Part One:
Genetic Testing for Inherited Marrow Failure

Bone marrow failure syndromes:
How can genetic testing help to distinguish and guide therapy?

What This Talk Will Cover
- Genetic testing for inherited marrow failure
  - General background: What are gene mutations?
  - Risk and severity: Do all people with inherited mutations develop disease?
  - How does medical genetic testing differ from commercially available testing?
- Cytogenetic testing in Bone Marrow Failure
- Genomic profiling of bone marrow cells in MDS and AA for acquired mutations
  Implications for diagnosis, prognosis and therapies
- Benefits and Limitations of Genetic Testing and Genomic Profiling

Living with Aplastic Anemia, MDS or PNH
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Katherine R. Calvo, M.D., Ph.D.
Department of Laboratory Medicine, Clinical Center
National Institutes of Health
Bethesda, Maryland

Personalized Medicine:
Genetic Testing and the Implications for Future Therapies

Genetic testing for inherited marrow failure
- General background: What are gene mutations?
- Risk and severity: Do all people with inherited mutations develop disease?
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  Implications for diagnosis, prognosis and therapies
- Benefits and Limitations of Genetic Testing and Genomic Profiling

What This Talk Will Cover

Bone marrow failure syndromes:
Autoimmune Disease:
MS, IBD, uveitis, DM type 1, etc.
Acquired AA

AA/PNH
PNH
LGL
AA
TELOMERE
GATA2
FA
MDS
AML

Acquired AA

Bone marrow failure syndromes:
How can genetic testing help to distinguish and guide therapy?
GENETIC TESTING – Background
The Basics: GENES are made of DNA

- Each person has a unique “Genome” made up of DNA that forms individual genes
- Humans have around 30,000 genes
- Genes encode the directions for making:
  - Proteins and everything that is you
  - Hair color/texture, eye color, skin color
  - All the cells in the blood, bone marrow, and every organ of the body

What is a mutation?

GENETIC TESTING – Background
The Basics: DNA is made up of Nucleotides (building blocks)

- DNA is made up of four types of building blocks (called nucleotides)
- These spell out the genetic “code” used in our genes
- We all have normal “variations” in our genetic code that make each one of us different or unique

GENETIC TESTING – Background
WHAT IS A MUTATION?

- A mutation is a change in the genetic code that can increase your risk of getting:
  - Disease
  - A major change in whatever the gene is responsible for
- Mutations usually occur in conserved regions of genome
WHAT ARE CHROMOSOMES?

- In the nucleus of cells DNA is tightly wound and forms Chromosomes
- Telomeres = protective caps at the ends of each chromosome

We normally have 23 pairs of chromosomes.
Each chromosome contains thousands of genes.
Chromosomes are numbered according to size.
Sex Chromosomes:
- XX female
- XY male

HOW DO WE GET MUTATIONS?

Inherited (Germline) Mutations

- Inherited mutations are present at birth and are in all of the cells of the body

Recessive mutations – Need two copies of the mutation (one from mom and one from dad) for disease

Dominant mutations – Only need one copy of the mutation (from mom or dad) for disease
GENETIC TESTING
Examines your DNA to look for mutations in the genetic code

“Next Generation Sequencing”

⇒ Panel of genes related to hereditary marrow failure

⇒ Usually testing is only needed once

⇒ Samples sent for testing can be: Saliva, Buccal Swab, Blood, Bone Marrow, Skin biopsy, or Hair follicle

How does medical genetic testing ordered by your doctor for bone marrow failure differ from commercially available DNA testing?

Ancestry DNA testing

Similar:
⇒ DNA sequencing techniques

Different:
⇒ The genes tested

Nuclear or mitochondrial DNA genes that vary based on:
• patterns of human migration
• ethnic populations

Examples:
National Geographic Genographic Project
Ancestry.com
23andMe

Commercially Available DNA testing – Genetic Traits

23andMe.com
Asparagus Odor Detection
Back Hair (available for men only)
Bald Spot (available for men only)
Bitter Taste Perception
Cheek Dimples
Cleft Chin
Earlobe Type
Earwax Type
Eye Color
Finger Length Ratio
Freckles
Hair Curliness
Light or Dark Hair
Male Hair Loss (available for men only)
Newborn Hair Amount
Photic Sneeze Reflex
Red Hair
Skin Pigmentation
Sweet Taste Preference
Toe Length Ratio
Unibrow
Widow’s Peak
Limited amount of information related to small number of gene mutations well studied by scientists

Nearly all of the genes related to BMF and MDS are not included (with the exception of FANCC)

Not sufficient for diagnosing hereditary marrow failure disorders

Commercially available DNA testing: Health

| 23andMe - 35 Health related Genes currently tested including:
<table>
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<tbody>
<tr>
<td>Fanconi Anemia Over 19 genes encoding FANC proteins AR, XLR</td>
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<tr>
<td>Telomopathies, Dyskeratosis cong. TERT, TERC, DRC and others AD, AR, XLR</td>
</tr>
<tr>
<td>Diamond Blackfan anemia RPS19, RPS24, RPL11 AD</td>
</tr>
<tr>
<td>Schwachman Diamond SBDS AR</td>
</tr>
<tr>
<td>Familial MDS/AML GATA2, RUNX1, DDX41 AD</td>
</tr>
<tr>
<td>Familial AA SRP72 AD</td>
</tr>
<tr>
<td>Familial Thrombocytopenia/AA/MDS ANKRD26, ETV6 AD</td>
</tr>
<tr>
<td>Familial AML, ET, CMML, PMF ATG2B, SKIP duplications AD</td>
</tr>
</tbody>
</table>

Genetic Mutations Causing Hereditary Bone Marrow Failure Disorders

- Fanconi anemia
- Telomopathies, Dyskeratosis congenita
- Diamond Blackfan anemia
- Schwachman-Diamond
- Familial MDS/AML
- Familial AA
- Familial Thrombocytopenia/AA/MDS
- Familial AML, ET, CMML, PMF

Bone marrow failure syndromes:
How can genetic testing help to distinguish and guide therapy?

- Autoimmune Diseases
- MS, IBD, uveitis, DM type 1, etc.
- Acquired AA
- AA/PNH
- PNH
- LGL
- AML
- MDS
- TELOMERES (DKC/BDS)
- GATA1
- FA
- SOS

Medical Genetic Testing for Bone Marrow Failure

Purpose:

- To diagnose hereditary bone marrow failure disorders
- Sequence genes known to cause BMF, looking for mutations
GENETIC TESTING – Reasons Your Doctor May Order Genetic Testing

If other family members have:
- Bone marrow failure
- MDS
- Leukemia
- Physical malformations: small height, abnormal fingers or nail growth, etc.
- Stiffening (fibrosis) of the lung and liver
- Early hair graying

=> Evaluate for an inherited genetic mutation

Most common onset of disease is in children and young adults

Why is it important to identify an inherited BMF gene mutation?

- Proper diagnosis
- Guides therapy
- Implications for family members
- Implications for future pregnancies
- Donor selection and screening for bone marrow transplantation

Genetic Testing - Informed Consent

Patient or guardian must fully understand:
- Testing procedure
- Benefits and limitations of the test
- Possible consequences of the test
- Implications for family members and future pregnancies
- Voluntary agreement to have the test done

Genetic Testing for Inherited Mutations

Examples of Genes Commonly Tested in Sequencing Panels for Bone Marrow Failure

| BRCA2 | CSFR3 | CT1C | DKC1 | ELANE | ERCC4 | FANCA | FANCB | FANCC* | FANCD2 | FANCE | FANCF | FANCG | FANCI | FANCL | FANCN | FANCM | G6PC3 | GATA2 | GF1 | HAX1 | MPL | NHP22 | NOP10 | PALB2 | RAD51C | RBM8A | RPL11 | RPL3A | RPL5 | RPS10 | RPS19 | RPS24 | RPS26 | RPS7 | RTEL1 | RUNX1 | SBDS | SBF2 | SLX4 | SLPF2 | TERC | TERT | TIN2 | TEL1 | XENC2 |
|--------|-------|------|------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|

* The only gene included in 23andMe testing
Example of Genetic Testing Results

<table>
<thead>
<tr>
<th>Patient 1 Result DNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
</tbody>
</table>

Zygozy:  
- Heterozygous: one copy of gene is mutated and the other copy is normal  
- Homozygous: both copies of the gene are mutated

Inheritance (pattern for the specific gene):  
- Recessive: need both copies of the gene mutated to have increased risk of disease  
- Dominant: only need one copy of the gene mutated for increased risk of disease

What does “Variant of unknown significance” mean?

Variant of Unknown Significance VUS: Not sure if the DNA change is normal variation, or if it is a mutation.

Do all family members with an inherited mutation develop disease?  

Answer: Not necessarily. It depends on the gene.

Question: Do all family members with an inherited mutation develop disease?  

Answer: Not necessarily. It depends on the gene.

Risk:  
- Proportion of people with a mutation that have signs of disease  
- Not all gene mutations have the same risk of disease  
- Some persons with mutations may be normal and not develop disease

Severity:  
- Variation in the degree of signs and symptoms of the disease  
- Very mild vs. severe  
- Symptoms differ among affected persons

Bottom Line: It’s complicated! Need genetic counselors and geneticists to help educate and advise
Part Two
Cytogenetic Analysis

What are acquired cytogenetic abnormalities in the bone marrow?

During the process of cell division in the bone marrow, problems can arise leading to loss or gain of chromosomes.

Examples:
- monosomy 7
- trisomy 8

20 bone marrow cells analyzed. Abnormalities are only in BM cells. Not inherited or passed. Ordered many times to monitor disease.

Abnormal Chromosomes can Function like Mutations Increasing Risk of Disease
Cytogenetic Analysis – how can this help diagnose disease in bone marrow cells?

In General

**AA and PNH:** Normal cytogenetics

**MDS:** Abnormal cytogenetics in 50%

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**FISH (Fluorescence In Situ Hybridization)** also detects cytogenetic abnormalities

*TRISOMY 8*

Bone marrow cell (blue)

Each pink dot inside the cell is one copy of chromosome 8 (too many)

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**Cytogenetic Abnormalities in MDS and Prognosis**

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Abnormalities</th>
</tr>
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<tbody>
<tr>
<td>Very Good</td>
<td>del(11q), -Y</td>
</tr>
<tr>
<td>Good</td>
<td>Normal, del(5q), del(12p), del(20q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>del(7q), +8, i(17q), +19, other</td>
</tr>
<tr>
<td>Poor</td>
<td>inv(3), t(3q), del(3q), -7, 3 abnormalities</td>
</tr>
<tr>
<td>Very poor</td>
<td>greater than 3 abnormalities</td>
</tr>
</tbody>
</table>

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What is “Clonal Evolution” and what does it mean for patients with BMF?
What is “Clonal Evolution” and what does it mean for patients with BMF?

Clonal evolution => detection of new cytogenetic abnormality or mutation that was not previously present

Monitored by cytogenetic analysis, FISH, or genomic testing for acquired mutations

May be insignificant, or may indicate progression or transformation of disease

Incidence:
10-20% of AA patients developed Cytogenetic abnormalities at 10 yrs

Prognosis:
Trisomy 8 Vs. Monosomy 7

Genomic profiling of bone marrow cells in AA and MDS for acquired mutations
What are acquired mutations and how do we get them?

What are ACQUIRED (aka SOMATIC) MUTATIONS?

ACQUIRED MUTATIONS:
- Not present at birth
- Only present in certain cells, not in all cells
- Can not be passed on to offspring
- Can be related to toxic exposure (smoking, radiation, benzene, asbestos, etc.)
- Environment (e.g. UV radiation from sun)
- Random
- Normal in aging population
- PNH – PIG-A mutations

May increase your risk for disease:
- MDS
- Neoplasia

PNH – PIG-A mutations

GENOMIC PROFILING for Acquired Mutations
Examines your DNA to look for mutations in the genetic code
- Genomic profiling of cells in bone marrow to characterize disease
- Sequence panels of genes related myeloid disease (MDS, AML, CMML)
- Repeated over time as disease changes, monitor for disease progression
- Major Area of Research and recently available for clinical testing
- Prognostic implications for outcome & response to therapy in MDS and AA

Mutations and Cytogenetic Abnormalities are common in MDS

Somatic point mutations in bone marrow cells are common in myelodysplastic syndromes (~90%) and are often associated with specific clinical features.
Specific Acquired Gene Mutations in BMF

- Area of active investigation
- Some doctors are using mutations profiles to help make decisions about treatment or determine eligibility on clinical trials
- Potentially useful in MDS for new inhibitors and clinical trials
- Not yet used for diagnosis -------- WHY?
- Not specific for MDS, can be seen in other diseases (MPN, AML, etc.) AND........

Acquired Mutations in MDS are also found in Healthy Aging Population

Effects of Time
MDS-associated mutations are also in healthy people

- 10% of people >70 years carry at least one (Genovese et al. NEJM 2014; Jaiswal et al. NEJM 2014)
- Present in fewer % of blood cells than MDS (<10% vs. 30%)
- Increased risk of subsequent hematologic malignancy (factor of 11)
- Increased risk of higher all-cause mortality (cardiovascular disease, factor 2.6)

What about acquired mutations in AA or PNH?

Hallmark of PNH => acquired mutations in PIG-A in bone marrow cells

Mutations in Aplastic Anemia

One-third of AA have at least 1 mutation

A SUBSET OF MUTATIONS in AA CORRELATE WITH SURVIVAL - FREE FROM CLONAL EVOLUTION

Good outcome
Part Four

Benefits and Limitations of Genetic Testing and Genomic Profiling

**GENETIC TESTING and GENOMIC PROFILING SUMMARY**

**Benefits:**
- Knowledge of underlying disease factors
- Making informed decisions about health care
- **Inherited mutations:**
  - Implications for family planning
  - Screening donor family members for patients undergoing BMT
- **Acquired mutations:**
  - Prognostic implications for outcome and response to therapy (BMT, HSCT, HMAs, etc)
  - Monitoring for disease progression over time, clonal evolution
  - Availability of targeted therapy for specific mutations and clinical trials with new drugs

**Drawbacks and Limitations:**
- Financial implications
- Emotional implications
- **Limitations of genetic testing:**
  - Inherited mutations:
    - Not everyone with a mutation may develop disease => value of discussing with genetic counselor
  - Acquired mutations:
    - Not necessarily specific for MDS/BMF
    - May be seen in other diseases
    - May be present in healthy individuals

**What is on the Horizon?**
- More research needed to understand the significance of acquired mutations and “VUS’s in MDS, AA and other myeloid diseases
  For diagnosis, treatment and prognosis
- Discovery: Growing list of genes/mutations related to BMF
- Development of new targeted therapies in the pipeline against specific mutations in MDS and other myeloid diseases
Thank you!

Specific Gene Mutations and Associations a few examples based on research studies

- SF3B1 and TET2 - each mutated in 20-25% of MDS cases, most common
- TET2 and DNMT3A predicts higher likelihood of response to HMAs
- SF3B1 – ring sideroblasts
- RUNX1 – thrombocytopenia
- TP53 – complex karyotype, therapy related disease, higher relapse after HSCT
- SRSF2, CBL - MDS/MPN overlap
- ASXL1 - CMML
- SETBP1 – atypical CML
- JAK2 and SF3B1 – RARS-T