This is Not Your Daddy’s Hematology

How Next Generation Sequencing is Changing the Diagnosis, Understanding and Treatment of Rare Hematologic Disorders
“Appreciation of DNA as a complex and dynamic molecular anthology is essential for the study of inherited and acquired biological processes.”

~ J. Johnsen et al.
“Please let Tony Blau know the hematology topic outside of your field of study that you would like to present...”

~ Scott O’Neill
**COMMENTARY**

They’re not your daddy’s inherited platelet disorders anymore

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**IN FOCUS**

GFI1B mutation causes a bleeding disorder with abnormal platelet function

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Overview / Objectives

- Give a very basic review/overview of NGS technology
- Review two papers that demonstrate how Next Generation Sequencing (NGS) is revolutionizing hematology
  - Rare Inherited Platelet Disorders and the GFI1B mutation
- Discuss how NGS may impact:
  - How we define disease
  - Diagnosis
  - Treatment
- Discuss obstacles to using this technology in the study of rare (and not-so-rare) hematologic and other diseases
Next Generation Sequencing

1953: MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

J. D. WATSON
F. H. C. CRICK

1977: Sanger sequencing technique developed

1990-2003: Sequencing of the Human Genome

2000: Massively Parallel/Next Gen Sequencing
Next Generation Sequencing

Johnsen et al. Blood 122 (19) :3268-3275
NGS & Identification of Variants
Approaches to Studying Inherited Genetic Disorders

- Inherited Disorders
  - NGS may be combined with pedigrees to hone in on causative alleles
  - Which individuals should be tested are dictated by inheritance pattern

Boycott et al. Nat Rev Gen 14, 681-691
Four generations

Symptoms
- Excessive bruising
- Prolonged bleeding from superficial injuries
- Bleeding after minor & major surgery
- Responsive to platelet transfusion
- Autosomal Dominant
Phenotype

- All affected family members
  - Moderate thrombocytopenia
  - Abnormal smears
  - Increased platelet volume
  - Increased RDW and abnormal RBCs
  - Prolonged bleeding\(^1\) and PFA closure times
  - Variable aggregation responses

Genotype

0 16 family members
0 SNP array
0 Haplotype of a single region on chromosome 9
   -- LOD score 4.51
   -- 367 genes
Targeted Candidate Region Sequencing

0 Pooled DNA of 6 affected and 6 unaffected family members

0 Variant calling → 28,566 variants

0 Filtering of non-coding, synonymous and common variants (>0.1% of the population) → 34 variants

0 Ranking → Single nucleotide insertion in exon 7: c.880-881insC
   0 Frameshift mutation
   0 Present in 34% of reads within the affected pool
   0 0 of the reads within the unaffected pool

0 All affected family members heterozygous for the mutation
**GFI1B Mutation**

- Cloning of cDNA from affected platelets and CD34+ progenitors of 1 family member
GFI1B Function

GFI1B and Transcriptional Regulation
GFI1B and Protein Expression

**A**
- P-selectin (CD62)
- Fibrinogen
- GPIIla (CD61)
- GPIIbα (CD42b)
- CD63

**B**
- GPIIla (CD61) Mean Fluorescence Intensity
- P-selectin (CD62) Mean Fluorescence Intensity

**C**
- GFI1b WT
- GFI1b mutant
GF11B and Platelet Structure
Summary

0 Previously undescribed, AUTOSOMAL DOMINANT inherited platelet disorder (?)

0 Identification of a mutation (never before linked to human disease) that may lead to abnormal hematopoiesis and platelet function
Gray Platelet Syndrome: Inherited platelet disorder resulting from poor production of alpha granules

Phenotype: low numbers of large, “gray” platelets, with variable bleeding tendency

Historically, autosomal recessive inheritance

Recent studies using NGS techniques identified missense mutations in the NBEAL2 gene to be associated with AR disease\textsuperscript{1,2,3}
Autosomal Dominant GPS
Identification of *GFI1B* Mutation

- Chromosome 9q34 identified through linkage analysis in 14 family members
- *GFI1B* targeted due to previously demonstrated role in megakaryopoiesis
- Nonsense mutation detected in exon 6 → early introduction of stop codon → *GFI1B*\textsuperscript{Trun}
- Complete segregation with affected family members
Functional Abnormalities

- Dysfunctional regulation (repression) of gene expression @ Gfi1 promoter site
- Inhibition of function of non-mutant GFI1B when co-expressed
Alterations in Structure

Affected Family Member

Mouse Bone Marrow
*GFI1B* and Protein Expression

- Decreased level of glycoprotein 1Bα-CD42B on platelets and megakaryocytes
- Strong expression of CD-34 detected on platelets and megakaryocytes from all affected family members
- Myeloid and erythroid lineages unaffected
Summary

- *GFI1B* mutation identified as in autosomal dominant Grey Platelet Syndrome

- Mutated *GFI1B* affects the development/maturation of megakaryocytes and platelets in a dominant negative fashion

- Altered expression of certain platelet surface proteins may be closely related to clinical features
  - Decreased platelet adhesion through 1Bα-CD42B
  - Large platelet size (GP1Bα/CD42B mutn in Bernard-Soulier)

- Future efforts to identify GFI1B target genes disrupted by GFI1BTr expression may open doors to understanding megakaryocyte and platelet biology.
Defining Disease in the NGS Era

0 Variant/mutation?
   0 Article 1: Frameshift mutation (ins C) in Exon 7
   0 Article 2: Nonsense mutation (C->T) in Exon 6

0 Abnormal protein?
   0 Normal: CQVCGKAFSQSSNLIITHSRKH
   0 Article 1: CQVCGKAFSQSSNLITPQPQA
   0 Article 2: CQVCGKAFS

0 Gene/Mechanism/Pathway?
   0 Both articles: Inability of GFI1B to repress transcription at multiple gene targets

0 Phenotype?

  Article 1:  

  Article 2:  

Defining Disease in the NGS Era

0 1 Phenotype, Many Genes: Fanconi Anemia
  0 Affected genes: > 15 genes, each with multiple mutations

0 1 Gene, Many Mutations, Same Phenotype: Hemophilia A
  0 > 2100 mutations discovered in FVIII gene
  0 Differential risk of inhibitor development?

0 1 Gene, Many Phenotypes: MPL
  0 Type 1: Congenital Amegakaryocytic Thrombocytopenia
  0 Type 2: Hereditary Thrombocytopenia
  0 Type 3: MPNs, RARS-t and AML

He et al. J of Hematology & Oncology 2013, 6:11
Clinical Utility of NGS

- NGS is emerging as a rapid and specific diagnostic tool
  - Characterization of previously undefined syndromes
  - Exclusion of diagnoses
    - Hermansky-Pudlak syndrome
    - Wiskott Aldrich syndrome

- Can it provide us ideas for how to treat rare diseases?
  - Improved characterization of rare phenotypes may reveal opportunities for treatment
    - X-linked hypoproliferative syndrome Type 2
  - Replacement or inhibition of relevant proteins
  - Targeting of affected pathways downstream
NGS Limitations

- Technical and analytical limitations
  - Improvement in sequencing and mapping
  - Standardized approaches to annotation of variants
  - Documenting functional impact
  - Development of strategies for determining disease risk

- Logistical
  - Poor funding for the study of rare diseases
NGS Limitations

- Ethical
  - Incidental findings of clinically important mutations
  - Sharing of information with test subjects
  - Counseling subjects regarding clinical & therapeutic significance

- Privacy
  - Identifying data in publically available databases
Conclusion

- Next generation sequencing will change the way we diagnose, understand and treat rare hematologic disorders.
- The use of Mendelian genetics and NGS will bring to light many new rare diseases and redefine many others.
- As the field progresses and technical limitations are reduced, increased focus should be placed on logistical and ethical hurdles.