Webinars

Constitutional thrombocytopenia

EuroBloodNet

INHERITED THROMBOCYTOPENIA
ILLUSTRATION THROUGH REAL-LIFE CLINICAL CASES

MC, ALESSI

Reference center

on constitutional platelet disorders (CRPP)

APHM, Timone Hospital

Laboratory of hematology

Aix Marseille University

Marseille, France

Aix*Marseille université

APHM Assistance Publique Hôpitaux de Marseille

EuroBloodNet

CRPP

European Reference Network for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)

Today objective

- ✓ **Inherited thrombocytopenia as a Gateway for complex diseases**

✓ Case illustration 1

Referred to our center for investigation of constitutional thrombocytopenia.

♀, 8 yrs

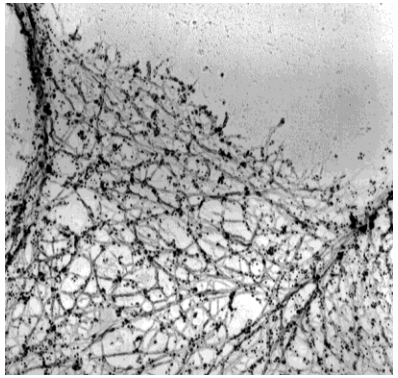
	Child	Mother
Platelet count	110 G/l	124 G/l
MPV (Advia)	10.4 fl	10.8 fl
Bleeding	Prolonged bleeding at cuts	Menorrhagia
Surgery history	Adenoidectomy tooth extraction under exacyl	No bleeding
Medical history	GO reflux Pervasive devpt disorders	Miscarriage Corticoid +IV IG

Gene Panel sequencing : **FLNA variant :exon7:c.1056del:p.Thr353LeufsTer32**

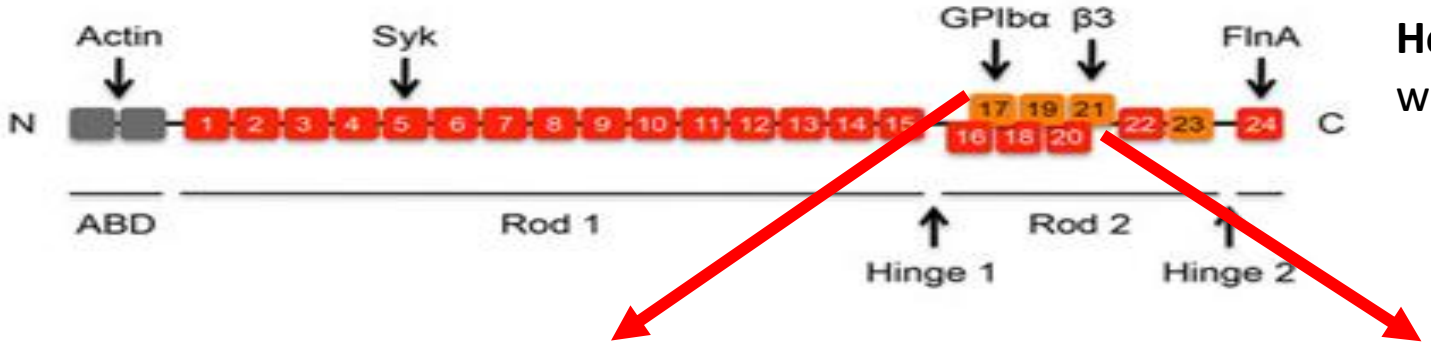
FILAMINOPATHY

Filamin A and platelets

interacts with components of the **cytoskeleton**

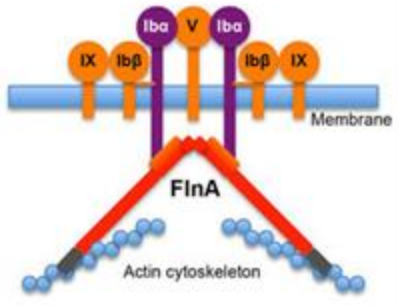


FLNa supports platelet activation induced by collagen by enabling the spatial localization of **Syk** to the cytoplasmic membrane and GPVI.

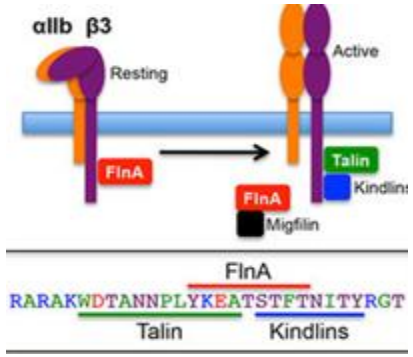


Homodimers
widely expressed

The FLNa–GPIbα interaction regulates downstream signaling and adhesion to vWF



FLNa maintains integrin αIIbβ3 in an inactive state,

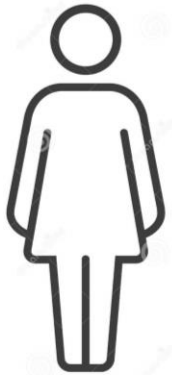


X-linked *FLNA* mutations (Xq28)

« FLNA deficiency syndrome »

Loss of function mutation
Truncation of Filamin A
Very rare missense variations

THROMBOCYTOPENIA

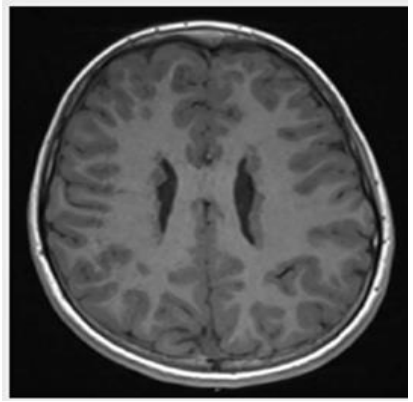


Gain of function mutation

Missense mutation

~~THROMBOCYTOPENIA~~

- Periventricular nodular heterotopia (FLNA-PVNH - OMIM#300049)
  - Seizures
- Bowel obstruction, short bowel
- Cardiovascular defects
  - Aortic aneurysm and patent ductus arteriosus
- Joint hyperlaxity and skin hyperelasticity
- Emphysema



- Otopalatodigital syndrome 1 & 2
  - Skeletal abnormalities, deafness, organ malformation, death
- Frontometaphyseal dysplasia
  - Skeletal dysplasia, deafness, urogenital defects
- Melnick Needles syndrome
  - Skeletal dysplasia, early death in males

What are the signs that may indicate a "FLNA related thrombocytopenia"

- Half of patients have seizures,
- Half of these patients have aortic aneurysm, a third have patent ductus arteriosus, atrial or ventricular septal defect
- More than a half exhibit joint hyperlaxity and more than a third skin hyperelasticity
- Emphysema is still underestimated in FLNA deficiency syndrome,
- **"All" these patients have pathognomonic cerebral MRI (PVNH)**

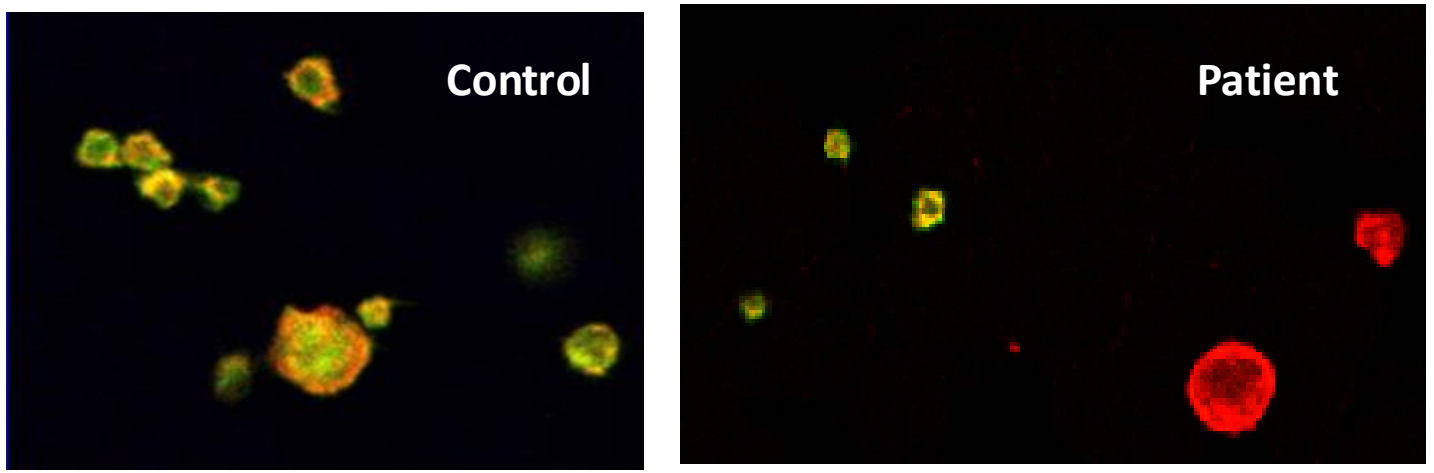
Platelet phenotype

Thrombocytopenia is associated with **enlarged, spherical** platelets

Abnormal **α-granule** distribution compared with controls.

Reduced aggregation in response to collagen (Diminished thrombus formation on collagen)

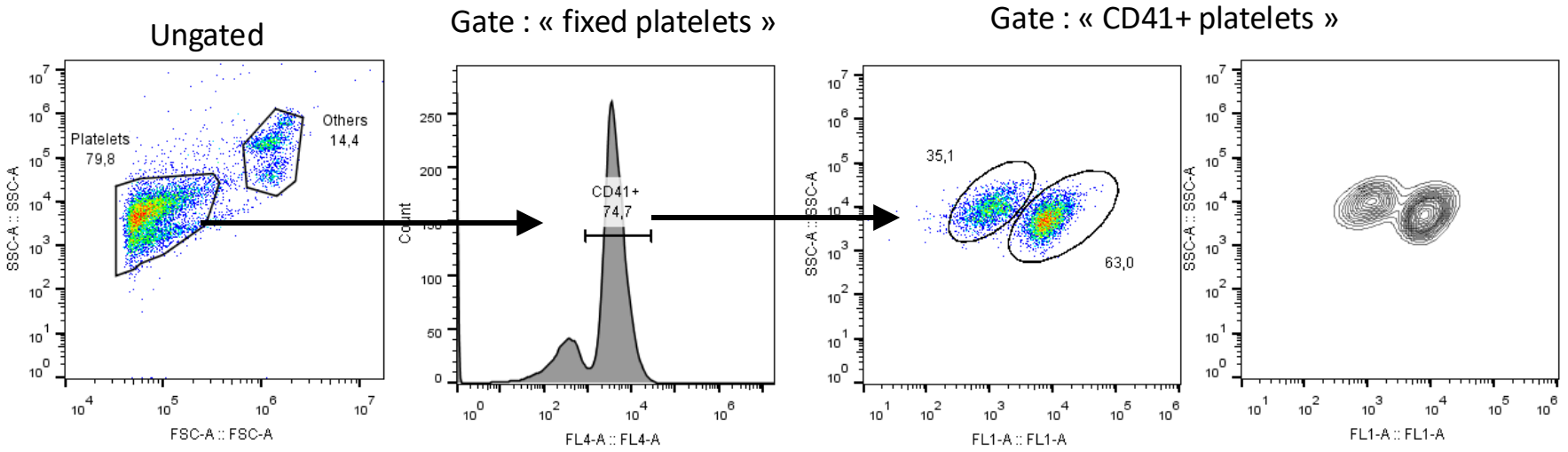
A subset of platelets are deficient in FLNA



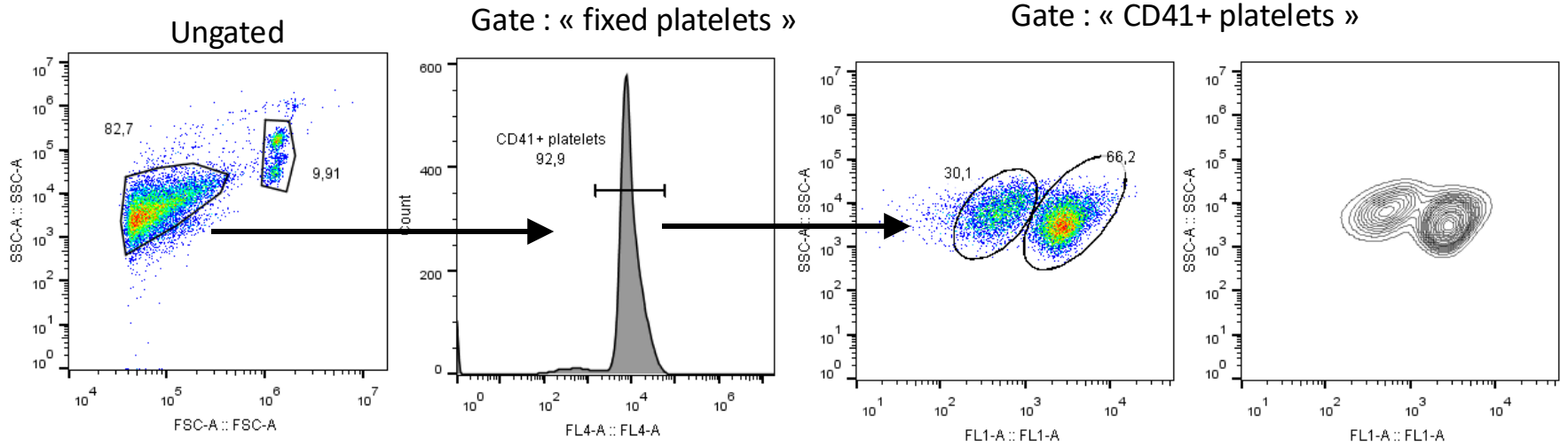
MKs generated ex vivo showed **incomplete maturation** and a reduced production of **platelets** associated with **dysregulated RhoA** activation.

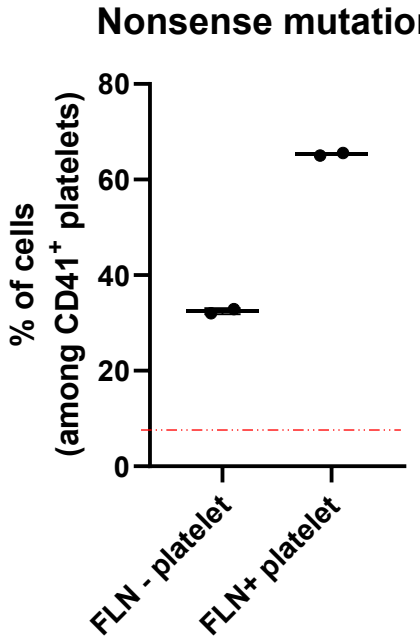
Quantification of intraplatelet Filamin A in severe cases

Thr353LeufsTer32 -



Val374SerFsTer2 -





✓ Case illustration 1

MOTHER

- Neurological :
  - ✓ Brain MRI: peri-ventricular heterotopia
  - ✓ Simple partial seizures requiring treatment
  - ✓ Neuropsychological evaluation evidenced auto-biographical memory disorders
- Vascular :
  - ✓ Aortic insufficiency
  - ✓ Venous insufficiency
  - ✓ Raynaud's syndrome
- Joint hyperlaxity: orthopedic management
- Obstetrical: Late miscarriage of male fetus ?

CHILD

- Neurological :
  - ✓ Brain MRI: peri-ventricular heterotopia
  - ✓ Learning disability
- Vascular :
  - ✓ Aortic Insufficiency
- Joint hyperlaxity: orthopaedic management

PVNH1
SEVERE MUTATIONS
Truncated protein

Deletion Exons 20-48
Deletion 31/32-48
Duplication Exons 1-28
Intragenic partial duplication

c.373+1G>A
Gln45Ter
Cys53Ter

Glu170ter
Gly199AlafsTer43
c.623-1G>A
c.720+2T>C
Trp235Ter

Asn296GlufsTer38
Lys331Ter
Lys331SerfsTer5
Val332Ter
Thr353LeufsTer32
Glu371ArffsTer14
Val374SerfsTer2
Glu 531 Ter
Glu541Ter
Ser565ArgfsTer11

Ser564ArgfsTer11
Phe597CysfsTer23
« Gln668Ter »
p.Y731AfsX10
c.1828+2T>G
c.1828+1G>T
c.1829-2A>G
c.1829-1G>C
Gln668Ter
Tyr731Ter
Val745SerfsTer2
Lys876SerfsTer11

Arg921GlnfsTer25
Val1016LeufsTer10
Val1052ArgfsTer17
Ile1195LeufsTer91
Pro1226LeufsTer38
Gln1248Ter
Gly1289Ter
Val1347CysfsTer3
Ala1383LeufsTer10
c.4143-12T>A
c.4143-2del
c.4303+4_4303+11del

Leu1483TyrfsTer19
Asn1501GlnfsTer3
Arg1515Ter
Tyr1525Ter
Ser1533GlufsTer12
Leu1560AlafsTer4
Thr1605GlnfsTer6
Pro1610ArgfsTer12
Gly1779ValfsTer2

c.5417-1G>T
c.5837_2del (??)
Ala1833AspfsTer3
His2029GlnfsTer32
Phe2089LeufsTer65
Gly2078MetfsTer56
[Glu2142AlafsTer22
Gly2127-Pro2167del]
His2196ProfsTer11
Arg2201AlafsTer14
Val2212AlafsTer2
c.6770-1del
Glu2258Ter
Tyr2305Ter
Gln2345Ter
Gln2385Ter

Leu2605TrpfsTer2
Trp2632Ter
[Pro2641Ala;
Tyr2642ThrfsTer63]

Val21dup
Ala39Glu
Ala39Gly
Gly74Arg
Leu80Val
Met102Val
Leu104Val
Ile 119Asn
Val122Gly
Lys127Asn
Ala128Val
Ile 129Met
Ser149Phe

Arg285Cys
Lys355lys
Pro469Lys
Asp535Gly

Lys936Arg

Arg1635Cys
Gly1728Gly

E1803K Ala 2257Pro
His2116Gln
Cys2160Arg (OPDSD)

MISSENSE MUTATIONS

FLNA related thrombocytopenia : remaining questions

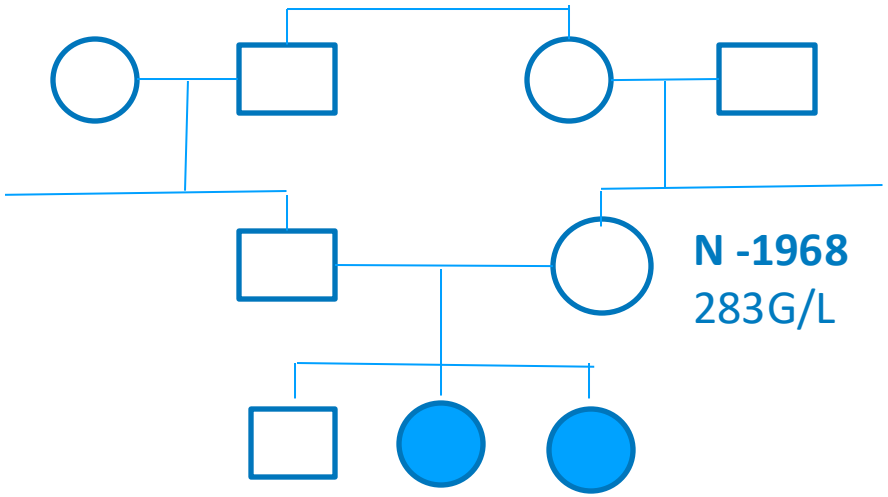
- Thrombocytopenia prevalence in patients with PVNH is unknow
- What is the specificity of platelet FLNA deficiency
  - Does thrombocytopenia exist without FLNA deficiency in platelets (qualitative defect)
- What is the specificity of PVNH
  - Does thrombocytopenia exist in carriers of FLNA mutations without PVNH (*1 case with FLNA variant +intestinal malrotation + thrombocytopenia-PVNH*)

Today objective

- ✓ **Familial thrombocytopenia can exceed the classical framework of inherited thrombocytopenia**

✓ Case illustration 2

Referred to our center for investigation of severe constitutional refractory thrombocytopenia.



Fluctuating thrombocytopenia (nadir 1G/l)
Spontaneous Bleeding +++
Mental retardation, facial dysmorphia
Recurrent febrile episodes
Cutaneous xerosis
Lymphopenia (reduced lymphocyte proliferation in response to several inducers)
Search for metabolic diseases and dyskeratosis : negative
Absence of genomic imbalance

Homozygous variation in TPP2 gene
Tripeptidyl peptidase 2

c.1001A>C
His334Pro

No match in gnomAD
Damaging (Sift, Polyphen, AlphaMissense
Revel, ClinPred, Mistic)

This rare genetic immunological disease early childhood or childhood, Immunodeficiency associated with recurrent viral, bacterial, and fungal infections, a severe autoimmune disease (RBC, platelets, neutrophils) mild to moderate developmental delay. Biological analyses reveal ***a decrease in circulating T, B, and NK*** *cells and hypergammaglobulinemia.*

Today objective

- ✓ **Inherited thrombocytopenia as a Gateway for complex diseases**

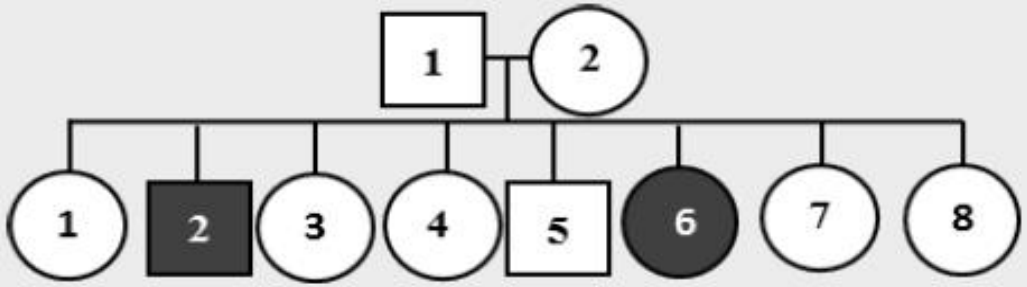
✓ Case illustration 3*

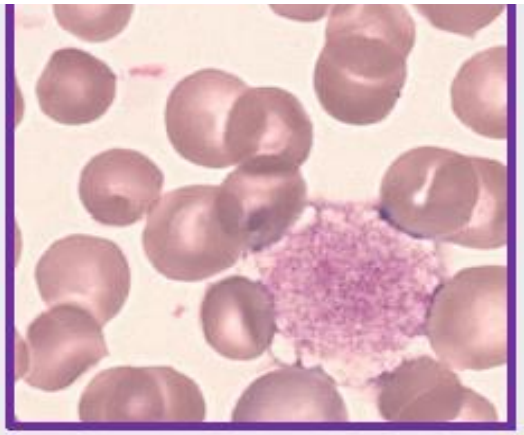
Man 48 years old

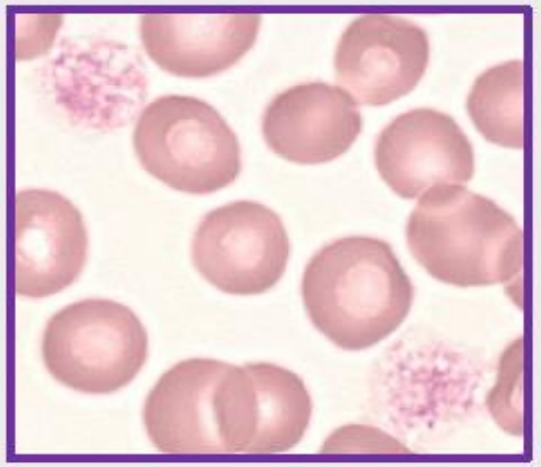
Platelet count 83G/L - (1 sister also affected).
40% of large platelets

Medical history : Several coronary bypass surgeries with hypercholesterolemia treated with ezetimibe
Family history of CV events

No consanguinity



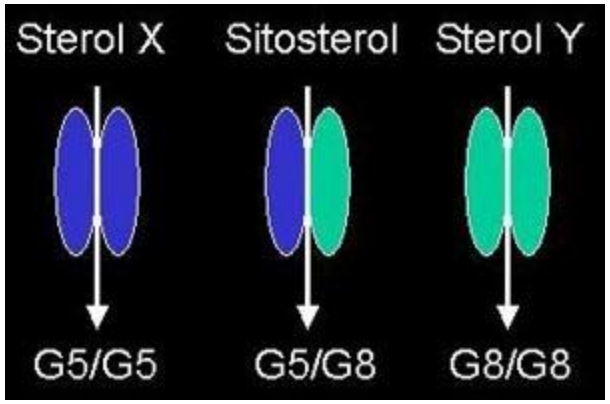




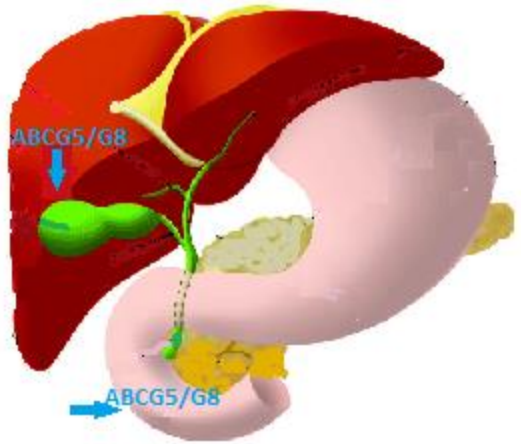
ABCG8
homozygous c.1270G>A,
p.Gly574Arg

* *explored in Paris*

✓ Case illustration 3

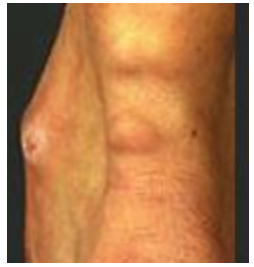


Promotes the secretion
of cholesterol and xenosterols
into the bile and the intestinal lumen

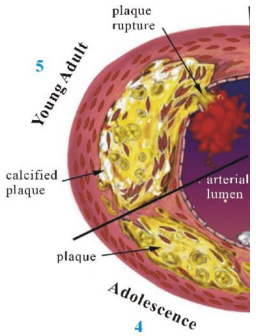


Homozygous/double heterozygous point mutation

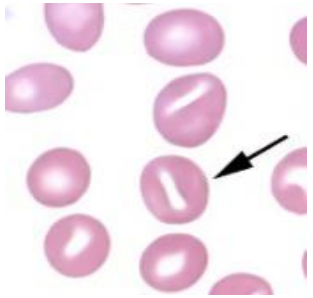
Deposition of phytosterols in various tissues



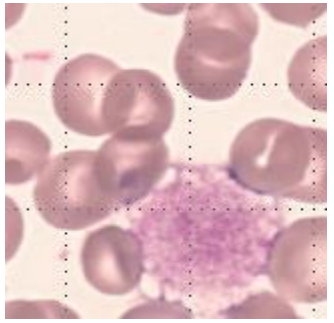
Xanthomas



Premature atherosclerosis



Stomatocyte
Hemolytic anemia



Macro thrombocytopenia

Few early warning signs

Increased in LDL Cholesterol is not always present
If present « poor response to statin treatment »
Clinical signs are not present at a young age
Sitosterolemia measurement (specialized laboratories)

Recommendation

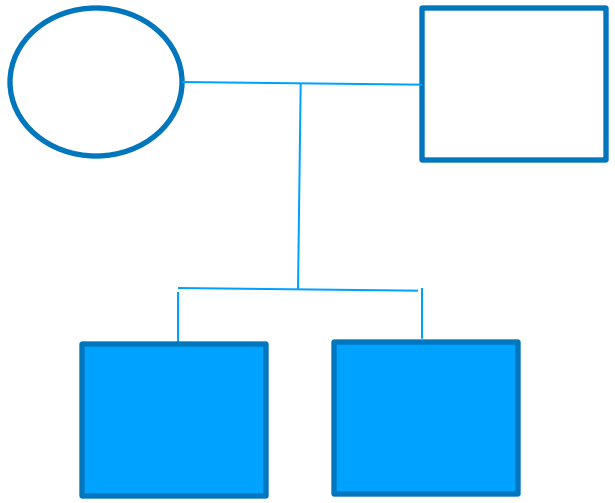
Early diagnosis (sequencing)
Inhibition of sitosterol absorption (ezetimide)
Diet recommendation

Today objective

- ✓ **Inherited thrombocytopenia as a Gateway for complex diseases**

✓ Case illustration 4

Referred to our center for investigation of chronic bleeding



1
Birth 1988

Hematomas
Bruises and easy bleeding
Nosebleeds cauterized

2
Birth 1992

Hematomas
Petechias with exertion
Tooth extraction and surgery for appendicitis
Under platelet concentrates

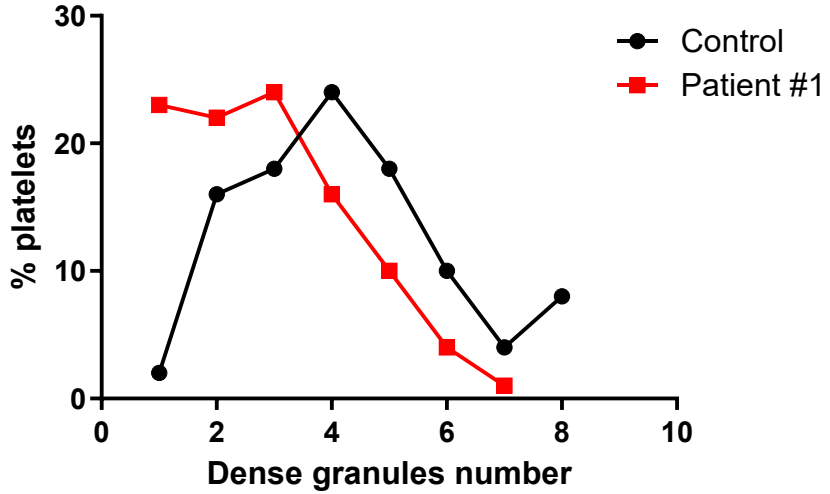
#child 1
Platelet exploration at 1.5 yrs old

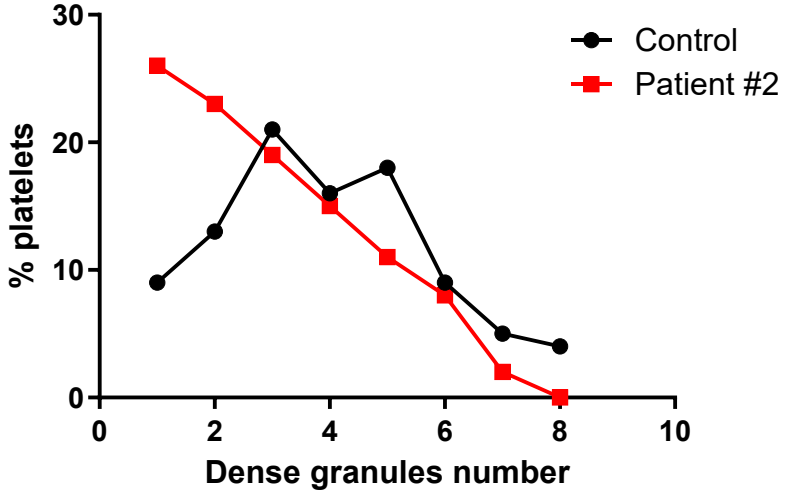
Blood CC	Normal
Platelets G/l	233
aPTT sec	30/31
PT (%)	95
vWF UI/ml	1.3
VIII UI/ml	0.95
Fibrinogen g/l	2.07
MPV (fl)	6.5
BT min	**>15**

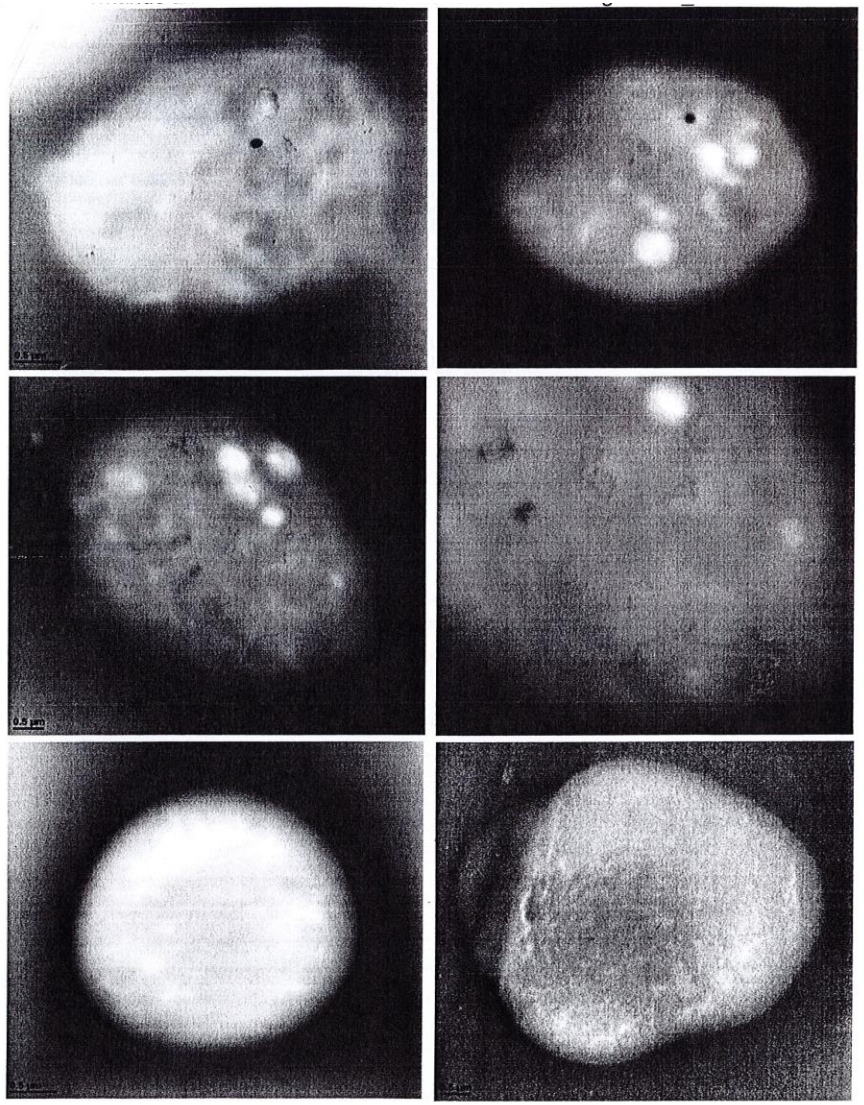
Light transmission aggregation (LTA)

LTA Maximal Intensity		Control 350G/L	Patient 380G/L
	Concentration		
ADP µM	**5**	68	**45**
	2.5	68	**25**
	1	45	**9**
Collagen µg/ml	**high**	63	**0**
	Low	60	**0**
Ristocetin mg/ml	**1.5**	76	67
	0.5	0	0
Arachidonic acid	**1mM**	69	**13**

Decreased in dense granules count



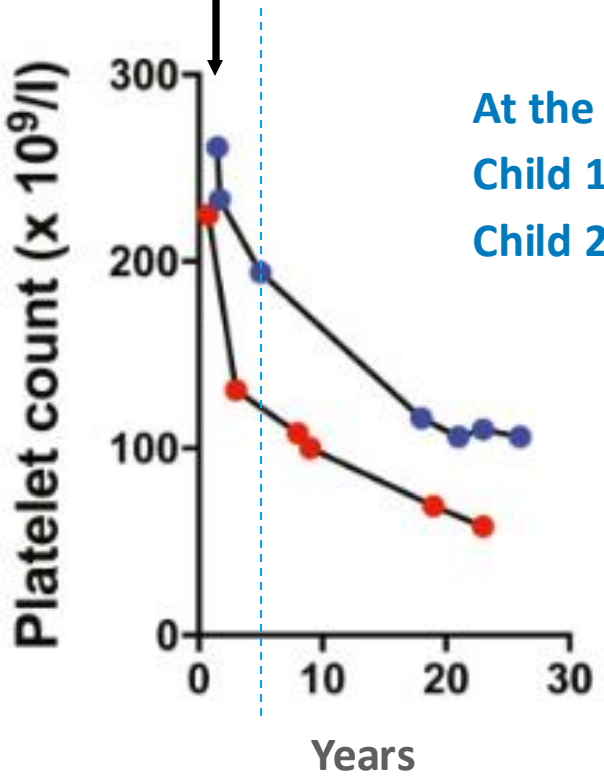




Whole mount : severe dense granule deficiency (65-90%)

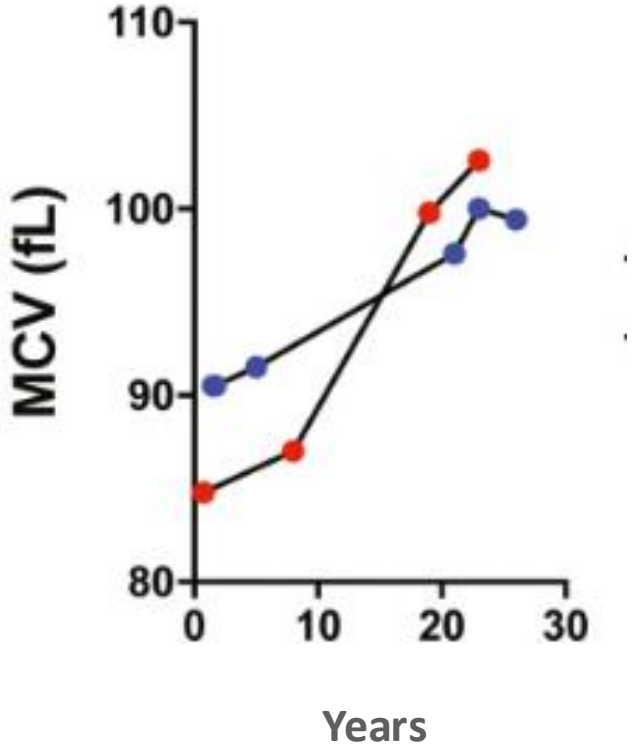
Thrombocytopenia was identified as the disease progressed

Normal platelet count
At the age of 1.5 yrs



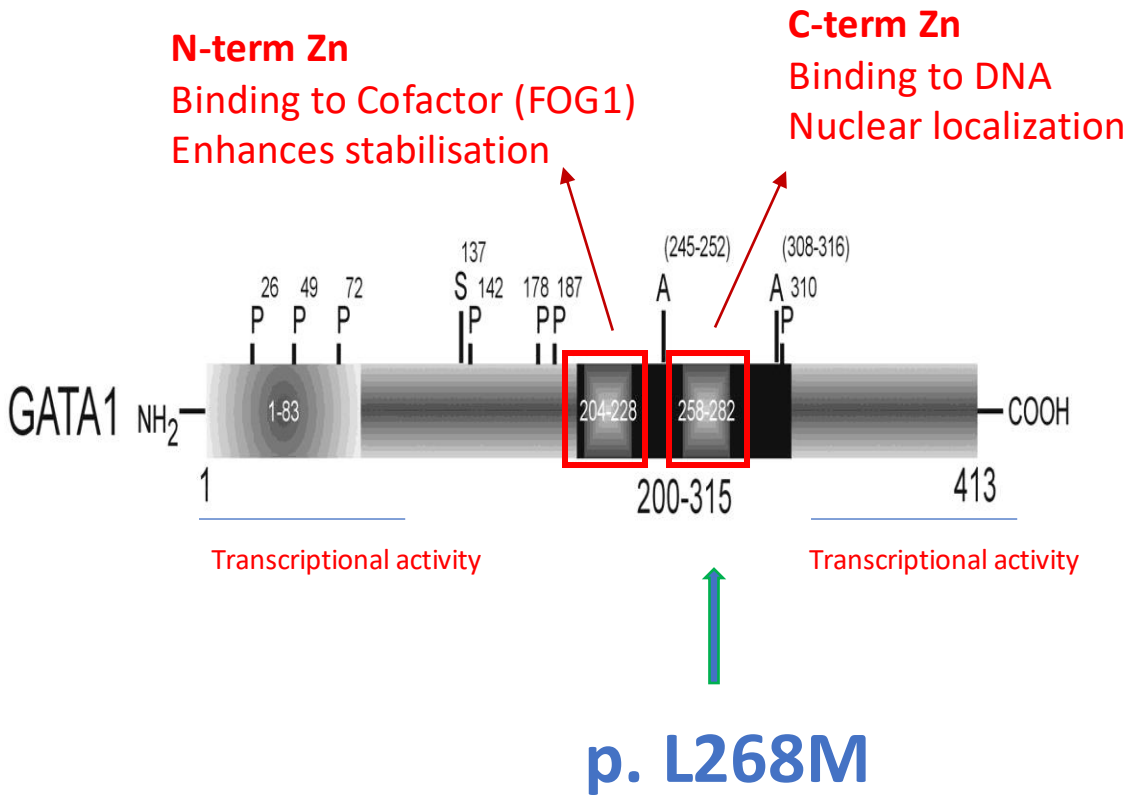
At the age of 5 yrs
Child 1 : 194G/L
Child 2 : 131 G/L

Presence of schizocytes
Persistence of HbF (25%)
Numerous red blood cells with a Heterogeneous distribution of HbF



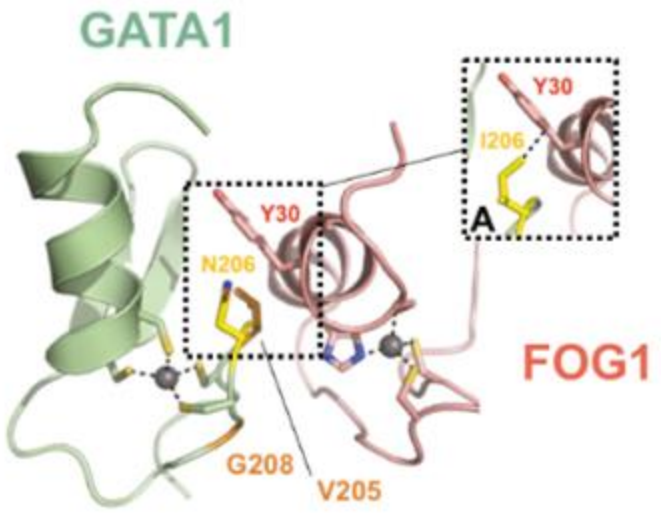
Genetic diagnosis

- Mutation **GATA1 Exon 5 (c.802C>A, p L268M)** **Chromosome Xp11,23**



Genotype-phenotype relationship depending on the mutation site

Disruption of FOG1 binding leads to severe forms



NMR structure of Zn fingers of GATA1 bound to FOG1

Zinc finger domain

	Disruption GATA1/FOG1	Anemia	Thrombocytopenia
V205M	+++	+++	+++
N206I	+++	-	+++
D218Y	+++	+++	+++
G208R	?	+	+++
G208S	+	-	++
D218G	+	-	+/++/+++
R216Q	-	+/-	+ granules
L268M	-	-	+ granules

Take home messages

Exploring even mild familial thrombocytopenia can reveal underlying pathologies that require multidisciplinary management. (FLNA, ABCG8)

Gene supporting syndromic thrombocytopenia may be outside the classical list of known gene involved in inherited thrombocytopenia (TPP2).

It is important to closely examine blood smears and hemocytometry results (schizocytes (GATA1), giant platelets with vacuoles and stomatocytes (ABCG8), MCV (GATA1)).

Thrombocytopenia cannot be present in young children and can evolve overtime (GATA1)

In cases of syndromic forms, the severity of accompanying symptoms may be influenced by the mutation location and the affected functional domain (GATA1).

Thank you for your attention !

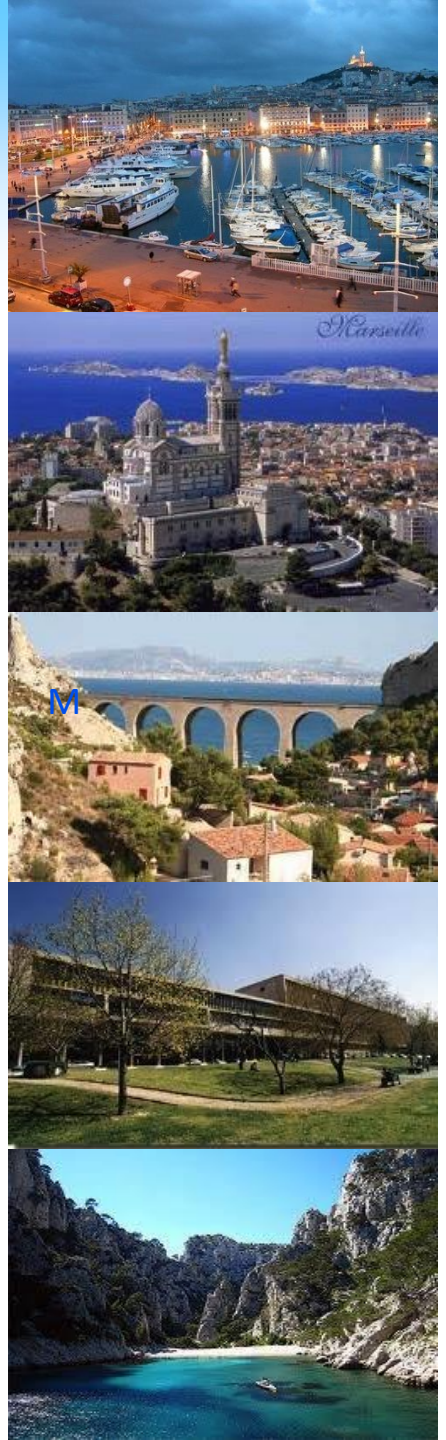
Marie-christine.alessi@univ-amu.fr
Paul.Sautier@ap-hm.fr
Celine.falaise @ap-hm.fr
Manal.ibrahim@ap-hm.fr

CHU Timone , Marseille, France

CRPP

Faculté de Médecine Aix*Marseille Université

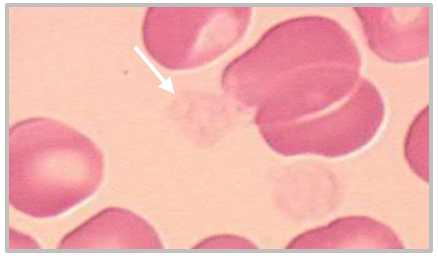
Assistance Publique Hôpitaux de Marseille



Contribution of blood smears

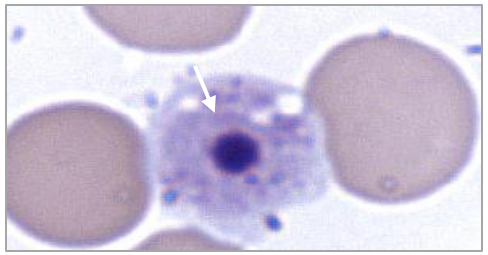
Platelets morphology

Absence of α granules



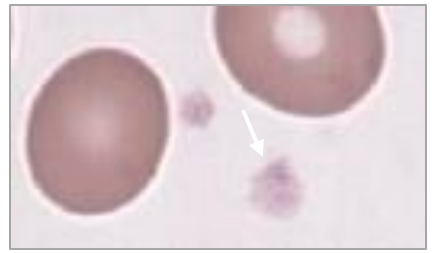
Grey syndrome

Giant granules α



Paris-Trousseau
Fli1 variants

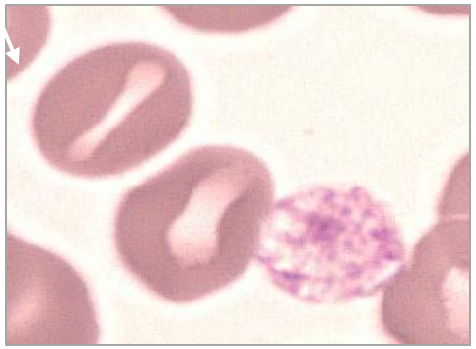
Defect in α granules



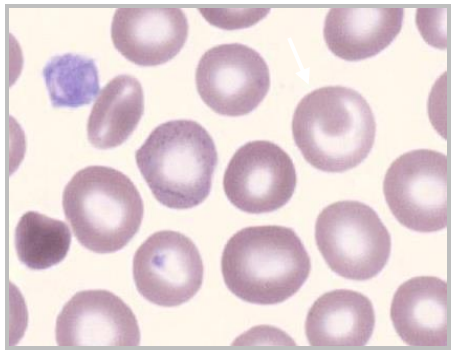
Thrombopénie
ANKRD26

Cytology of RBC and leukocytes

Stomatocytes

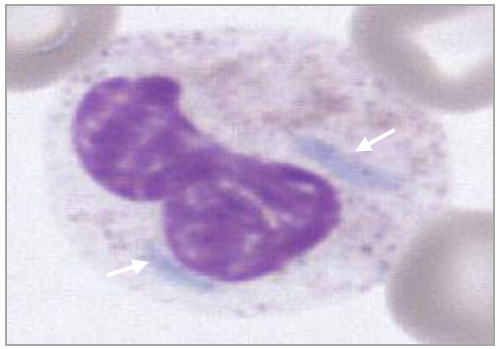


« signs of β thalassemia »



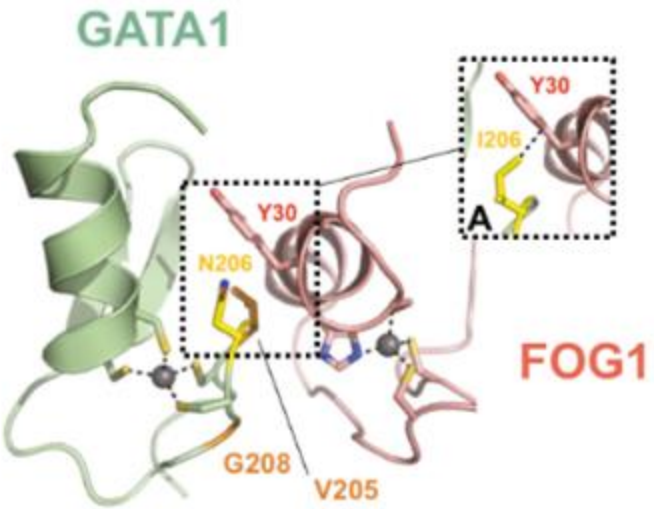
Red Blood cells

Döhle bodies



Neutrophils

Disruption of FOG1 binding leads to severe forms



NMR structure of Zn fingers of GATA1 bound to FOG1

Zinc finger domain

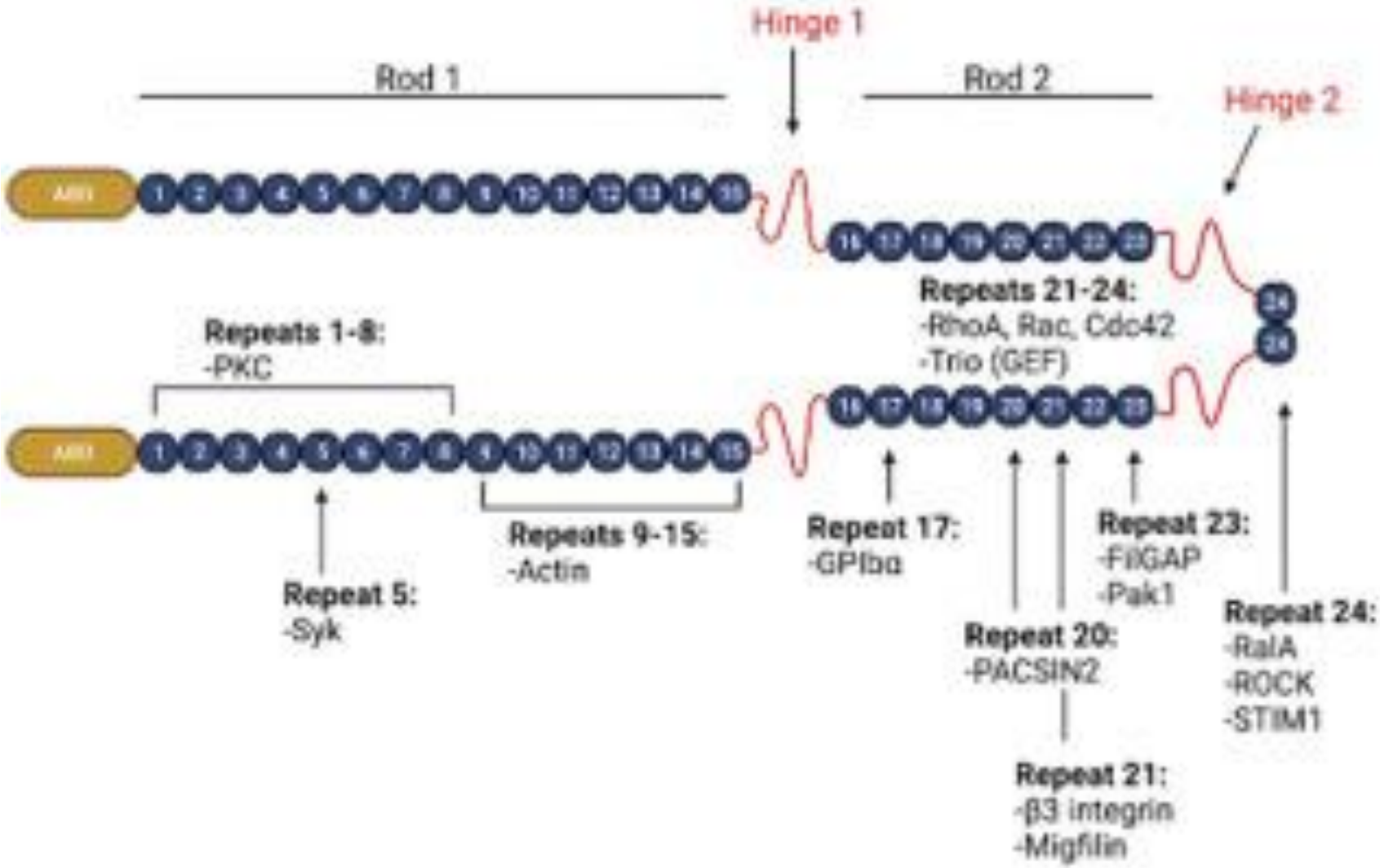
	Disruption GATA1/FOG1	Anemia	Thrombocytopenia
V205M	+++	+++	+++
N206I	+++	-	+++
D218Y	+++	+++	+++
G208R	?	+	+++
G208S	+	-	++
D218G	+	-	+/++/+++
R216Q	-	+/-	+ granules
L268M	-	-	+ granules

V74L	Macrocytic anemia, neutropenia, platelet dysfunction
F172S	Pancytopenia
Splice 5'UTR	Dyserythropoiesis, thrombocytosis
L387LfsTer62	Congenital anemia

Other domains

Germinal mutation generating short GATA1 isoform (GATA1s). Anemia and risk to develop acute megakaryoblastic leukemia

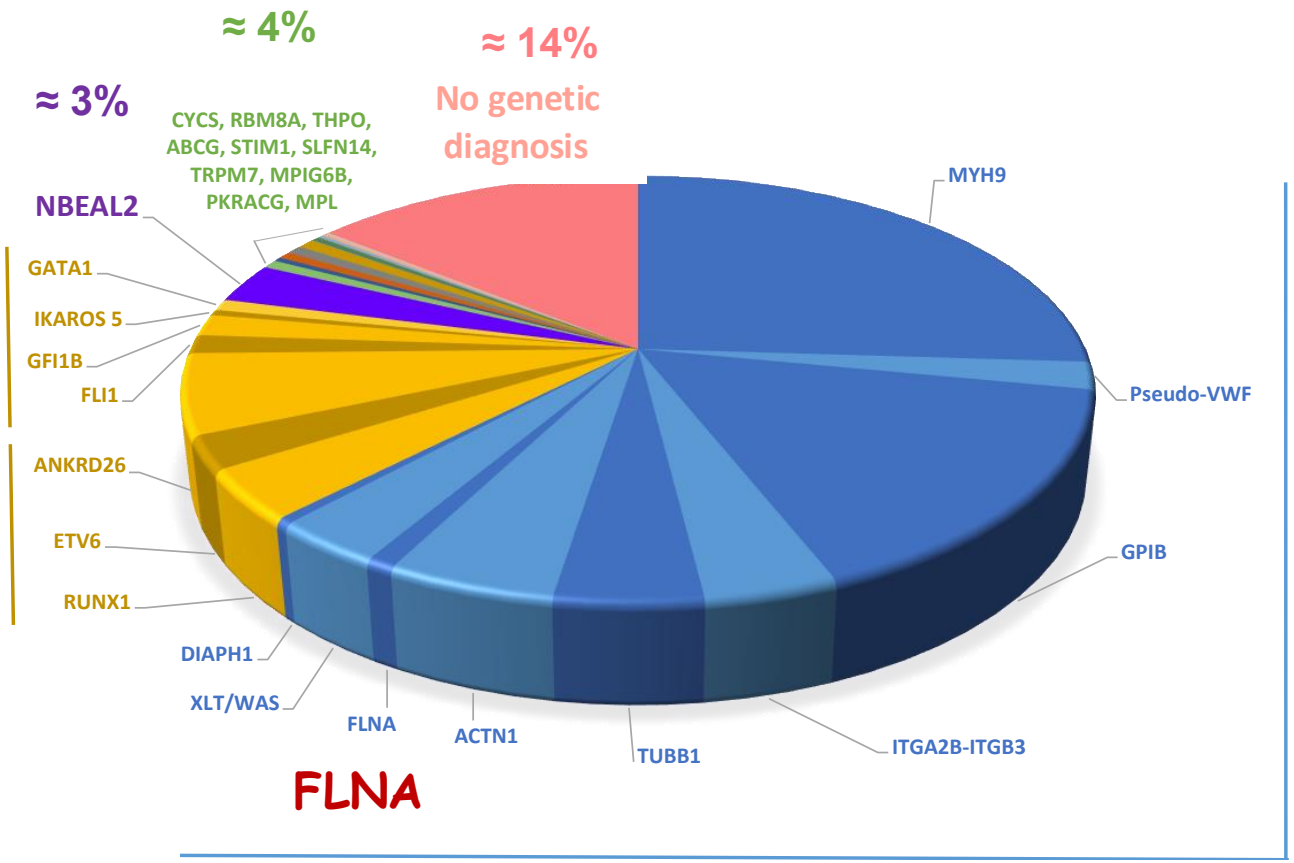
A


Hinge 1
Rod 1
Rod 2
Hinge 2
Repeats 21-24:
-RhoA, Rac, Cdc42
-Trio (GEF)
Repeats 1-8:
-PKC
Repeat 5:
-Syk
Repeats 9-15:
-Actin
Repeat 17:
-GPIbα
Repeat 20:
-PACSIN2
Repeat 21:
-β3 integrin
-Migfilin
Repeat 23:
-FilGAP
-Pak1
Repeat 24:
-RalA
-ROCK
-STIM1

FRENCH REGISTER OF INHERITED THROMBOCYTOPENIA

Thrombocytopenia
n=920

CRPP



≈ 4%

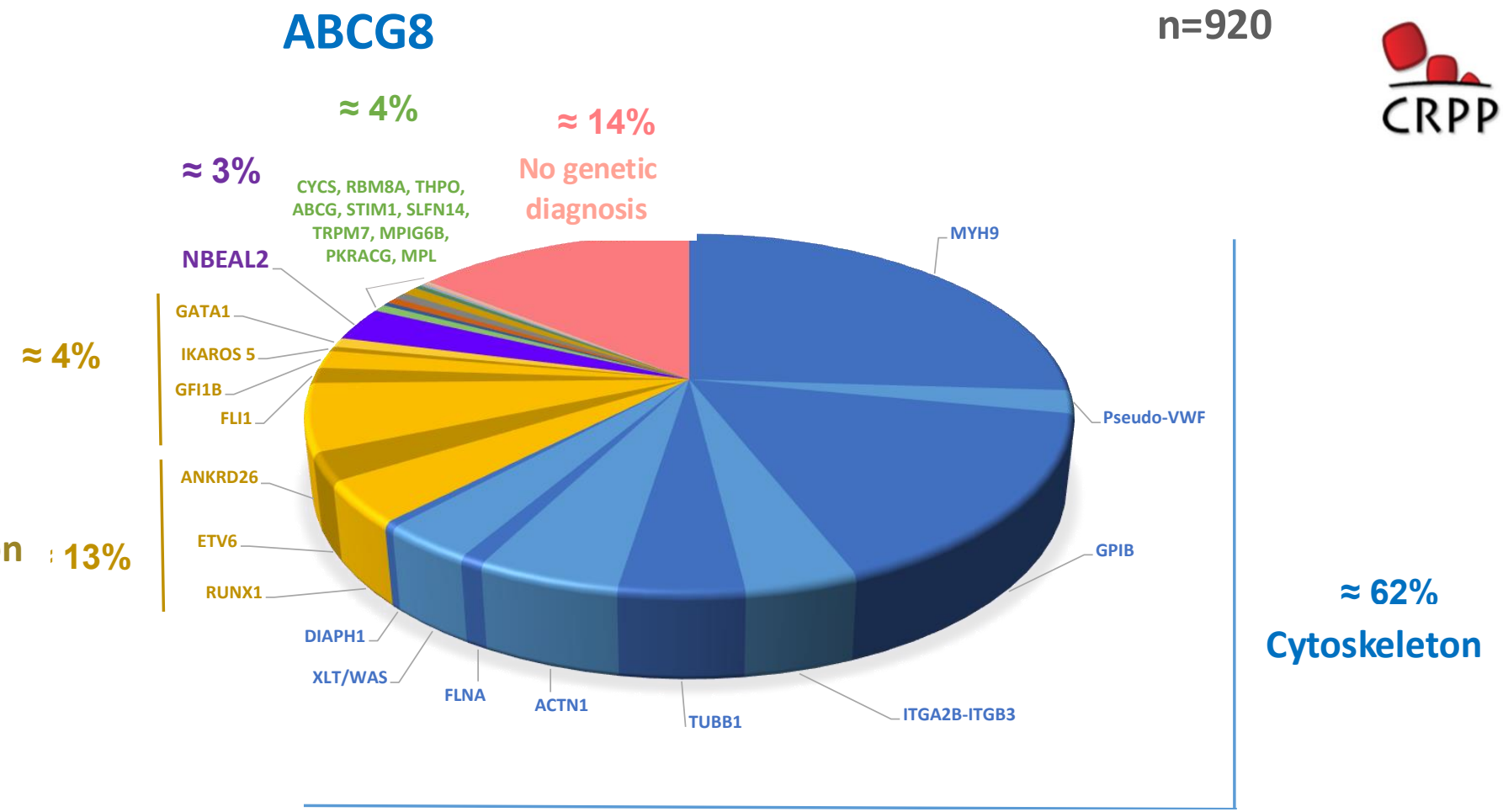
Leukemia predisposition ≈ 13%

≈ 62%
Cytoskeleton

FRENCH REGISTER OF INHERITED THROMBOCYTOPENIA

Thrombocytopenia
n=920

CRPP



FRENCH REGISTER OF INHERITED THROMBOCYTOPENIA

C Thrombocytopenia
n=920

CRPP

≈ 4%

≈ 14%
No genetic diagnosis

≈ 3%

CYCS, RBM8A, THPO, ABCG, STIM1, SLFN14, TRPM7, MPIG6B, PKRACG, MPL

NBEAL2

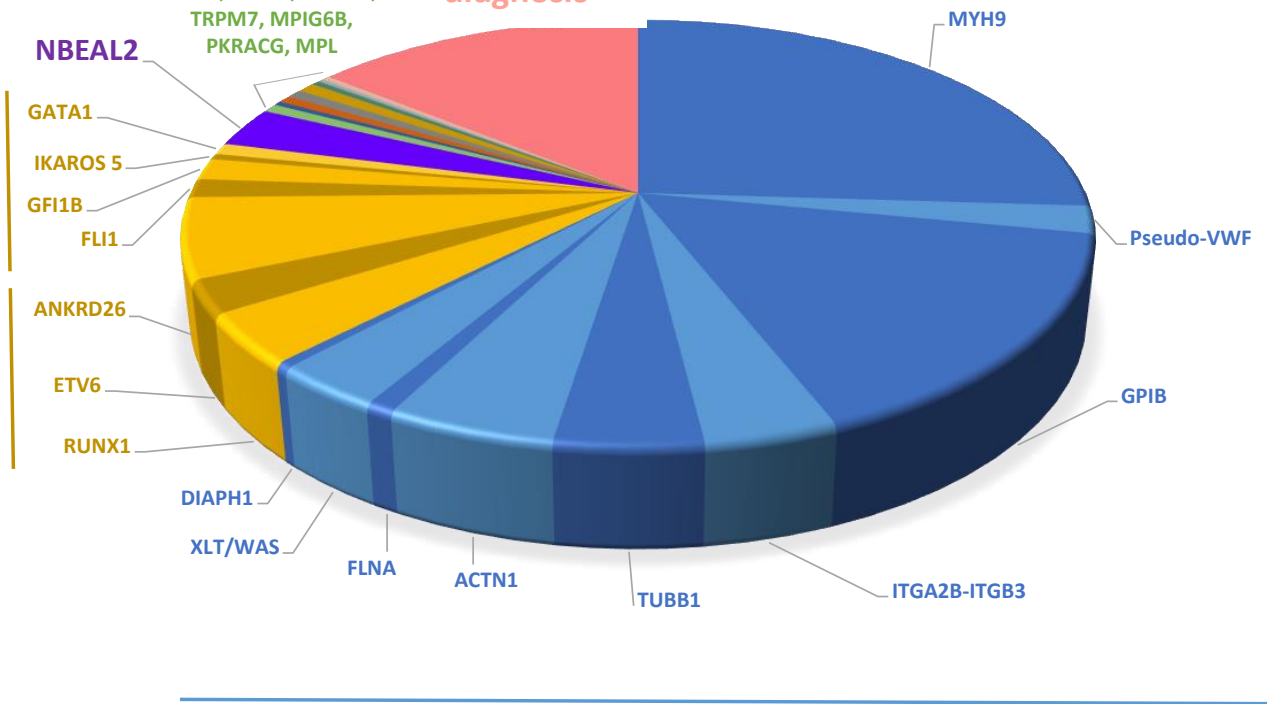
Transcription factors

GATA1

≈ 4%

Leukemia predisposition

13%



≈ 62%
Cytoskeleton