Unexpected genetic architectures underlying rare blood diseases: Lessons learned from the BRIDGE project

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Expanding phenotype & (epi)genotype of rare inherited platelet defects

Why platelets?
Platelets are the cells that make the blood clot and underlie the Number 1 killer with a lethal heart attack every 8 seconds in Europe.

- Cardiovascular disease causes over 4 million deaths in Europe.
- 40% of all deaths in Europe are cardiovascular related.
- Cardiovascular diseases are the main cause of death in women in Europe.
The BRIDGE Platelet project builds on the strategic investment by Wellcome Trust, National Institute for Health Research and Medical Research Council in genomics.
Data sharing: 1000 patients with platelet disorders will be enrolled. Clinical data from all KU Leuven patients are coded in the central BRIDGE database.

Abnormal platelet formation and/or function (IPD) associated with/without other phenotypes: 276 cases

1. IPDs with Bleeding or Thrombosis:
   - 29 Storage Pool Disease
   - 59 Thrombocytopenia
   - 88 Others

2. IPDs & Bone pathology:
   - 26 patients with decreased bone mineral density with/without fractures

3. IPDs & Neuropathology:
   - 23 Autism
   - 37 Mental retardation and/or epilepsy (CHR 6q22 and RAB27B)

4. IPD & Endocrinopathy
   - 4 Pseudo-Hypoparathyroidism Ib MIM603223

5. IPD & Immune Deficiency
   - 4 Roifman syndrome MIM300258

6. IPD & Extra Cellular Matrix
   - 3 Caffey & 2 Ehlers Danlos-like syndrome (COL1A1 & CHR 15q15) MIM114000 & 601776

7. Vascular lymphatic disease
   - 6 Gorham’s disease MIM123880

178 bleeding/thrombosis (KU Leuven) Increased to 1000 samples from 34 centres from Europe & USA.
Sequencing of the exomes of 10 cases resolved the genetic basis of two rare bleeding disorders known since the Seventies.

100% heritability because of mutation from mum and dad with a large effect on clinical phenotype.

Grey Platelet Syndrome
A bleeding disorder with no $\alpha$ granules

TAR
Too a low number of platelets with skeletal abnormalities
The genetic architecture of Grey Platelet Syndrome was classic “autosomal recessive mutations” altering the function of a novel protein NBEAL2
The discovery for the TAR syndrome was less simple. Discovery was possible because of the functional annotation of the DNA of the cell that makes platelets.

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

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Data sharing via open databases is essential because it showed that a microdeletion is obligatory but not sufficient for TAR.
The functional annotation of the epigenomes of 40 different blood cells types to IHEC standard allows for the discovery of regulatory variants that are causative of rare diseases.
Two rare sequence variants at base pairs that do not code for protein but that regulate the transcription of the *RBM8A* gene are causative of TAR.

The variant that causes TAR is present in 20 people in the audience but it only causes TAR if combined with an extremely rare deletion at the same location.

*Gene has 6 exons*

*Gene is transcribed in the platelet precursor*

“Blue and black hump” signatures indicate a regulatory element and that the area of genome is “open for business”.

Computer predicted binding sites of transcription factors
A protein that regulate gene transcription

named EVI1 binds to DNA if there is an A base pair but not if there is a G base pair

The presence of the A base pair leads to less effective transcription of the RBM8A gene
RBM8A encodes for the protein Y14 and platelets from TAR children have less of the Y14 protein

Albers et al., Nature Genetics, 2012: 44, 435–439
More EpigenOMICS and rare disease
testing for DNA methylation of the GNAS gene

Pronounced brachydactyly (X rays), short stature (not SGA, GH therapy), and platelet Gsalpha hypofunction $N=17$ (*mild AHO – Albright’s Hereditary Dystrophy*)
The health and ill-health of platelets can be tested by simple and reliable laboratory tests thus linking genotype to phenotype.

Heterozygous loss of function mutations in Gsalpha AHO + platelet Gs hypofunction
Beyond rare platelet diseases

three of the five drugs given after a heart attack are used to inhibit platelets

1.2M death of stroke per year in Europe

- **Unmet Need**
  - Anti-platelet drugs used in heart attacks do not work for the treatment of thrombotic stroke

- **Blockbusters and Rare Disease Research**
  - Three of the “blockbuster” drugs that have led to the dramatic reduction of death by heart attacks were developed on basis of discoveries in the rare disease field

- **BRIDG-ing Rare Diseases with Common Ones**
  - BRIDGE will improve the diagnosis and treatment of rare platelet disorders
  - BRIDGE may also contribute to the development of better and safer treatments for stroke and heart attacks

- Platelet inhibitors
- Lipid and blood pressure lowering
IRDiRC and BRIDGE

- UK-led BRIDGE studies will contribute at least 20,000 sequenced cases to IRDiRC by 2016

- Regulatory mechanisms may underlie a large fraction of unresolved Rare Diseases

- Therefore exploring the epigenomic space will become an integral part of Rare Disease gene discovery