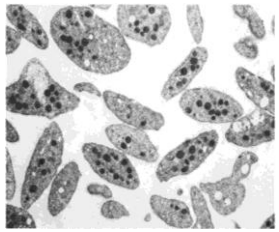
inherited thrombocytopenias

not diagnosed during childhood:

an update

Thomas LECOMPTE

WPH / March 2017

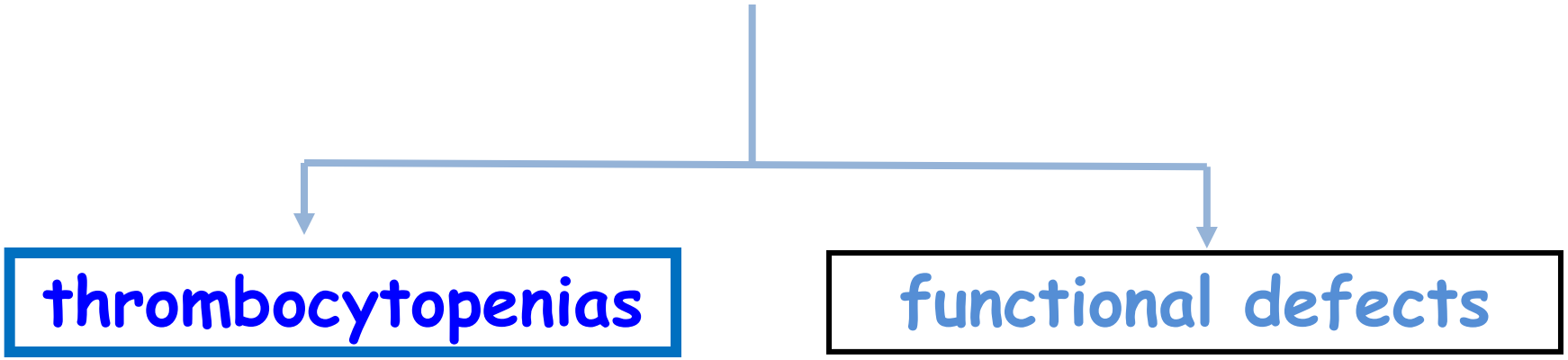


Hôpitaux Universitaires Genève

Geneva Platelet Group

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

inherited platelet disorders predisposing to bleeding



both can be present

thrombocytopenia + functional defect

Bernard Soulier Syndrome (biallelic)

grey platelet syndrome

platelet-type pseudo-VWD

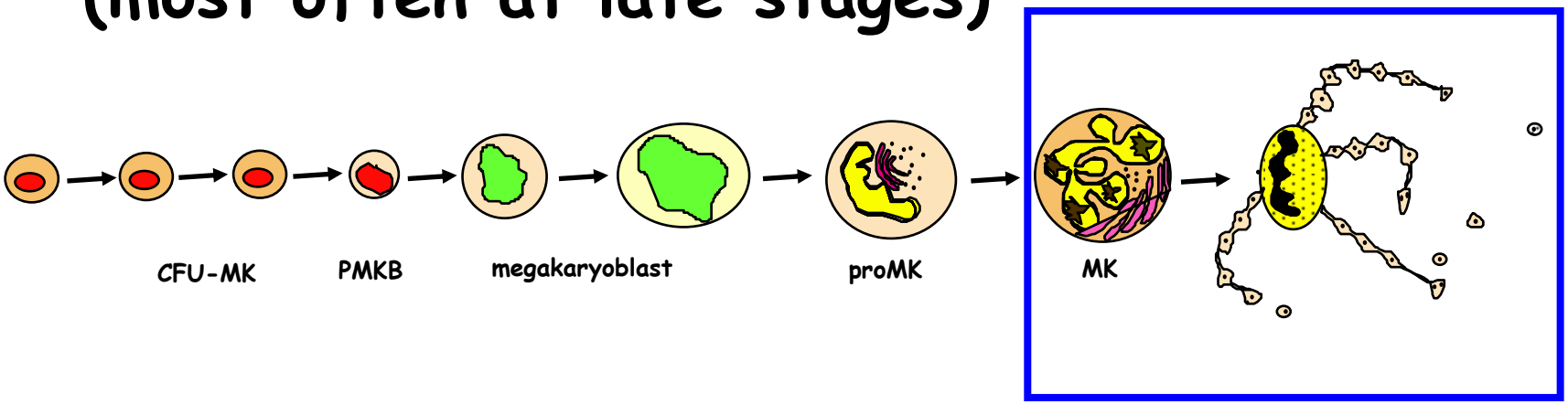
FPD-AML

…

inherited thrombocytopenias

- unfrequent to exceedingly rare
- at least 20 identified entities
- deleterious variations in genes
- very heterogeneous
- defective production

  (most often at late stages)



inherited thrombocytopenias:

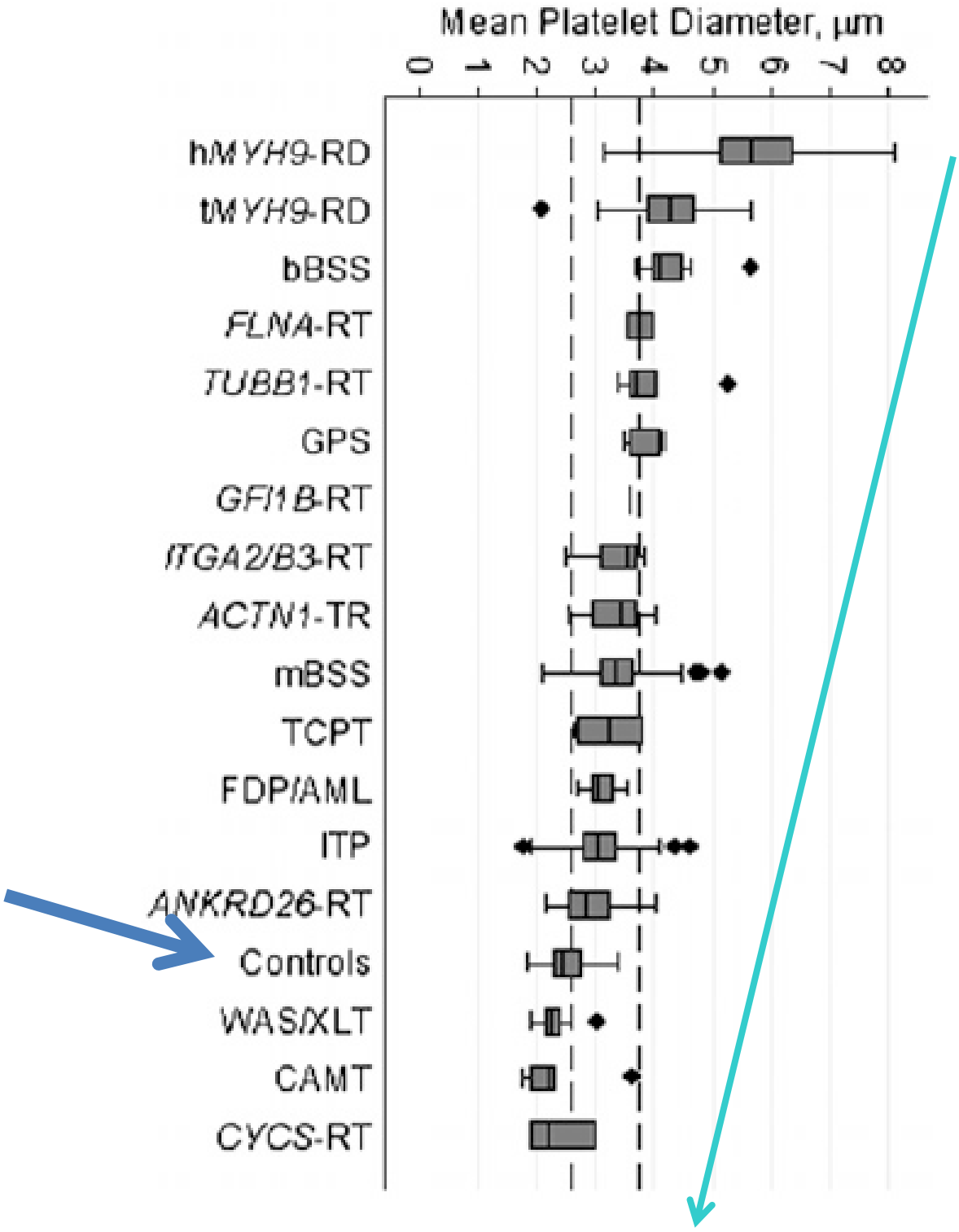
clues for the correct diagnostic orientation

- PC never normal
- family history

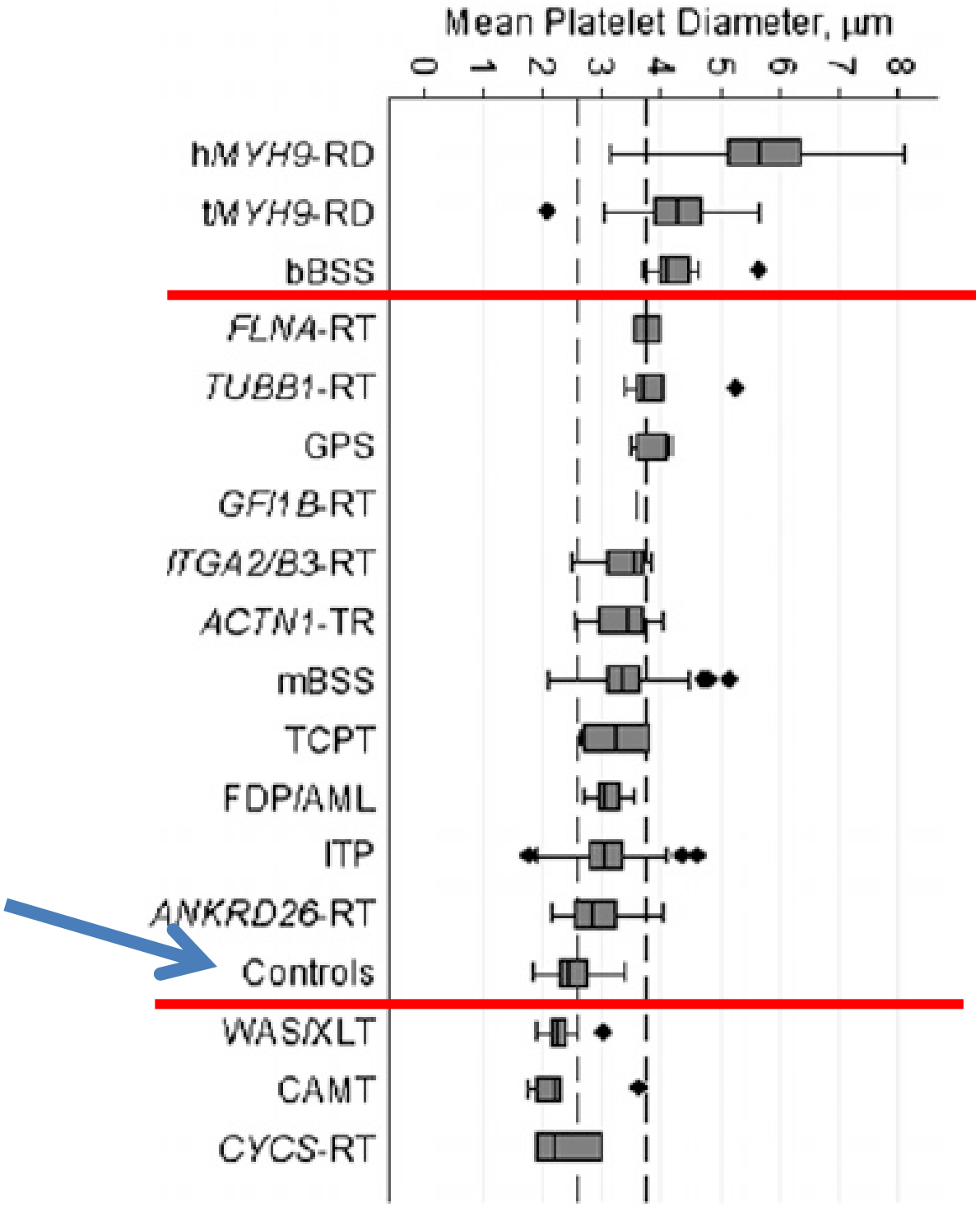
- abnormal platelet morphology

ITs with giant platelets	*MYH9*-RD Biallelic BSS
ITs with large platelets	*TUBB1*-RT GPS *FLNA*-RT *GFI1b*-RT Monoallelic BSS *ITGA2B/ITGB3*-RT *ACTN1*-RT
ITs with normal/slightly increased platelet size	FDP-AML TCPT *ANKRD26*-RT
ITs with normal/reduced platelet size	CAMT *CYCS*-RT WAS XLT

membrane proteins, cytoskeleton, α-granules, transcription factors, TPO-receptor


Mean Platelet Diameter, µm
0
1
2
3
4
5
6
7
8
hMYH9-RD
tMYH9-RD
bBSS
FLNA-RT
TUBB1-RT
GPS
GFI1B-RT
ITGA2/B3-RT
ACTN1-TR
mBSS
TCPT
FDP/AML
ITP
ANKRD26-RT
Controls
WAS/XLT
CAMT
CYCS-RT

Noris 2014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Controls
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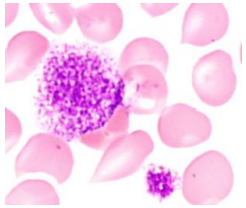
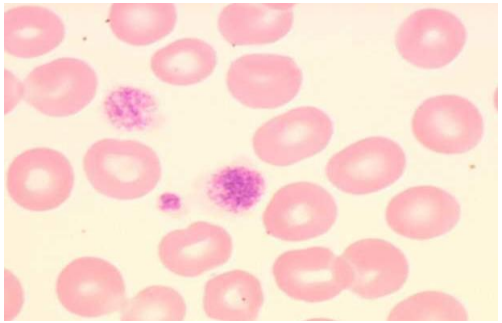
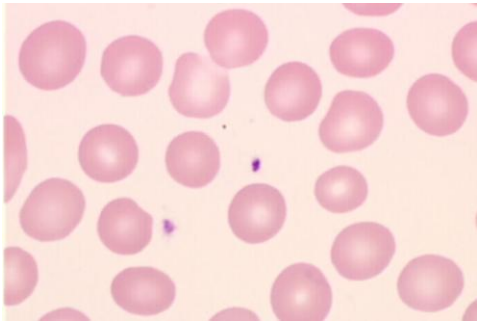
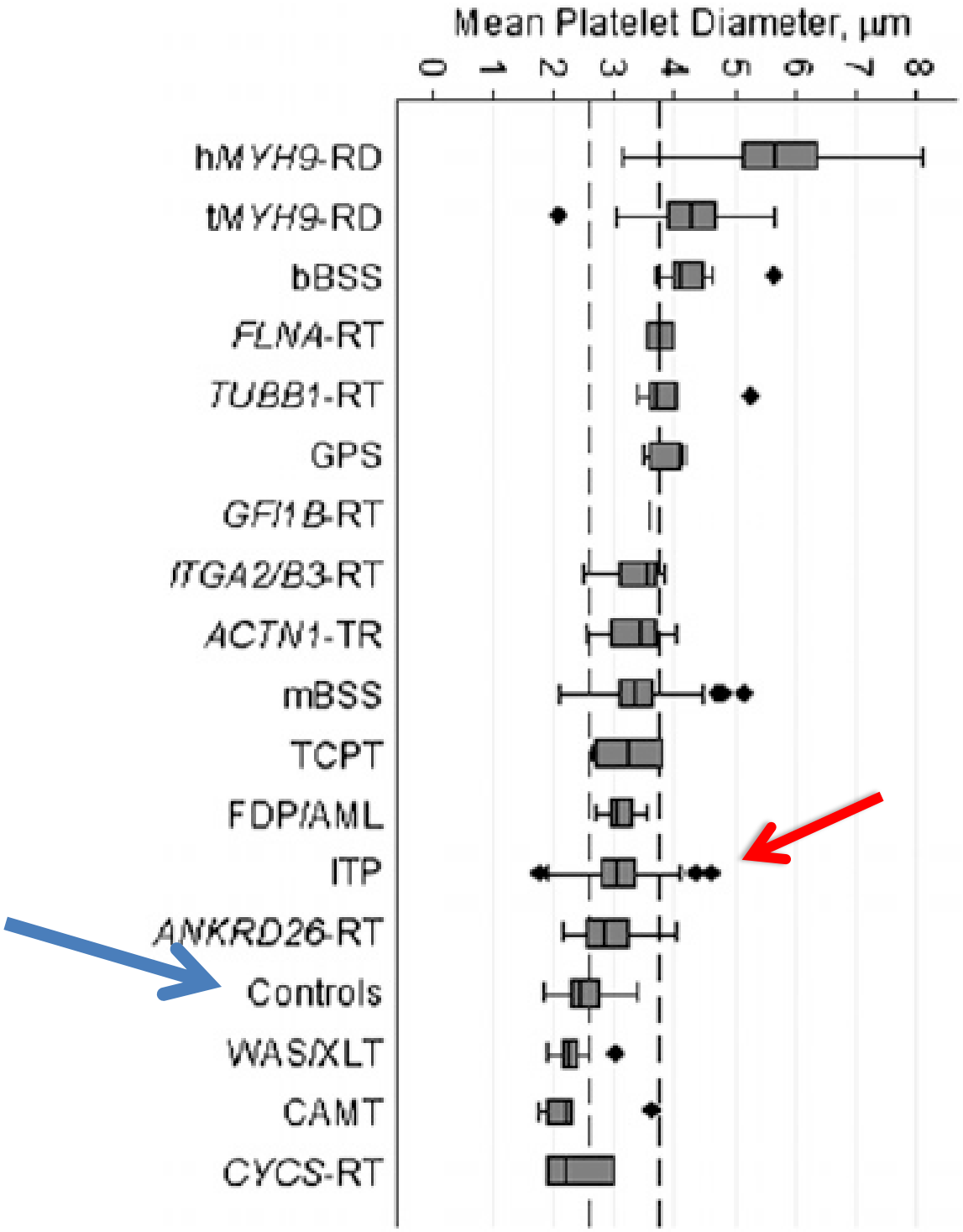
Noris 2014

ITs with giant platelets 	*MYH9*-RD Biallelic BSS
ITs with large platelets 	*TUBB1*-RT GPS *FLNA*-RT *GFI1b*-RT Monoallelic BSS *ITGA2B/ITGB3*-RT *ACTN1*-RT
ITs with normal/slightly increased platelet size	FDP-AML TCPT *ANKRD26*-RT
ITs with normal/reduced platelet size 	CAMT *CYCS*-RT WAS XLT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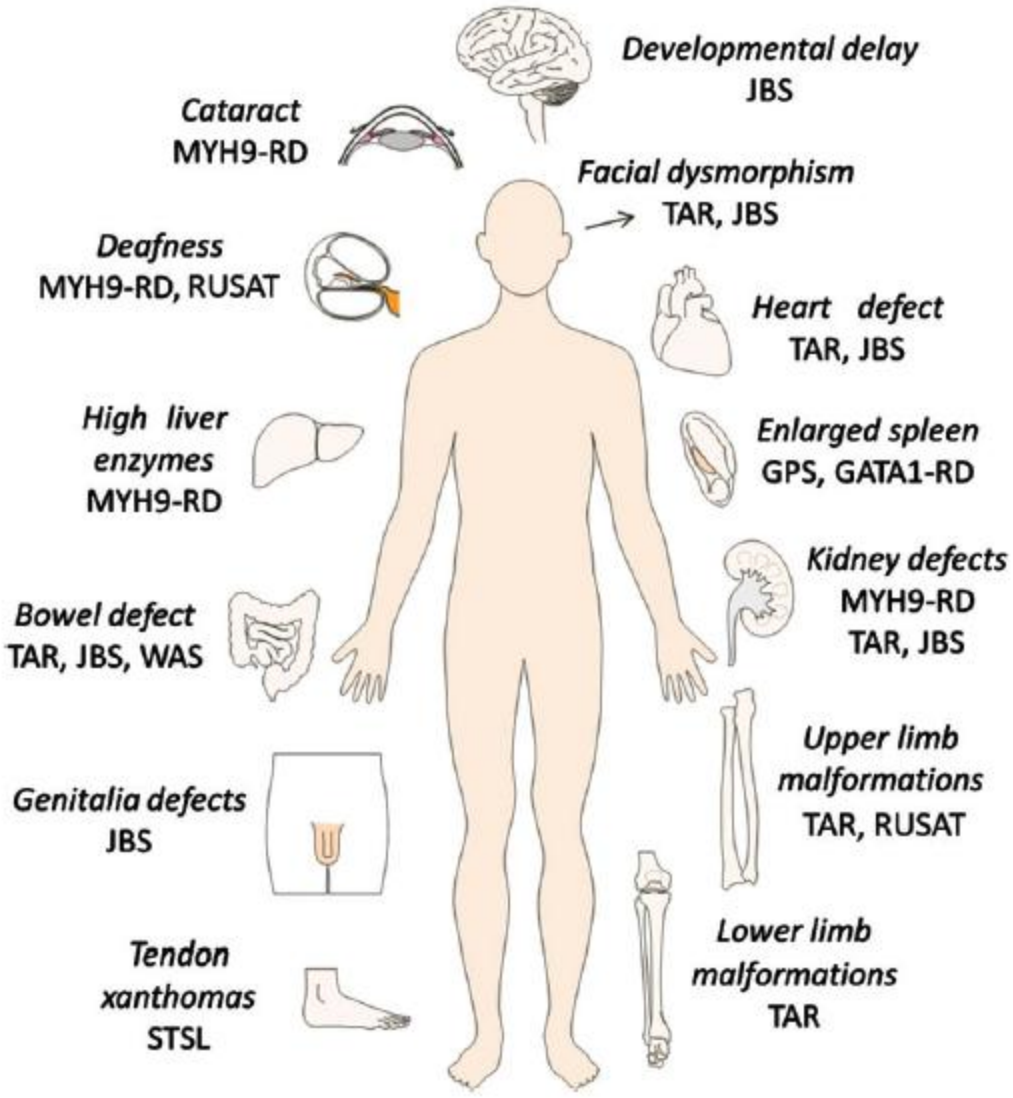
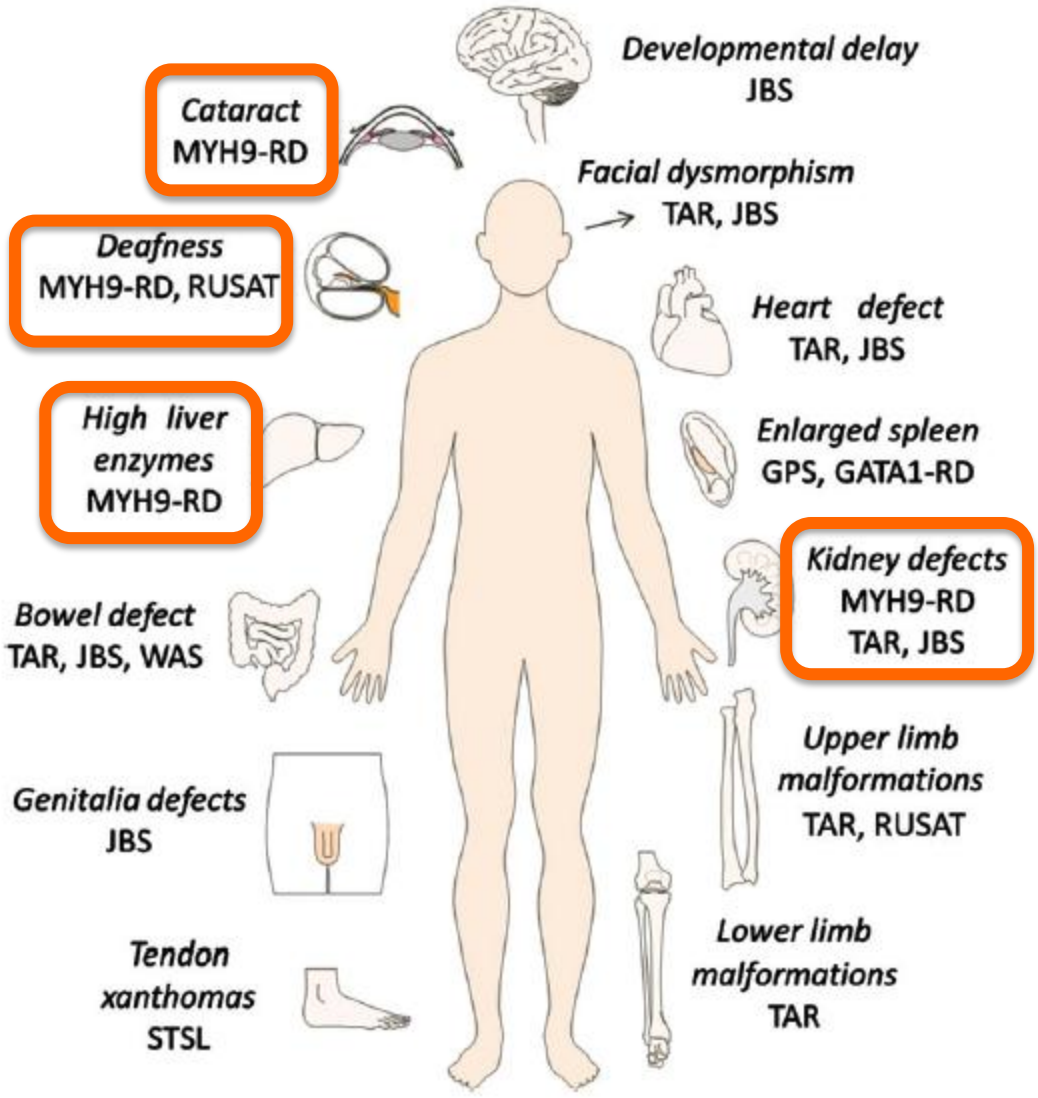
Controls
WAS/XLT
CAMT
CYCS-RT

Noris 2014

Blood cell abnormality	Inherited thrombocytopenia
Platelets	
Giant platelets and >40% of platelets larger than half a red blood cell	*MYH9*-RD, biallelic BSS
Small platelets and/or <10% of platelets larger than half a red blood cell	WAS, XLT, CAMT, *CYCS*-RT
Agranular ('pale') platelets (with large platelets)	GPS, *GFI1b*-RT
Hypo-granular platelets (with normal-sized platelets)	*ANKRD26*-RT
Some platelets have one single giant granule	TCPT, JBS
Vacuolated platelets	XLTT
Leukocytes	
Basophilic 'Döhle-like' inclusions in neutrophils	*MYH9*-RD
Red blood cells	
Anisopoikilocytosis or anisocytosis	*GATA1*-RD, *GFI1b*-RT
Stomatocytosis	Sitosterolemia
Anisopoikilocytosis with dacryocytosis	GPS


Developmental delay
JBS
Cataract
MYH9-RD
Facial dysmorphism
TAR, JBS
Deafness
MYH9-RD, RUSAT
Heart defect
TAR, JBS
High liver enzymes
MYH9-RD
Enlarged spleen
GPS, GATA1-RD
Kidney defects
MYH9-RD
TAR, JBS
Bowel defect
TAR, JBS, WAS
Upper limb malformations
TAR, RUSAT
Genitalia defects
JBS
Lower limb malformations
TAR
Tendon xanthomas
STSL

MYH9-RD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STSL

inherited thrombocytopenias associated with a risk of hematologic malignancies

FPD-AML *RUNX1*

more recently: *ANKRD26*
ETV6

transcription factors

inherited thrombocytopenias: the least rare

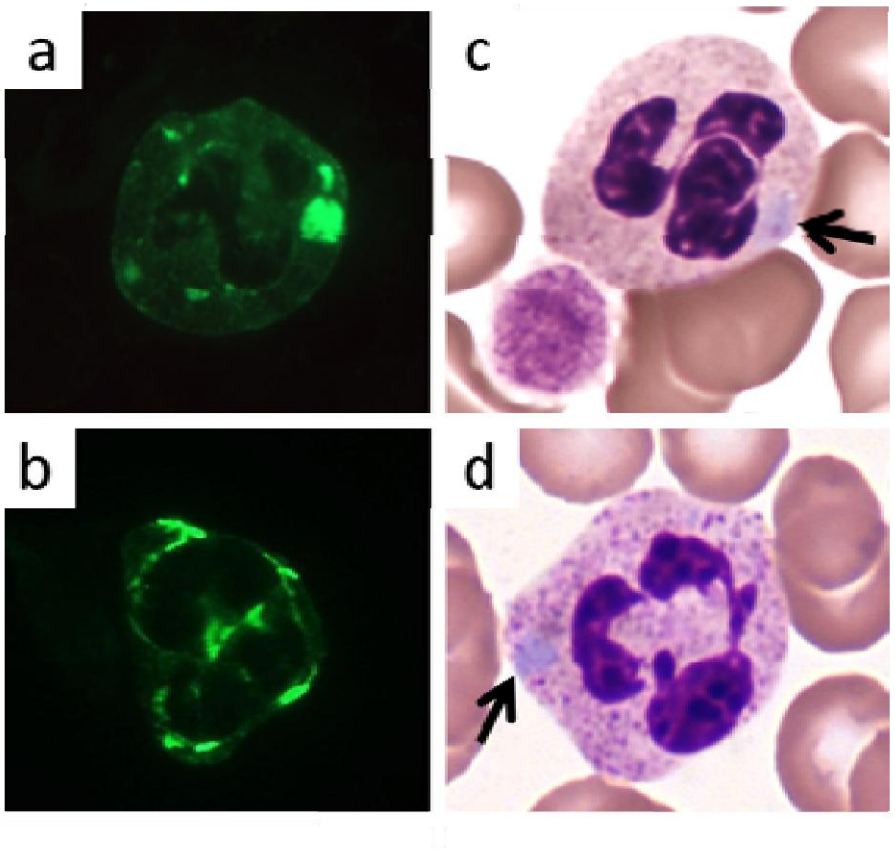
4 identified disorders often diagnosed in adults:

- *MYH9*-related disease
- monoallelic 'Bernard-Soulier syndrome'
- *ANKRD26*-related thrombocytopenia
- familial platelet disorder
  with predisposition to acute leukaemia

C.L. Balduini, A. Savoia, M. Seri JTH 2013

inherited thrombocytopenias: diagnosis

suggestive phenotype?



Clumps of NMMHC-IIA in neutrophils: a distinguishing feature of *MYH9*-RD

IF with antibodies against myosin-9

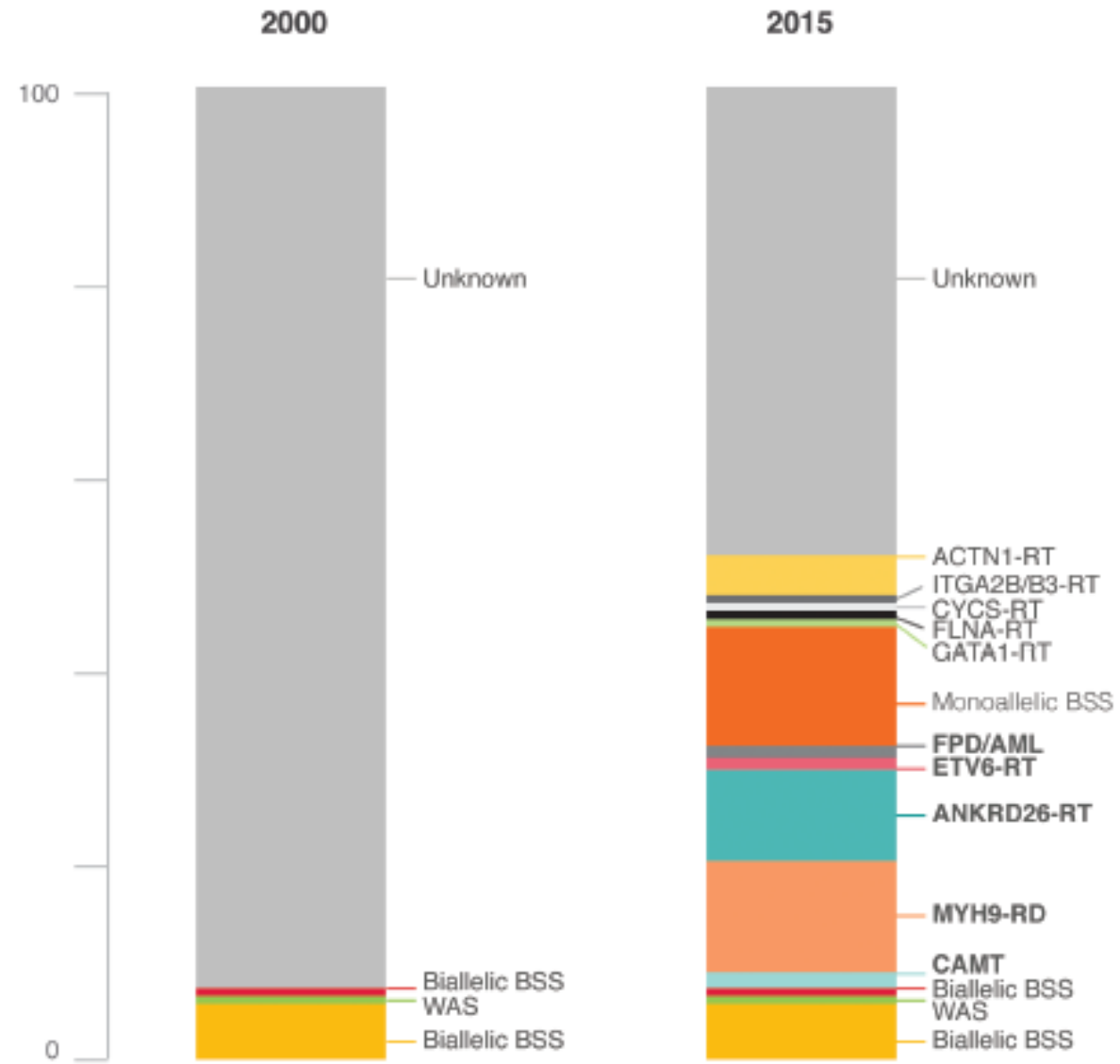
inherited disorders of platelets: genetic diagnosis

Most families have private mutations.

Gene variants may

prevent protein synthesis,
abrogate function,
result in aberrant activated proteins.

Bleeding severity: difficult to predict by genotype alone



BSS: Bernard-Soulier synd.
WAS: Wiskott-Aldrich synd.
GPS: gray platelet synd.
ACTN1-RT: *-related tcp*
ITGA2B/B3-RT: *-related tcp*
CYCS-RT: *-related tcp*
FLNA-RT: -related tcp
GATA1-RT: *-related tcp*

Carlo L. Balduini and Patrizia Noris *Internal Medicine, IRCCS Policlinico San Matteo Foundation and University of Pavia, Pavia, Italy*
based on the database comprising 274 consecutive families and 566 patients
does not include all known disorders, since many of them are exceedingly rare

inherited thrombocytopenias: recent studies with NGS

Next-Generation Sequencing (NGS) technology

- revolutionized the scale and cost-effectiveness of genetic testing
- emerged as a valuable tool for IPD

- diagnostic tool to streamline detection of causal variants in known IPD genes

- vehicle for new gene discovery

To cite this article: Annabel Maclachlan, Steve P. Watson & Neil V. Morgan (2017) Inherited platelet disorders: Insight from platelet genomics using next-generation sequencing, Platelets, 28:1,

Gene Associated phenotypes	Genomic variation	Protein effect	Variation type
NBEAL2 Thrombocytopenia, large platelets, lack of α-granules.	c.256 A>G	p.Ile86Val	Missense
	c.1163T>C	p.Leu388Pro	Missense
	c.1928 A>T	p.Glu643Val	Missense
	c.2044 >T	p.Ile682Phe	Missense
	c.6299 C>T	p.Pro2100Leu	Missense
	c.6802-2 A>AGGAGT		Splice site
	c.6806 C>T	p.Ser2269Leu	Missense
	c.7658 G>A	p.Gly2553Glu	Missense
ACTN1 Thrombocytopenia, large platelets .	c.64 G>A	p.Asp22Asn	Missense
	c.94 C>A	p.Gln32Lys	Missense
	c.136 C>T	p.Arg46Trp	Missense
	c.137 G>A	p.Arg46Gln	Missense
	c.313 G>A	p.Val105Ile	Missense
	c.673 G>A	p.Glu225Lys	Missense
	c.751 G>C	p.Gly251Arg	Missense
	c.2210 C>A	p.Thr737Asn	Missense
	c.2212 C>T	p.Arg738Trp	Missense
	c.2255 G>A	p.Arg752Gln	Missense
	c.2289 G>A	p.Gly764Ser	Missense
	c.2305 G>A	p.Glu769Lys	Missense
SRC Thrombocytopenia, sometimes large platelets, lack of α-granules.	c.1579 G>A	p.Glu527Lys	Missense
SLFN14 Thrombocytopenia, reduced δ-granules.	c.652 A>G	p.Lys218Glu	Missense
	c.657 T>A	p.Lys219Asn	Missense
	c.659 T>A	p.Val220Asp	Missense
	c.667 C>T	p.Arg223Trp	Missense
ETV6 Thrombocytopenia, hematologic malignancy.	c.641 C>T	p.Pro214Leu	Missense
	c.1106 G>A	p.Arg369Gln	Missense
	c.1195 C>T	p.Arg399Cys	Missense
FYB Thrombocytopenia, small platelets.	c.393 G>A^{Hom}	p.Trp131Stop^{Hom}	Nonsense
PRKACG Thrombocytopenia, giant platelets.	c.222 C>G^{Hom}	p.Ile74Met^{Hom}	Missense
RBM8A Thrombocytopenia, absence of radius bones.	c.-21 G>A		Regulatory SNP
	c.67+32G>C		Regulatory SNP

inherited thrombocytopenia with secondary qualitative function defects potentially damaging variants in novel candidate genes

Family	Gene	Variant	Protein effect	Prevalence	PhyloP	PhastCons	Mutation taster	SIFT	Provean	PolyPhen-2	ACMG	Classification
30	PADI2	c.1496A>G	p.Lys499Arg	0.000008681	1.647	1	Diseas-e causing	Tolerated	Neutral	Benign	PM (segregation)	Uncertain significance
	TTF2	c.3265C>G	p.His1089Asp	1.65E-05	5.131	1	Disease-causing	Damaging	Deleterious	Damaging	PM (segregation) , PP3	Uncertain significance
35	*ANKRD18A*	c.2395_2397del^{hom}	p.Glu799del^{hom}	Novel	0.772	0.965	Polymorphism	NA	Deleterious	NA	PM2, PP (segregation), PM6	Uncertain significance
	GNE	c.1339G>A^{hom}	p.Gly447Arg^{hom}	Novel	5.343	1	Disease-causing	Damaging	Neutral	Damaging	PM2, PP (segregation), PM6	Uncertain sgnificance
	FRMPD1	c.1526C>T^{hom}	p.Ala509Val^{hom}	0.0003708	-1.459	0	Polymorphism	Tolerated	Neutral	Benign	PP (segregation), PM6	Uncertain significance
37	MKL1	c.1723G>A	p.Val575Met	0.0007718	3.358	1	Disease-causing	Damaging	Neutral	Damaging		Uncertain significance

When a variant has been previously observed it is annotated in the prevalence column with the database in which it is included. PhyloP scores vary between -14 and +6 and measure conservation at each individual base, sites predicted to be conserved are assigned a positive score, fast evolving sites are assigned a negative score. Mutationtaster uses a Bayes classifier to predict the effect of a mutation. SIFT damaging prediction score= <0.05. Provean deleterious score = <-2.5. PolyPhen-2 predictions are appraised qualitatively as benign or damaging. The ACMG consensus guidelines, including supporting evidence, are also shown.

Johnson on behalf of the UK GAPP Study Group Haematologica 2016

inherited platelet disorders: diagnostic work-up

why should genetic studies be performed?

Inherited thrombocytopenias: genotyping?

genetic nature of thrombocytopenia

and some implications

MYH9 → prediction of adverse phenotypes (nephropathy, cataract, hearing defect)

RUNX1 → risk of leukaemia

ANKRD26

ETV6 → intrafamilial donor for HSCT

Inherited thrombocytopenias: genotyping?

genetic nature of thrombocytopenia

and some implications

RUNX1
ANKRD26
ETV6
→ risk of leukaemia

watchful waiting

annual CBC & CD34+ circulating cells?

inherited platelet disorders: diagnostic work-up

why should genetic studies be performed?

may also help choose
thrombopoietin mimetics
to increase platelet counts
in settings such as before surgery

inherited platelet disorders: diagnostic work-up

when should genetic studies be performed?

and how?

inherited thrombocytopenias: genotyping?

- after careful phenotyping
- patient's informed consent

inherited platelet disorders: diagnostic work-up

when should genetic studies be performed?

and how?

- targeted sequencing

- whole exome / NGS

INHERITED THROMBOCYTOPENIAS

awareness

clinical features

family history

platelet phenotype (morphology, function)

other blood cells

proper diagnosis in specialized – experienced centres