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HEALTH PROFESSIONALS

EHA&EuroBloodNet Spotlight on Congenital BMF syndromes

NGS in Hypoproductive Anemias

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Conflicts of Interest

Novo Nordisk (Consultant)

Agios Pharmaceuticals (Research support)

Hereditary red blood cell defects (H-RBCDs)

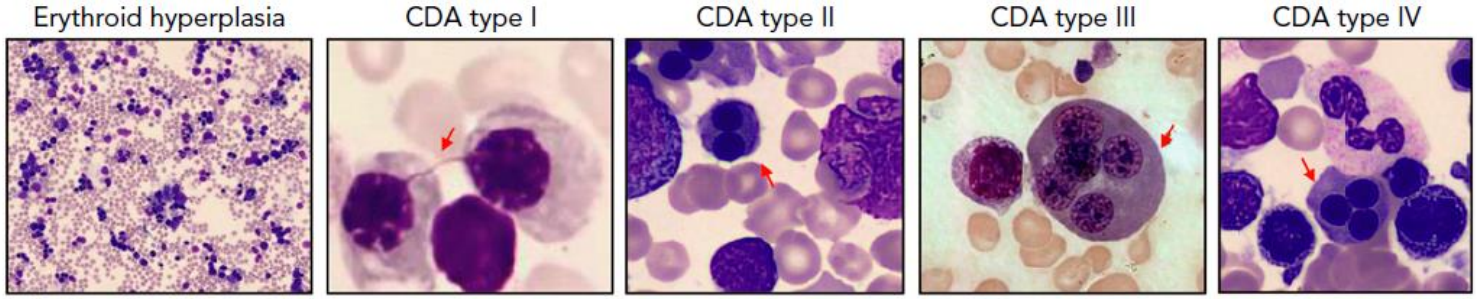
- ✓ Anemia affects 1.6 billion people worldwide
- ✓ About 10% of these individuals are affected by rare anemias of which >80% are hereditary

Acquired (<20%)
Erythropoietic defects (non-regenerative anaemias)
▪ Bone marrow aplasia (BMA) ▪ Pure red cell aplasia (PRCA) ▪ Myelodysplastic syndromes (MDS)
RBC defects (regenerative anaemia)
▪ Paroxysmal nocturnal hemogobinuria (HPN)
Blood plasma abnormalities (regenerative anaemias)
▪ Autoimmune haemolytic anaemia (AIHA)

- **Hypoproductive anemias due to ineffective erythropoiesis**
- Hemolytic anemias due to red cell membrane defects
- Hemolytic anemias due to enzymatic defects
- Anemias due to defects in iron metabolism genes

Hereditary (congenital) Dyserythropoietic Anemias

- ✓ CDAs are Mendelian diseases affecting the normal differentiation-proliferation pathway of the erythroid lineage
- ✓ They belong to a subtype of **bone marrow failure syndromes** characterized by **monolineage** involvement and morphological abnormalities in **erythroid** precursor cells



Erythroid hyperplasia with specific morphological alterations involving late erythroblasts

Williams Hematology, 10th Edition, Chapter 40 by A. Iolascon, R. Russo, R. Khoriaty
Iolascon A, Andolfo A, Russo R. Blood 2020
Russo, Iolascon, Andolfo, Marra, Rosato. Int J Lab Hematol 2024

Physiopathology of CDAs

- Hemolytic anemia
- **Reduced retics count**
- Jaundice
- Splenomegaly
- **Hemosiderosis**
- Gallstones
- TD (≈ 20%)

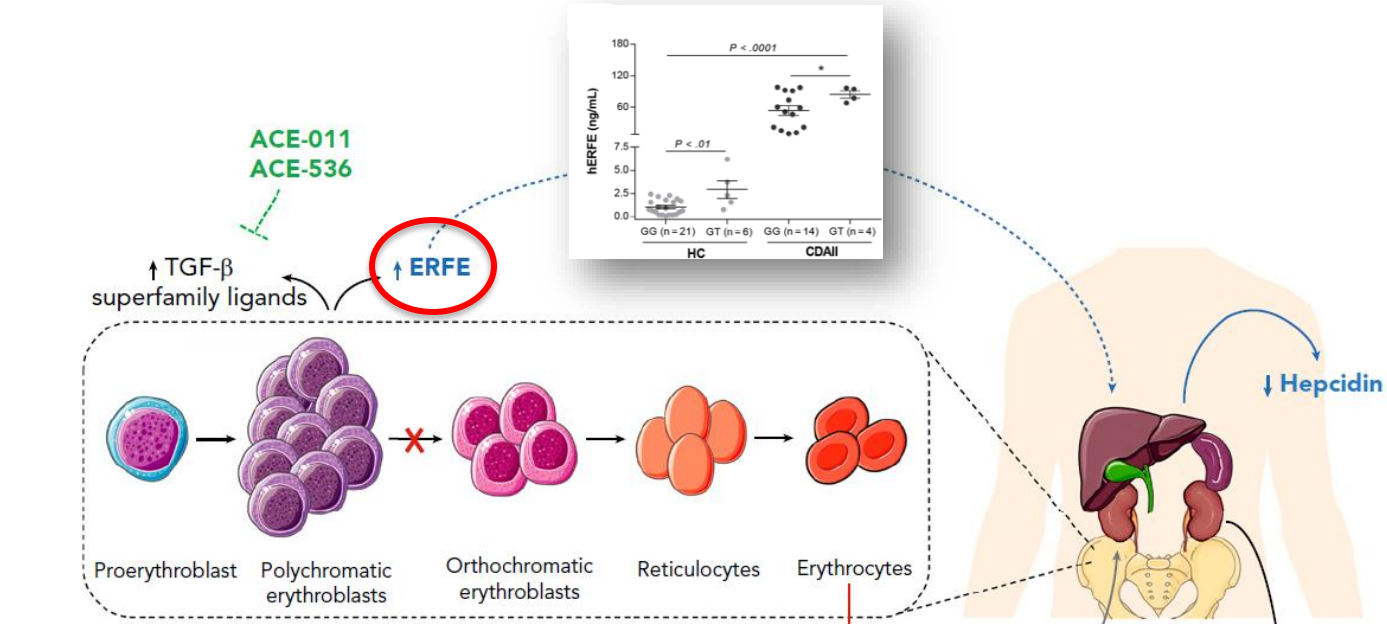
Anemia with reduced reticulocyte count

Increased levels of **EPO** (unable to increase the production of RBCs)

Increased levels of **erythroferrone** (ERFE)

Reduced expression of hepatic hormone **hepcidin**

Increased iron absorption and tissue distribution

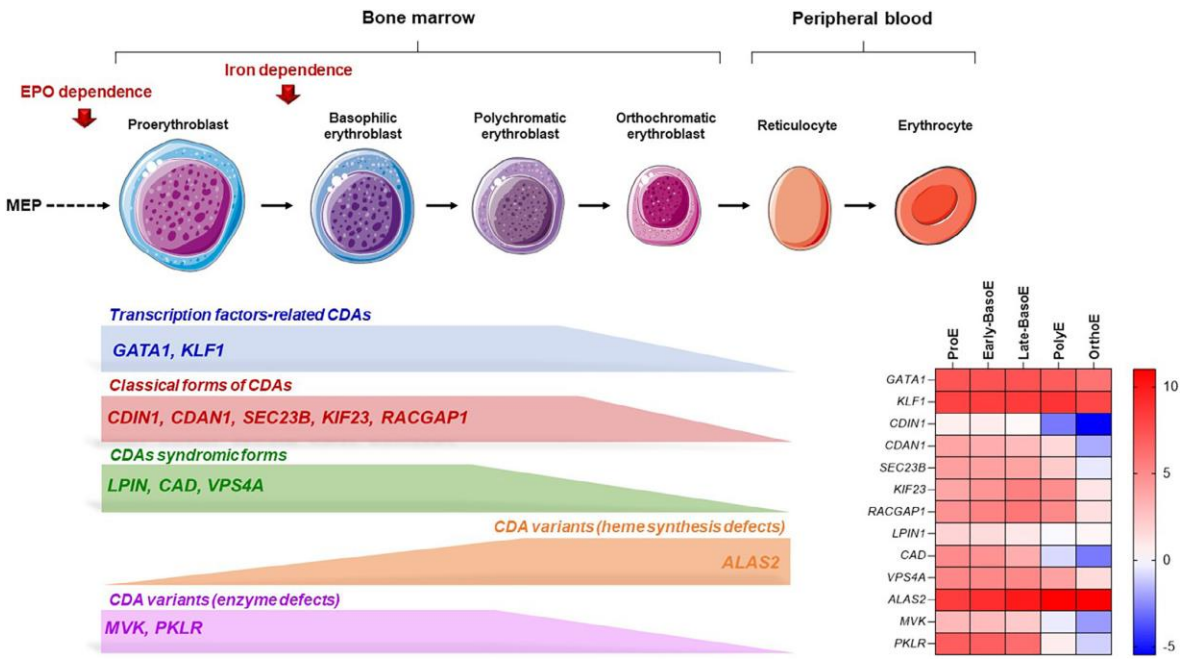


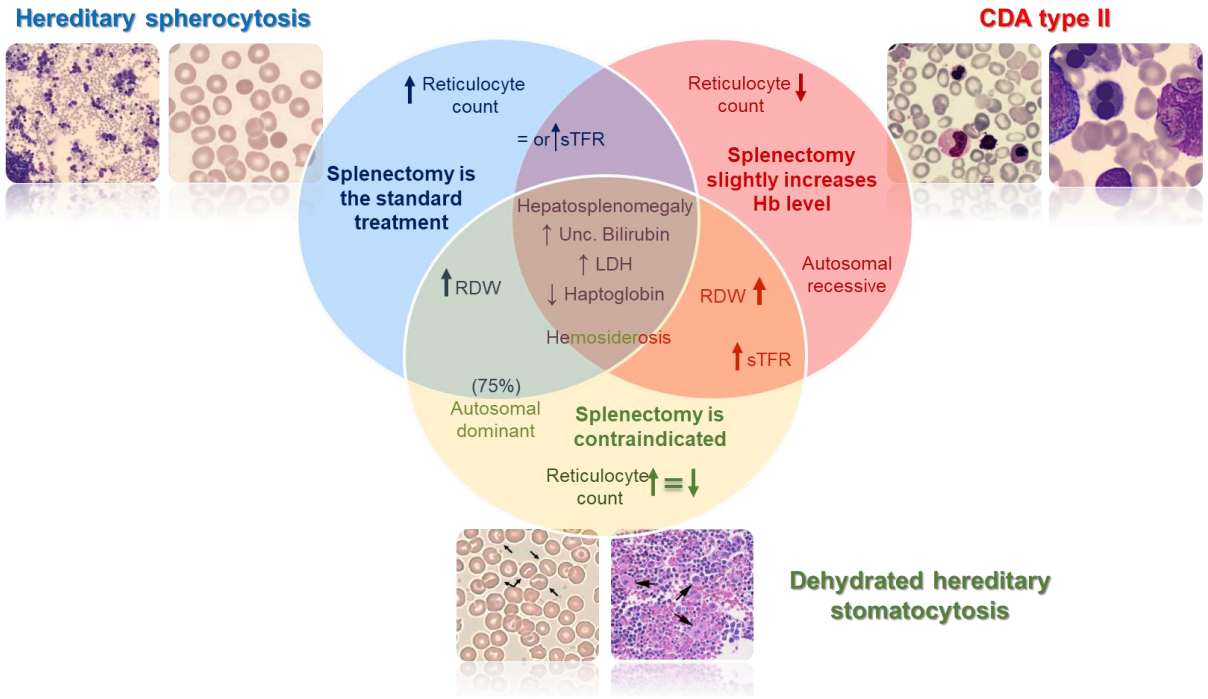
Complete blood count	
RBC (10^6/μl)	3.2 ± 0.1 (1.2–5.8; 93)
Hb (g/dl)	9.6 ± 0.2 (3.6–16.4; 161)
Ht (%)	28.4 ± 0.7 (10.2–49.3; 95)
MCV (fl)	87.3 ± 1.0 (60.0–114.0; 98)
MCH (pg)	29.4 ± 0.4 (20.4–47.5; 89)
MCHC (g/dl)	33.5 ± 0.3 (24.3–38.6; 89)
Retics abs count (x10^3/μl)	88.9 ± 4.9 (6.3–265.7; 115)
Retics %	3.1 ± 0.2 (0.2–13.4; 113)
Reticulocyte Index	1.7 ± 0.1 (0.1–5.1; 90)
PLT (10^3/μl)	360.8 ± 27.0 (96–1065; 64)

Iolascon A, Andolfo A, Russo R. Blood 2020

Genetic and phenotypic heterogeneity of CDAs

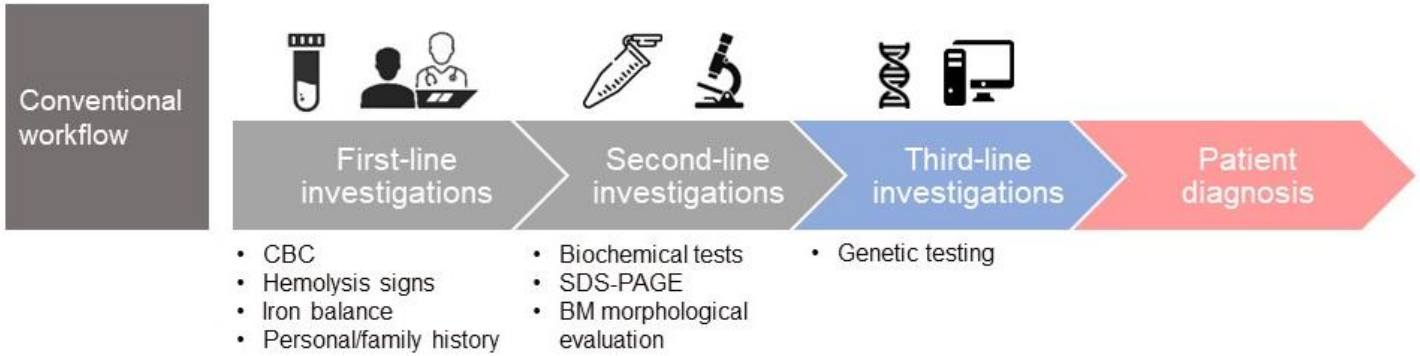
- ✓ > 10 genes associated with CDAs
- ✓ Overlapping clinical features

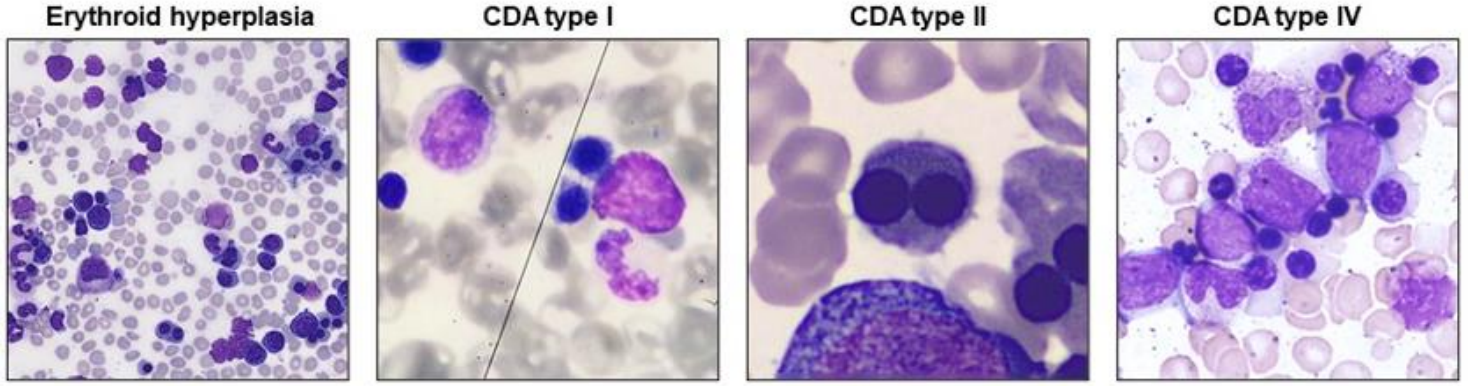




Russo, Iolascon, Andolfo, Marra, Rosato. Int J Lab Hematol 2024

Diagnostic workflow



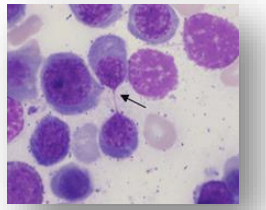
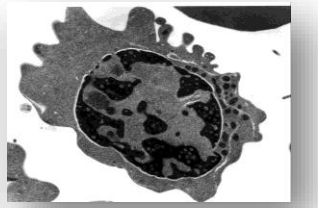
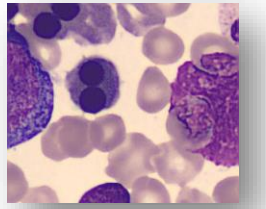
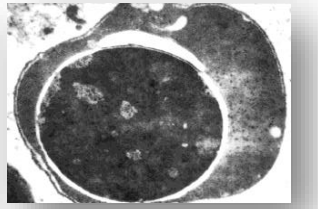
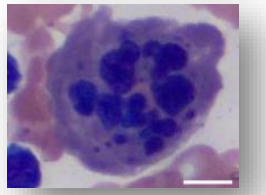


- ✓ Traditional diagnostic techniques rely heavily on heuristic approaches, coupling clinical experience from prior rare disease presentations with the medical literature
- ✓ Bone marrow dyserythropoiesis is a morphological feature common to several conditions

Russo, Iolascon, Andolfo, Marra, Rosato. Int J Lab Hematol 2024

Morphological classification of CDAs

Disease symbol	*Gene* location	Inheritance	Phenotype MIM number[a]	Main clinical and laboratory features	Bone marrow morphological features
Classical forms of CDAs					
CDA Ia	*CDAN1* 15q15.2	AR	224 120	Anemia typically of moderate severity (Hb 8–10 g/dL), often macrocytic. Dysmorphic features present in 4%–14% of individuals: syndactyly, phalangeal hypoplasia, extra metatarsal bones, clubfoot, short stature, thoracic dysplasia, short limbs.	Erythroid hyperplasia with binucleate polychromatic erythroblasts (3%–7%); thin chromatin bridges between nuclei of erythroblasts (1.4%–7.9%). *EM*: spongy heterochromatin (or "Swiss cheese appearance") in up to 60% of early and late polychromatic erythroblasts.
CDA Ib	*CDIN1* 15q14	AR	615 631		
CDA II	*SEC23B* 20p11.23	AR	224 100	Anemia of variable degree, usually moderate (Hb 8–10 g/dL) and normocytic/slightly macrocytic. Hypoglycosylation of the erythrocyte protein band 3.	Binucleated intermediate/late erythroblasts (10%–30%); rare multinucleated erythroblasts. Karyorrhexis; Gaucher-like cells in ~60% of patients. *EM*: double plasma membrane of the erythroblasts.
CDA IIIa	*KIF23* 15q23	AD	105 600	Anemia typically mild or absent. Macrocytosis and poikilocytosis. Hemolysis, jaundice, and cholelithiasis are common. Serum thymidine kinase is markedly increased. Co-occurrence of myeloma and monoclonal gammopathy.	Giant multinucleated (up to 12 nuclei) in 16%–23% of marrow erythroblasts. *EM*: clefts within heterochromatin, autophagic vacuoles, iron-laden mitochondria, myelin figures in the cytoplasm.
CDA IIIb	*RACGAP1* 12q13.12	AR	619 789	Moderate-to-severe macrocytic anemia and hepatosplenomegaly.	Multinucleated erythroblasts and gigantoblasts.

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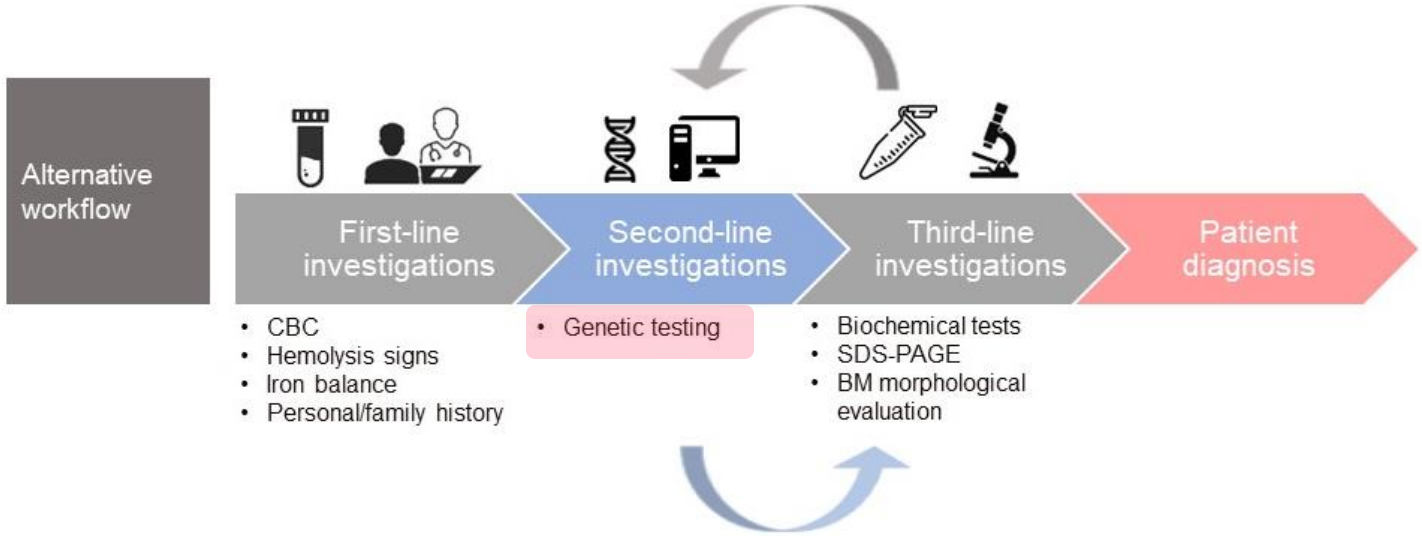
Iolascon A, Andolfo A, Russo R. Blood 2020

Diagnostic workflow



- ✓ Currently, **genetic testing** is used early in the diagnostic workflow when:
- ✓ The clinical data do not suggest a specific suspicion
- ✓ The patient is transfusion-dependent
- ✓ The sample is shipped from other countries (long shipment)

NGS in clinical settings

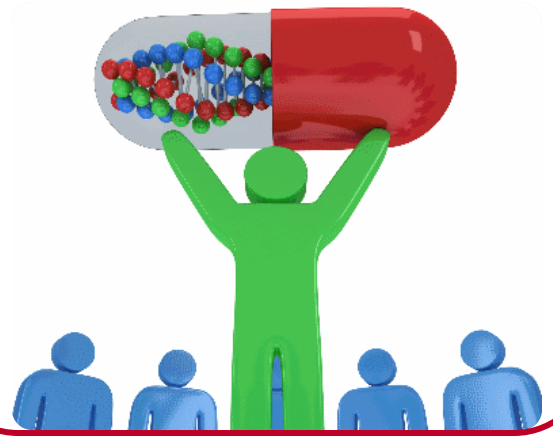
- ✓ Custom or in silico (WES-based) gene panels (100-200 genes)
- ✓ Diagnostic yield: 50-70% of analyzed patients
- ✓ **Modified clinical diagnosis in 10-40% of cases**

Russo, Iolascon, Andolfo, Marra, Rosato. Int J Lab Hematol 2024
Roy NBA, Da Costa L, Russo R. Br J Haematol. 2022
Russo R, Marra R, Rosato BE, Iolascon A, Andolfo I. Front. Physiol. 2020

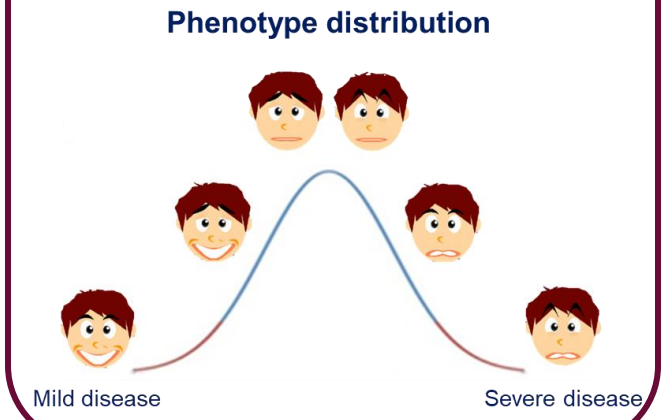
Strengths and opportunities of NGS testing

- ✓ High-throughput sequencing has revolutionized the framework of rare disease diagnosis

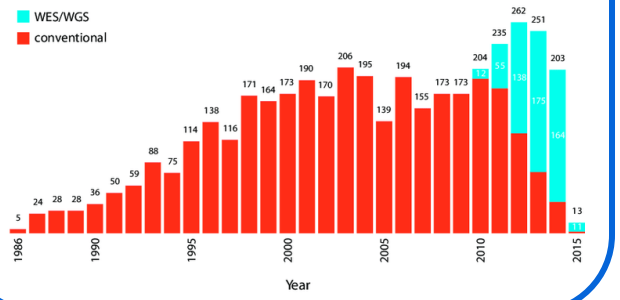
Differential diagnosis for personalized medical care



Genotype-phenotype relationship



Identification of new causative genes



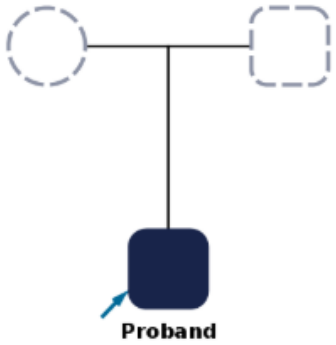
Challenges and limitations of NGS$_{(short-read)}$ testing

Detection

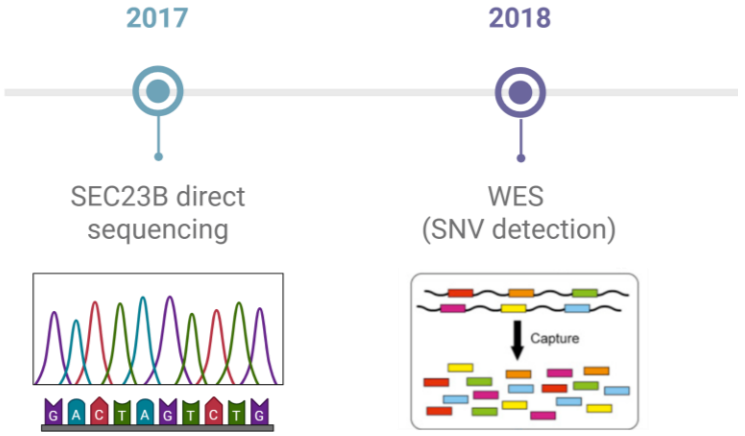
- Sensitivity of mutation detection → somatic variants
- High sequence similarity → e.g., *RPS17* - *RPS17L* (DBA); *SBDS* - *SBDSP1* (SDS)
- **Chromosome imbalance and rearrangements**

Interpretation

- Phenotyping
- Variant interpretation
- Complex inheritance



- Age 1: Microcytic anemia with spherocytes
- Age 7: Splenectomy performed; anemia persisted despite the procedure
- Age 7: Bone marrow aspiration (clinical suspicion: CDA II)
- First access to the laboratory: 2017



- Identification of the heterozygous pathogenic variant **R14W** in *SEC23B* gene
- No second variant identified

Manno, et al. 2025 (in submission)

Challenges and limitations of NGS$_{(short-read)}$ testing

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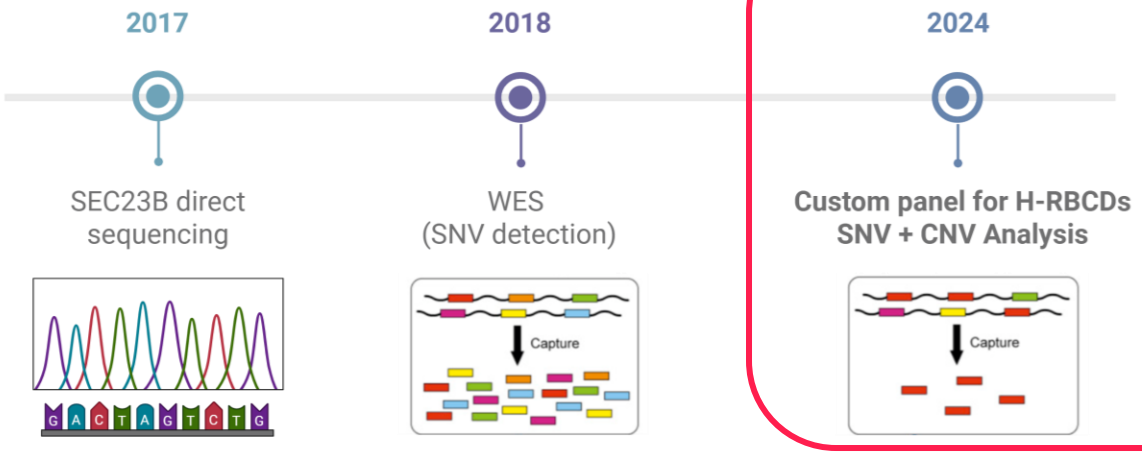

- Heterozygous R14W variant in *SEC23B*
- CNV involving exons 8-11 of *SEC23B*

Manno, et al. 2025 (in submission)

Challenges and limitations of NGS$_{(short-read)}$ testing

- Detection
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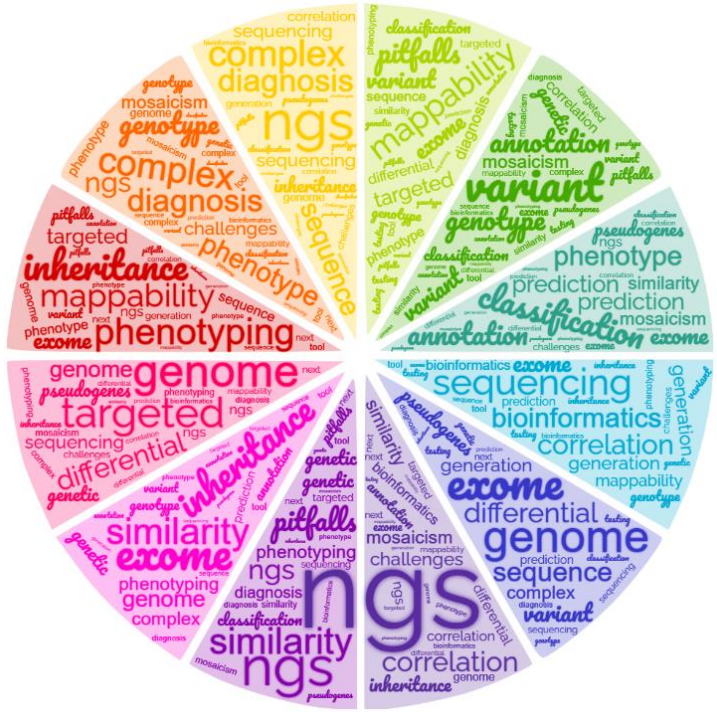
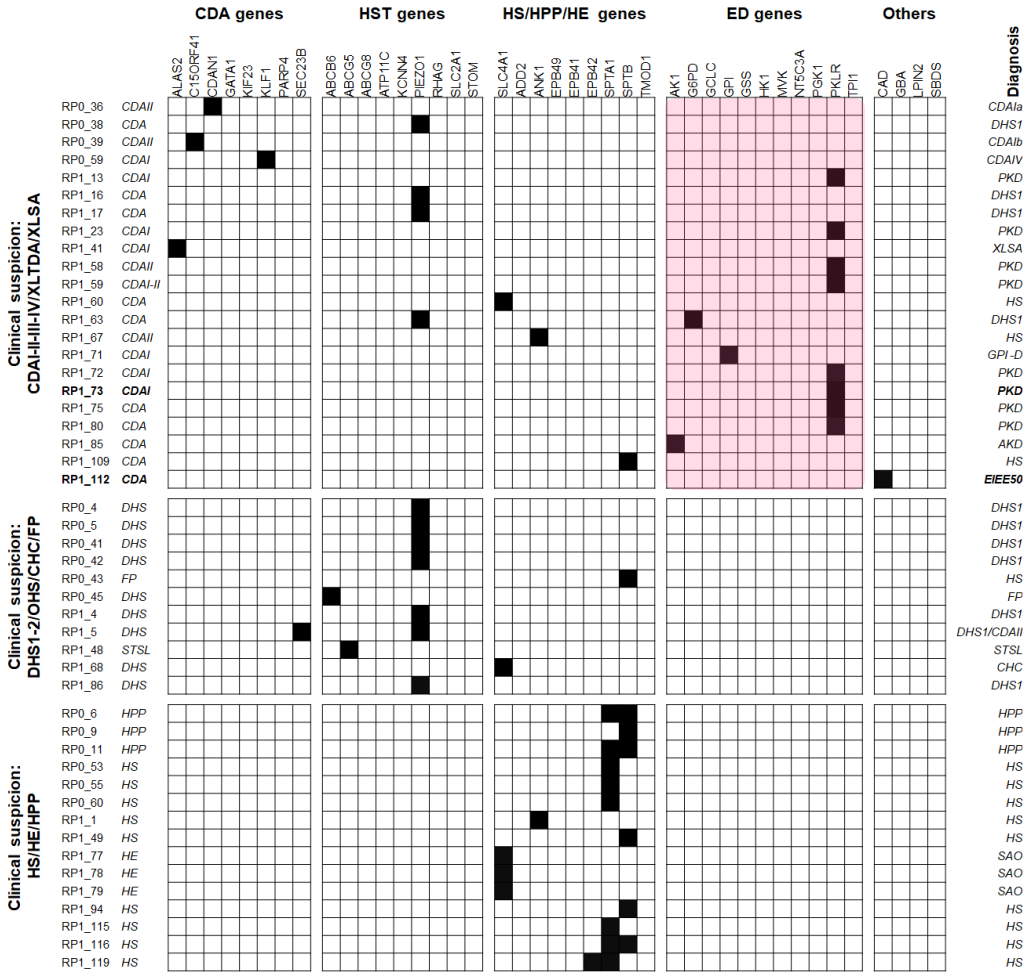
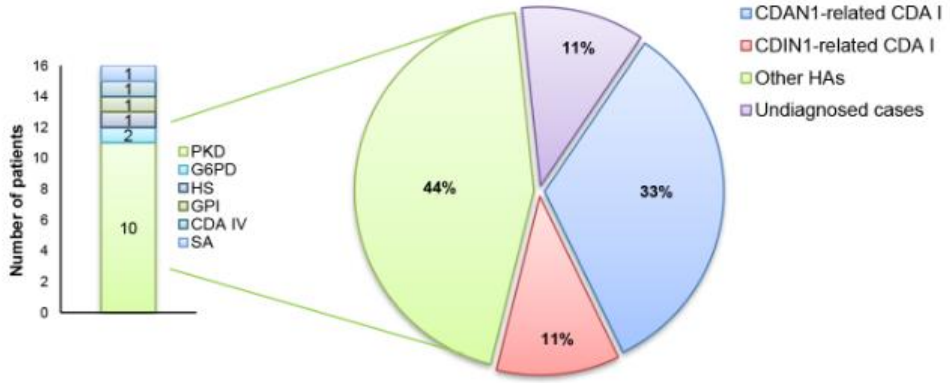


Overlapping genetic features



- ✓ The multi-gene approach modified the original diagnosis in **45.8%** of H-RBCD patients (**non-matched phenotype-genotype**)
- ✓ 81.8% of non-matched patients were clinically suspected to suffer from CDA

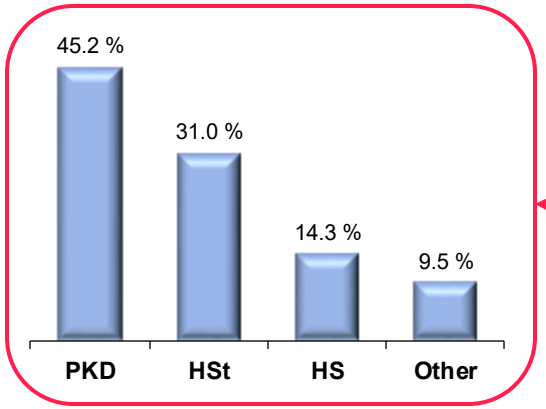
Retrospective cohort study of 36 patients suspected of CDA I

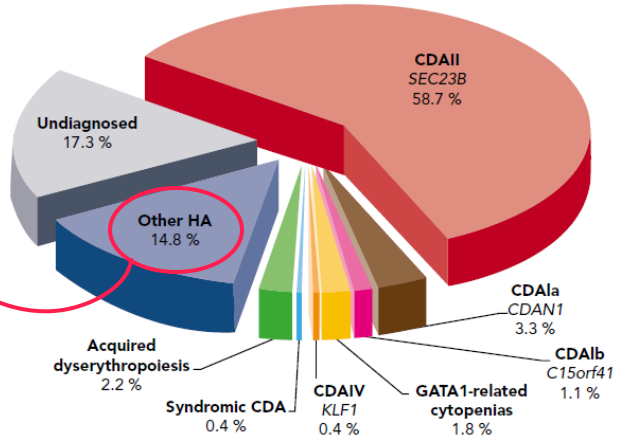


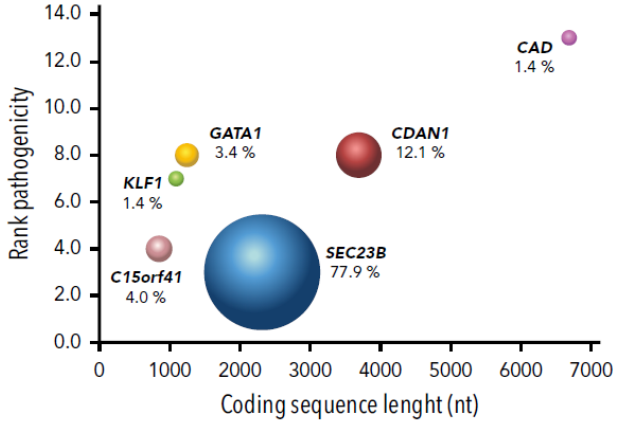
Russo R, et al. Am J Hematol. 2018
Marra R, Nostroso A, ... Andolfo I, Russo R. Am J Hematol 2024

To define the molecular genetics of CDAs

218 patients clinically suspected of CDA
enrolled by the Medical Genetics Unit of Naples







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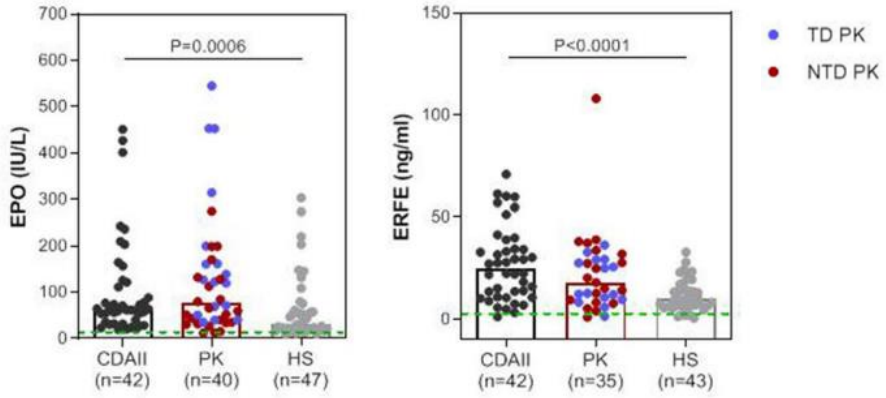
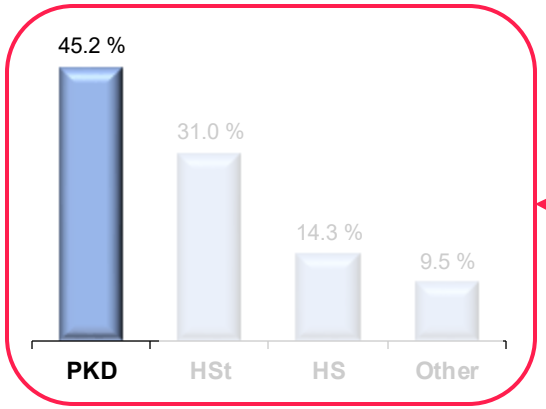
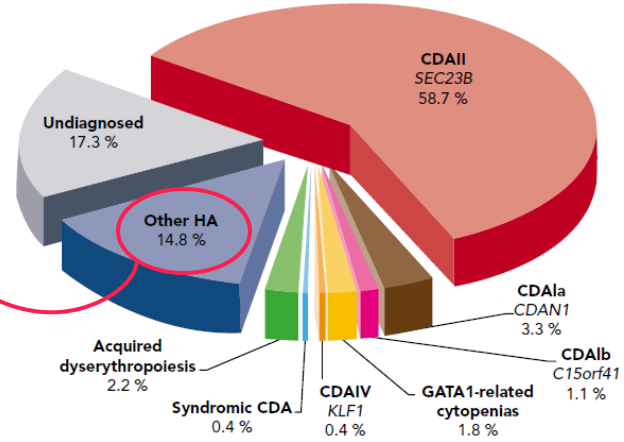


- Approximately 7% of suspected CDAs show *PKLR* gene variants
- CDA patients show similarities with those affected by PKD
- **Therapeutic implications**: Mitapivat (AG-348), a small-molecule activator of PK

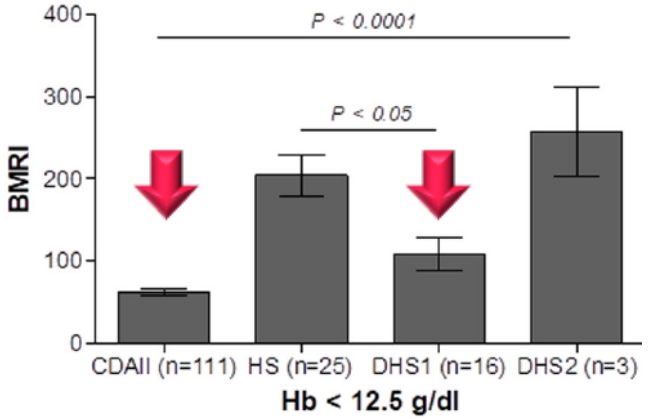
218 patients clinically suspected of CDA enrolled by the Medical Genetics Unit of Naples





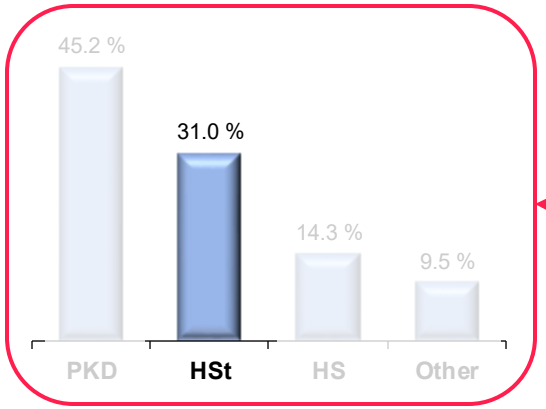
Zaninoni A, Marra R, ... Bianchi P, Russo R. Sci Rep. 2023
Iolascon A, Andolfo A, Russo R. Blood 2020

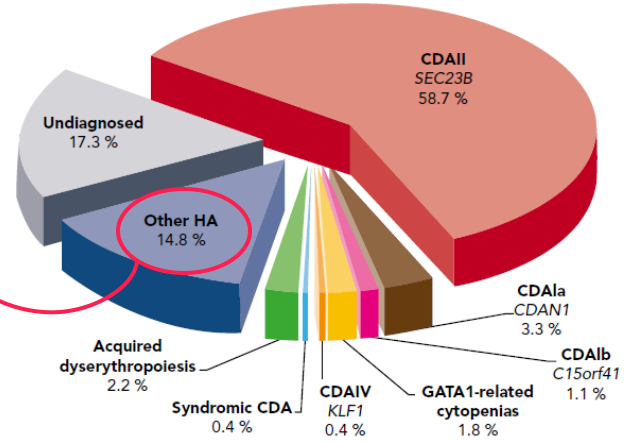
To define the molecular genetics of CDAs



- Approximately 5% of suspected CDAs show pathogenic variants in the *PIEZO1*, the causative gene of dehydrated hereditary stomatocytosis (DHS1)
- Accordingly, a subset of DHS1 patients show dyserythropoietic features at bone marrow analysis, as erythroid hyperactivity and double nuclearity in the erythroid lineage

218 patients clinically suspected of CDA enrolled by the Medical Genetics Unit of Naples





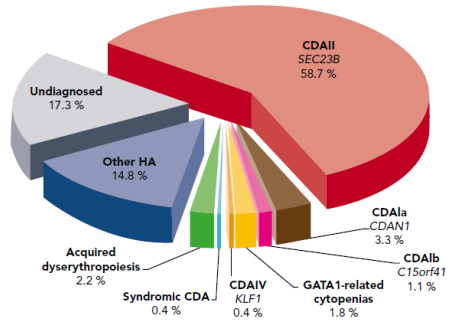
Iolascon A, Andolfo A, Russo R. Blood 2020
Andolfo I, Russo R, et al. Am J Hematol. 2018

To define the molecular genetics of CDAs: *non-classical forms of CDA*

Disease symbol	Gene Inheritance	Main clinical features	Bone marrow morphological features
CDA IV	*KLF1* Autosomal dominant	Hemolytic anemia, generally severe, with normal or slightly increased reticulocyte count, and markedly elevated fetal hemoglobin levels	Erythroid hyperplasia with bi- or multi-nucleated erythroblasts; immature erythroid progenitors with atypical cytoplasmic inclusions, invagination of the nuclear membrane, and marked heterochromatin
XLTDA	*GATA1* X-linked recessive	Macro-thrombocytopenia, bleeding tendency, and mild-to-severe anemia	Erythroblasts with megaloblastic features, bi- and multi-nucleation, and nuclear irregularities; small dysplastic megakaryocytes with signs of incomplete maturation and reduced number of alpha granules
MJDS	*LPIN2* Autosomal recessive	Hypochromic microcytic anemia; chronic recurrent multifocal osteomyelitis and inflammatory dermatosis	Microcytosis and dyserythropoiesis
EIEE50	*CAD* Autosomal recessive	Autism, developmental delay, and generalized epilepsy; mild CDA II-like anemia with marked anisopoikilocytosis and abnormal glycosylation of the erythrocyte proteins band-3 and RhAG	Erythroid hyperplasia with dyserythropoiesis, bi- and tri-nucleated erythroblasts, prominent cytoplasmic bridging
-	*VPS4A* De novo autosomal dominant	Microcephaly, hypotonia, global developmental delay, structural brain abnormalities, cataracts; hemolytic anemia	Dyserythropoiesis with bi-nucleated erythroblasts and cytoplasmic bridges
-	*ALAS2* X-linked dominant	Macrocytic anemia with iron overload in female individuals	Erythroid hyperplasia with dyserythropoiesis; rare erythroblasts with siderotic granules (no excess iron or sideroblasts)
-	*COX4I2* Autosomal recessive	Exocrine pancreatic insufficiency, dyserythropoietic anemia, and calvarial hyperostosis	Erythroid hyperplasia with dyserythropoiesis
MEVA	*MVK* Autosomal recessive	Mevalonate kinase deficiency associated to CDA II-like anemia	CDA II-like morphological abnormalities of erythroblasts

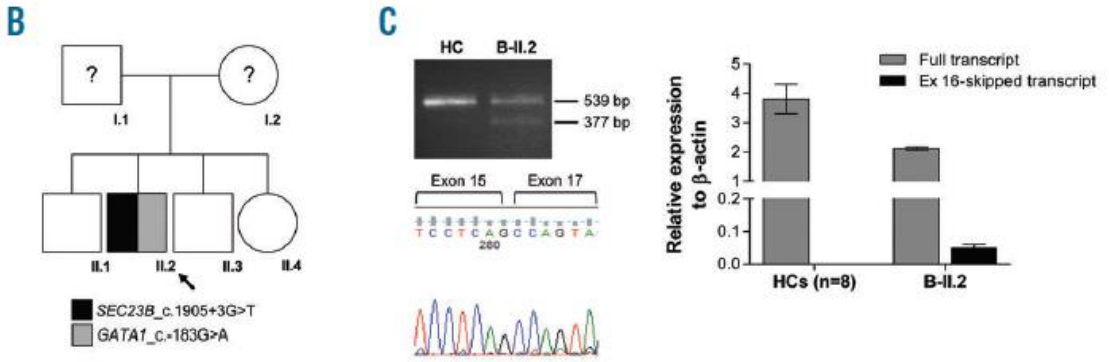
CDA IV, CDA type IV; XLTDA, X-linked thrombocytopenia with or without dyserythropoietic anemia; MJDS, Majeed syndrome; EIEE50, early infantile epileptic encephalopathy-50; MEVA, mevalonic aciduria.

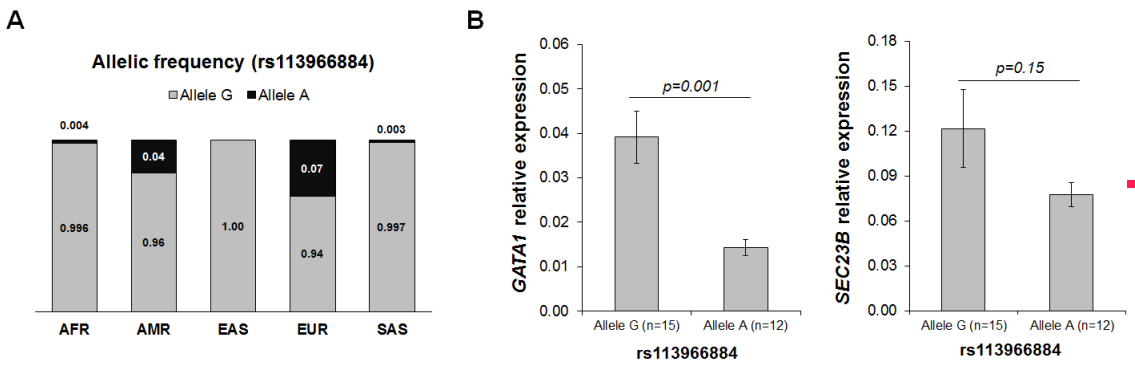
- Transcription factor-related CDAs
- CDA syndromic forms



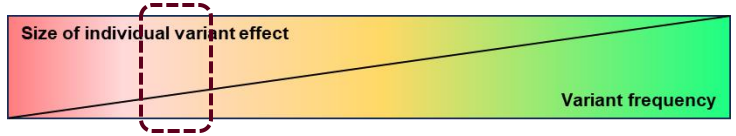
Patterns of digenic inheritance: CDA II

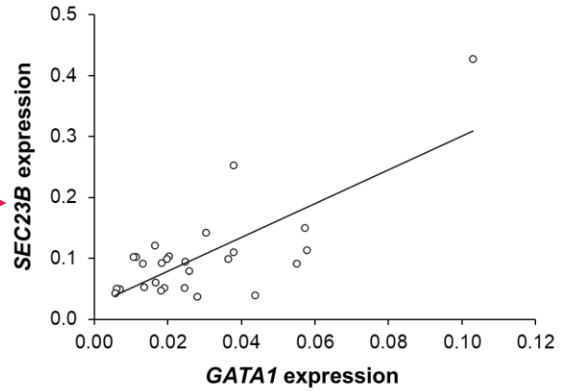
- ✓ A CDA type II patient with:
  - a splice site mutation in ***SEC23B*** gene
  - a non-coding variant in the 5'upstream region of ***GATA1*** gene





A. Directly interacting genes/proteins
B. Indirectly interacting genes/proteins
C. Common pathway
D. Co-expression (RNA)
E. Similar function of genes/proteins
F. No obvious link in genes/proteins (different pathways)



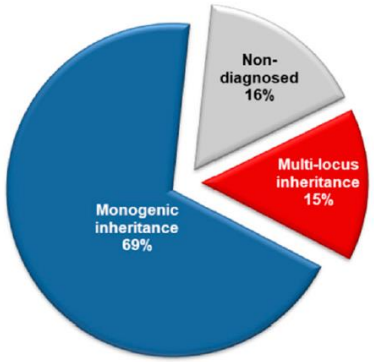


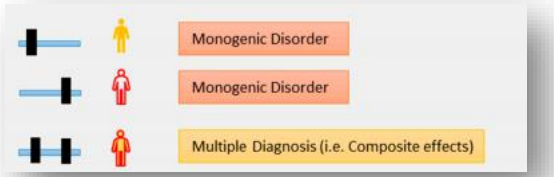
Russo R et al, Haematologica 2017

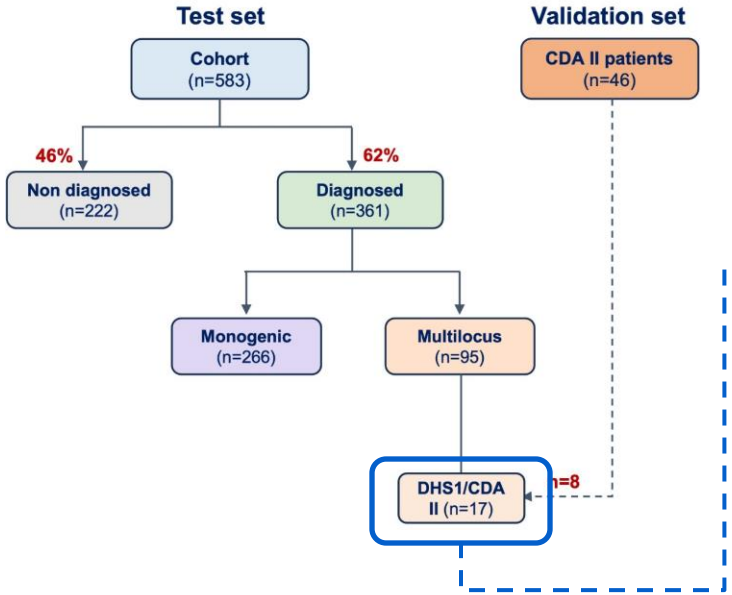
Dual (multiple) inheritance

NGS-based genetic testing defined that:

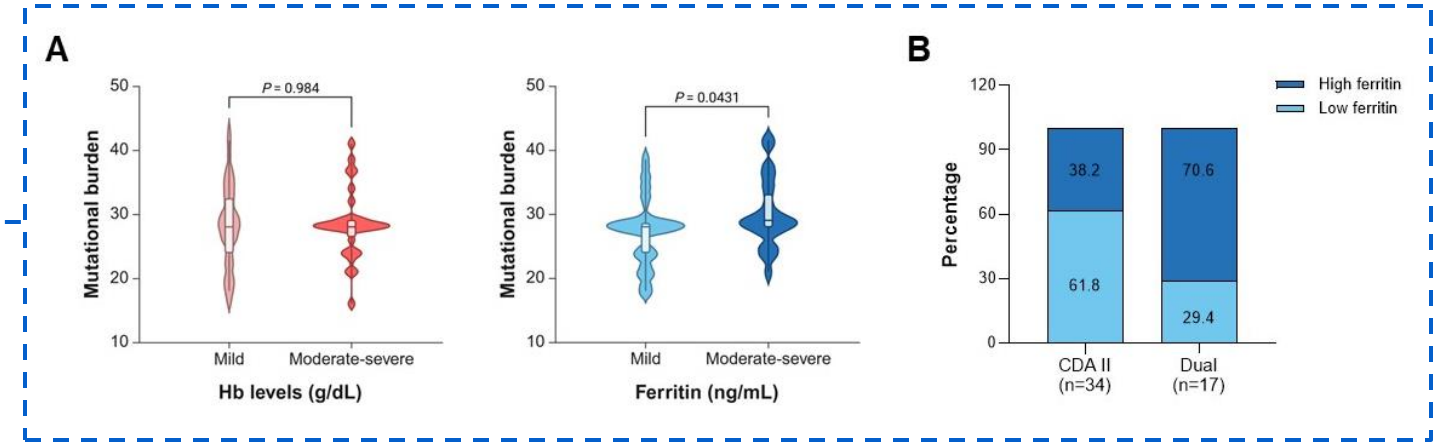
- ✓ Dual inheritance accounts for at least **4%** of analyzed cases
- ✓ Higher rates for case series with **selected phenotypes** (**12%**)
- ✓ Multiple inheritance has been estimated to occur in **15%** of H-RBCDs







Dual ***SEC23B-PIEZO1*** inheritance does not impact Hb levels but is associated with increased ferritin levels



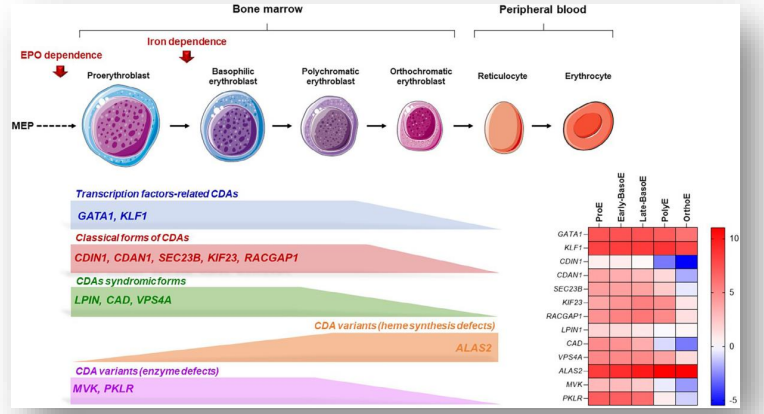
Andolfo I, Martone S, … Iolascon A, Russo R. Genes, 2021
Nostroso A, et al. 2025 (submitted)

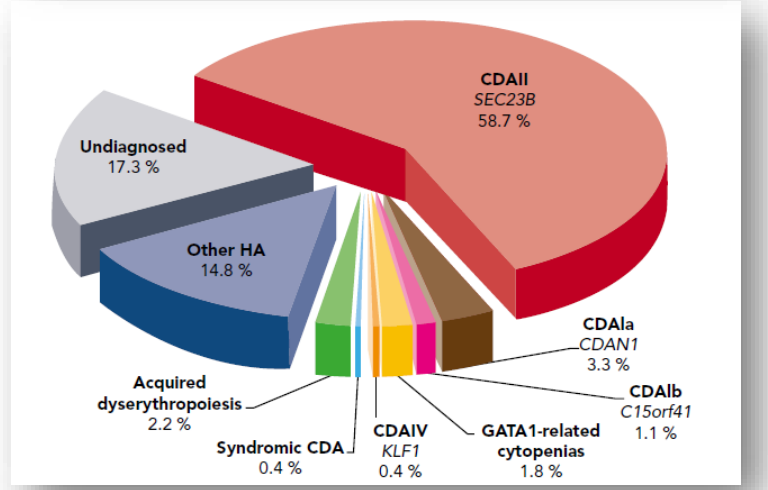
Take home messages

- CDAs encompass a wide group of disorders characterized by high phenotypic heterogeneity, which often complicates accurate clinical diagnosis

- Genetic classification of these disorders enables appropriate patient management and an understanding of the underlying pathogenic mechanisms

- NGS-based genetic testing enables:
  - Differential diagnoses, particularly in cases involving erythrocyte enzyme defects (PKD)
  - Identification of genetic modifiers influencing phenotypic variability
  - Discovery of new genes and pathogenic mechanisms





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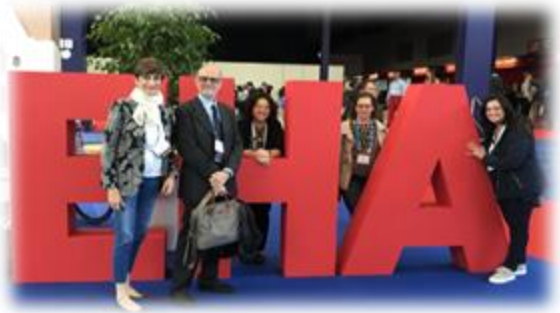
Patients and their families



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HEALTH PROFESSIONALS

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European Hematology Association (EHA)

European Hematology Association

@EHA_Hematology Youtube channel

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www.eurobloodnet.eu

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ERN-EuroBloodNet's EDUcational Youtube channel

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webinar

HEALTH PROFESSIONALS

EHA&EuroBloodNet Spotlight on Congenital BMF syndromes

NGS in Platelet Production (inherited thrombocytopenia)

Speaker Kathleen FRESON
Organisation University of Leuven

12 May 2025

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Conflicts of Interest

Unrestricted research grant from SOBI

Heterogeneous genetic causes of thrombocytopenia

Thrombopoietin pathway
THPO
MPL

Transcription regulation
GATA1
FLI1
GFI1B
HOXA11
MECOM
RUNX1
ANKRD26
ETV6
ERG
IKZF5

Intracellular signalling
SRC
STIM1
MPIG6B
RAP1B

Glycosylation/plasma lipids
GNE ABCG5/ABCG8

RNA metabolism
SLFN14
RNU4ATC
RBM8A

Pathway incomplete
CYCS PLAU
KDSR

Cytoskeletal organization
MYH9
FLNA
ACTN1
TUBB1
ARPC1B
CDC42
FYB
DIAPH1
TPM4
WAS
ATCB

Glycoprotein/integrin signalling
GP1BB
GP9
GP1BA
VWF
ITGB3
ITGA2B
RASGRP2
FERMT3
GP6

Granule biogenesis and secretion
NBEAL2
HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1
BLOC1S3, BLOC1S5, BLOC1S6, AP3D1, LYST,
NBEA, VPS33B, VIPAS39, STXBP2

Procoagulant activity
ANO6

GPCR and Intracellular signalling
P2RY12
TBXA2R
TBXAS1
ABCC4
PTGS1
PLA2G4A

Thrombocytopenia (and dysfunction)

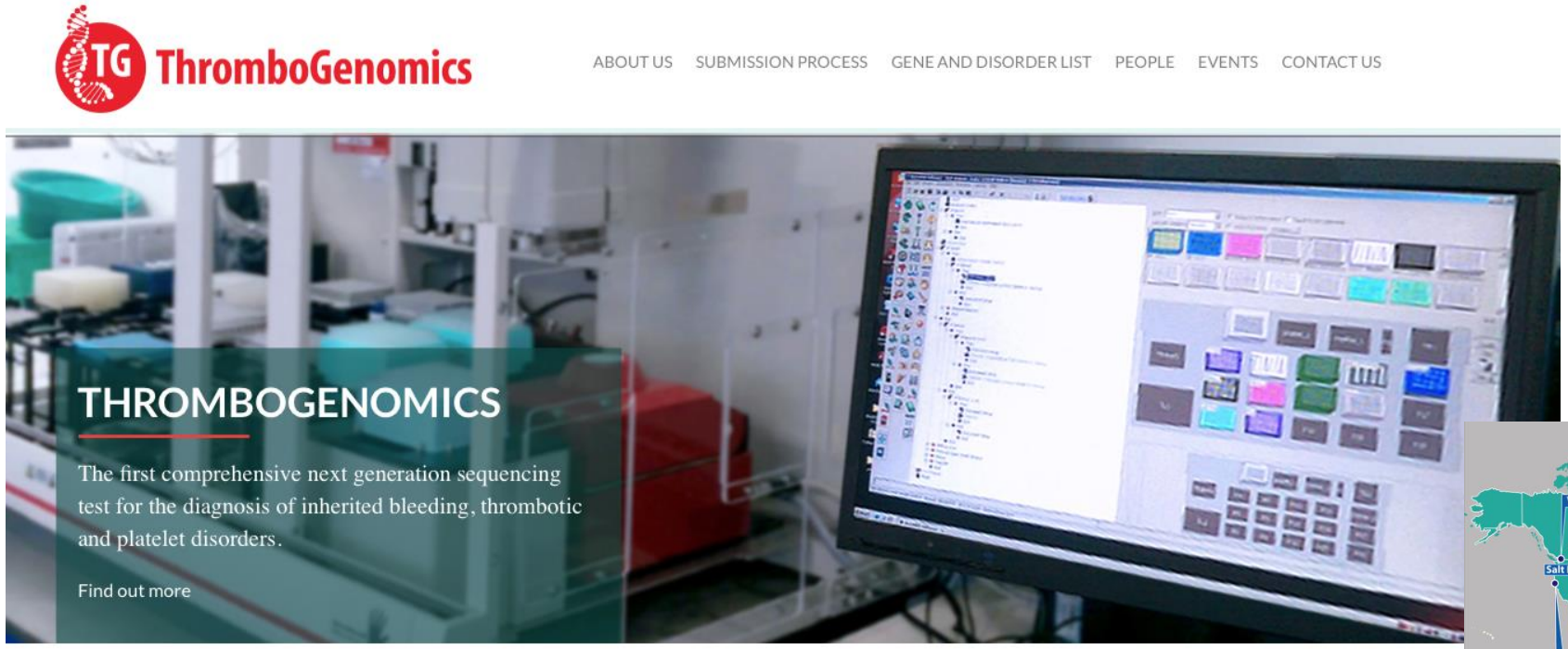
Platelet dysfunction-only

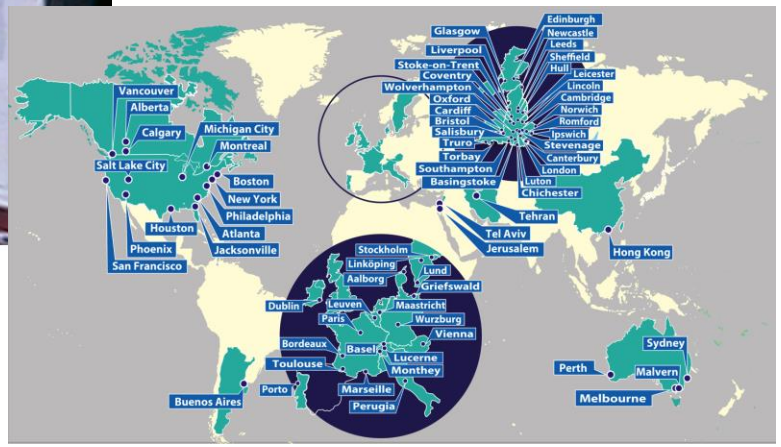
At least 50% overlap with panels for BMF disorders

Ver Donck F et al. RPTH 2021

ERN | EuroBloodNet

ThromboGenomics study: international initiative to test a gene panel for bleeding, platelet and thrombotic disorders





Simeoni I, et al. Blood. 2016

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Gene curation to define diagnostic-grade genes (TIER1)

RECOMMENDATIONS AND GUIDELINES

J Thromb Haemost. 2019;17:1253–1260.

Curated disease-causing genes for bleeding, thrombotic, and platelet disorders: Communication from the SSC of the ISTH

Karyn Megy[1,2,3] | Kate Downes[1,2,3] | Ilenia Simeoni[1,2,3] | Loredana Bury[4] | Joannella Morales[5] | Rutendo Mapeta[1,2,3] | Daniel B. Bellissimo[6] | Paul F. Bray[7] | Anne C. Goodeve[8] | Paolo Gresele[4] | Michele Lambert[9,10] | Pieter Reitsma[11] | Willem H. Ouwehand[1,2,3] | Kathleen Freson[12] | on behalf of the Subcommittee on Genomics in Thrombosis and Hemostasis

SSC Scientific and Standardization Committee

- www.isth.org/page/GinTh_GeneLists
- Yearly updates during the SSC session

ORIGINAL ARTICLE

J Thromb Haemost. 2024;22:645–665

Evaluating the clinical validity of genes related to hemostasis and thrombosis using the Clinical Genome Resource gene curation framework

Justyne E. Ross[1] | Shruthi Mohan[1] | Jing Zhang[2] | Mia J. Sullivan[3] | Loredana Bury[4] | Kristy Lee[1] | Isabella Futchi[1] | Annabelle Frantz[1] | Dara McDougal[1] | Juliana Perez Botero[3,5] | Marco Cattaneo[6] | Nichola Cooper[7] | Kate Downes[8] | Paolo Gresele[4] | Catriona Keenan[9] | Alfred I. Lee[10] | Karyn Megy[o] | Pierre-Emmanuel Morange[11,12] | Neil V. Morgan[13] | Harald Schulze[14] | Karen Zimowski[15] | Kathleen Freson[16] | Michele P. Lambert[17,18]

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Clinical Domain Working Groups

Hemostasis/Thrombosis Gene Curation Expert Panel

Affiliated to Hemostasis/Thrombosis CDWG

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Thrombocytopenia screening is part of the platelet defects gene panel

Panel name	Diagnostic-grade (TIER1) genes
Platelet defects gene panel *Including genes for thrombocytopenia*	ABCC4, ABCG5, ABCG8, ACTB, ACTN1, ANKRD26, ANO6, AP3B1, AP3D1, ARPC1B, BLOC1S3, BLOC1S5, BLOC1S6, CDC42, CYCS, DIAPH1, DTNBP1, ERG, ETV6, FERMT3, FLI1, FLNA, FYB1, GATA1, GFI1B, GNE, GP1BA, GP1BB, GP6, GP9, HOXA11, HPS1, HPS3, HPS4, HPS5, HPS6, IKZF5, ITGA2B, ITGB3, KDSR, LYST, MECOM, MPIG6B, MPL, MYH9, NBEA, NBEAL2, P2RY12, PLA2G4A, PLAU, PTGS1, RASGRP2, RAP1B, RBM8A, RNU4ATAC, RUNX1, SLFN14, SRC, STIM1, STXBP2, TBXA2R, TBXAS1, THPO, TPM4, TUBB1, VIPAS39, VPS33B, VWF, WAS
Bleeding and Thrombosis gene panel (anti)Coagulation genes	ADAMTS13, F12, F10, F11, F13A1, F13B, F2, F5, F7, F8, F9, FGA, FGB, FGG, GGCX, HRG, KLKB1, KNG1, LMAN1, MCFD2, PIGA, PLG, PROC, PROS1, SERPINC1, SERPIND1, SERPINE1, SERPINF2, THBD, VKORC1, VWF
Unexplained bleeding gene panel	ACVRL1, CHST14, COL1A1, COL3A1, COL4A1, COL4A2, COL5A1, COL5A2, ENG, GDF2, SMAD4

STH gene panels (Version 2024): www.isth.org/general/custom.asp?page=GinTh_GeneLists

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Who to test for the thrombocytopenia gene panel ?

- Chronic thrombocytopenia (‘since birth’: not always possible as the diagnosis can be an incidental finding)
- Exclusion of acquired causes (especially in adults)
- For children: useful to diverse between ITP and inherited thrombocytopenia
- Syndromic thrombocytopenia
- Thrombocytopenia with immune deficiency
- Thrombocytopenia with leukemia trait in the family

ThromboGenomics Version 1

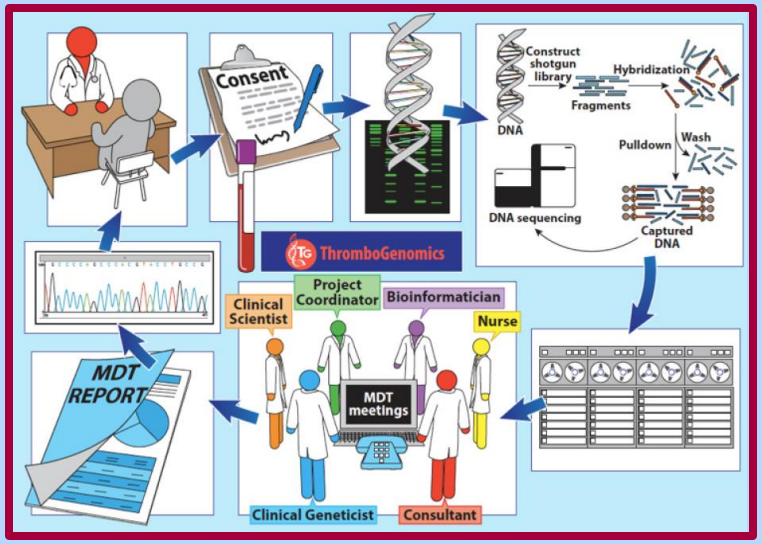
blood

Regular Article

THROMBOSIS AND HEMOSTASIS

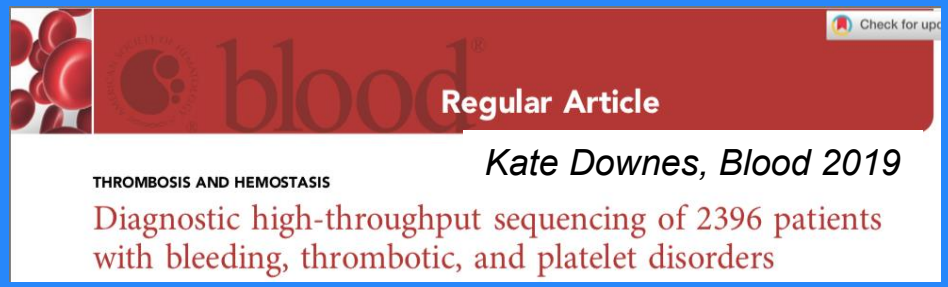
Ilenia Simeoni , Blood 2016

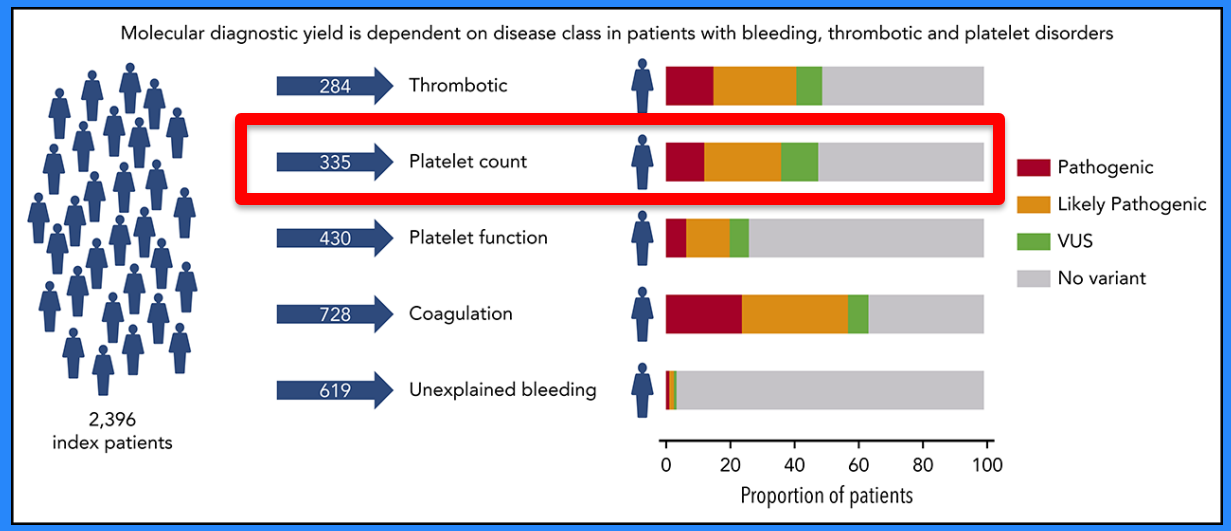
A comprehensive high-throughput sequencing test for the diagnosis of inherited bleeding, thrombotic, and platelet disorders



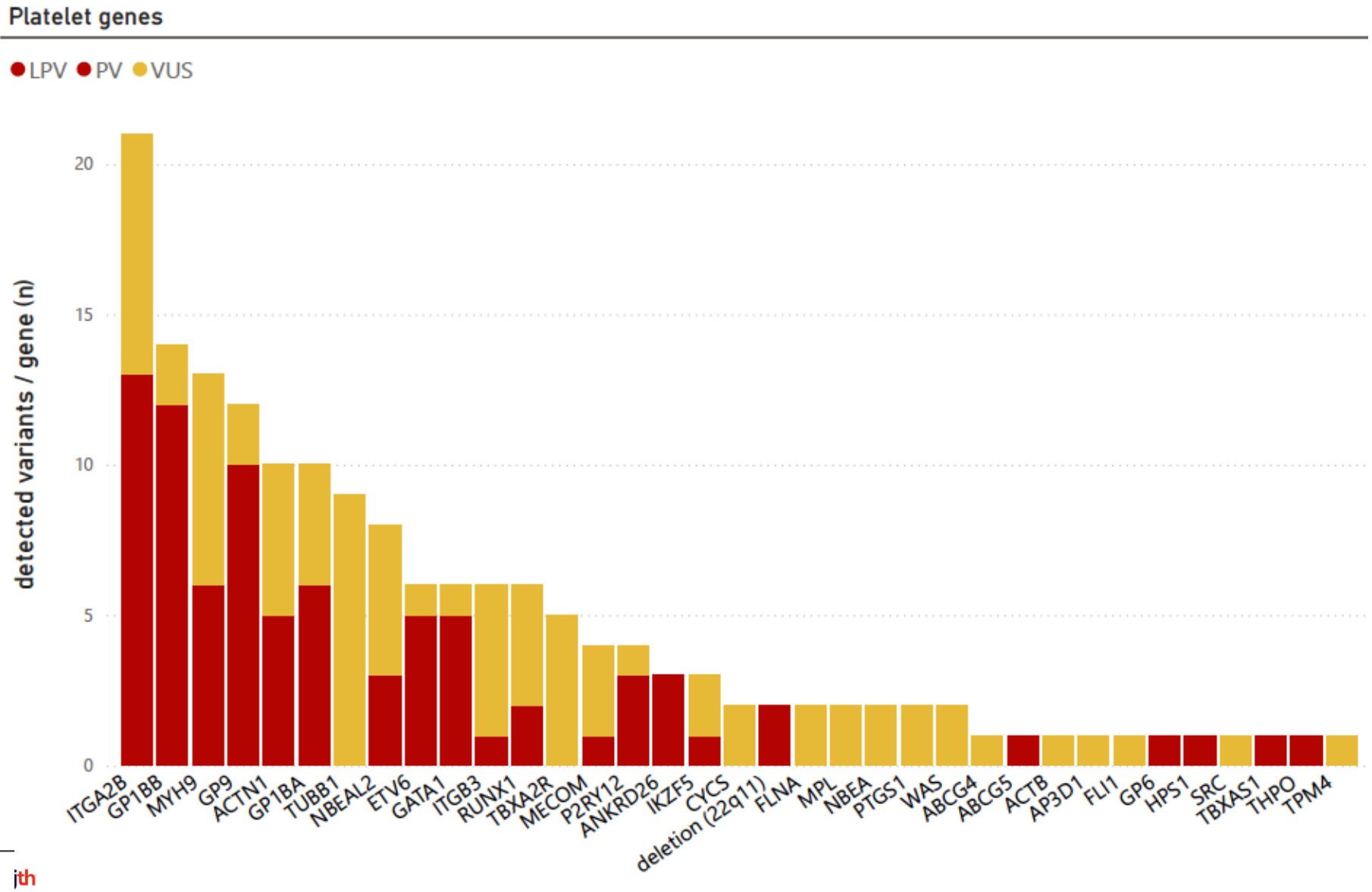
- Targeted approach with coverage: 99 – 98 %
- Detection indels (no inversions)
- Mean of 5.34 variants/case after filtering
- Multiplexing 24 (later 48) samples

ThromboGenomics Version 2





Diagnostic rate for thrombocytopenia is 50%



https://doi.org/10.1016/j.jtha.2022.12.

J Thromb Haemost. 2023;21:887–895

BRIEF REPORT

Clinical application of multigene panel testing for bleeding, thrombotic, and platelet disorders: a 3-year Belgian experience

Christine Van Laer[1,2] | Marc Jacquemin[1,2] | Sarissa Baert[3] | Veerle Labarque | Chantal Thys[1] | Thomas Vanassche[1,5] | Chris Van Geet[1,4] | Peter Verhamme | Karen Willekens[3] | Anniek Corveleyn[3] | Kathelijne Peerlinck[1,5] | Kathleen Freson[1]

journal of thrombosis and haemostasis

IST

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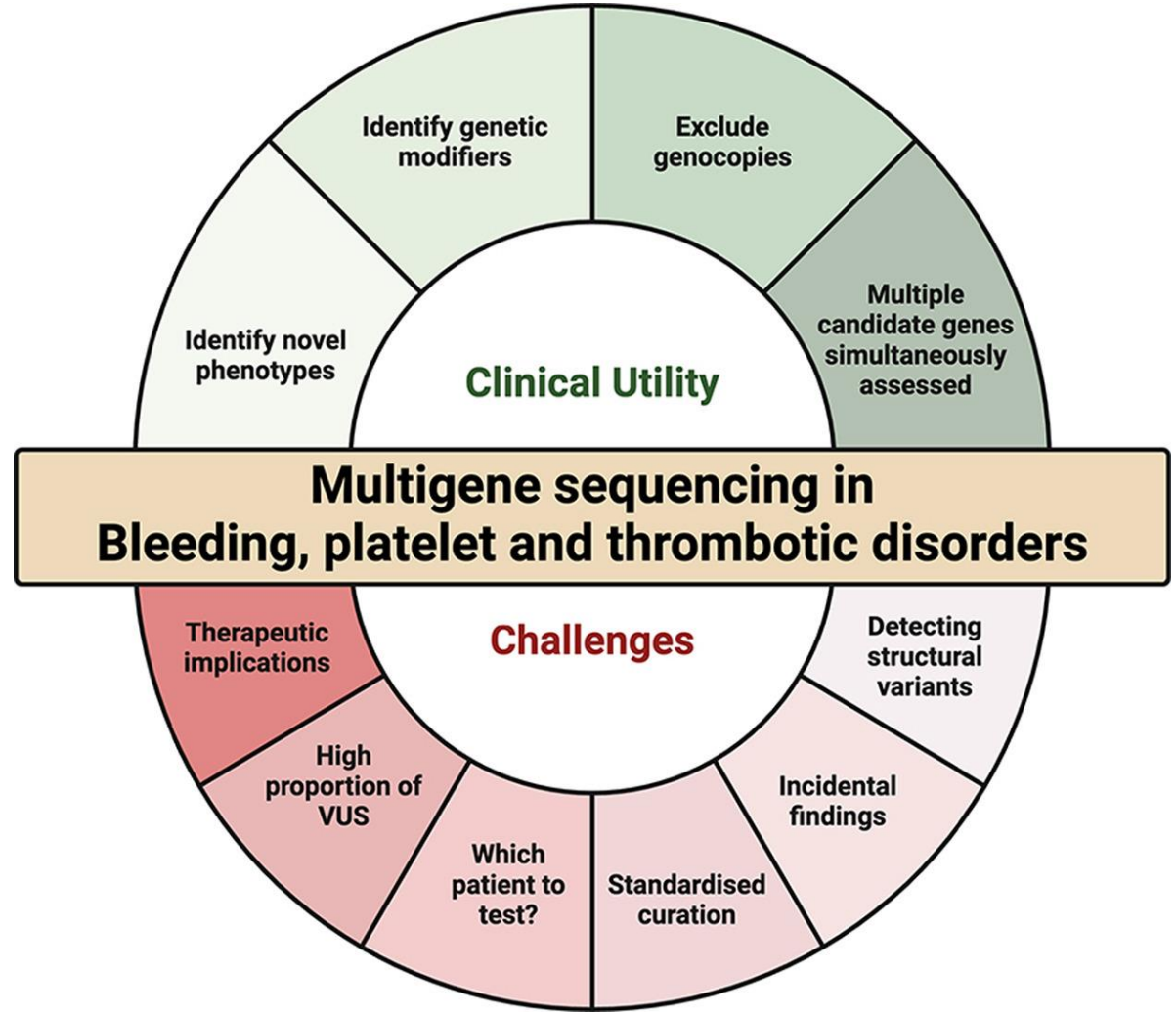
REVIEW · Articles in Press, May 07, 2025

Implementation and clinical utility of multigene panels for bleeding, platelet, and thrombotic disorders

Radha Ramanan [1,4,5] · Andreas Verstraete [1,3] · Christine Van Laer [1,2] · Kathleen Freson [1]



What do I tell the patient about NGS testing?

SSC Scientific and Standardization Committee

RECOMMENDATIONS AND GUIDELINES | Open Access

Clinical management, ethics and informed consent related to multi-gene panel-based high throughput sequencing testing for platelet disorders: Communication from the SSC of the ISTH

Kate Downes, Pascal Borry, Katrin Ericson, Keith Gomez, Andreas Greinacher, Michele Lambert, Eva Leinoe, Patrizia Noris, Chris Van Geet, Kathleen Freson ✉, Subcommittee on Genomics in Thrombosis, Hemostasis ... See fewer authors

First published: 08 July 2020 | https://doi.org/10.1111/jth.14993

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ebn ERN | EuroBloodNet

Risk of unsolicitated findings: an example

Index case, 35 y

Mucocutanous bleeding symptoms

Platelet count 145 - 161 K, normal size

Platelet delta storage pool disease

RUNX1 p.Glu5ValfsTer5

BRIEF REPORT | DECEMBER 12, 2013

Enrichment of *FLI1* and *RUNX1* mutations in families with excessive bleeding and platelet dense granule secretion defects

Brief Report

Jacqueline Stockley, Neil V. Morgan, Danai Bem, Gillian C. Lowe, Marie Lordkipanidzé, Ban Dawood, Michael A. Simpson, Kirsty Macfarlane, Kevin Horner, Vincenzo C. Leo, Katherine Talks, Jayashree Motwani, Jonathan T. Wilde, Peter W. Collins, Michael Makris, Steve P. Watson,
Martina E. Daly on behalf of the UK Genotyping and Phenotyping of Platelets Study Group

Blood (2013) 122 (25): 4090–4093.

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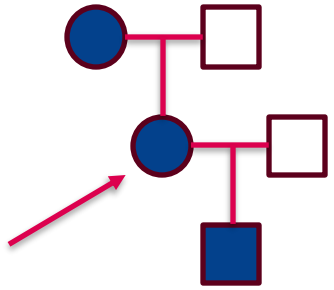
Detection of a missed diagnosis: an example



Index case, 28 y

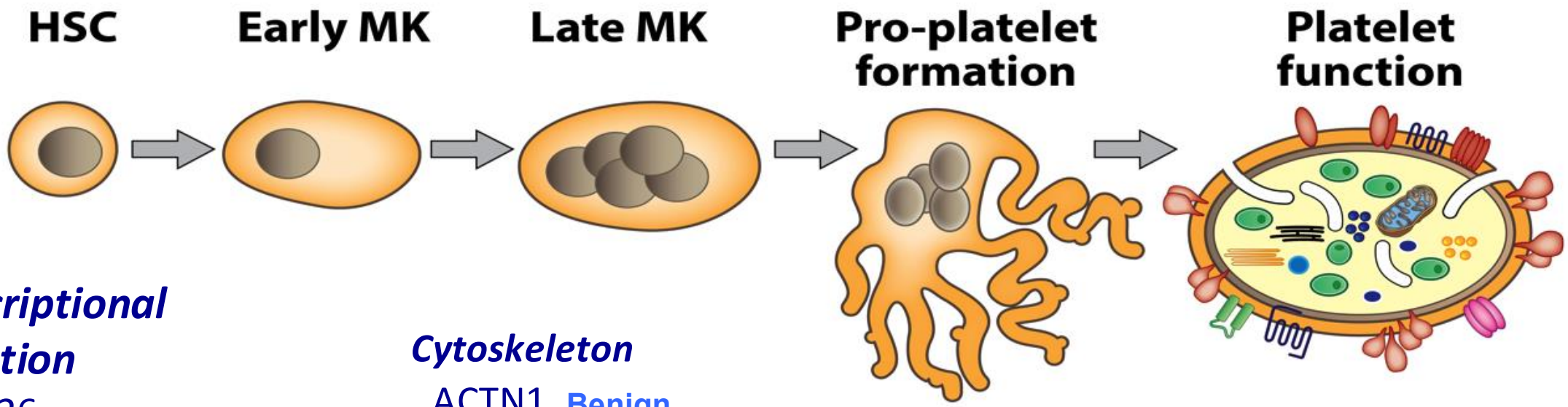
Mild bleeding symptoms

Platelet count 120 K, MPV 13

Autosomal dominant TP

GATA1 p.Arg216Gln (X-linked)

Precision diagnosis of thrombocytopenia can influence management



Transcriptional regulation

ANKRD26
RUNX1 — Leukaemia risk
ETV6
GATA1 — Dyserythropoiesis/ anaemia
GFI1B

Cytoskeleton

ACTN1 — Benign
TUBB1
MYH9 — Genotype/phenotype hearing loss/ kidney failure
DIAPH1 — Hearing loss/ TPO mimetics
WAS — Infections/transplant

TPO signaling

MPL — Pancytopenia /transplant
THPO — Pancytopenia /TPO mimetics

Lambeigne C, et al. Blood 2016;127:2814-23

Key messages for use of an NGS panel test for diagnostics

- ✓ A (virtual) panel test is fast (TAT 3 months) and cheap
- ✓ It detects unexpected phenotype-genotype associations (including unsolicitated findings)
- ✓ Panel test is typically ordered by specialist with knowledge of the complexity of such test and its inclusion/exclusion criteria. Patients should be aware of what this test means
- ✓ Sufficient phenotype information should be provide to allow variant classification
- ✓ Variants of Unknown clinical Significance need further research (improved variant databases)

The use of a panel test for thrombocytopenia is useful for counceling, therapy, and management.

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UZ LEUVEN

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Co-funded by the European Union

webinar

HEALTH PROFESSIONALS

EHA&EuroBloodNet Spotlight on Congenital BMF syndromes

Integrating NGS in Clinical Practice

Speaker **Erika MASSACCESI**
Organisation IRCCS Institute G. Gaslini Children's Hospital

12 May 2025

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Conflicts of Interest

No disclosure

INTRODUCTION

Inherited bone marrow failure syndromes (IBMFS)
- heterogeneous group of rare blood disorders due to hematopoiesis impairment
- various pathogenic mechanisms
- heterogeneous clinical phenotype, from very severe to mild or silent forms

→ **diagnosis can be very challenging**

- Classical IBMFS
FA, TBD, SCN, SDS, DBA….

- Non classical IBMFS
Overlap BMF/immune dysregulations
GATA2, DADA2, SAMD9/SAMD9L, TACI…

The **correct genetic diagnosis** of IBMFS is crucial
- to predict the disease course
- to provide genetic counseling
- to select the most appropriate treatment, including HSCT from healthy donors

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DIAGNOSIS

Suggestive clinical findings

! sometimes the clinical phenotype does not allow a straightforward diagnosis

+ Functional analysis

! not available for all genes/proteins

+ Genetic screening

- Next-generation sequencing (NGS) techniques
i.e. targeted gene sequencing (panels), WES, and WGS
→ More comprehensive method

- Gene-to-gene study techniques
i.e. Sanger sequencing
→ Best suited for validation

NGS - LIMITATIONS

- genes not yet described in the literature
- defects located in regulatory regions not sequenced by targeted panels and/or
- missed detection of CNVs and regions of homozygosity or large structural chromosomal variants, translocations, and aneuploidy (unless they have been specifically designed for such a purpose).

Thus, it **should be combined** with

- other molecular approaches: CGH array, qPCR, or MLPA
- studies of familial segregation and functional analyses to confirm pathogenic role of VUS/new variants →

can provide useful additional information on the significance of the variant

Gálvez E, et al. Hemasphere. 2021 Mar 9;5(4):e539. doi: 10.1097/HS9.0000000000000539. PMID: 33718801; PMCID: PMC7951136.

How can all this work in practice?

Some explanatory clinical cases....

Ali, M, dob 20/02/1995

14 yrs and 17 yrs: multiple episodes of severe transfusion-dependent hyporegenerative anemia
Since 26 years:
PRCA → evolution to severe trilinear marrow failure + polyclonal lymphoid infiltration (CD4- and CD8-negative T cells)
Lymphoproliferation (splenomegaly)
Positive autoimmunity screening
Acute hyperinflammatory event

➢Early genetic testing not consistent with clinical phenotype and plasma assay.
➢Proceed with further genetic investigations if the clinic is suggestive.

1) NGS panels congenital dyserythropoietic anemia and myeloid transformation: negative
2) NGS panel for 160 genes related to immune and haematological disorders: negative

Immune dysregulation + marrow failure + hyperinflammatory event → overlap syndrome?

3) Functional ADA2 test: completely absent enzymatic activity
But
4) ADA2/CECR1 gene Sanger sequencing: negative.

5) Further genetic evaluation (more in-depth reading): biallelic synonymous variants on CECR1/ADA2 gene,
leading to a new donor splice site secondary to the nucleotide change and, as a consequence, an altered splicing. Complementary DNA analysis confirmed a 94bp deletion -> altered catalytic domain altered aminoacidic sequence.

This finally supported a clinical and functional, but also genetically confirmed, diagnosis of DADA2

Luisa, F, dob 19/07/2023

Proband:
IUGR, 37 w, SGA
3 months: bronchiolitis, failure to thrive, slight transaminitis
1 year: mild/moderate neutropenia, exocrine pancreatic dysfunction
No dysmorphic features

Genetic test: Sanger sequencing of SBDS exon 2 → SDS DIAGNOSIS
3 pathogenic variants inherited from parents

➢Clear phenotype, rapid diagnosis.

The test was then extended to the rest of the family

Siblings
- A brother: JMML at 1 year, died for HSCT complications
- A sister: 12 yrs, regular weight-for-height development, normal pancreatic and liver function, no dysmorphic features, only slight neutropenia (1320 N).

Sanger sequencing: positive for the same pathogenic variants of the proband

➢Mild phenotype, delayed diagnosis
➢Oligo/non-classical symptomatic relatives should undergo genetic screening

Beatrice, F, dob 17/04/2013

- Celiac disease
- Mild to moderate thrombocytopenia since 4 yrs
- Mild elevation of transaminases (idiopathic)
- Nail dystrophy , skin dyschromia, facial telangiectasias
- Hypocellular BM
- Cerebellar hypoplasia, nucleobasal calcifications
- Blepharitis
- Mild liver fibrosis
- Restrictive lung disease, severe intrapulmonary-shunt

➢Mosaicism can occur as a consequence of molecular events generating a correction of a mutation in a HSC or in a lymphocyte progenitor.

➢Genetic test on peripheral blood may be negative or with low VAF → if the suspicion persists, the test should be performed on "nonblood" chromosomes (skin fibroblasts or hair follicles).

1) Telomere length analysis: TL < 1st p

2) NGS panel for 160 genes related to immune and BMF disorders, analysis on peripheral blood: pathogenic heterozygous de novo variant (c.845G>A) on TINF2 gene **(VAF 12%** ← Low VAF, variant considered **"not reliable"** by the software used!)

3) Skin fibroblast analysis: presence of the same variant with VAF 50%

TAKE HOME MESSAGE

- Diagnosis of IBMFS can be very challenging
- **Impact** on prognosis, surveillance, treatment, management of complications and on appropriate family genetic counseling
- **Accurate, extensive, and integrated** diagnostic work-up is mandatory
- **NGS** should be used as an **initial screening test** to provide precise and clinically relevant molecular diagnoses in a timely manner and at a reduced cost
- New genetic tools surely **improve the diagnostic efficiency** but at the moment can **not completely** resolve the issue
- Clinical suspicion, functional tests and genetic investigations should be **complementary**

EXTENSIVE DIAGNOSTIC WORK-UP FOR BMF

3 pillars: Suggestive clinical findings + Functional analysis + Genetic screening

Integration of several biochemical, instrumental, and genetic tests

Blood Cells, Molecules and Diseases 108 (2024) 102860

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Diagnosis and management of acquired aplastic anemia in childhood. Guidelines from the Marrow Failure Study Group of the Pediatric Haemato-Oncology Italian Association (AIEOP)

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Table 2a
Mandatory diagnostic tests.

Mandatory diagnostic tests	Information on
• Full blood count	• Diagnosis and definition of severity
• Reticulocyte count (with automatic coulter or manual)	• Diagnosis and definition of severity
• Peripheral blood smear	• Differential diagnosis
• Liver function tests	• Association with hepatitis
• Serology and viral genome research of hepatitis viruses (HAV, HBV, HCV, HDV, HEV, HGV), CMV, EBV, Parvovirus B19, HIV, HHV6.	• Association with viral hepatitis or other viral infection
• Bone marrow aspirate for morphology, immunophenotype, standard karyotype	• Diagnosis, differential diagnosis, prognosis
• BM and PB flow cytometry analysis of B and T lymphocytes, NK cells and monocytes. Monoclonal populations and/or blasts search, evaluation of abnormal maturation/differentiation patterns as a sign of dysplasia	• Differential diagnosis with myeloid neoplasms, PID/PIRD association with peculiar genetic variants. Association with lymphoma
• Bone marrow trephine biopsy with immuno-staining for CD34 and CD117 antigens and iron staining	• Diagnosis, differential diagnosis, prognosis
• Chromosomal fragility test (MMC or DEB). In case of doubtful result these tests should be done on fibroblasts to identify somatic mosaicism	• Differential diagnosis of FA
• Flow FISH analysis for TL measurement	• TL < 1st centile in lymphocytes is strongly indicative of TBD. Shortened telomere, usually above 1st centile in lymphocytes and granulocytes may be seen in AA because of increased cell turnover. However, TL between 1st and 10th percentile in lymphocytes indicates probable telomeropathy but can also be seen in other congenital BMFs (i.e DBA)
	• Differential diagnosis with constitutional marrow failure syndromes:

"Functional tests"

• Sequencing of major genes associated with bone marrow failure and WES/NGS panels including genes involved in TBD, FA, DBA, SBDS, CAMT, SCN, *GATA2* deficiency, *SAMD9/SAMD9L*, *ETV6*, *MECOM* and other congenital marrow failures; DADA2, immune dysregulation syndromes/PID. WGS, if NGS/WES turns out negative and suspicion persists	1. Consider TERC, as it is frequently associated (1-10%) with idiopathic AA with no somatic abnormalities. 2. If short telomeres are associated with stigmata of dyskeratosis, candidate genes are *DKC1*, *TINF2*. 3. If thrombocytopenia with absence of megakaryocytes, candidate gene is c-*Mpl*. 4. If monocytopenia, papillomavirus infections, lymphedema, candidate gene is *GATA2*. 5. If metabolic/pancreatic disorders, consider *SDS*. 6. If history of vascular disease/inflammation, consider *ADA2*.
• Flow cytometry (FLAER) for PNH clones	• Detection of PNH clones: diagnosis, differential diagnosis, prognosis
• Screening for autoantibodies (panel according to clinical presentation), including core and DNA antibodies. Double-negative T lymphocyte sub-populations, Tregs analyses, Ig, and vaccine titers.	• Association with autoimmune diseases, immunodeficiencies, immune dysregulation syndromes: diagnosis, differential diagnosis, prognosis
• Vitamin B12, folic acid, thyroid function assay	• Exclusion of vitamin deficiency or hypothyroidism
• Fibrinogen, ferritin	• Differential diagnosis with hemophagocytic syndrome
• Fecal pancreatic elastase, serum amylase and lipase	• Differential diagnosis with SDS
• Serum bilirubin, LDH, HbF assay	• Nonspecific indices. May increase in mild ineffective erythropoiesis.
• Chest x-ray	• Exclusion of infections or displacement of the mediastinum due to proliferative diseases

"Genetic tests"

Table 2a (*continued*)

Mandatory diagnostic tests	Information on
• Abdominal ultrasound and echocardiogram	• Increased volume of spleen and/or lymph nodes (acute lymphoproliferation from hematological neoplasm or chronic benign lymphoproliferation due to autoimmune lymphoproliferative syndrome/ALPS). Organ malformation or malposition of internal organs (FA)

Table 2b
Ancillary diagnostic tests.

Ancillary diagnostic tests	Information on
• Bone marrow culture and stain for acid-alcohol resistant bacilli	• Presence of mycobacterial infections (especially atypical mycobacteria, less frequently TB)
• FISH analysis on BM for monosomy 7, trisomy 8, deletion of 5q, etc.	• Diagnosis, differential diagnosis, prognosis
• Clonogenic tests on BM (not standardized results, cannot be done in all centers)	• Differential diagnosis between BMF and MDS
• STIR or traditional MRI of skeletal segments (spine)	• Inhomogeneity of bone marrow content due to bone marrow replacement by adipose tissue (probable AA)

Acquired AA versus IBMFS

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