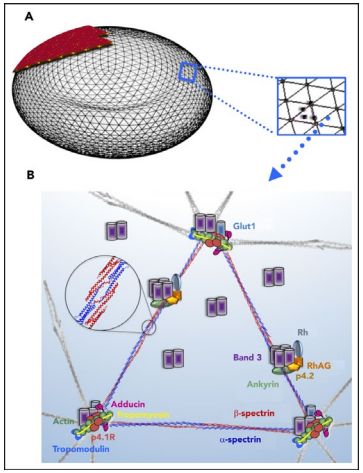
Hereditary Elliptocytosis



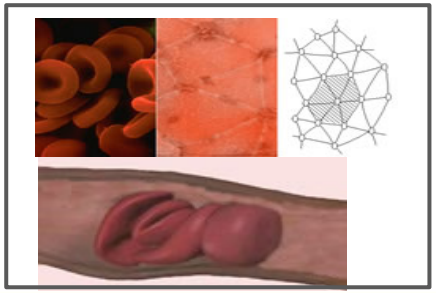
Hereditary Stomatcytosis

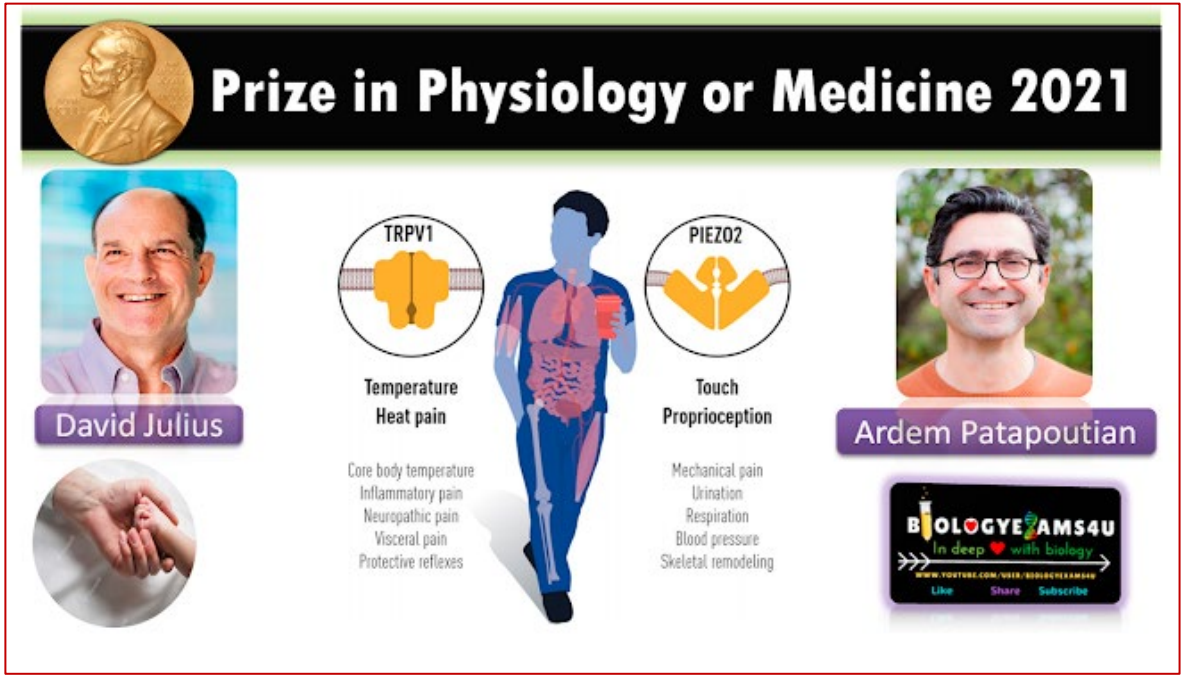


Jansen et al, 2021



Risinger M, Blood, 2020




Prize in Physiology or Medicine 2021
David Julius
Ardem Patapoutian
TRPV1
Temperature
Heat pain
Core body temperature
Inflammatory pain
Neuropathic pain
Visceral pain
Protective reflexes
PIEZO2
Touch
Proprioception
Mechanical pain
Urination
Respiration
Blood pressure
Skeletal remodeling
BIOLOGYEXAMS4U
In deep with biology
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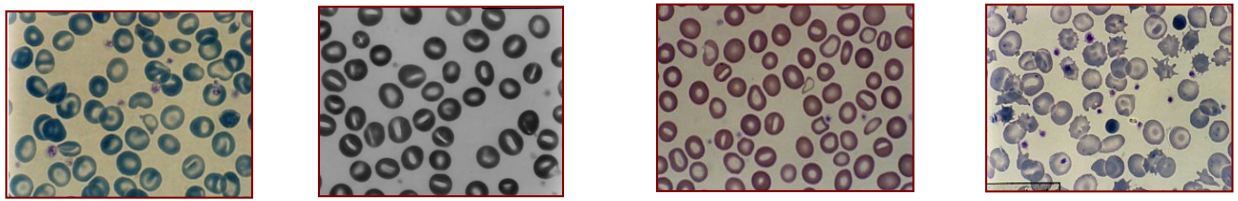
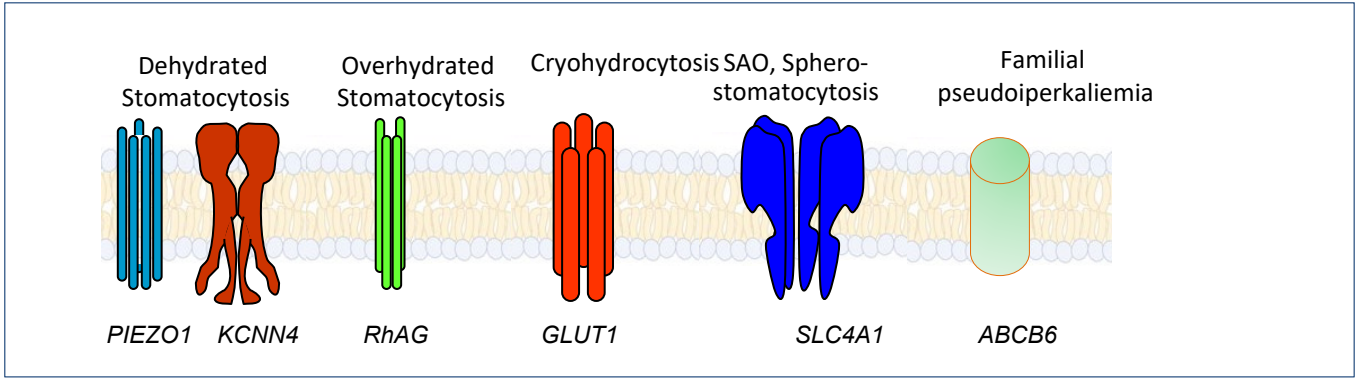
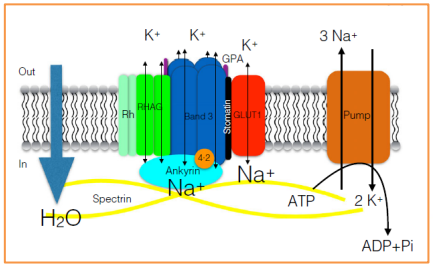
HEREDITARY STOMATOCYTOSIS

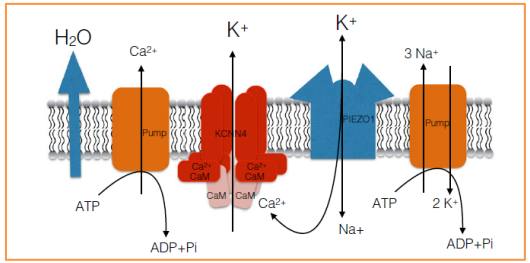




Overydrated stomayocytosis

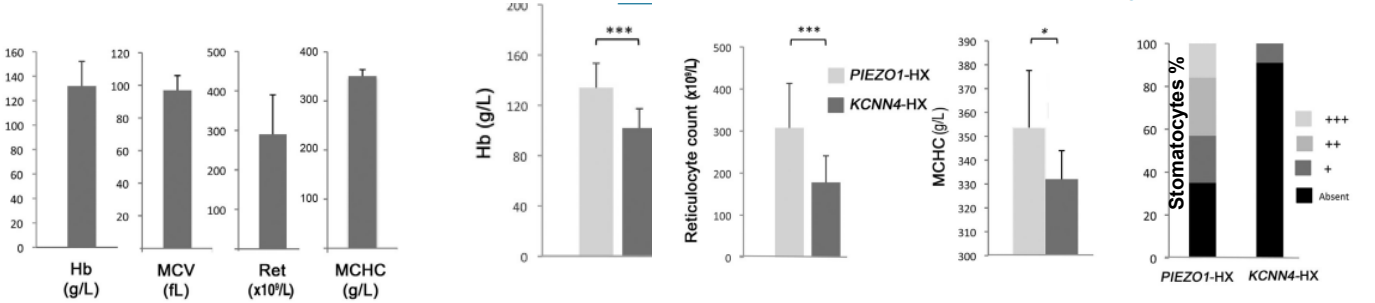


Dehydrated stomayocytosis

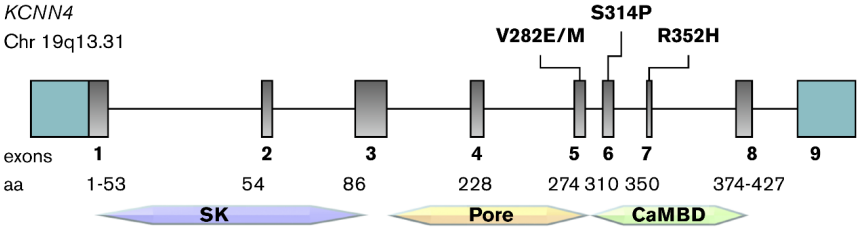


PIEZO1 variants vs KCNN4 Gardos Channelopathy

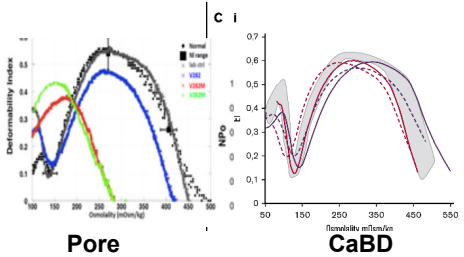
Clinical and biological features in PIEZO1-HX and Gardos channelopathy:
a retrospective series of 126 patients



Picard et al, 2019

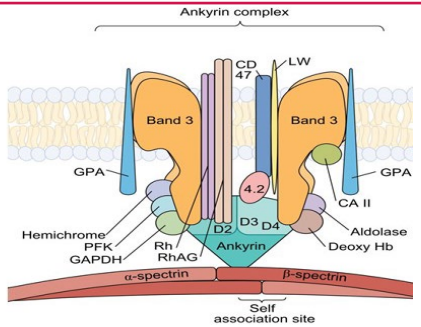


aa 12-122	SK	Potassium channel, calcium-activated
aa 231-289	Pore	Two pore domain potassium channel domain
aa 304-377	CaMBD	Calmodulin-binding domain

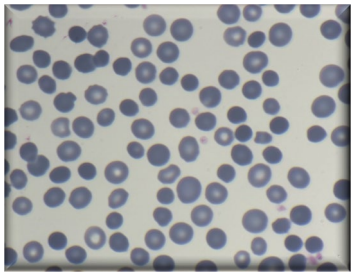


Fermo et al 2017, Fermo et al 2018, Andolfo et al 2018, Rivera et al, 2019, Fermo et al 2020

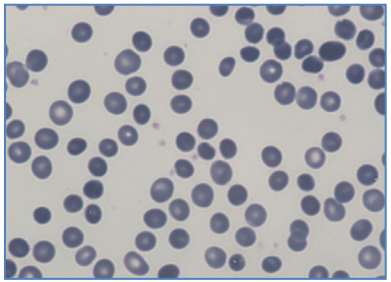
HEREDITARY SPHEROCYTOSIS



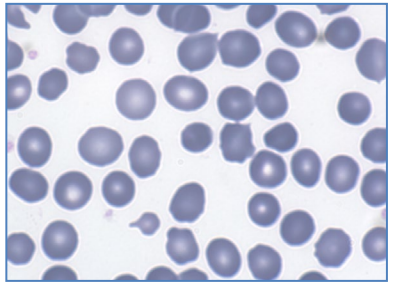
- ✓Prevalence 1:2000
- ✓Worldwide distribution
- ✓Dominant/recessive transmission
- ✓Variable severity
- ✓Presence of spherocytes
- ✓ Genes involved SLC4A1, EPB42, SPTA1, ANK1
- ✓Complete response to splenectomy



AIHA



CDA

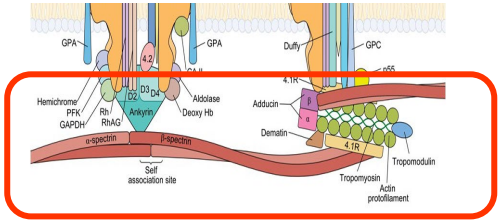


Demographic features and common complications of 446 patients with hereditary spherocytosis at diagnosis

Characteristics	At diagnosis ($N = 446^a$)
Male/female (*N*)	239/207
Median age at diagnosis (y, range)	22 (0.1–80)
<18 years old ($n = 186$)	7 (0.1–17)
≥18 years old ($n = 260$)	36 (18–80)
Median Follow-up (y, range)	–
Splenomegaly (*N*, %)	$300/374^b$ (80)
Gallstones (*N*, %)	$148/353^c$ (42)
Neonatal jundice (*N*, %)	133/446 (30)
Transfused patients (*N*, %)	130/446 (29)
Cholecystectomy (*N*, %)	93/446 (21)
Splenectomized (*N*, %)	72/446 (16)
Exchange transfusion (*N*, %)	20/446 (4.5)
Aplastic crises (*N*, %)	21/446 (5)
Infections (*N*, %)	13/446 (3)
Thromboses (*N*, %)	4/446 (0.9)

Vercellati et al, 2022

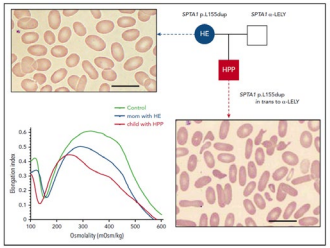
HEREDITARY ELLIPTOCYTOSIS (classical form)



- ✓Prevalence: 1/1000 -1/4000
  up to 1/50 in malaria endemic areas like west and central Africa.
- ✓Dominant transmission
- ✓Anemia: Compensated – Mild
- ✓Genes involved: SPTA1, SPTB; EPB41
- ✓No therapies required

HEREDITARY PYROPOIKILOCYTOSIS

Allele α LELY SNP: c.6531-12C/T, c. 5572C>G
Leu1858Val



Risinger M, Blood, 2020

- ✓ Recessive transmission
- ✓ Severe hemolytic anemia – tx dependent
- ✓ Altered morphology, mimiking heat lability
- ✓ Within a family, HE and HPP may both be present
- ✓ Genes involved : SPTA1, SPTB, EPB41

RBC disorders: molecular heterogenity

Protein	Gene	Position	Function	Phenotype
a-spectrin	SPTA1	1q23.1	Membrane skeletal network	HS HE/HPP
b-spectrin	SPTB	14q23,3	Membrane skeletal network	HS HE
Ankyrin	ANK1	8p11.21	Vertical interactions	HS
Protein Band 3	SLC4A1	17q21.31	•Anion exchange channel •Link to glycoltytic enzymes •Veritcal interactions	HS SAO HSt
Protein 4.2	EPB42	15q15.2	Stabilize band3/ankyrin complex	HS
Protein 4.1	EPB41	1p35.3	Stabilize spectrin-ankyrin contact	HE
Glycophorin C	GYPC	2q14.3	Gerbich - blood group	HE
FAM38A	PIEZO1	16q24.3	Mechanosensitive ion channel	DHst/ Polycythemia
Gardos channel KCa3.1	KCNN4	19q13.31	Potassium Calcium-Activated Channel	DHSt
Rh associated Glycoprotein	RHAG	6p12.3	Rh -blood group	OHSt
GLUT1	SLC2A1	1p34.2	Glucose transporter	CHC
ABC transporter Superfam	ABCB6	2q35	Porphyrin transporter	Fam. PHYK
Flippase	ATP11C	Xq27.1	Phosphatidylserine (ATPase) flippase	Hemolytic anemia

Need of new nomenclature ?

Diagnostic tools

Osmotic fragility (OF) test (Parpart et al, 1947)	68%
Acidified glycerol lysis test (AGLT) (Zanella et al, 1980)	95%
The Pink test (Vettore & Zanella, 1984)	91%
Hypertonic cryohaemolysis test (Streichman & Gescheidt, **1998**)	91%
Eosin-5-maleimide (EMA) binding (King et al, 2000)	93% Se 98% Sp

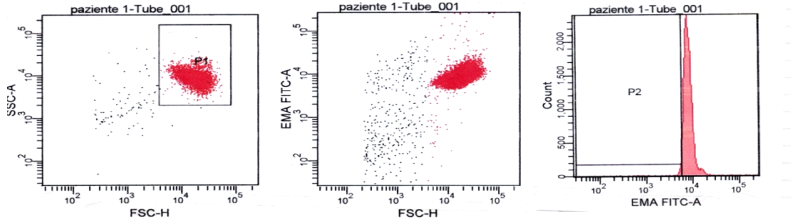
British Journal of Haematology, 2000, **111**, 924–933

Rapid flow cytometric test for the diagnosis of membrane cytoskeleton-associated haemolytic anaemia

MAY-JEAN KING,[1] JUDITH BEHRENS,[2] CHRIS ROGERS,[3] CLARE FLYNN,[4] DAVID GREENWOOD[5] AND KEITH CHAMBERS[6]
[1]*International Blood Group Reference Laboratory, Bristol,* [2]*Department of Haematology, St. Helier Hospital, Carshalton,* [3]*Research and Development Support Unit, Southmead Hospital, Bristol,* [4]*Department of Haematology, St. Mary's Hospital, London,* [5]*Department of Haematology, Southmead Hospital, Bristol, and* [6]*Department of Haematology, Leicester Royal Infirmary, Leicester, UK*

Received 12 June 2000; accepted for publication 13 July 2000

Sensitivity = 92,7%
Specificity = 99,1%.



EJHaem. 2021 Sep 9;2(4):716-728. doi: 10.1002/jha2.277. eCollection 2021 Nov.

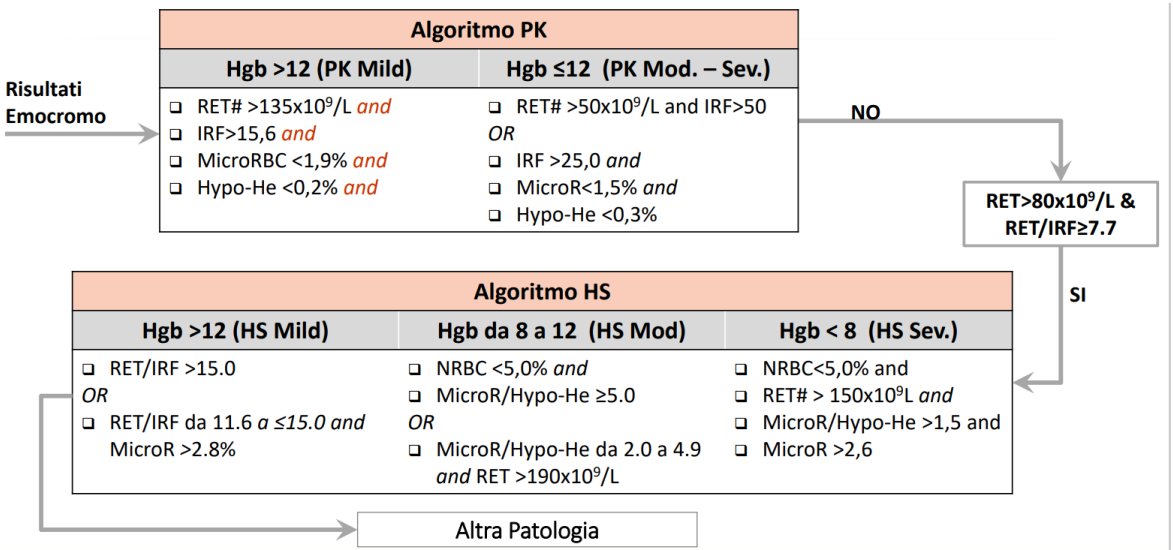
Facilitating EMA binding test performance using fluorescent beads combined with next-generation sequencing

Andreas Glenthøj [1], Christian Brieghel [1], Amina Nardo-Marino [1], Richard van Wijk [2], Henrik Birgens [1], Jesper Petersen [1]

Automated red cell parameters in the prediction of congenital hemolytic anemias

M. Chiron, et al 1999	HS samples MSCV < MCV *Mean Spherized Corpuscular Volume, assessed during the retics count procedure under hypoosmotic conditions)*	Sensitivity 100% Specificity 93.3%
Danise et al 2001	RDW/HDW ratio significantly greater in CDA II than HS CHDW/CHDWr ratio significantly lower in CDA II than HS *RDW= anisocytosis; HDW= anisochromia; CHDWr= cell Hb content of reticulocytes*	p<0.0002 p<0.0002
Da Costa et al 2001	Reticulocyte volume <100fL HS (except for neonates) Advia H*3 Bayer	
Brosèus, et al 2010	Delta MCV-MSCV >9.6fL Beckman coulter	
Mullier F. et al 2011	Hs screening index: RET ≥80x109/L and RET/IRF >7.7 Ret/IRF; %MicroR; %MicroR%/HypoHe *%MicroR: % erythrocytes <60 fL; %Hypo-He: % of erythrocytes Hb<17g/dL)* (30HS) Sysmex XE-5000	Sensitivity 100% Specificity 99.3%
Persijn L et al 2012	Modification of Mullier algorithm (25 HS) Sysmex XE-5000	Sensitivity 100% Specificity 99%
Lazarova , et al 2014	MRV (mean reticulocyte volume) IRF ; Delta MCV-MSCV Beckman Coulter	Sensitivity 100% Specificity 88%
Bobée V et al, 2018	Hs screening index: RET ≥80x109/L and RET/IRF >9.1 Ret/IRF; %MicroR; %MicroR%/HypoHe (47 HS, 17 PKD) Sysmex XE-5000	Sensitivity 100% Specificity 92.1%
Sottiaux JF et al, 2020	Hs screening index: RET ≥80x109/L and RET/IRF >7.7 Ret/IRF; %MicroR; %MicroR%/HypoHe (20 HS) Sysmex- XN	Sensitivity 94,6% Specificity 96,7%

Patologie	MILANO	Literature	TOTALE
HS	81	65	**146**
PKD	18		**18**
AEA	50		**50**
CDA-I, CDA-II	9		**9**
HSt (PIEZO1 +KCNN4)	9		**9**
Talassemie, HbS, HbC, HbH	118	4	**122**
Altre anemie emolitiche	34	29	**63**
TOTALE	**319**	**94**	**413**



Bianchi et al, in prepraration

PKD

Tot. pazienti	VP	VN	FP	FN	SE	SP	VPN	VPP
366	14	348	3	1	93,3	99,1	99,7	82,3

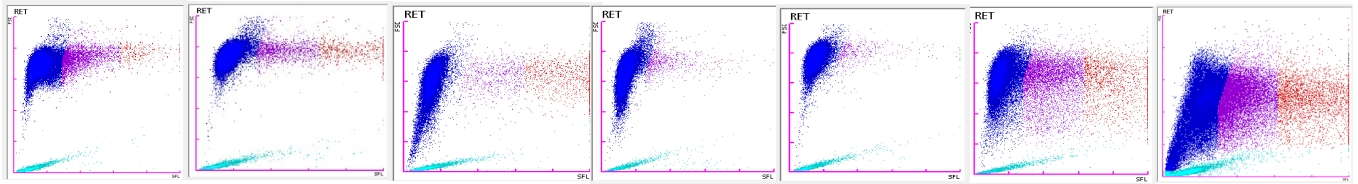
HS

Tot. pazienti	VP	VN	FP	FN	SE	SP	VPN	VPP
366	116	232	17	1	99,1	93,2	99,6	87,2

HS

Algoritmo	VP	VN	FP	FN	SE (%)	SP (%)	VPN (%)	VPP (%)
«Milano»	116	232	17	1	99,1	93,2	99,6	87,2
Mullier	103	227	22	14	88,0	91,2	94,2	82,4
Persijn	99	230	19	18	84,6	92,4	92,7	83,9
Sottiaux	109	224	25	8	93,2	90,0	96,6	81,3
Bobée	97	199	50	20	82,9	79,9	90,9	66,0

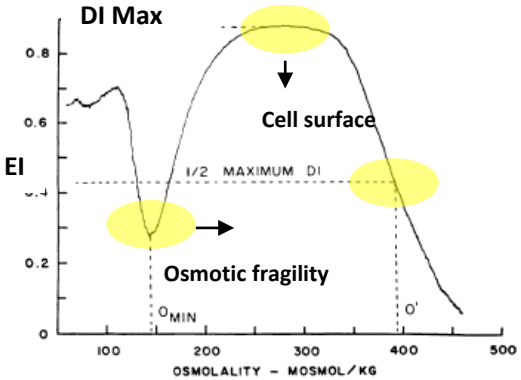
Anche l'occhio vuole la sua parte……



HS | PKD an. lieve | PKD an. grave | AEA | CDAII | MDS | Thal mayor

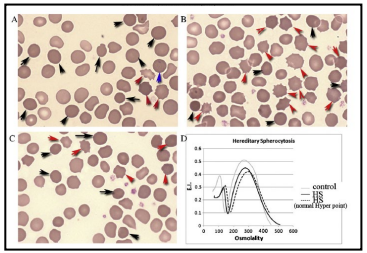
Bianchi et al, in prepraration

Laser-assisted Optical Rotational Cell Analyzer

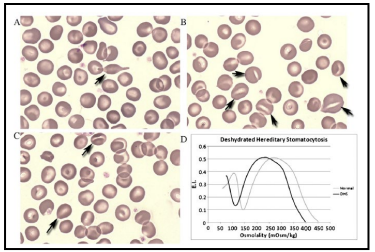


Clark et al, Blood 1984

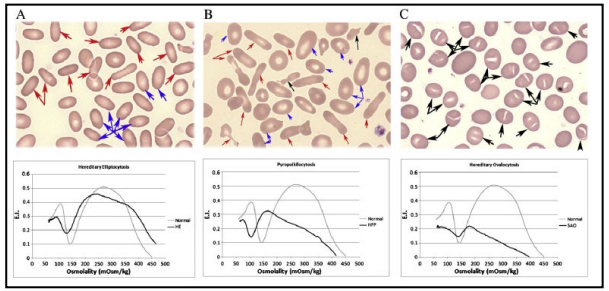
Hereditary Spherocytosis



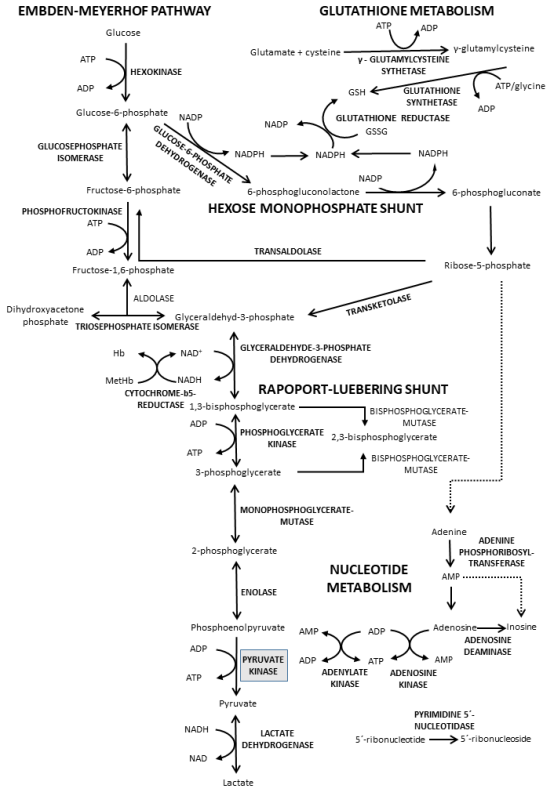
Dehydrated Stomatocytosis



Hereditary Elliptocytosis



Da Costa L et al , 2013, 2016
Lazarova E, 2017
Zaninoni et al, 2016

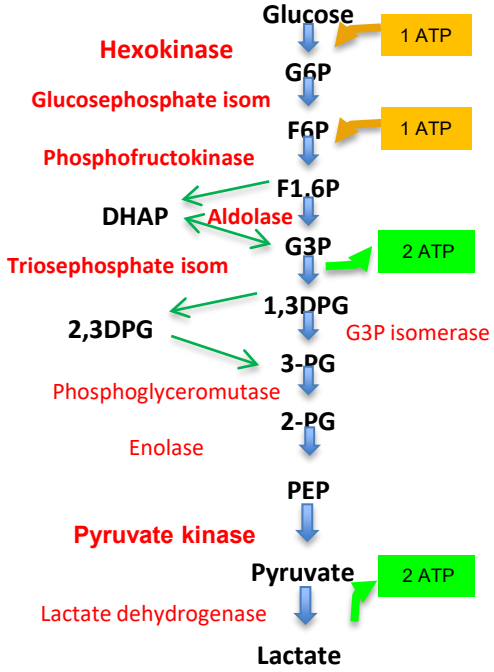


Congenital hemolytic anemias due to RBC enzyme defects

Methemoglobinemia Erythrocytosis

Hemolytic anemia
(acute o chronic)

The Embden-Meyerof pathway



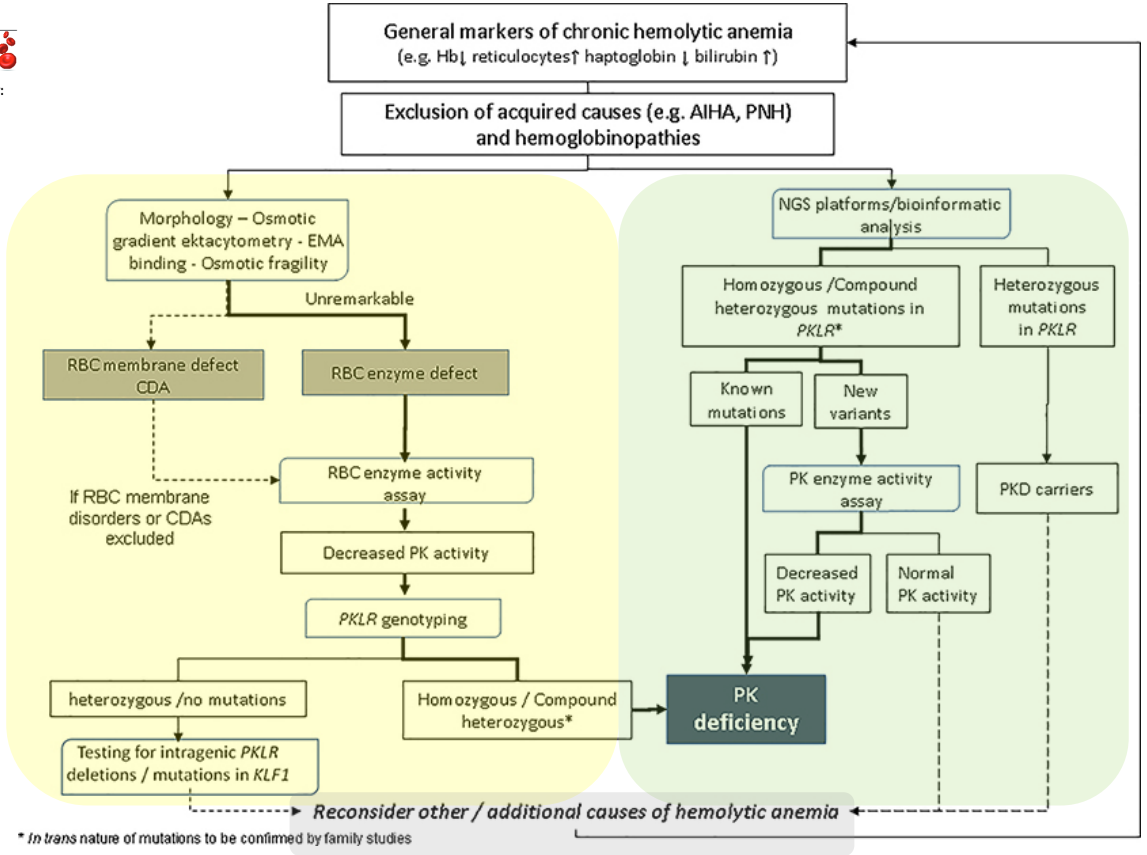
Enzyme	Gene	Position	N. of cases	Phenotype
Embden-Meyerof pathway				
Hexokinase	*HK1*	10q22.1	20 cases	CNSHA
Glucosephosphate isomerase	*GPI*	19q13.11	>50 fam	CNSHA Mental retardation?
Phosphofructokinase	*PFK-M* *PFK-L*	12q13.11 21q22.3	~75 cases	Erythrocytosis, minimal hemolysis, Tarui disase, muscle disease
Aldolase	*ALDOA*	16p11.2	6 cases	CNSHA, mental retardation Dysmorphism
Triosephosphate isomerase	*TPI1*	12p13	~75 cases	CNSHA, neuromuscular disease, Infections
Phosphoglycerate kinase	*PGK1*	X13.3	40 cases	CNSHA, neuromuscular disease
Pyruvate kinase	*PKLR*	1q22	>500 fam	CNSHA

Received: 19 July 2018 | Revised: 19 October 2018 | Accepted: 20 October 2018
DOI: 10.1002/ajh.25325

WILEY AJH

TEST OF THE MONTH

Addressing the diagnostic gaps in pyruvate kinase deficiency: Consensus recommendations on the diagnosis of pyruvate kinase deficiency



Bianchi at al, 2019

Sensitivity PK activity enzyme assay

41 PKD patients

90% sensitivity [95% confidence interval (CI) 77-97%] when performed following reccomendations (method, population not transfused for ≥90 days before sampling

→ **98%** (95% CI 87-100%) considering young cell age % (reticulocyte count) PK/HX ratio

Al-Samkari, et al 2021

45 PKD patients

80% when performed following recommendation, not taking in consideration transfusion state/ time from last transfusion or reticulocytosis

Dongerdiye, et al 2023

NGS apport to diagnosis of chronic hemolytic anemias

	N. of genes analysed	N. of cases	Overall sensitivity	Sensitivity in hemolytic patients with no previous diagnosis
Agrawal, et al 2023	28	450 (CHA)		24%
More et al, 2023	5	26 HS	80%	Not studied
Nieto et al, 2022	48	165 (HS)	83%	35%
Fermo, et al. 2021	48	122 (CHA)	74%	35%
Morado et al, 2021	40	99 (CHA)	78%	n.a.
Chonat, et al. 2019	32 (membrane defects)	11 (HS)	100%	Not studied
van Vuren, et al.2019	7 (membrane defects)	95 (HS)	89%	Not studied
Xue, et al. 2019	10 (membrane defects)	10 (HS)	90%	Not studied
Peng, et al, 2018	n.a.	51 (HS)	72%	Not studied
Li, et al., 2018.	217	46 (CHA)	60.9%	n.a.
Russo et al., 2018	34 and 71	74 (CHA)	64.9%	45.8%
Agarwal et al., 2016	28	17 (CHA)	70%	70%
Roy et al., 2016	33	57 (CHA)	38.6%	11%

CHA = Chronic hemolytic anemias, HS = Hereditary spherocytosis, n.a. = not available

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GUIDELINE

BJHaem

The use of next-generation sequencing in the diagnosis of rare inherited anaemias: A Joint BSH/EHA Good Practice Paper*

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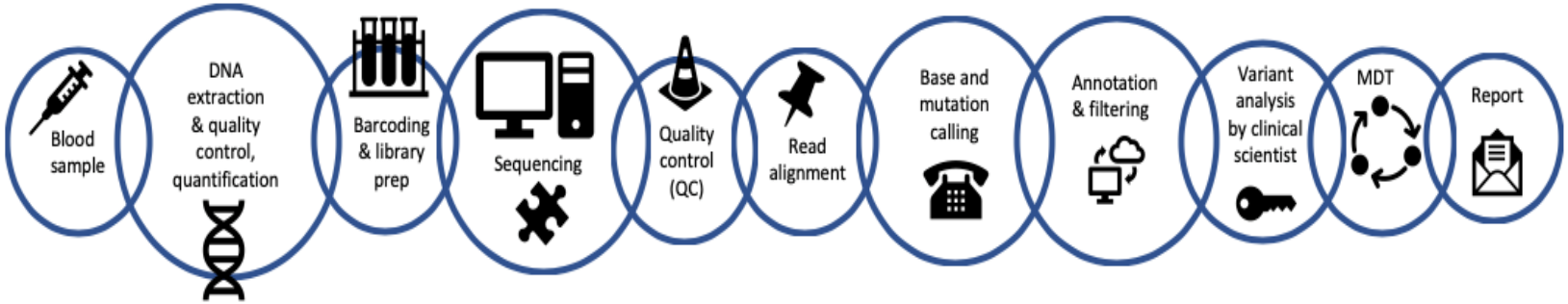


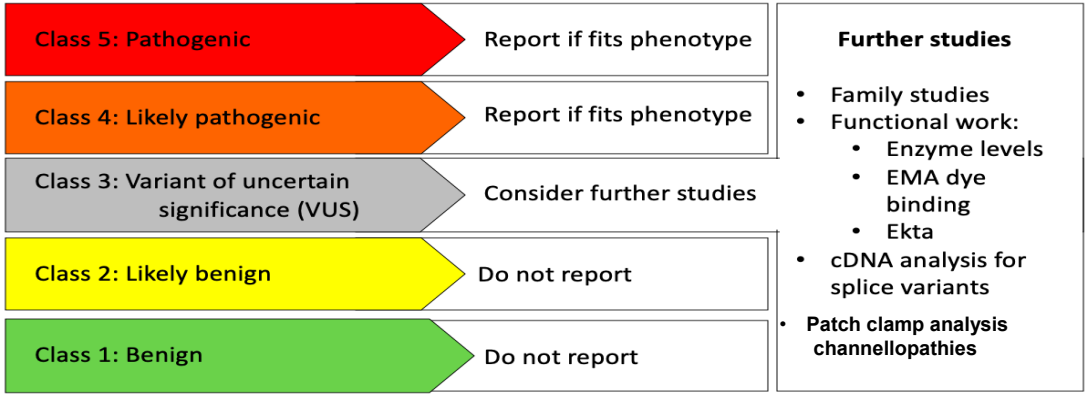
TABLE 2 Comparison of different types of next-generation sequencing

	Targeted resequencing panels (t-NGS)	Whole exome sequencing (WES)	Whole genome sequencing (WGS)
Target of sequencing; size (base pairs [bp])	Exons of 20–200 genes with some intron/exon boundaries for splice site mutations; 500 000 bp	The 'exome'- ~30 000 exons of known coding genes (~1.5% of genome but 80%–90% of known disease-causing mutations) with some intron/exon boundaries for splice mutations; 2×10^7 bp	The whole genome (coding and non-coding space) 3×10^9 bp
Method	Capture of chosen exons, amplification steps and sequencing or amplification of chosen exons and sequencing	Capture all the exons, amplification step and sequencing	DNA is fragmented randomly, ligation of adaptors and direct sequencing (no capture or amplification)
Advantages	Cost, relative ease of interpretation, few unsolicited findings, more challenging to identify CNVs	Cost lower than WGS	Entire genome interrogated including non-coding region; more potential to identify CNVs. Can add genes to virtual panel. Relatively even coverage
Disadvantages	Will only identify mutations in targeted regions, coverage is often uneven, so mutations may be missed. Harder to detect some CNVs	Interpretation can be challenging, high chance of unsolicited findings, will only find mutations in coding regions, coverage is often uneven, may not detect CNVs. Ethical issues of incidental findings in genes that predispose to serious illness.	Interpretation challenging unless there is a trio, non-coding region cannot easily be interpreted. Ethical issues of incidental findings in genes that predispose to serious illness. Cost.

Abbreviation: CNV, copy number variant.

Roy et al, 2023

Variant classification (ACMG)



Roy et al, 2023

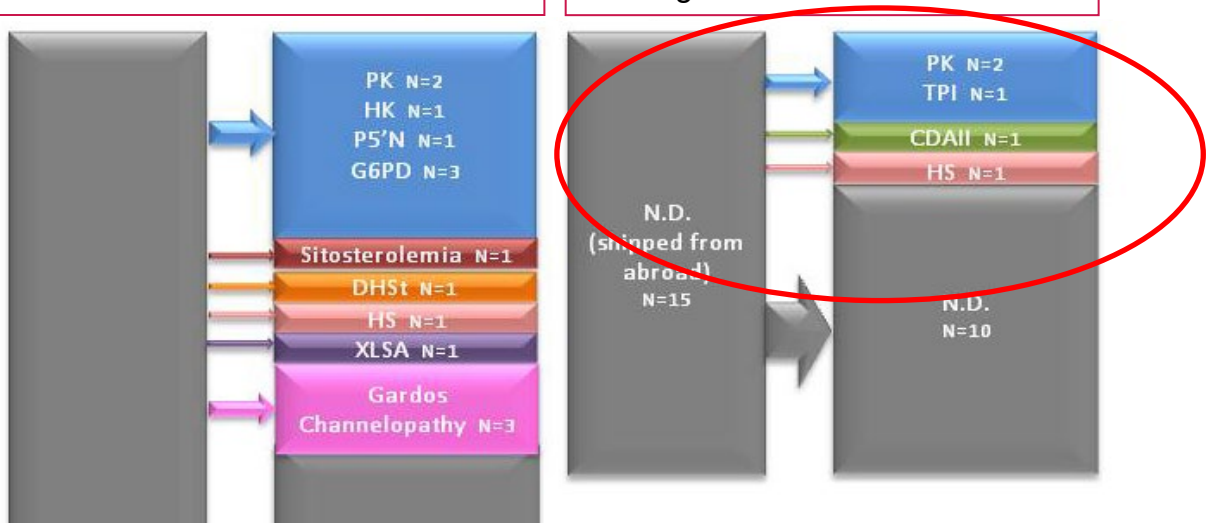
Targeted Next Generation sequencing and diagnosis of congenital hemolytic anemias: a three years experience monocentric study

48genes t–NGS platform

2017-2019
122 patients -105 unrelated families

60 patients who reached a diagnosis after first and second level hematologic investigations, to be confirmed at molecular level

62 patients with unexplained chronic haemolytic anemia after extensive hematologic investigations.



Sensitivity 74 %

Sensitivity 35 %

T-NGS panel vs laboratory testing

	Laboratory testing	Molecular analysis (NGS)
HS	EMA-binding test Ectacytometry Others	High molecular heterogenity Consistency with clinical and laboratory features required
HE	Osmotic fragility tests Ectacytometry RBC morphology	High molecular heterogenity Consistency with clinical and laboratory features required
HSt- *PIEZO1*	Rbc morphology; Ektacytometry Always requiring molecular testing to confirm diagnosis	Highly polymorphic gene Functional tests mandatory in presence of new variants
HSt-*KCNN4*	Absence of specific laboratory markers	
RBC enzyme defects	RBC enzyme assay. Always requiring molecular testing to confirm diagnosis	
Familial sitosterolemia	Complete blood count	
Atypical conditions		

Fermo et al, 2021

M.C., 54-year-old male

Referred to our Pathophysiology of Anemia Unit in May 2016 for chronic hemolytic anemia present since childhood

Past medical history:
- Prolonged jaundice at birth
- Moderate anemia since childhood:
    - Hb 9-9.5 g/dL, MCV 105 fL, PLT 312 x10^9/L, WBC 7.8 x10^3/mcL (normal differential count)
    - Unc Bil 3.7 mg/dL, Hp <20 mg/dL, retics 190 x10^9/L
- Excluded nutrient deficiency anemia, acquired causes of hemolysis (DAT neg, PNH neg, no cardiac valvular defects)
- Normal morphology of RBCs, screening for membrane/enzymatic RBC defects: unremarkable
- Splenomegaly (16 cm)
- Cholecystectomy for gallstones (16 years old)
- Bone marrow evaluations (at 6 and 25 years): erythroid hyperplasia
- Non-cirrhotic liver steatosis with liver iron overload, treated with oral iron chelator (*HFE*: heterozygous H63D)

Family history:
- No history of anemia, transfusions, gallstones, cholecystectomy at young age, splenomegaly/splenectomy

	Pt #1	Reference values
Hb (g/dL)	10.3	13.4-17.5
MCV (fL)	105	80-94
MCHC (g/dL)	34.8	31-37
Reticulocytes (x10^9/L)	171	20-100
Unconj. Bilirub. (mg/dL)	3.91	<1
Serum ferritin (ng/mL)	650	30-400
AGLT	189	>900
NaCl osmotic fragility	normal	
EMA binding test	normal	

	Pt #1	Reference values
HX	2.3	0.79 – 1.33
GPI	65	55.0 – 73.0
PFK	8.7	8.7 – 11.7
GAPD	414	228 – 328
PGK	447	280 – 429
PK	14.7	11.9 – 16.5
G6PD	8.6	7.1 – 9.7
AK	303	229 - 342

Conclusion: chronic hemolytic anemia of unknown origin since childhood

Indication to perform NGS studies (congenital anemias platform)

Gene	Ref. Sequence	Gene	Ref. Sequence
ABCB6	NM_005689	GSS	NM_000178
ABCG5	NM_022436	HK1	NM_033497
ABCG8	NM_022437	KCNN4	NM_002250
ALAS2	NM_001037967	KIF23	NM_138555.2
AK1	NM_000476	KLF1	NM_006563.3
ALDOA	NM_000034	NT5C3A	NM_016489.12
BPGM	NM_001293085	PFKL	NM_001002021
C15ORF41	NM_001130010	PFKM	NM_000289.5
CDAN1	NM_138477	PGK1	NM_000291.3
CYB5R3	NM_000398	PGM1	NM_001172819
ENO1	NM_001201483	PKLR	NM_000298.5
EPB41	NM_004437.3	PIEZO1	NM_001142864.2
EPB42	NM_000119.2	RHAG	NM_000324.2
G6PD	NM_000402	SEC23B	NM_006363.4
GATA1	NM_002049	SLC2A1	NM_006516
GCLC	NM_001498.3	SLC4A1	NM_000342.3
GCLM	NM_001308253	SLC25A38	NM_017875.2
GPI	NM_000175.3	SPTA1	NM_003126.2
GPX1	NM_000581.2	SPTB	NM_000347.5
GSR	NM_000637	TPI1	NM_000365.5

Gene	Mutation (HGVS)	Effect	HOM/HET	ID variant	Pathogenity
PKLR	NM_000298: c.1456C>T	P.Arg486Trp	HET	rs116100695	Pathogenic
PKLR	NM_000298:c.1151C>T	p.Thr384Met	HET	rs74315362	Pathogenic

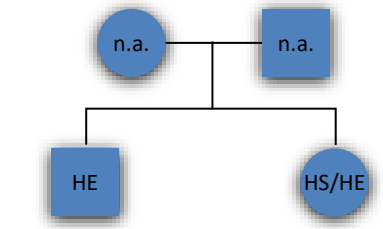
Patient management after diagnosis

June 2019: enrolled in **phase 3 clinical trial AG348-C-006** (oral mitapivat vs placebo)
Day +21: Hb 11.2 g/dL
Day +85: Hb 13.7 g/dL, hemolysis normalized
Month +4: deferasirox stopped due to normalization of iron indices and no iron overload at liver T2* MRI

Last follow-up visit (March 2022): Hb 15.1 g/dL

PK/HX ratio: 6.8 (v.n. 11.40-17.65 n=165)
(HS. 7.44-16.61 n=62)

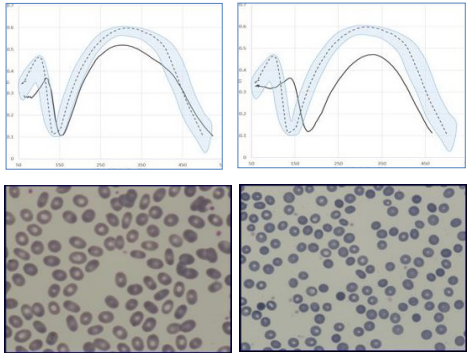
t-NGS Illumina MiSeq - HaloPlex Target Enrichment System – Agilent Tecnologies. Total amplicons 16596, median coverage 99.62 %

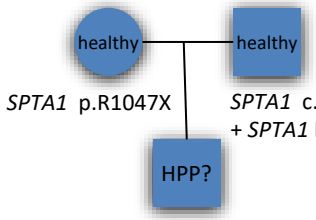


38-1
SPTA1 p.L155dup/wt

38-2
SPTA1 p.L155dup/wt
SLC4A1 p.S510R/wt

#38-1	#38-2
EMA binding: normal	EMA-bind: ↓↓
SDS-PAGE: normal	SDS-PAGE: spectrin ↓



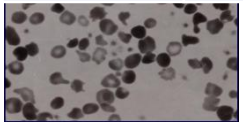


SPTA1 p.R1047X

SPTA1 c.3477+1g>c
+ *SPTA1* Lely

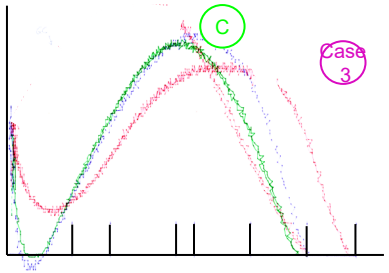
20-1
SPTA1
c.3477+1g>c/p.R1047X

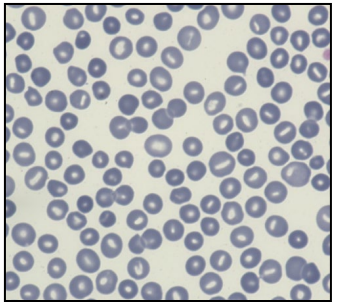
	#20-1
Hb (g/dL)	7.8 *- 11.2
Retics (x10^9/L)	415
MCV (fL)	73
Osm. fragility	↓
EMA binding	↓↓
SDS-PAGE	a-spectrin 68% ↓ Ankirin 56% ↓
Transfusions	Regular until splenectomy
Splenectomy (age)	Yes (7 yrs)



RBC morphology

1999 – 27yrs Diagnosis HS and piastrinopenia





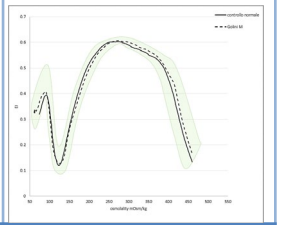
RhAG mutations: negative

ABCG8: c.788G>A; p.Arg263Gln

Donna 22 anni
Non familiarita', 1 sorella in buona salute
Anemia dalla nascita' – necessita' trasfusioni
21 anni 2 ricoveri per anemizzazione - trasfusa

Functional evidences/family studies!

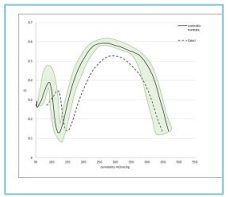
Diagnosi di invio: Sospetta anemia diseritropoietica congenita?

	Pt #3	Reference values
Morfologia: sferociti (15%), stomatociti (4%), emazie fungo		
Hb (g/dL)	10.4	13.4-17.5
MCV (fL)	104	80-94
Reticulocytes (x10^9/L)	102	20-100
Unconj Bilirub. (mg/dL)	2	<1
Aptoglobin (g/L)	<1	
DAT	normal	
NaCl osmotic fragility	normal	
EMA binding test	0%	
SDS-Page	Normal	

Gene	Mutazione (HGVS)	Effetto	HOM/HET	ID variante	Patogenicità
CDAN1	NM_138477.4 c.2804A>G	p.Glu935Gly abn splic?	HET	Rs371548844 GnomAD 0,001	Probabilmente patogenica
CDAN1	NM_138477.4 c.2164C>T	p.Arg722Cys	HET	Rs140014115 Gnom AD 0,002	VOUS*

→ Richiesta di studi funzionali per frequenza delle varianti

Bimbo 7 anni
Anemia dalla nascita
Familiarita' per sferocitosi (mamma affetta e splenectomizzata a 12 anni)

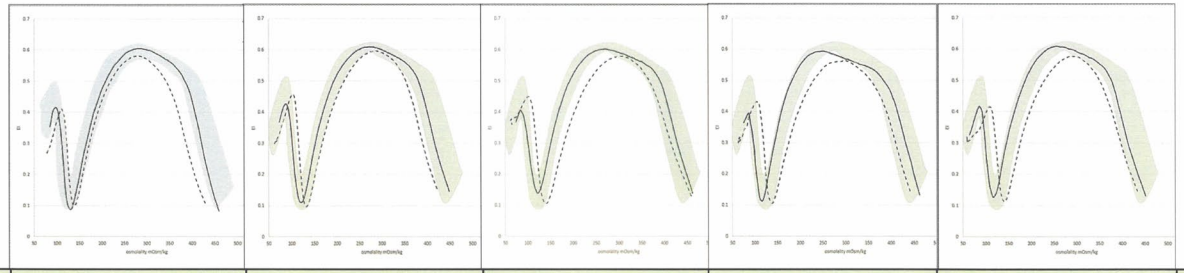
	Pt #3	Reference values
Morfologia: sferociti (15%), stomatociti (4%), emazie fungo		
Hb (g/dL)	10.7	13.4-17.5
MCV (fL)	84	80-94
Reticulocytes (x10^9/L)	307	20-100
Unconj Bilirub. (mg/dL)	2	<1
Aptoglobin (g/L)	<1	
DAT	normal	
NaCl osmotic fragility	Increased	
EMA binding test	-22%	

Gene	Mutazione (HGVS)	Effetto	HOM/HET	ID variante	Patogenicità
CDAN1	NM_138477.4 c.2804A>G	p.Glu935Gly abn splic?	HET	rs371548844	Probabilmente patogenica
CDAN1	NM_138477.4 c.2164C>T	p.Arg722Cys	HET	rs140014115	VOUS*

Trasmissione in CIS delle due varianti?

When alpha spectrin null alleles meet low expression alpha spectrin polymorphisms.

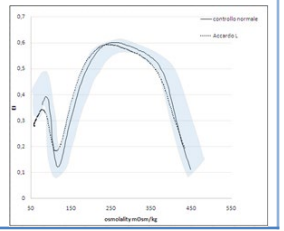
Diagnosis in reference centers



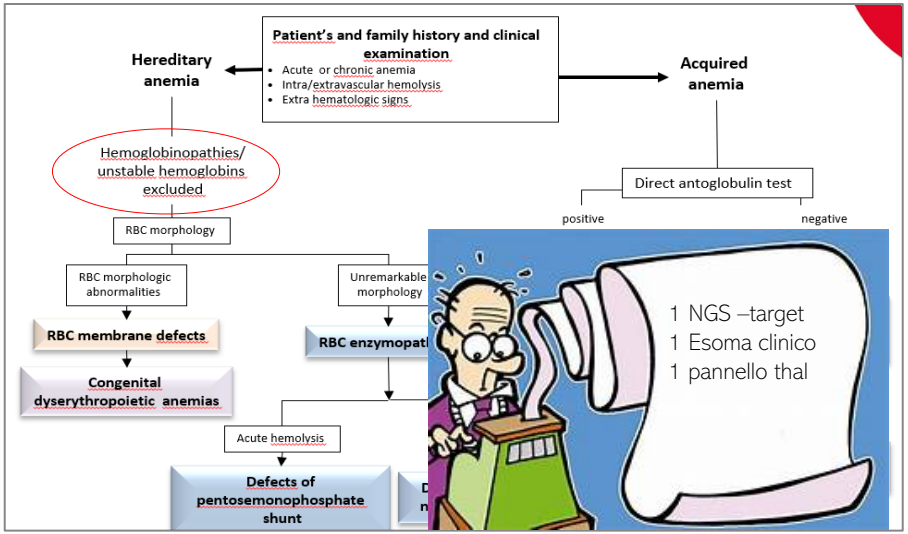
Osmoscan parameters	II.1	I.1	2	3	4	Ref. Values*
EI min	0.099	0.097	0.106	0.106	0.113	0.08-0.14
O min	138	132	139	137	141	116-141
EI max	0.581	0.597	0.579	0.566	0.577	0.59-0.62
O (EI max)	281	287	303	297	288	260-302
EI hyper	0.290	0.298	0.289	0.283	0.288	0.3-0.31
O hyper	388	390	417	413	399	412-470
''Ohypo''	168	168	176	170	175	156-168
Area	120.4	125.8	130.8	127.8	122.5	144-164

*Ref values are calculated as the mean of 200 normal controls +1SD. ''Ohypo'' ref values are calculated as the mean of 30 normal controls + 1SD

Donna 50 anni
Anemia dal' infanzia – no trasfusioni o splenectomia
Splenomegalia
Hb 7-8 g/dL
Terapia: dall' eta' 6 anni folina, B12
Diagnosi: deficit di piruvato chiansi eritrocitaria (no documentazione)

	Pt #2	Reference values
Morfologia: stomatociti 7%, ellissociti 7%, dacriociti 4%, schisto 6%		
Hb (g/dL)	7,5	13.4-17.5
MCV (fL)	78	80-94
Reticulocytes (x10^9/L)	110	20-100
Tot Bilirub. (mg/dL)	2,2	<1
LDH	408	<214
DAT	normal	
NaCl osmotic fragility	Normal	
EMA binding test	Normal	
PK actvivity	Normal	

Follow a diagnostic flowchart!



Take home message:

- Rispettare una flowchart diagnostica di primo livello prima di rivolgersi NGS
- NGS deve essere accompagnato da informazioni anamnestiche cliniche e di laboratorio (studio familiari)
- Valutare il migliore approccio diagnostico (valutare sempre costi e tempistiche)
- Non sempre 1 = 1
  Utilita' eseguire diagnosi NGS in centri di riferimento

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OSPEDALE MAGGIORE POLICLINICO
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