Personalized Medicine:
Genetic Testing and the Implications for Future Therapies

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Molecular Geneticist, PreventionGenetics

· Founded in 2004 in Marshfield Wisconsin by Dr. Jim Weber
· Comprehensive source for clinical germline genetic testing
· Largest test menu options in the U.S. offering testing for nearly all clinically relevant genes
· Only test samples from physicians, genetic counselors, or other healthcare professionals; we do not offer direct-to-consumer testing
· Offer DNA banking services
· Have tested many patients for different bone marrow failure disorders

BONE MARROW FAILURE DISORDERS

OBJECTIVES
- Introduce Genetic Testing and define bone marrow failure
- Genetics 101 – brief introduction to DNA, Chromosomes, Genes, and Types of Mutations
- Define the types of mutations found in bone marrow failure disorders
- Discuss the clinical utility of genetic testing and how genetic testing applies to personalized care for bone marrow failure disorders
- Discuss the process of genetic testing and the caveats and challenges of genetic testing

GENETIC TESTING IS USED FOR MANY DISORDERS

TYPES OF GENETIC TESTING

Clinical Genetic Testing
- Diagnostic Testing
- Carrier Testing
- Prenatal Screening
- Preimplantation Testing
- Newborn Screening
- Predictive/Presymptomatic Testing

Other DNA Analyses
- Paternity
- Forensic
- Ancestry

BONE MARROW FAILURE DISORDERS

Questions we will focus on answering:

How are genes associated with bone marrow failure?

How does analyzing our genes help with diagnoses and treatment of bone marrow failure syndromes?
UTILITY OF GENETIC TESTING

- End the diagnostic odyssey
  - Average time to diagnosis for many rare disorders is 7 years
- Influence treatment and management of disease
- Prevent harm from genetic conditions and improper treatment
- Personalize medicine
  - Specific drugs and therapies may be available based on genetic findings

TESTING FOR BONE MARROW FAILURE (BMF)

- Many different types of tests used for diagnosing BMF
  - Flow cytometry – immunophenotypic analysis
  - Morphology – visual analysis of blood cells / bone marrow
  - Biochemical – study amount or activity level of proteins
  - Cytogenetics – Chromosomal Analysis
  - Genetics – really just one tool available for diagnosis

WHAT IS THE FUNCTION OF BONE MARROW?

Hematopoiesis

Formation and renewal of blood cells from a single hematopoietic stem cell.

3 main blood cells
1. Red Blood Cells
2. Platelets (thrombocytes)
3. White Blood Cells

Key: Healthy bone marrow makes healthy blood cells

Aplastic Anemia (AA) – failure to produce progenitor and mature blood cells
Paroxysmal Nocturnal Hemoglobinuria (PNH) – destruction of RBC
Myelodysplastic Syndrome (MDS) – production of ineffective progenitor cells
Myeloproliferative Neoplasms (MPN)
Acute Myeloid Leukemia (AML) – over production of progenitor cells

So how are genes related to bone marrow failure?
GENES ENCODE PROTEINS; PROTEINS MAKE OUR BODIES WORK

Key: When proteins do not work properly, cells do not work properly.

DNA STRUCTURE

Genes are packaged in Chromosomes
- 22 pairs autosomes
- Except sperm and egg cells, all cells in our bodies have 23 pairs = 46 total
- 1 pair determines the individual’s sex
- Many individual genes on each chromosome
- Get 1 chromosome from each pair from each parent

DNA STRUCTURE

- ~ 3 Billion base pairs
- DNA sequence in any two people is ~ 99.9% identical and only 0.1% unique

DNA STRUCTURE

- Section of DNA on a chromosome that have specific instructions
- Humans have about 21,000 genes
- ~2,500–3,000 are currently associated with human disease
- We have two copies of every gene – one from mother one from father

DNA IS A RECIPE BOOK FOR OUR BODIES

All DNA in our Cells
- Chromosomes
- Genes
- Nucleotides

Book
- Chapters
- Sentences
- Letters

TYPES OF MUTATIONS
TYPES OF MUTATIONS - CHROMOSOMES

- 5q minus syndrome
  - deletion of part of chromosome 5
  - associated with acquired MDS

**Quick Summary**

How are genes associated with bone marrow failure?
- Genes are segments of DNA that encode proteins our bodies need.
- Mutations in DNA may cause “bad” proteins to be made.
- Bad proteins in bone marrow cells may cause bone marrow failure.
- Mutations in DNA can occur on a large scale – chromosomes, or on a small scale – nucleotides.
- Mutations can be inherited from our parents or mutations can be acquired sporadically.

How does analyzing our genes help with diagnoses and treatment of bone marrow failure syndromes?

**Types of Mutations – Nucleotides**

**Origins of Mutations – Hereditary vs. Acquired**

Hereditary mutations are inherited from a parent and are present throughout a person’s life in virtually every cell in the body.

Acquired (sporadic or somatic) mutations occur at some time during a person’s life and are present only in certain cells, not in every cell in the body.

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APLASTIC ANEMIA (AA)

- Acquired – events lead to immune response attacking blood progenitor cells, the cause in most cases is unknown
- Hereditary – disease is passed from parents to children and usually diagnosed early in childhood. In many cases, other symptoms precede AA.

4 Diseases Commonly Associated With Hereditary AA
- Fanconi anemia (FA)
- Shwachman – Diamond syndromes (SDS)
- Dyskeratosis congenita (DKC)
- Diamond – Blackfan anemia (DBA)

Diseases Commonly Associated With Hereditary AA
- Fanconi anemia (FA) – disease of DNA repair
- Shwachman – Diamond syndrome (SDS) – disease of telomeres, chromosome maintenance
- Dyskeratosis congenita (DKC)
- Diamond – Blackfan anemia (DBA) – disease of ribosomes, “reading” the DNA book

Key: All have distinct clinical characteristics and all conditions are associated with AA and predisposition for MDS / AML

APLASTIC ANEMIA

Fanconi anemia (FA) – is an inherited anemia associated with bone marrow failure
- FA is characterized by a range of physical abnormalities, pancytopenia, and predisposition to acute myelogenous leukemia (AML), and solid tumors of the gynecologic and GI tract and of the head and neck (Auerbach 2009).
- FA patients are up to 800 fold more susceptible to AML than the general population with a median age of onset of 13 years (Rosenberg et al. 2003).

FA - Importance of Early Diagnosis
- Prevent inappropriate management of hematologic disease (cytopenias, aplastic anemia, leukemia)
- Permits consideration of Bone Marrow Transplant (BMT), androgens, hematopoietic growth factors, and surgical intervention for solid tumors
- BMT can cure the blood phenotype, but patients are still at risk for developing solid tumors
- Identify carriers for surveillance, donor identification, and family planning purposes

FA - 21 Genes Associated with FA account for >95% of FA cases

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Fanconi anemia chromosomal breakage test
Lisa Moreau, Dana Farber Cancer Institute, Boston, MA.
APLASTIC ANEMIA

FA - 21 Genes Associated with FA account for >95% of FA cases
- FANCA, FANCC, and FANCG account for ~ 86% of FA cases

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* Mutation in FANCA is associated with Fanconi anemia-like syndrome (OMIM 612390)
* FANCC mutations in GNAS1 cases are associated with retinoblastoma (OMIM 277720) and WEE syndrome (OMIM 607233)

http://www2.rockefeller.edu/fanconi/

CASE STUDY #1 – FA

- Patient: 2 month old with atresia of esophagus and duodenum, renal anomaly, polydactyly
- Positive chromosomal breakage (+DEB)

- Ordered Sequencing of FANCA, FANCC, FANCG

CASE STUDY #1 – FA

- Found 2 mutations in the FANCA gene

Mutation 1: Deletion of 4 nucleotides
AGTTCAGTTCCTCATGTTCAGATTGTTCTCAGAGG
AGTT

Mutation 2: Missing a large part of Chromosome 16 and FANCA
The piece that is missing contained part of the FANCA gene

CASE STUDY #1 – FA, Importance of early diagnosis

- Carrier testing showed mother carried the large deletion, father carried the small deletion.
- Patient has a healthy brother who can be tested to see if a viable donor for BMT
- Patient’s aunt (mother’s sister) is pregnant
- Carrier testing of aunt reveals she is a carrier for the large deletion as well, therefore fetus is a potential carrier of a FANCA mutation
- Could test aunt’s partner for FANCA carrier status

UTILITY OF TESTING FOR GENETIC CAUSES OF FA

- Confirmation patient has FA and not acquired AA due to autoimmune response, so would not necessarily treat this FA patient with immunosuppressive Therapy
- Large patient has very significant risk of early onset MDS / AML, and development of solid tumors
- FA is a DNA repair disease, so standard transplant conditioning regimens may be highly toxic for this patient and patient may require reduced intensity conditioning
- Inform donor choice
- Family counseling – both the patient’s nuclear family and extended family

Same process can be applied to the other hereditary forms of AA
- Shwachman – Diamond syndromes (SDS) – SBDS
- Dyskeratosis congenita (DKC) > 11 genes
- Diamond – Blackfan anemia (DBA) > 18 genes
- Severe congenital neutropenia (SCN) > 24 genes
- Congenital amegakaryocytic thrombocytopenia (CAMT) - MPL
- GATA2 gene deficiency

Key: Hereditary BMF disorders often have distinct clinical features and we know many of the genes associated with many of these disorders and can tailor treatment
APLASTIC ANEMIA

Acquired – in many cases the cause of AA is unknown, but recently found that ~30% of patients with acquired AA have somatic mutations in myeloid cancer genes
• Mutations are clustered in many of the same genes in different patients with acquired AA
• Only a subset of cells have the mutations initially, and this population grows over time – Clonal Hematopoiesis

Yoshizato et al. NEJM 2015

REMEMBER: BM is insufficient Hematopoiesis

Mutations in the progenitor cells...

...result in insufficient mature cells.

Yoshizato et al. NEJM 2015

APLASTIC ANEMIA

Model of Disease Progression

APLASTIC ANEMIA

Model of Disease Progression

APLASTIC ANEMIA

Model of Disease Progression

M-YELODYSPLASTIC SYNDROME (MDS)

Myelodysplastic Syndrome – production of ineffective progenitor hematopoietic cells

• Both Acquired and Hereditary forms
  • Hematopoietic stem cell clonal disorder with morphologic dysplasia and peripheral cytopenias. Mostly sporadic and late onset, but more occurrences of familial MDS are being identified.

• Early onset MDS is strongly associated with cytotoxic exposures, hereditary bone marrow failure syndromes (e.g. FA, SDS, DBA, DKC) and other non-syndromic familial MDS/AML syndromes.

MDS

Hereditary MDS

Like AA, there are specific genes and disorders associated with MDS

Clinical presentation and genetics can help determine prognosis and care

Yoshizato et al. NEJM 2015

Yoshizato et al. NEJM 2015

MDS

Hereditary MDS

Like AA, there are specific genes and disorders associated with MDS

Clinical presentation and genetics can help determine prognosis and care

Bannon and DiNardo, 2016

Bannon and DiNardo, 2016
CASE STUDY #2 – MDS

- Patient: 19 year old with Thrombocytopenia and Neutropenia
- Ordered sequencing for 12 genes associated with thrombocytopenia

Mutation in the RUNX1 gene predicted to alter splicing

TTTCAAGTACTGGCCCG

TTTCAAG TACTGGCCCG

UTILITY OF TESTING FOR GENETIC CAUSES OF MDS

- Confirmed patient has a mutation in the RUNX1 gene which is associated with familial thrombocytopenia and predisposition to MDS / AML.
- The exact mutation found in our patient was reported in other patients with thrombocytopenia and a family history of progression to MDS / AML.
- Treating patient for thrombocytopenia and neutropenia may not be sufficient for this patient; have to consider their increased risk of MDS / AML.
- Sample tested was blood, RUNX1 mutations are found in both hereditary and acquired MDS – consider testing another sample type like skin fibroblasts.
- What is patient’s family history? And should other family members be tested?

REMEMBER: BMF is insufficient hematopoiesis

Mutations in the progenitor cells...

...result in insufficient mature cells.

MDS

Model of Disease Progression – similar as AA, but typically considered more aggressive

- Germline RUNX1 mutation
- Somatic mutations driving clonal expansion
- MDS/AML initiating mutations

Age

clonal expansion

Key: MDS progresses in a similar manner as AA, but different combinations of mutations arise over time.
MDS
Chromosomal analysis also plays a key role in diagnosis of MDS:
- 5q, +8, -7, -Y, del(20q)

http://dna-explained.com/2012/09/27/x-marks-the-spot/

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)
- Phagocytosis of red blood cells
- Acquired – clonal disease in which the PIGA gene is mutated within hematopoietic progenitor cells resulting in loss of proteins that protect red blood cells from complement-mediated destruction
- PIGA gene is located on the X - chromosome
- Males – XY
- Females – XX (one inactivated)

Karyotype

How does analyzing our genes help with diagnoses and treatment of bone marrow failure syndromes?
- Determination of acquired or hereditary disease
- Informs treatment options
- Considerations for HSCT
  - Timing of transplant
  - Choice of conditioning regimen
  - Donor selection
  - Unique transplant-related complications
- Prognosis
  - Tumor surveillance – hematologic and other solid tumors
  - Pre-implantation, prenatal genetics
  - Genetic counseling of patient and family – knowledge of conditions and options
  - Establish patient cohorts for clinical trials

GENETIC TESTING APPROACH FOR BMF SYNDROMES
A recent study on inherited BMF syndromes analyzed 72 genes in 158 patients

GENETIC TESTING PROCESS – ADVANCED TECHNOLOGY
- Extract DNA
- Massively Parallel Gene Sequencing
- Analyze Genes
- Find Mutations
- Deliver Report
- Follow up with Physician
- Benign
- Likely Benign
- Uncertain
- Likely Pathogenic
- Pathogenic

CHARACTERIZE MUTATIONS
GENETIC TESTING PROCESS – ADVANCED TECHNOLOGY

- Extract DNA
- Analyze Chromosomes
- Find Mutations
- Characterize Mutations
- Deliver Report
- Follow up

- Benign
- Likely Benign
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- Pathogenic

CONSIDERATIONS FOR GENETIC TESTING

- Other clinical symptoms
- Personal / family history
- Onset of disease
- Tissue being tested – will it reveal inherited and/or acquired mutations
- Gene panel or specific gene?
- How will results affect patients and their families?

CHALLENGES OF GENETIC TESTING FOR BMF SYNDROMES

- Incomplete knowledge/variants of unknown significance
- Benign variants misclassified as pathogenic
- True pathogenic variants that have not been previously described
- Mutation outside of NGS capture area
- Mutation in a gene not known to be linked to a disease
- Mutation in a known gene linked to the disease, but outside of the region of NGS capture
- Mutation in a distant regulatory region
- Technical limitations
  - Inadequate sequencing depth over the pathogenic region
  - Failure to detect large insertions or deletions
- Wrong tissue tested
  - False-negative in peripheral blood of a patient or sibling with reversion mosaicism (e.g. FA)
- Penetrance and expressivity
  - Disease may manifest differently or not at all in family members with a common mutation

SUMMARY

- Genetic abnormalities in hematopoietic stem cells can cause bone marrow failure syndromes.
- Genetic testing of chromosomes and genes is available for patients suspected of having a bone marrow failure syndrome and can help establish a diagnosis and treatment options.
- Results from genetic testing can lead to a faster diagnosis, and inform personalized care.
- Our knowledge of the genetic components of disease is expanding at a rapid pace with new, relevant discoveries being reported daily.

ONLINE RESOURCES

- AA-MDS
  - https://www.mds.org/
- Genetics Home Reference
- Fanconi Anemia Research Fund
  - https://www.fanconi.org/
- OMIM
- Global Genes
  - https://globalgenes.org/
- Prevention Genetics
  - https://www.preventiongenetics.com/
- Be The Match
  - https://beathematch.org/