Anemie miste: casi clinici

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XIV CONGRESSO NAZIONALE SITE

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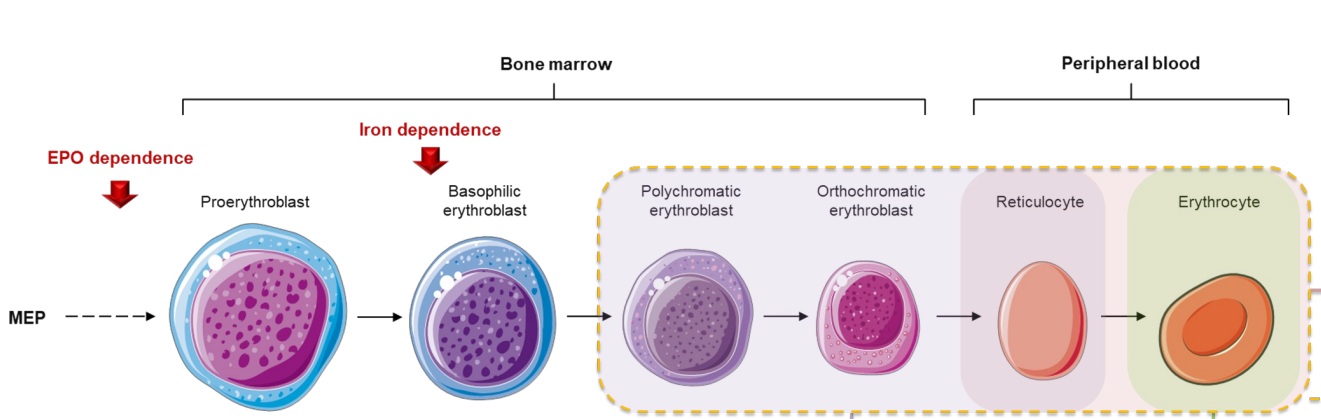
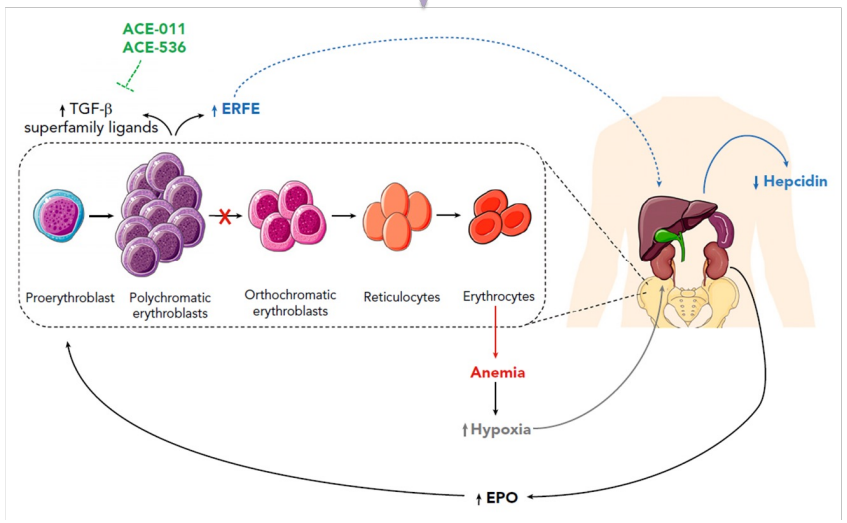
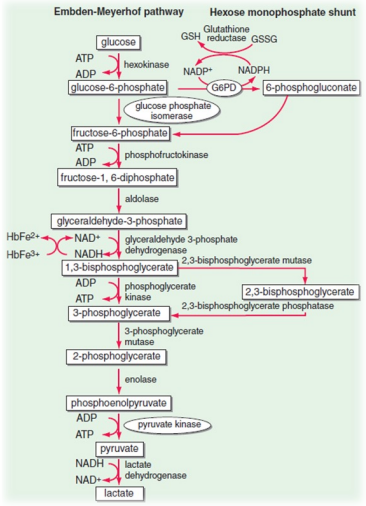
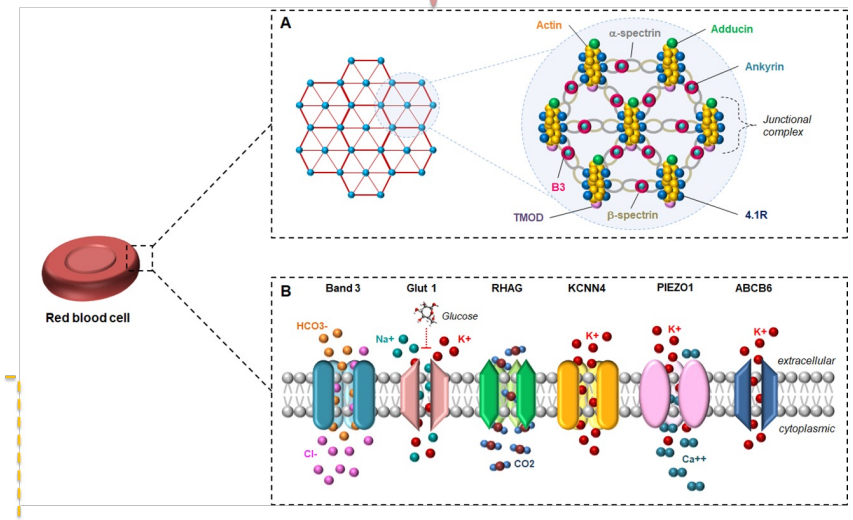
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
BMS					x	x	
Vertex			x				

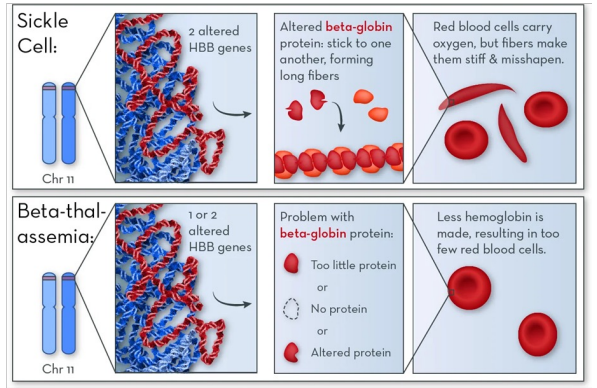
Difetti ereditari del globulo rosso











Iolascon A, Andolfo I and Russo R, BJH 2019
Iolascon A, Andolfo A, Russo R. Blood 2020
Luzzatto L, De Franceschi L. Chapter 100 . Hemolytic Anemias - Harrison's Hematology and Oncology 2023
Locatelli F, Cavazzana M, Frangoul H, Fuente J, Algeri M, Meisel R. Mol Ther. 2024

1. Paziente con beta trait, alt. indici emolitici, splenomegalia

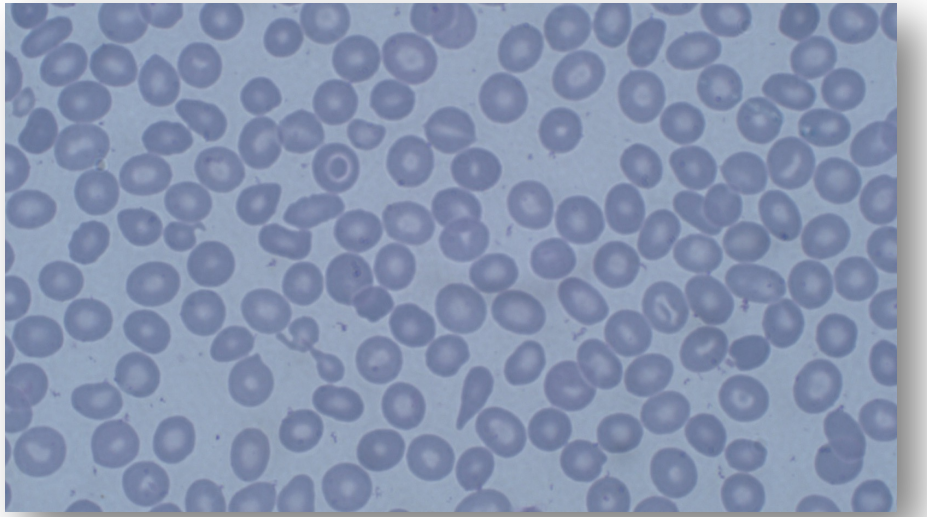
Anamnesi familiare: 5 fratelli, 4 riferiti beta trait ed 1 non beta trait con splenomegalia
1 figlio con beta trait e splenomegalia

Gender	†Age (y)	RBCs (×106/µL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	TS (%)	Ferritin (ng/mL)
M	49	6.22	11.9	60.8	19.2	31.6	15	1.3	1.0	194	38	47	248

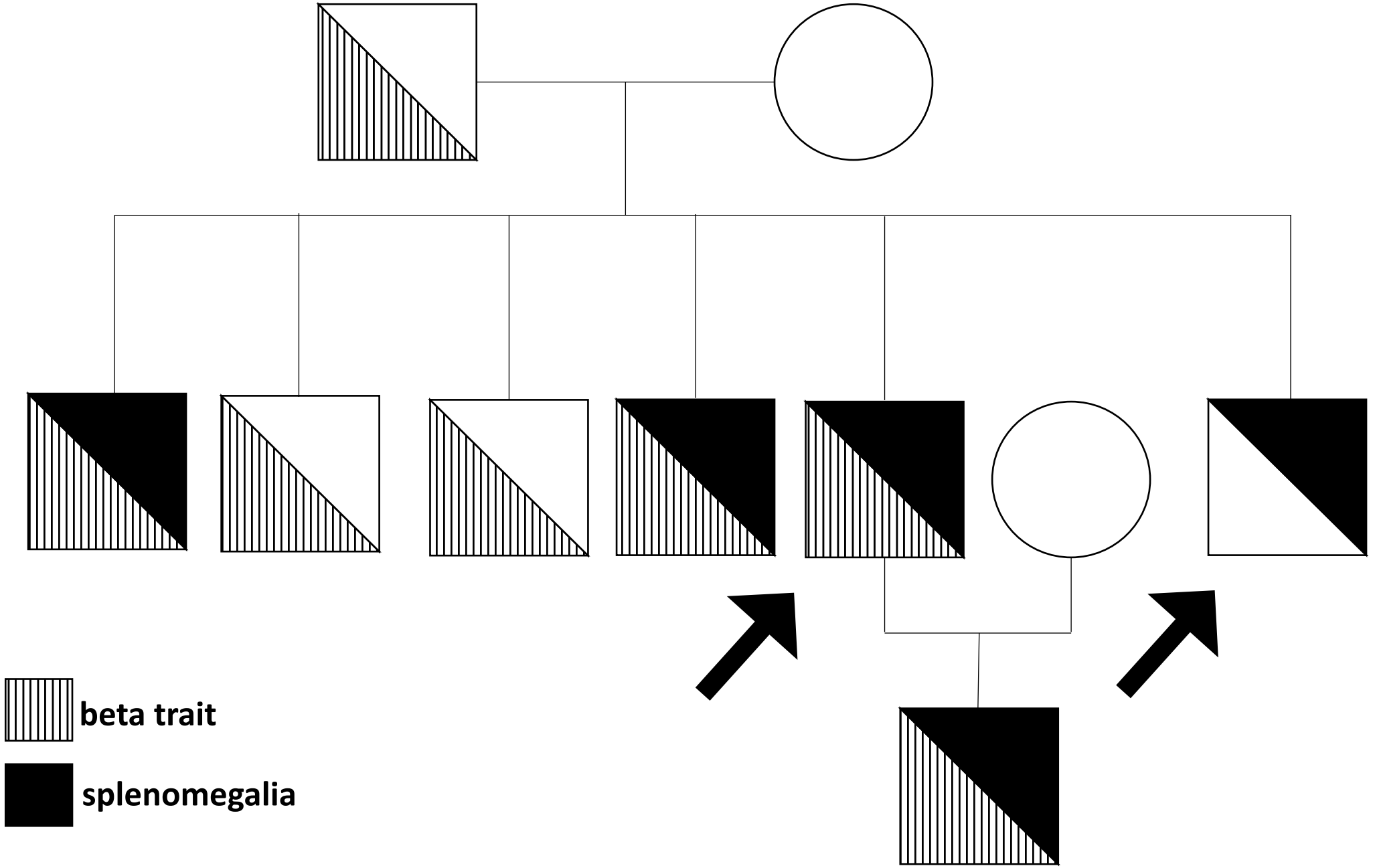
RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Tb, total bilirubin; LDH lactate dehydrogenase; TS, transferrin saturation
†Age at diagnosis

HPLC	*HBA1/HBA2*	Clinical manifestation
HbA2 5.4% HbF 1.5%	No variants	Splenomegaly (18 cm), gallbladder stones

HPLC, high-performance liquid chromatography



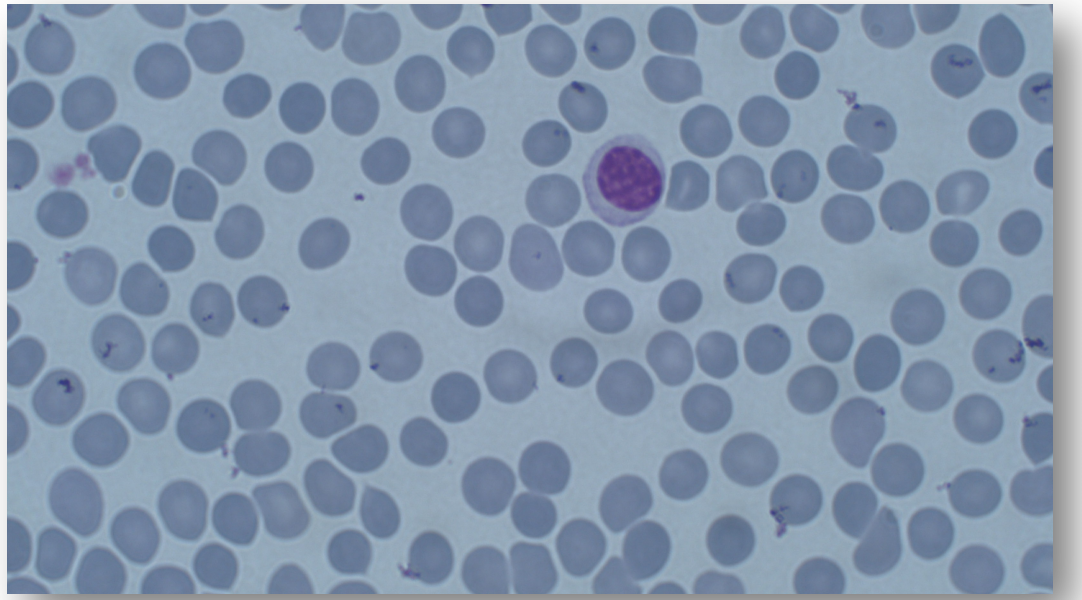
Pinto VM, Russo R*, ... Iolascon A, Gian Luca Forni, Andolfo I. AJH 2023*


beta trait
splenomegalia

1. Fratello non beta trait con splenomegalia

Gender	†Age (y)	RBCs (×106/µL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	TS (%)	Ferritin (ng/mL)
M	57	5.29	16.1	86.1	30.5	35.4	12.9	1.5	0.6	188	92	43	153

RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Tb, total bilirubin; LDH lactate dehydrogenase; TS, transferrin saturation
†Age at diagnosis



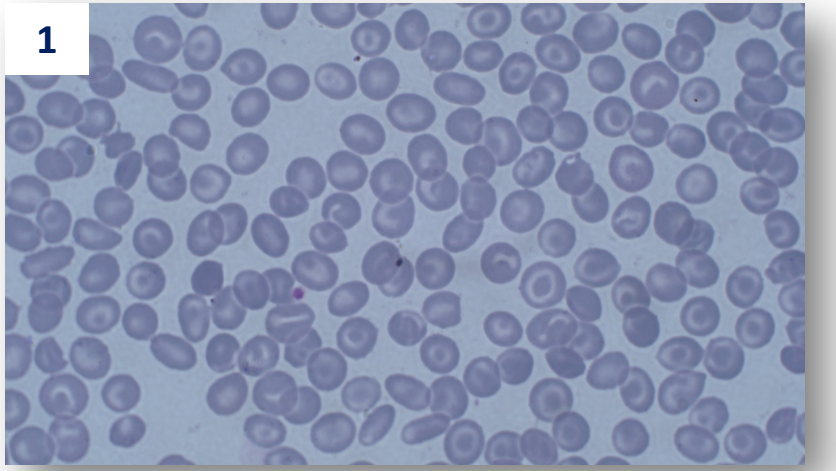
HPLC	Clinical manifestation
HbA2 2.6% HbF 0.2%	Splenomegaly (14 cm)

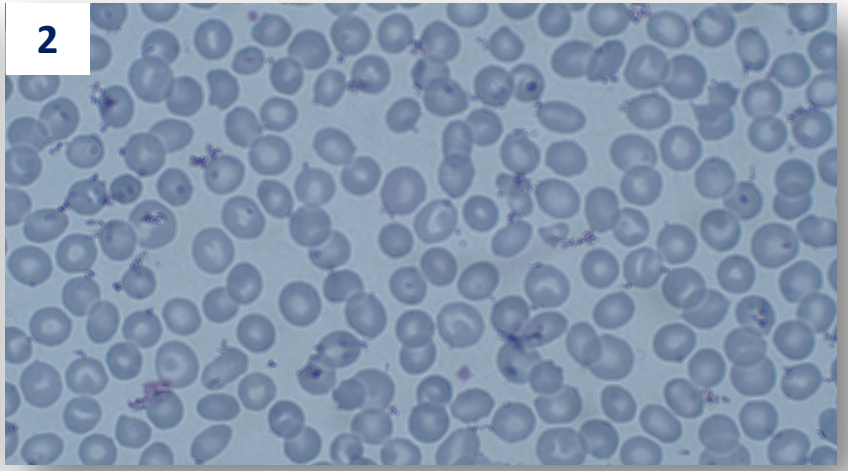
HPLC, high-performance liquid chromatography

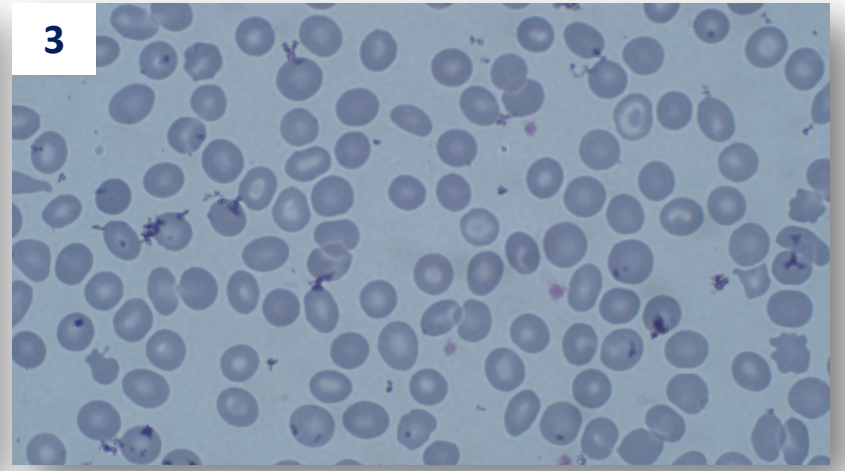
1. Figlio e fratelli con beta trait

	†Age (y)	RBCs (×106/µL)	Hb (g/dL)	HT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	TS (%)	Ferritin (ng/mL)	HPLC	Additional features	Peripheral blood smears
1	19	7.07	13.3	41.9	59.3	18.8	31.7	18	1.9	1.47	282	5.2	38	145	HbA2 6.0% HbF 0.6%	splenomegaly (17 cm)	anisopoikilocytosis stomatocytes
2	54	6.84	13.8	42.8	62.5	20.1	32.2	14.4	1.6	0.8	203	52	30	187	HbA2 5.6% HbF 0.6%	splenomegaly (15.4 cm)	stomatocytes
3	88	5.63	11.8	36.3	64	20.9	32.5	14.3	0.6	0.6	185	143	28	345	HbA2 5.5% HbF 0.3%	no splenomegaly	stomatocytes target cells
4	51	6.54	14.4	42.9	65.5	22	33.5	13.9	0.9	0.5	160	142	42	335	HbA2 5.7% HbF 0.5%	no splenomegaly, accessory spleen	anisopoikilocytosis
5	59	6.33	13.8	41.9	66.3	21.8	32.9	14.1	1.3	0.9	195	40	35	107	HbA2 5.7% HbF 0.4%	splenomegaly (15.5 cm)	anisopoikilocytosis stomatocytes

RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Tb, total bilirubin; LDH lactate dehydrogenase; TS, transferrin saturation; †Age at diagnosis



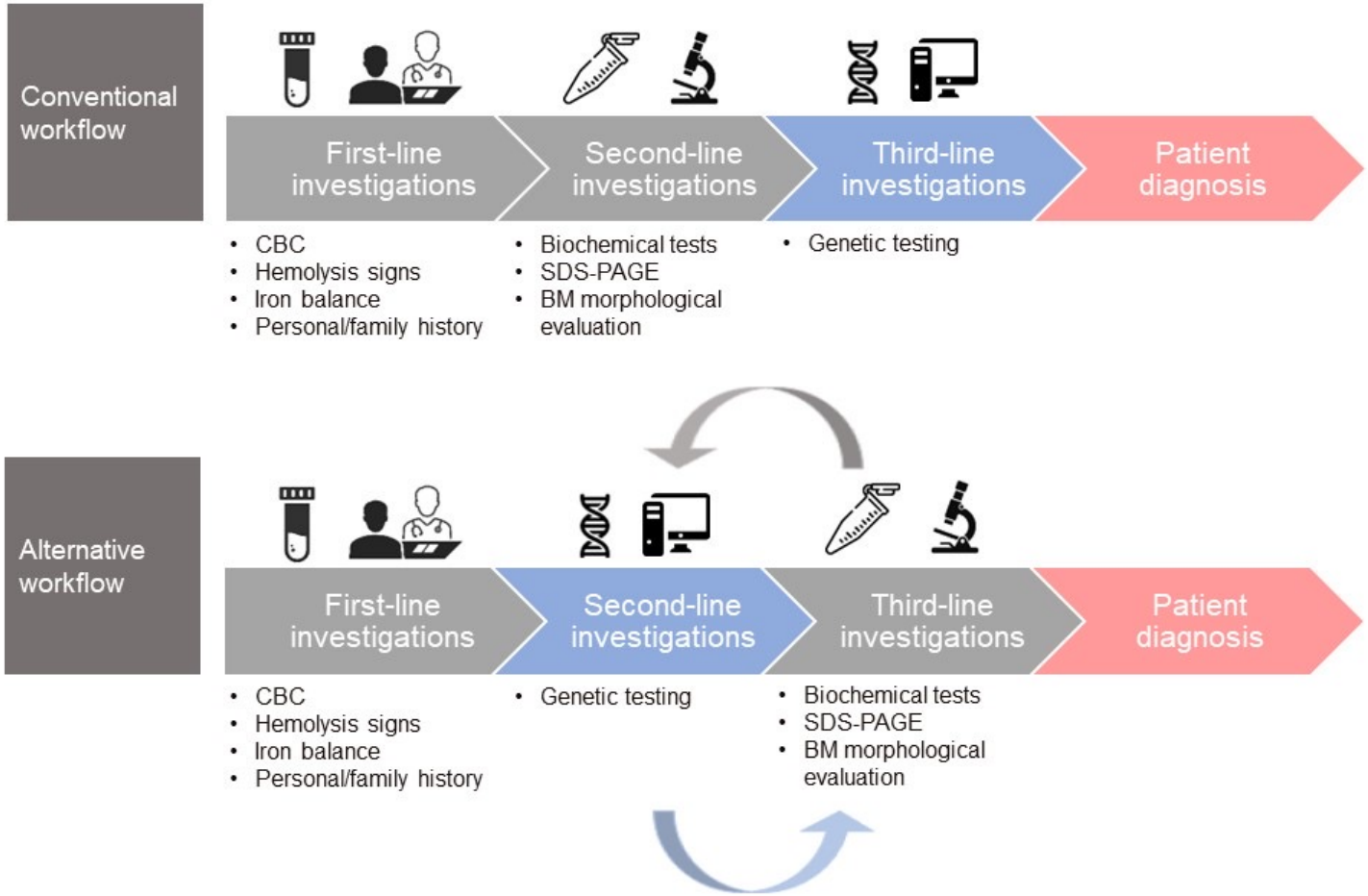


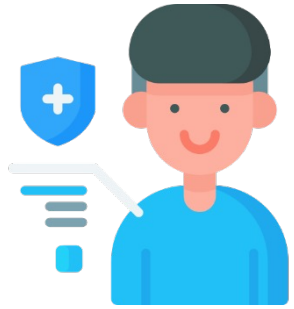


Gender	†Age (y)	RBCs (×106/µL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	Clinical manifestation
M	49	6.22	11.9	60.8	19.2	31.6	15	1.3	1.0	194	38	splenomegaly (18 cm)
M	57	5.29	16.1	86.1	30.5	35.4	12.9	1.5	0.6	188	92	splenomegaly (14 cm)

Manifestazioni cliniche più espresse nel portatore per dimensioni spleniche (18 vs 14), per alterazioni parametri emolitici, per valore di Hb

Workflow diagnostico





Attualmente, i test genetici vengono utilizzati precocemente nel flusso diagnostico quando:

- ✓ i dati clinici non suggeriscono un sospetto specifico
- ✓ il paziente è trasfusione-dipendente
- ✓ il campione deve affrontare un lungo percorso di spedizione

Russo, Iolascon, Andolfo, Marra, Rosato. Int J Lab Hematol 2024
Russo et al, Front Physiol 2020

Workflow diagnostico

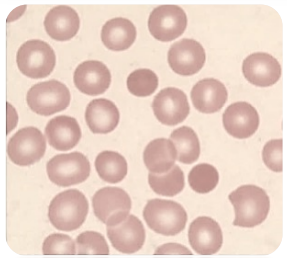
First-line investigations:
1. CBC + retics count
2. peripheral blood (PB) smear
3. family history

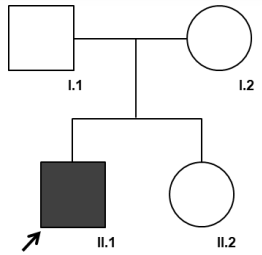
Second-line investigations:
1. Osmotic fragility (OF), AGLT50, Pink, EMA tests, SDS-PAGE
2. Ektacytometry

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

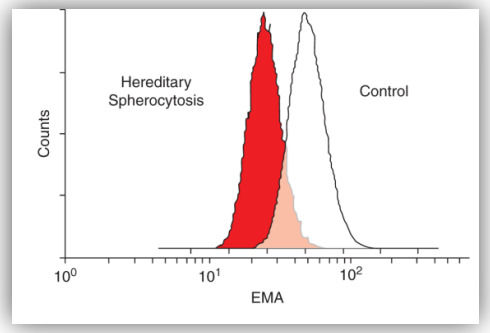
Complete Blood Count

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

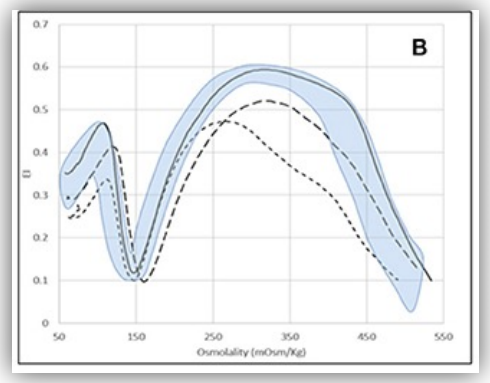




EMA test



Ektacytometry

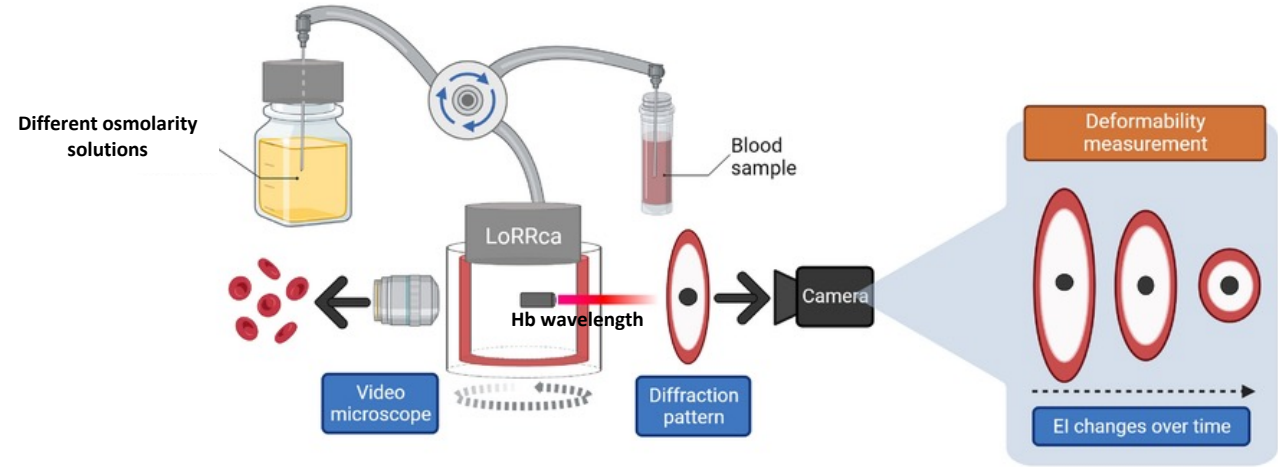


Russo et al, AJH 2018; Andolfo et al, Genes 2021; Zaninoni et al, Front Physiol 2018

Seconda linea di investigazione: ectacitometria

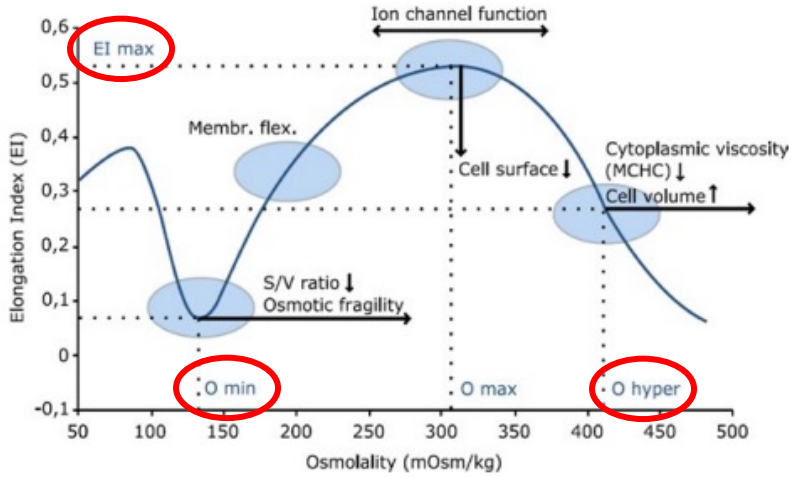
Second-line investigations:

1. Osmotic fragility (OF), AGLT50, Pink, EMA tests, SDS-PAGE
2. Ektacytometry



La curva ectacitometrica a gradiente osmotico fornisce informazioni su

- deformabilità dei globuli rossi (**EI**)
- fragilità osmotica (**Omin**)
- idratazione cellulare (**Ohyper**)



Seconda linea di investigazione: ectacitometria

Second-line investigations:

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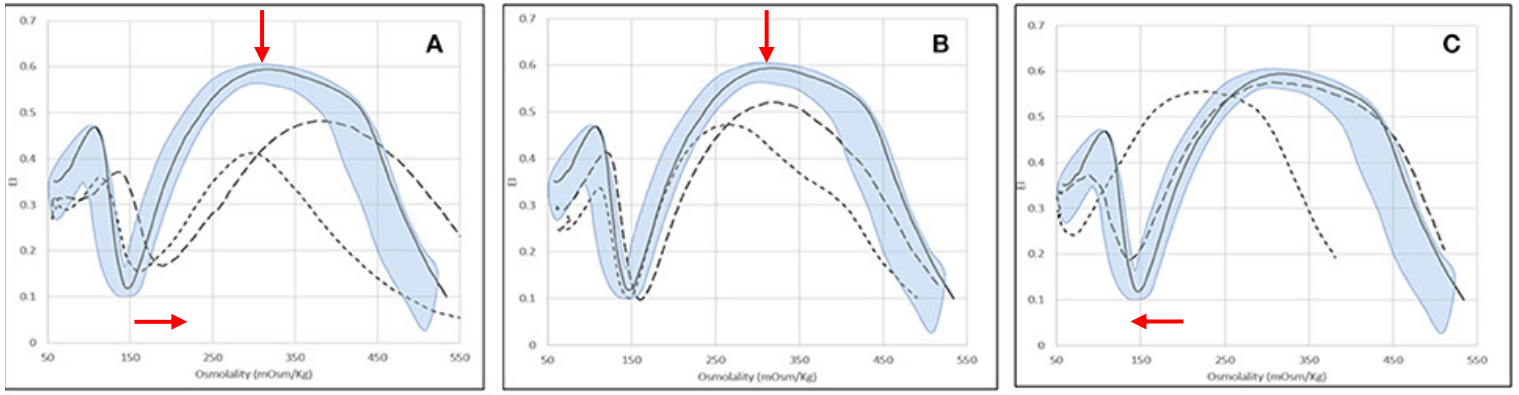


Hereditary spherocytosis | **Hereditary elliptocytosis** | **Dehydrated hereditary stomatocytosis**

- Diminuzione di Dimax (↓ superficie cellulare, deformabilità)
- Spostamento a destra della curva di osmolarità (↓ superficie/volume, fragilità osmotica e iperidratazione)
- Spostamento a sinistra della curva di osmolarità (↑ superficie/volume, fragilità osmotica e disidratazione)

Caveat

→ Sovrapposizione di andamenti in casi di concomitanza di più difetti

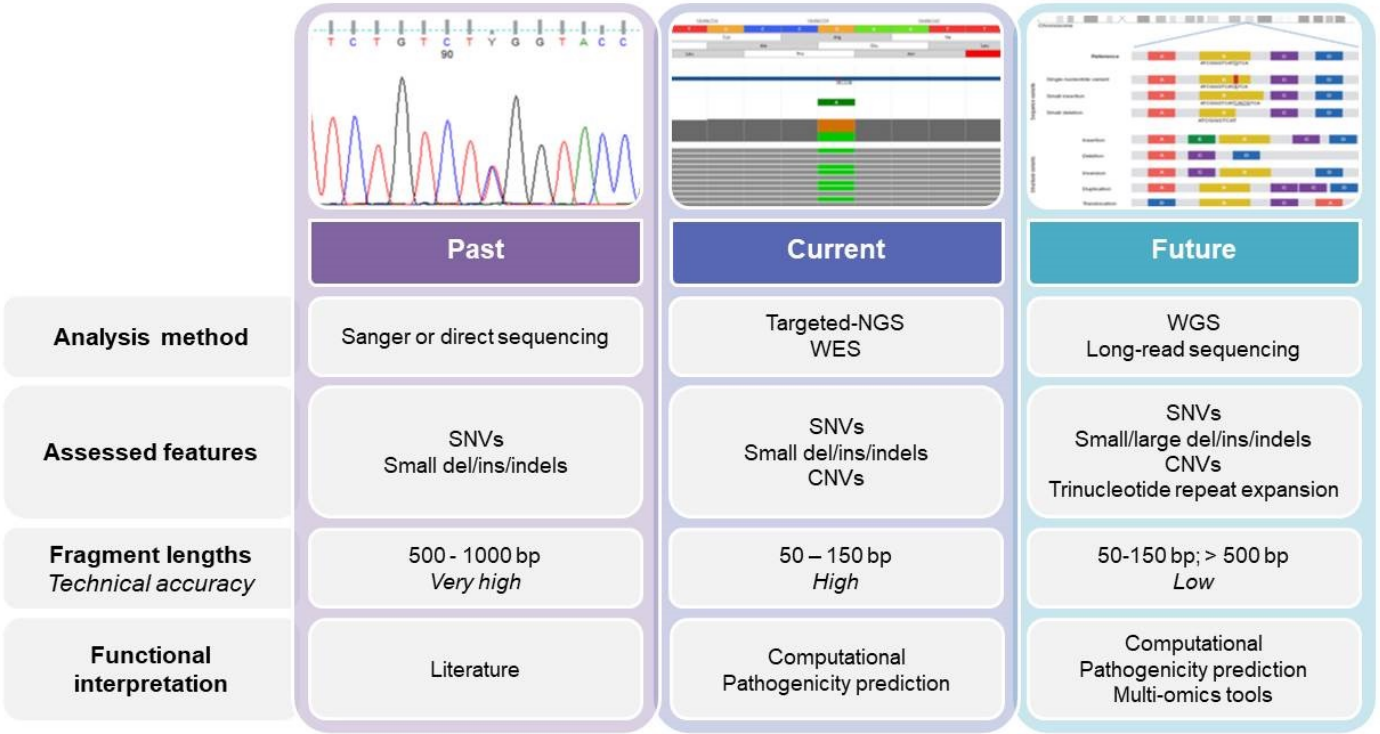
→ Il dato ottenuto va valutato in relazione ai dati clinici e/o genetici

Zaninoni et al, Front Physiol 2018

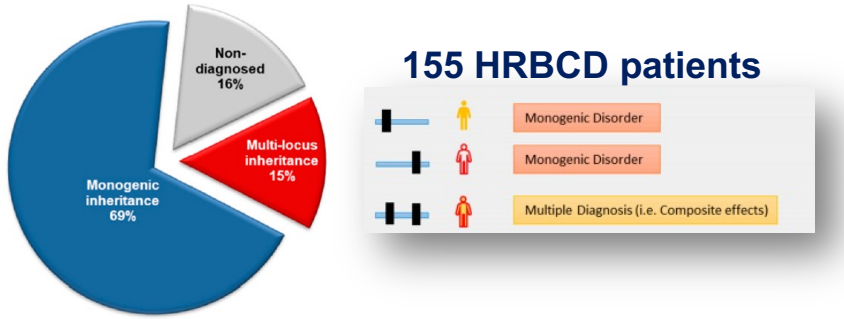
Terza linea di investigazione: test genetico

NGS in ambito clinico

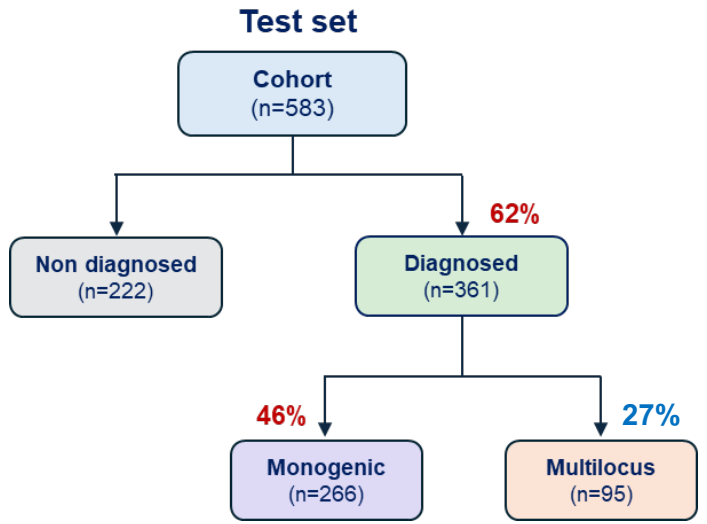
- Pannelli genici personalizzati (100–200 geni)
- Resa diagnostica: **50-70%** dei pazienti analizzati
- Modifica della diagnosi clinica nel **10-40%** dei casi



	Past	Current	Future
Analysis method	Sanger or direct sequencing	Targeted-NGS WES	WGS Long-read sequencing
Assessed features	SNVs Small del/ins/indels	SNVs Small del/ins/indels CNVs	SNVs Small/large del/ins/indels CNVs Trinucleotide repeat expansion
Fragment lengths *Technical accuracy*	500 - 1000 bp *Very high*	50 – 150 bp *High*	50-150 bp; > 500 bp *Low*
Functional interpretation	Literature	Computational Pathogenicity prediction	Computational Pathogenicity prediction Multi-omics tools



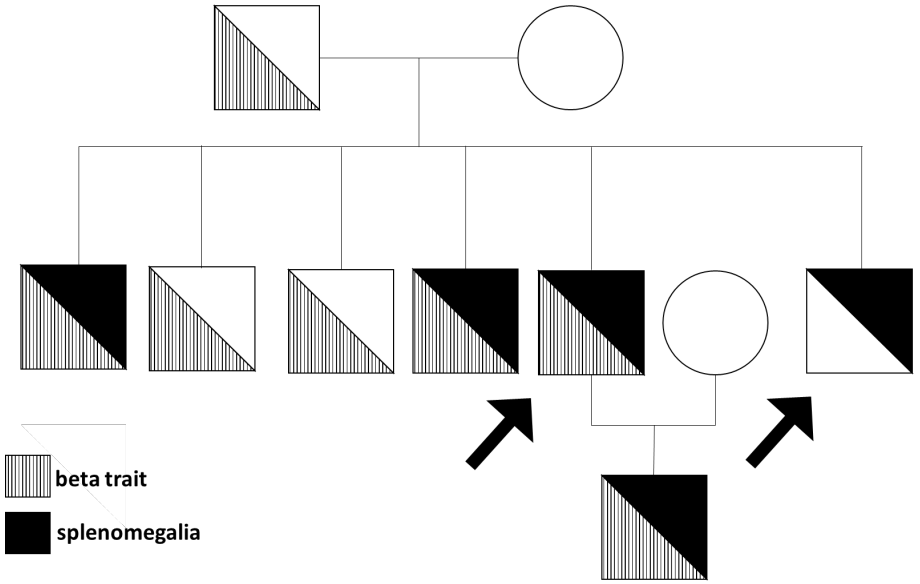
Andolfo I, Martone S, … Iolascon A, Russo R. Genes, 2021



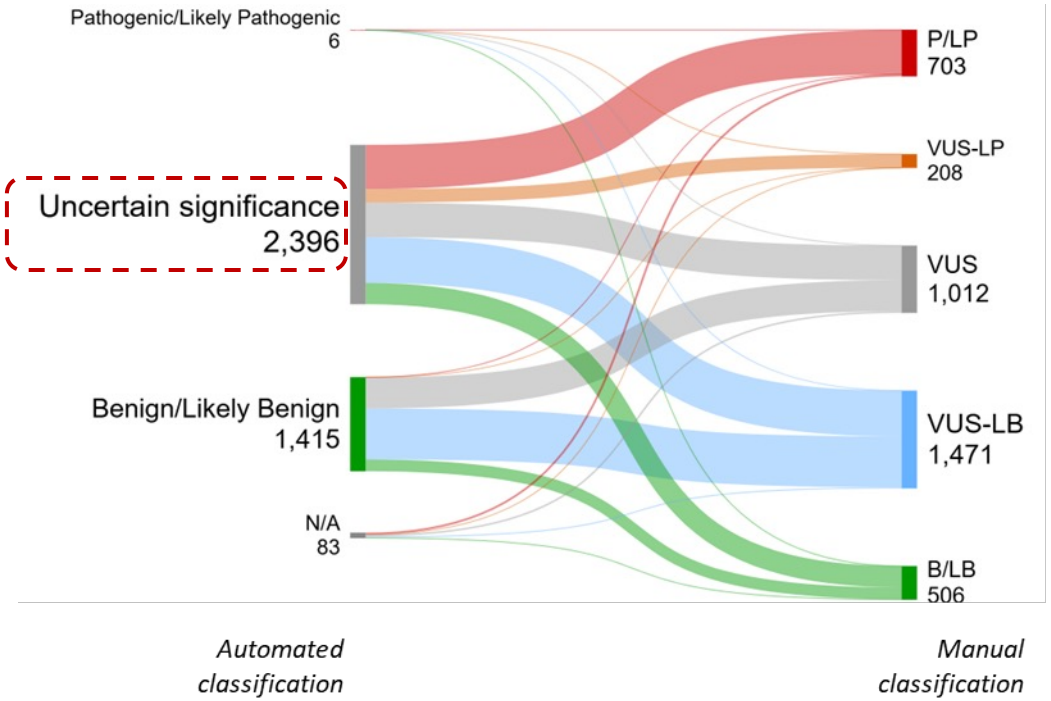
Nostroso A, Marra R, … Andolfo I, Russo R. HemaSphere 2025 (under revision)

Roy NBA, Da Costa L, Russo R. Br J Haematol. 2022
Russo R, Marra R, Rosato BE, Iolascon A, Andolfo I. Front. Physiol. 2020

1. Approfondimento diagnostico



Gene	HGVS cDNA	HGVS proteina	Zigosità	RefSeq ID	AF GnomAD	ACMG
PIEZO1	NM_001142864.4:c.7219G>C	p.Glu2407Gln	Eterozigote	rs200291894	-	VUS



Ulteriore approfondimento?

Analisi ectacitometrica

Rosato BE, Marra R, … Russo R, Andolfo I. AJHG 2025 (submitted)

2. Famiglia con beta trait e presunta diagnosi di «sferocitosi»

→ Revisione di diagnosi per mancata risposta ematologica alla splenectomia e comparsa di complicanze

- ❑ Probandi (madre e figlio) dopo splenectomia mostravano scarsa risposta in termini di risalita del valore di Hb
- ❑ La probanda ha sviluppato dopo splenectomia complicanze di tipo tromboembolico (evento ischemico cerebrale, ipertensione polmonare)
- ❑ All'anamnesi familiare una figlia non beta trait con lieve splenomegalia e stomatociti all'osservazione microscopica dello striscio di sangue periferico

Patient	Splenectomy	†Age (y)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	HPLC	*HBA1/HBA2*
Proband	no	42	9.8	67	22	33.7	18.8	3.1	4.6	544	7	HbA2 5.2% HbF 0.8%	No varianti
Proband	yes	43	9.9	67.4	22	32.6	19.3	7	3.1	180	34		
Son	no	7	9.0									HbA2 3.7% HbF 5.4%	No varianti
Son	yes	16	8.6	66	23.4	35.4	20.7	11.7	2.88	170	14		
Daughter	no	28	12.2	83.1	32.7	39.4	13.3	9.2	1.78	137	76	HbA2 2.8% HbF 0.4%	No varianti

RBC, red blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Tb, total bilirubin; LDH lactate dehydrogenase; TS, transferrin saturation; †Age at diagnosis

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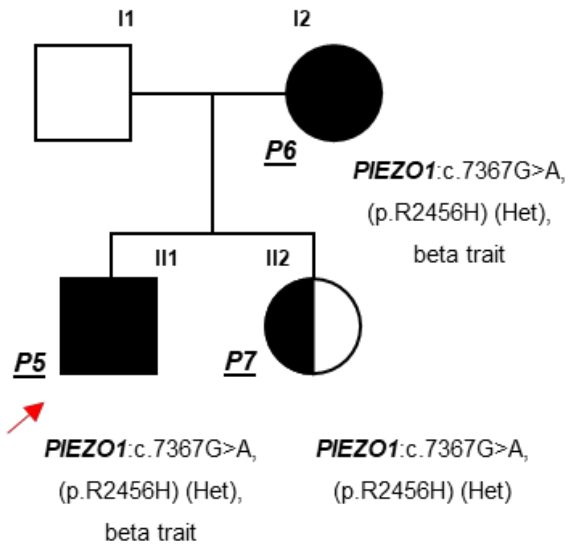
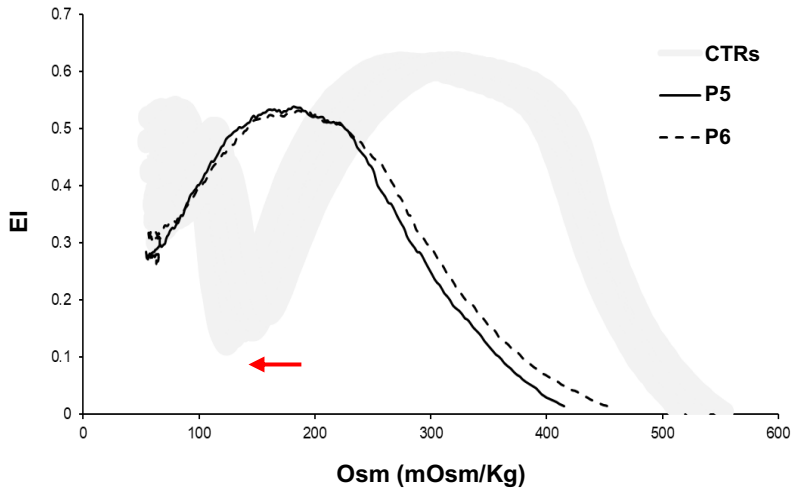
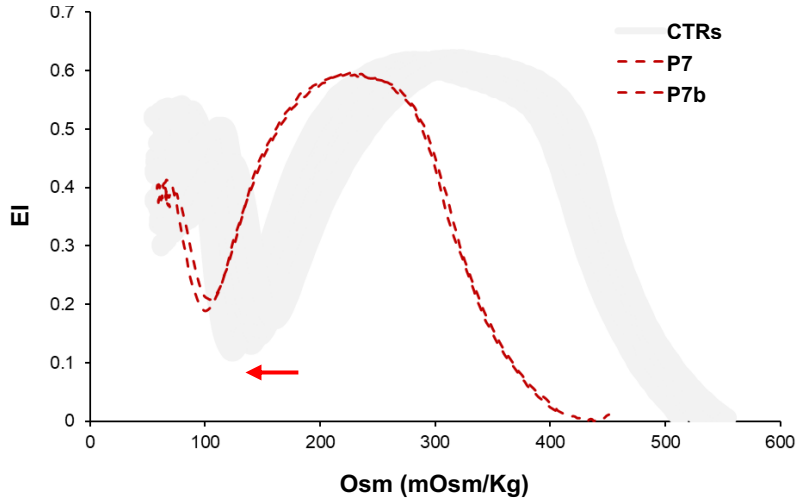
2. Approfondimento diagnostico



Gene	HGVS cDNA	HGVS proteina	Zigosità	RefSeq ID	AF GnomAD	ACMG
PIEZO1	NM_001142864.4:c.7367G>A	p.Arg2456His	Eterozigote	rs587776988	T=0.000002	P

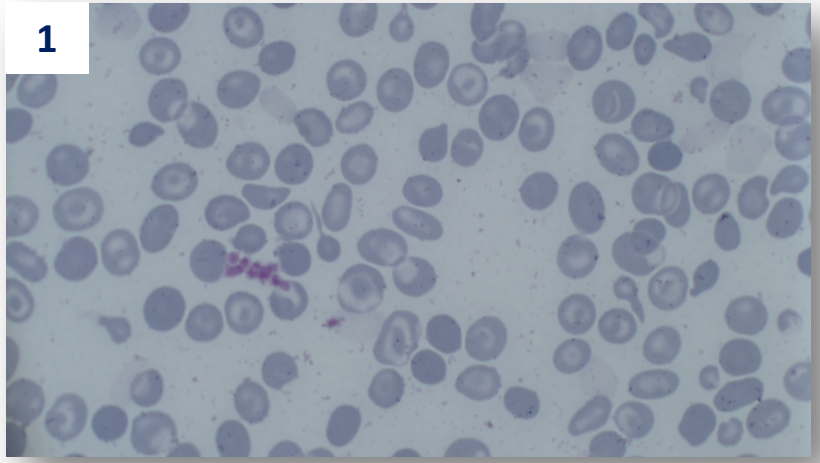


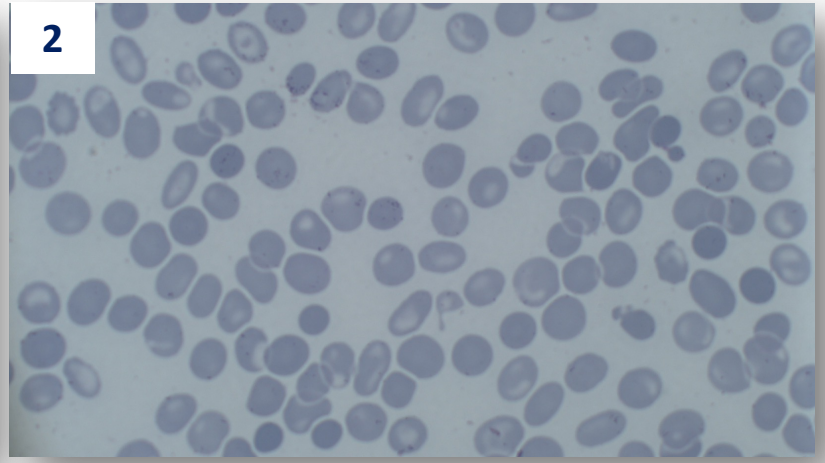


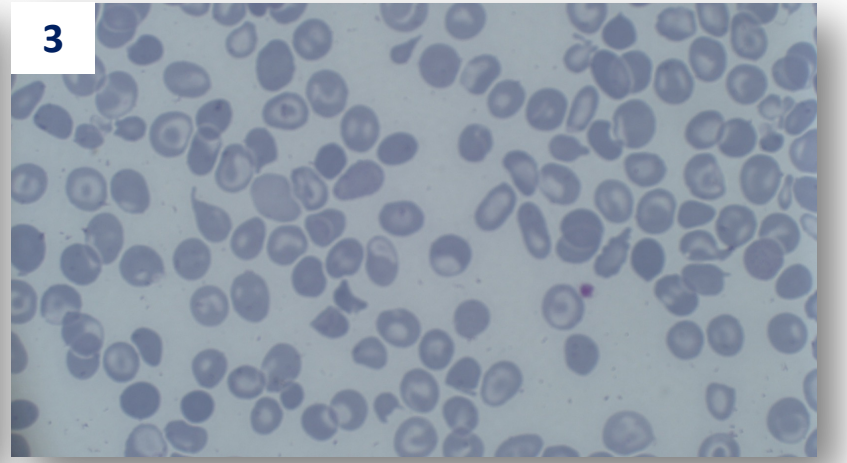
→ La Arg2456His è una delle più frequentemente identificata in pazienti DHS1 e si associa ad un fenotipo tendenzialmente più marcato

3. Famiglia con beta trait, anemia e splenomegalia

Patient	Gender	†Age (y)	RBC (×106/µL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW %	Retics (%)	Tb (mg/dL)	LDH (U/L)	Haptoglobin (mg/dL)	*HBA1/HBA2*	Clinical manifestations
Proband	F	38	**5.44**	**9.3**	**53.9**	**17.1**	317	21	**2.2**	1.0	216	**28.4**	No varianti	splenomegaly (15 cm)
Daughter	F	16	**5.66**	**10**	**55.7**	**17.7**	31.7	**19.9**	1.1	0.65	202	43.4	No varianti	splenomegaly (15 cm)
Son	M	12	**6.06**	**9.9**	**51.8**	**16.3**	**31.5**	**21.6**	1.9	1.1	245	0	No varianti	splenomegaly (12.5cm)







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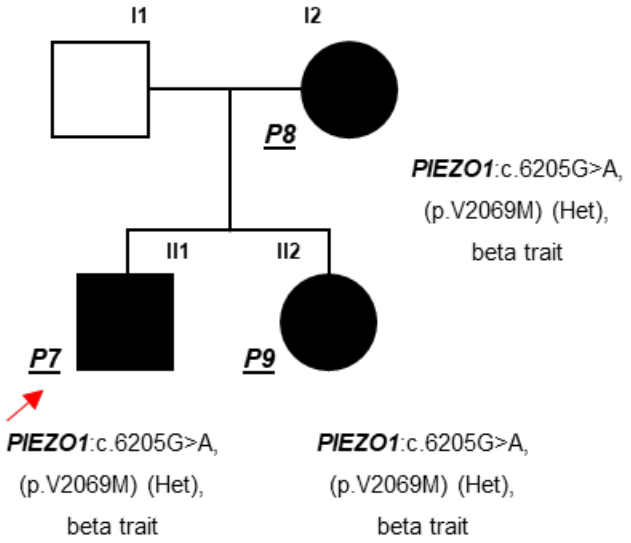
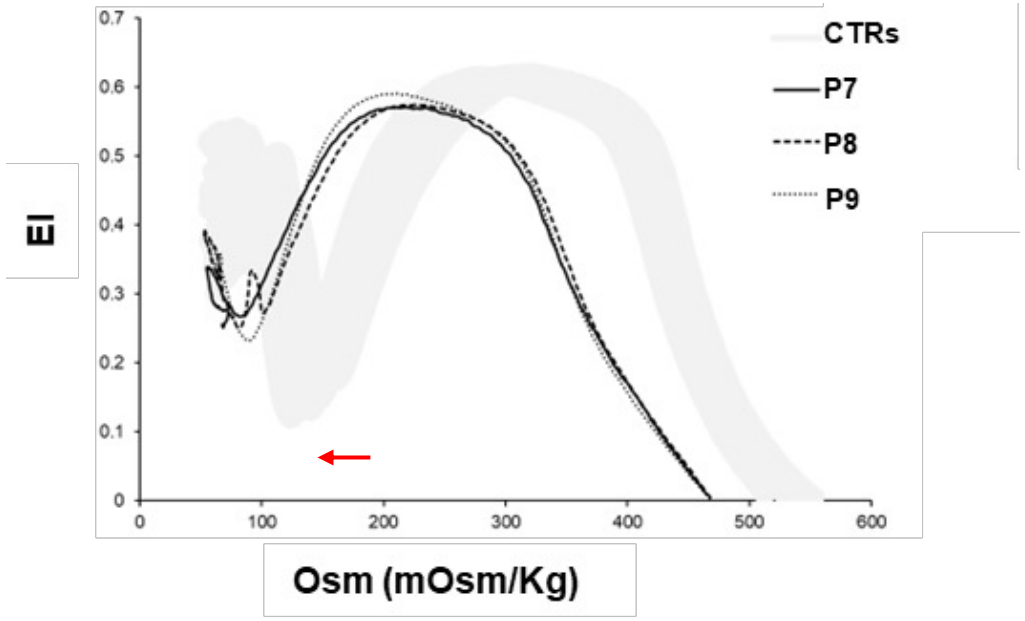
3. Approfondimento diagnostico

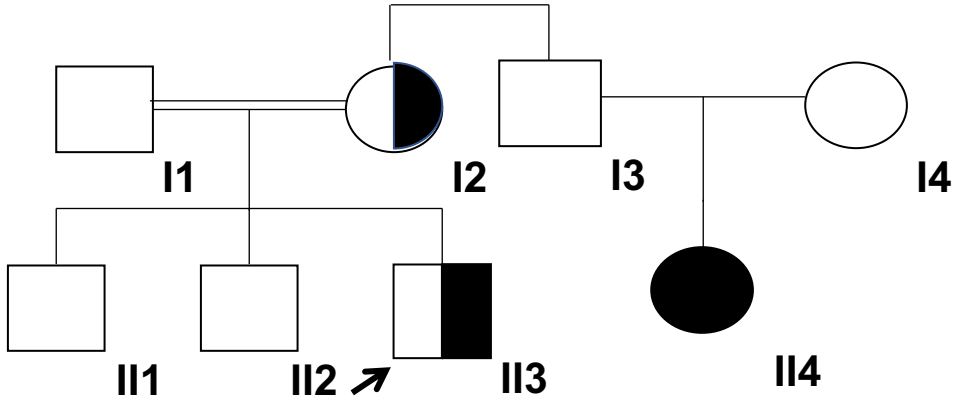


Gene	HGVS cDNA	HGVS proteina	Zigosità	RefSeq ID	AF GnomAD	ACMG
PIEZO1	NM_001142864.4:c.6205G>A	p.Val2069Met	Eterozigote	rs199752762	T=0.000596	LP



4. Paziente con beta trait, emolisi, splenomegalia

→ **Anamnesi familiare**: cugina affetta da talassemia trasfusione dipendente



→ **Anamnesi remota**

- ❑ all'età di 2 anni diagnosi di anemia microcitica emolitica con necessità di supporto trasfusionale fino ai 7 anni, frequenti crisi emolitiche in concomitanza ad eventi infettivi
- ❑ 37 aa diagnosi di deficit PK (dosaggio enzimatico); *HBA1/2, HBB*: b0 eterozigote
- ❑ 37 aa splenectomia (milza 18 cm) + colecistectomia

- ❑ nessun miglioramento → re- start trasfusioni
- ❑ 42 aa: terapia ferrochelante

Analytics	Value
RBC 10*6/uL	3,55
Hb g/dL	**7,4**
HCT %	22,3
MCV fL	**62,8**
MCH pg	20,9
MCHC g/dL	33,3
RDW %	17,6
PLT 10*3/uL	109
WBC 10*3/uL	4,3

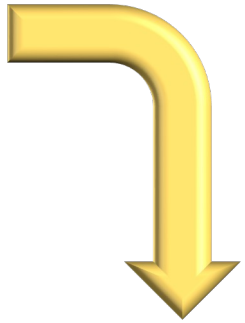
Analytics	Value
Reticolociti %	**12,07**
Reticolociti 10*3/uL	428
Bil totale mg/dl	**7,6**
Bil diretta mg/dl	1,4
Bil indiretta mg/dl	**6,2**
LDH U/L	**1180**
Aptoglobina	**0**

Pre-splenectomy

Paolo Ricchi – AORN Cardarelli
Achille Iolascon – UOC Genetica Medica AOU Federico II

4. Paziente con beta trait, splenomegalia

Approfondimento diagnostico per conferma diagnostica



Gene	HGVS cDNA	HGVS proteina	Zigosità	RefSeq ID	AF GnomAD	
PKLR	NM_000298.6:c.1456C>T	p.Arg486Trp	Omozigote	rs116100695	A=0.0029704	P

La mutazione 1456T (M/M) rappresenta la più comune nel Sud Europa

Le mutazioni M/M in genere hanno un fenotipo «più lieve» rispetto a NM/NM e la splenectomia tende in genere a migliorare il quadro clinico, sebbene in maniera variabile da paziente a paziente

«Peso» del beta trait nell'aggravare il fenotipo della malattia e nel limitare la risposta alla splenectomia

Paolo Ricchi – AORN Cardarelli
Achille Iolascon – UOC Genetica Medica AOU Federico II

Beta trait + difetti ereditari del globulo rosso: risultati preliminari

- Studio su una coorte di 20 BT carriers sintomatici da 16 famiglie non imparentate
- Identificate varianti causative di anemie ereditarie in 15/20 pazienti (**75%**)
  - 66.7% (10/15) → difetti monogenici
  - 33.3% (5/15) → difetti multi-locus

(A)

20 symptomatic BT

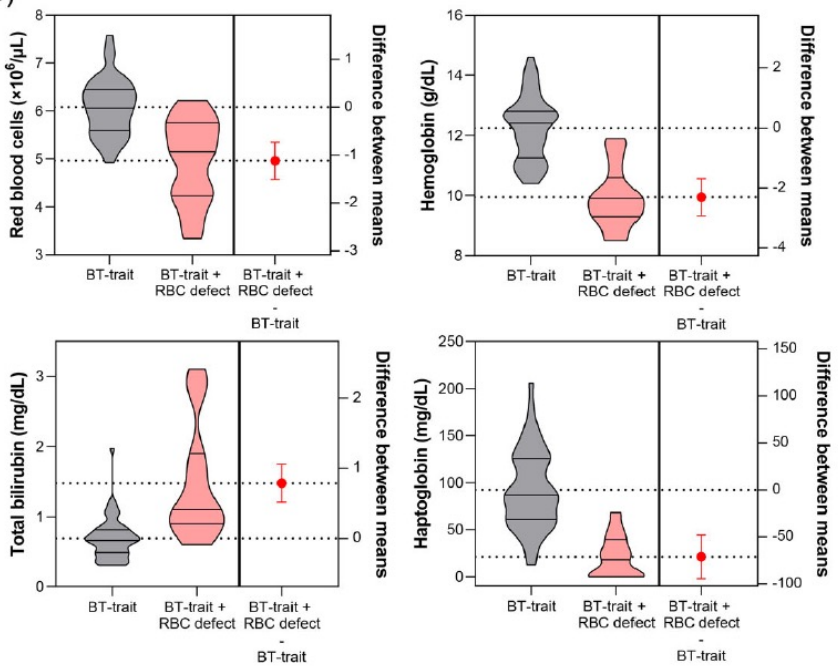
Heterozygous subjects for *HBB* gene mutations in absence of *HBA* pathogenic SNV/CNV with anemia, splenomegaly, and alteration of hemolytic indices

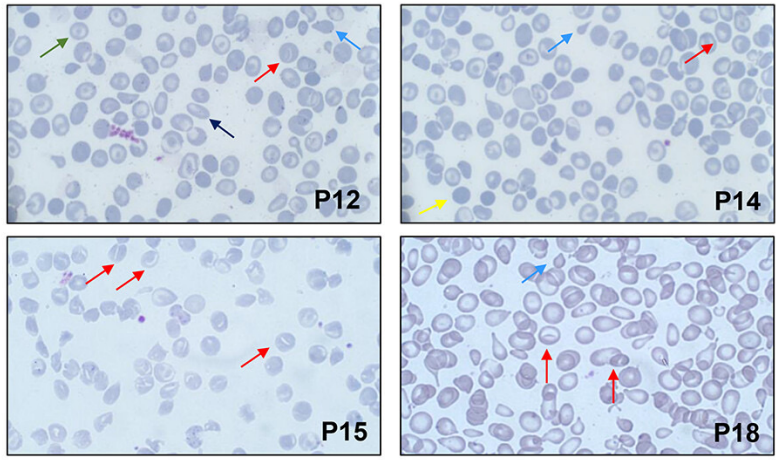
Evaluation of the clinical phenotype clear demonstrated the worsening of the phenotype in the **20 symptomatic BT compared to 53 asymptomatic BT**

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

(B)

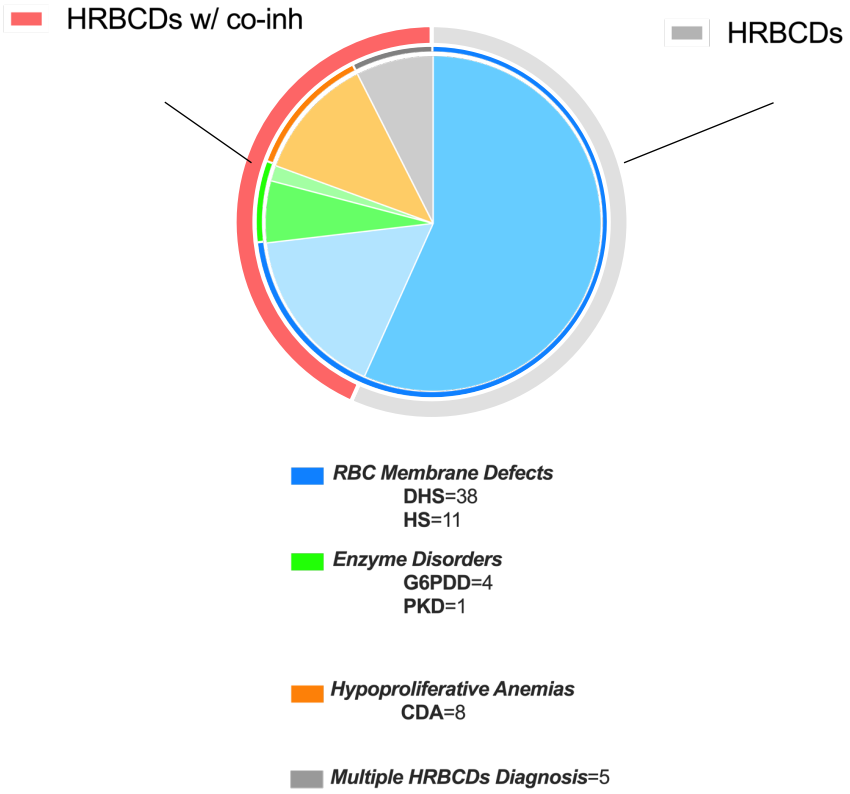




Pinto VM, Russo R*, ... Iolascon A, Gian Luca Forni, Andolfo I. AJH 2023*

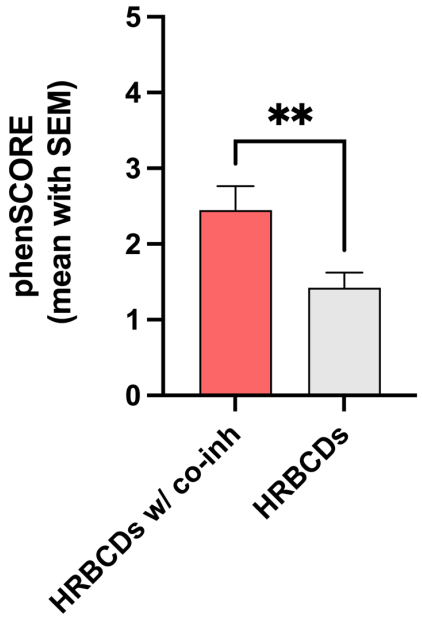
Beta trait + difetti ereditari del globulo rosso: update

- 2023-2025: studio di 67 BT carriers sintomatici
- Identificate varianti causative di difetti di membrana del globulo rosso in 49/67 pazienti (**73%**)
- La maggior parte delle varianti è a carico del gene ***PIEZO1*** (38/67, **57**%)



PhenSCORE (pSCORE)

- Hematological parameters:
  RBC, Hb, MCV, MCHC
- Score construction:
  - **Mean normalization** based on reference values from the general population*
  - **Adjustment** for age and gender
  - Hb values **weighted according to anemia severity** (WHO criteria**)

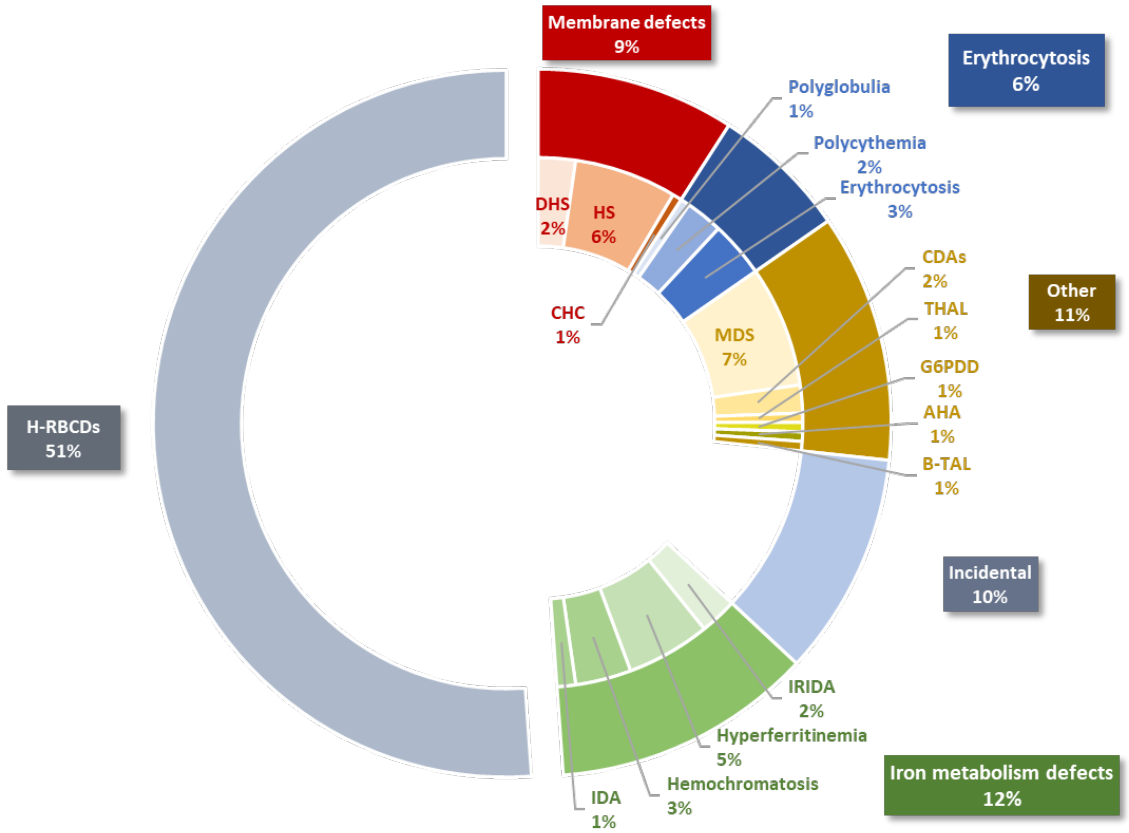


Russo F, Iscaro A, et al. In preparation

*Reference Values from DAIMedLab, **Azienda Ospedaliera Universitaria "Federico II" di Napoli**, ***2024***.
Guideline on haemoglobin cutoffs to define anaemia in individuals and populations. Geneva: **World Health Organization; ***2024***.

Beta trait + difetti ereditari del globulo rosso: update

- Prevalentemente co-eredità di difetti di membrana del globulo rosso
- La maggior parte delle varianti è a carico del gene ***PIEZO1***



Rosato BE, Marra R, … Russo R, Andolfo I. AJHG 2025 (submitted)

- Varianti a carico di ***PIEZO1*** si associano a molteplici sospetti clinici, anche fenotipicamente distanti tra loro
- Varianti in ***PIEZO1*** sono identificate **incidentalmente** nel **10**% dei pazienti analizzati

Corretto inquadramento diagnostico

Corretto inquadramento diagnostico

Anamnesi familiare

Parametri emocromocitometrici completi

Studio della morfologia eritrocitaria

Test di 2°livello (ectacitometria, EMA test)

Test di 3°livello: test genetico (NGS) come conferma

In prima istanza nel nel caso di pazienti trasfusione dipendenti o nel caso di dati clinici non informativi

Corretto inquadramento diagnostico

=

Corretta gestione della patologia

Es: Definire correttamente il difetto di membrana prima di considerare una splenectomia

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