Platelet inherited disorders

Chris Van Geet, Kathleen Freson
BSH 2014
Hereditary thrombocytopenia: introduction

• Thrombocytopenia: hereditary versus acquired
• Detailed medical and family history
• Detailed pedigree
• Diagnosis
  – symptomatic patients
  – detected by chance: routine automated platelet counting and MPV
• Underlying molecular mechanism
Classification for diagnosis

• Platelet size
  – Normal platelet volume
  – Microthrombocytes
  – Macrothrombocytes

• Mode of inheritance

• Associated features

• Molecular defect
<table>
<thead>
<tr>
<th></th>
<th>X-linked recessive</th>
<th>Autosomal dominant</th>
<th>Autosomal recessive</th>
</tr>
</thead>
</table>
| **Micro-thrombocytopenia**        | Wiskott-Aldrich syndrome  
WASP (Xp11)  
X-linked microthrombocytopenia  
WASP (Xp11) |                    |                     |
| **Macro-thrombocytopenia**        | X-linked macrothrombocytopenia  
GATA1(Xp11) | May-Hegglin anomaly  
MYH9(22q12-13)  
Fechtner syndrome  
MYH9(22q12-13)  
Sebastian platelet syndrome  
MYH9 (22q12-13)  
Epstein syndrome  
MYH9 (22q12-13) | Bernard-Soulier syndrome  
GP Ibα-gene (7p13)  
Or GP Ibβ-gene (22q11)  
Or GP IX-gene (3q21) |                     |
| **Thrombocytopenia with**         | VVD 2B  
VWF-gene  
Platelet-type VWD  
GP Ibα-gene (17p13)  
22q11 microdeletion  
GP Ibβ-deletion  
Montreal platelet syndrome  
Calpain defect?  
Paris-Trousseau thrombocytopenia/Jacobsen syndrome del 1q23-ter | Factor V Quebec  
?  
Familial platelet disorder with predisposition to acute myelogenous leukaemia (Fpd/AML)  
CBFA2(21q22) | Congenital amegakaryocytic thrombocytopenia  
c-Mpl gene (1p34)  
TAR-syndrome  
RBM8A (1q21.1) |
Normal platelets (x14,500)
Autos. dominant thrombocytopenia

*Macrothrombocytopenia*

- **May-Hegglin anomaly:**
  - Macrothrombocytopenia, Döhle-like inclusions in leukocytes

- **Epstein syndrome:**
  - Alport variant (renal disease, deafness) + macrothrombocytopenia
Neutrophils

May-Hegglin

Fechtner
Autos. dominant thrombocytopenia

Macrothrombocytopenia (cont.)

• Fechtner syndrome
  – Epstein syndrome + leucocyte inclusion bodies

• Sebastian platelet syndrome
  – Fechtner syndrome without Alport

mutations in MYH9-gene

224-kD nonmuscle myosin heavy chain IIA

MYH9-RD: 30% ESRF, 16% Cataract, 60% Deafness
MYH9 disorders - genotype/phenotype relation? Non muscle myosin, heavy chain 9
X-linked recessive thrombocytopenia

Microthrombocytopenia (cont.)

• **X-linked microthrombocytopenia (XLT)**
  – No eczema, no infections
  – Platelet count moderately to severely reduced
  – Normal megakaryocytes in bone marrow
  – Other mutations in the same WASP gene
  – Type of mutation determines severity
WASP: involved in cell motility via actin remodeling
X-linked thrombocytopenia

Macrothrombocytopenia

• X-linked macrothrombocytopenia
  – giant platelets and dyserythropoiesis
  – Transcription factor GATA1 mutation
X-linked macrothrombocytopenia / mild dyserythropoiesis without anemia

GATA1 - D218G
GATA1
X-linked transcription factor

Deep macrothrombocytopenia with anemia

Blood, 2001
Hum Genet, 2002
Hum Mol Genet, 2003

GATA-1<-/
D218Y
Shivdasani

Stem Cells, 2001

Diagram showing the differentiation process from Hematopoietic stem cell to MK-producing proplatelets, with key transcription factors and genes involved at different stages.
X-linked transcription factor GATA1

• member of C4-type zinc finger transcription factor family
  (A/T GATA A/G)

• expressed in erythroid and megakaryocytic cells

• two zinc fingers: C-finger for direct DNA binding
  N-finger for indirect DNA binding

• N-finger: interaction with a GATA1 cofactor FOG1
  (Friend Of GATA1)

• FOG1 and GATA1(-/-) mice: embryonic lethality
  (erythroid defect)
control

dysmorphic red blood cells

D218G

macrothrombocytopenia
giant round platelets
cytoplasmic clusters
Platelet formation defects

Proplatelet-forming MK

HSC  MEP  CFU-MK  MB

Transcriptional regulation

TPO signalling

Cytoskeletal organization

Apoptosis

Granulopoiesis

GP receptors

GPCR receptors

Others

Platelet formation

Functional defects

Platelet formation defects

Proplatelet-forming MK

HSC  MEP  CFU-MK  MB

Transcriptional regulation

TPO signalling

Cytoskeletal organization

Apoptosis

Granulopoiesis

GP receptors

GPCR receptors

Others

Platelet formation

Functional defects
**1. TRANSCRIPTION FACTORS**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disorder</th>
<th>Dysfunction</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA1</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>XLinked</td>
</tr>
<tr>
<td>FLI1</td>
<td>Thrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AD (11Q)</td>
</tr>
<tr>
<td>RUNX1</td>
<td>Thrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AD</td>
</tr>
<tr>
<td>GFI1B</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AD</td>
</tr>
</tbody>
</table>

**2. TPO regulation**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disorder</th>
<th>Phenotype</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>cMPL</td>
<td>Thrombocytopenia</td>
<td>NL</td>
<td>AR</td>
</tr>
<tr>
<td>RBM8A</td>
<td>Thrombocytopenia (age)</td>
<td>NL</td>
<td>AR (SNP)</td>
</tr>
<tr>
<td>ANKRD26</td>
<td>(micro)Thrombocytopenia</td>
<td>granules</td>
<td>AD (5’UTR)</td>
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### 3. CYTOSKELETAL ORGANIZATION

<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Dysfunction</th>
<th>Inheritance</th>
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<tbody>
<tr>
<td>MYH9</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AD</td>
</tr>
<tr>
<td>FLNA</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>X-linked</td>
</tr>
<tr>
<td>TUBB1</td>
<td>Macrothrombocytopenia</td>
<td>NL</td>
<td>AD</td>
</tr>
<tr>
<td>WAS</td>
<td>Microthrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>X-linked</td>
</tr>
<tr>
<td>ACTN1</td>
<td>Macrothrombocytopenia</td>
<td>NL</td>
<td>AD</td>
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### 4. APOPTOSIS

<table>
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<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Dysfunction</th>
<th>Inheritance</th>
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</thead>
<tbody>
<tr>
<td>CYCS</td>
<td>Thrombocytopenia</td>
<td>NL</td>
<td>AD</td>
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</table>

### 5. GRANULOPOIESIS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Syndrome</th>
<th>Dysfunction</th>
<th>Inheritance</th>
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<tbody>
<tr>
<td>NBEAL2</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AR</td>
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<tr>
<td>VSP33B</td>
<td>NL</td>
<td>Platelet dysfunction</td>
<td>AR</td>
</tr>
<tr>
<td>VIPAS39</td>
<td>NL</td>
<td>Platelet dysfunction</td>
<td>AR</td>
</tr>
<tr>
<td>HPS(1-10)</td>
<td>NL</td>
<td>Platelet dysfunction</td>
<td>AR</td>
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<tr>
<td>LYST</td>
<td>NL</td>
<td>Platelet dysfunction</td>
<td>AR</td>
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### 6. GLYCOPROTEIN RECEPTORS - SIGNALLING

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Position</th>
<th>Condition</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>GPIIb/IIIa</td>
<td>NL</td>
<td>(AD – MacroTP)</td>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>GPIb/IX/V</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AR</td>
</tr>
<tr>
<td>VWF-2B</td>
<td>Macrothrombocytopenia</td>
<td>Platelet dysfunction</td>
<td>AD</td>
</tr>
<tr>
<td>GP6</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
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### 7. GPCR AND SIGNALLING

<table>
<thead>
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<th>Protein</th>
<th>Position</th>
<th>Condition</th>
<th>Phenotype</th>
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</thead>
<tbody>
<tr>
<td>TBXA2R</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
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<tr>
<td>TBXAS1</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>PLA2G4A</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>P2Y12R</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
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<tr>
<td>GNAS</td>
<td>NL</td>
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<td>Platelet dysfunction</td>
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</table>

### 8. OTHERS

<table>
<thead>
<tr>
<th>Protein</th>
<th>Position</th>
<th>Condition</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAU</td>
<td>NL</td>
<td>(some TP)</td>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>ANO6</td>
<td>NL</td>
<td></td>
<td>Platelet dysfunction</td>
</tr>
</tbody>
</table>
Patients with Inherited Bleeding and Platelet Disorders (BPD)

• Bleeding of unknown etiology

• +/- Defect in platelet function, morphology and/or number

• Inherited
  – Onset at infancy or childhood
  – Familial bleeding disorder

• Exclusion of all acquired causes of platelet dysfunction (renal failure, liver failure)
• Exclusion of primary hemostatic defects of the vascular type (eg Hereditary Hemorrhagic Teleangectasia; Rendu Osler Weber, ‘Classic’ Ehlers Danlos Syndrome but not all !)
Past: diagnosis of BPDs
Platelet testing & Candidate gene screening

Holy grail: Functional Platelet testing
Aggregations Secretion Microcopy (PFA100) FACS

Candidate gene screening
Glanzmann Thrombastenia Bernard Soulier P2Y12 (6) GP6 (2) TXA2R (2)

Genetic Diagnosis Of BPD
BPD cases – UZ Leuven (K Peerlinck/C Van Geet):
Abnormal platelet formation and/or function
associated with/without other clinical phenotypes:
296 BPD WITHOUT clinical diagnosis

1. BPDs with Bleeding or Thrombosis:
   - 29 SPD
   - 59 THROMBOCYTO-PENIA
   - 88 Others

2. BPDs & BONE
   - 26 cases with decreased BMD with/without fractures

3. BPDs & NEURON
   - 37 Mental retardation and/or epilepsy

4. BPD & ENDOCRINE
   - 24 PseudoHypoPara-thyroidism
     MIM603223

5. BPD & IMMUNE
   - 4 Roifman syndrome
     MIM300258

6. BPD & Extra Cellular Matrix
   - BONE MARROW
     - 3 Caffey & 2 EDS(COL1A1 & CHR 15Q15)

7. Vascular:lymphatic disease
   - 6 GORHAM
     MIM123880

ONLY 1/3 ISOLATED thrombopathy
Future: diagnosis of BPDs
Next Generation Sequencing Approaches

Holy grail: Functional Platelet testing
  Aggregations
  Secretion
  Microcopy (PFA100)
  FACS

Candidate gene screening
  Glanzmann Thrombastenia
  Bernard Soulier
  P2Y12 (6)
  GP6 (2)
  TXA2R (2)

RNA Exome (Whole genome) sequencing
  NBEAL2
  RBM8A
  ACTN1
  GFI1B
NGS identified RBM8A as cause for Thrombocytopenia with Absent Radius syndrome

Compounding inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome

Cornelis A Albers1,3,17, Dirk S Paul3,17, Harald Schulze4,5,17, Kathleen Freson6, Jonathan C Stephens1,2, Peter A Smethurst1,2, Jennifer D Jolley1,2, Ana Cvejic1,3, Myrto Kostadima7, Paul Bertone7, Martijn H Breuning8, Najet Debili9, Panos Deloukas3, Rémi Favier9, Janine Fiedler5,10, Catherine M Hobbs1,2, Ni Huang3, Matthew E Hurles3, Graham Kiddle1,2, Ingrid Krapels11, Paquita Nurden12, Claudia A L Ruivenkamp8, Jennifer G Sambrook1,2, Kenneth Smith13,14, Derek L Stemple3, Gabriele Strauss15, Chantal Thys6, Chris van Geet6,16, Ruth Newbury-Ecob13,14,18, Willem H Ouwehand1,3,18 & Cedric Ghevaert1,2,18

- Hypomegakaryocytic thrombocytopenia (< 50 x10³)
  severe bleedings early in life
- Bilateral absence of the radius +/- shortening/aplasia of ulna and/or humerus (phocomelia)
- Dislocation of the hips, subluxation of knees
- Cardiac abnormalities, Cow milk allergy, Leukemia
1q21 microdeletion (incl RBM8A)

&

5’UTR or intronic SNP
Y14 part of Exon Junction Complex

mRNA uptake
non-sense mediated RNA decay

Link with MK and/or PLT formation completely unknown

Candidate gene screening would have never identified this gene
For TAR syndrome
What to do with 296 BPD WITHOUT clinical diagnosis?

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Genetic heterogeneity
Development in diagnosis of BPDs

Next Generation Sequencing Approaches

Holy grail: Functional Platelet testing
Aggregations
Secretion
Microcopy (PFA100)
FACS

Thrombogenomics
NGS diagnostic platform with 94 GENES (platelets/coagulation)

Candidate gene screening
Glanzmann Thrombasthenia
Bernard Soulier
P2Y12 (6)
GP6 (2)
TXA2R (2)

BRIDGE-BPD
genome wide exome/genome sequencing
ThromboGenomics

Streamlining the genetic diagnosis of patients with inherited bleeding and thrombotic disorders

https://haemgen.haem.cam.ac.uk
/thrombogenomics

94 genes

54 Platelet Genes

50 Coagulation Genes
Exome sequencing for rare disorders consortium: BRIDGE-BPD study
Grey Platelet Syndrome
NBEAL2
Bleeding Disorder with no alpha Granules
*Albers et al, Nat Genet 2011*

TAR Syndrome
RBM8A
Thrombocytopenia with Skeletal Abnormalities
*Albers et al, Nat Genet 2012*

Lethal neurodegeneration syndrome
TSP family gene
Platelet disorder with defective dense granules
*To be submitted*

EDS-like syndrome
CHST14
Severe Bleeding Disorder with defective alpha granules
*To be submitted*
Rare inherited Bleeding and Platelet disorders: EDS-like disease

1. IPDs with Bleeding or Thrombosis:
   - 29 SPD
   - 59 THROMBOCYTOSIS and PLT dysmorphosis
   - 88 Others

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   - 26 cases with decreased BMD with/without fractures

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   - BONE
   - MARROW
   - 3 Caffey & 2 EDS(COL1A1 & CHR 15Q15)

7. Vascular:lymphatic disease
   - 6 GORHAM
   - MIM123880

Platelet formation defect
THROMBOCYTOSIS and PLT dysmorphosis

Genetic heterogeneity
BRIDGE-BPD gene: Ehlers Danlos-like syndrome (EDS)
Autosomal recessive mutations in CHST14

Severe bleeding problems
Mild thrombocytosis
450 x 10^9/L
volume 8fL
EM aberrant alpha granules
Abnormal CD62P FACS
Abnormal TRAP aggregation

Skin hyperlaxity
Clubfoot
Hydrocephaly
Dandy Walker malformation
Arthrogryposis

Scalp hematoma
Reduced TSP1 levels in platelets
Abnormal wound healing
Co-culture: CHST14 regulates structuring of collagen I (fibroblasts) 

Hypermegakaryopoiesis with shedding of vWF+ alpha granules

Co-culture: Fibroblasts control + MK control

Control MKs - vWF

Co-culture: Fibroblasts patient + MK patient

Patient MKs - vWF
Conclusion

Clinical and good clinical phenotyping (standard)
Bleeding and Platelet disorders

Thrombogenomics
NGS diagnostic platform with 94 GENES (platelets/coagulation)

BRIDGE-BPD genome wide exome/genome sequencing

Holy grail: Functional Platelet testing
Aggregations
Secretion
Microscopy (PFA100) FACS

OMICS platforms
- Transcriptomics
- Proteomics
- Epigenomics
- Functional genetics
  - KO mice, Zebrafish
  - CD34 HSC (IPS)/MK cultures
UNIVERSITY of LEUVEN, BELGIUM
Center for Molecular and Vascular Biology

Benedetta Izzi
Chantal Thys
Anouck Wijgaerts
Sophie Louwette
Anne Rochtus
Christine Wittevrongel
Michela Di Michele
Kathleen Freson

Department of Pediatric
Department Vascular Biology
UZ Leuven

Chris van Geet
Kathelijne Peerlinck