Personalized Medicine:
Genetic Testing and the Implications for Future Therapies

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Molecular Geneticist, PreventionGenetics

AA•MDS INTERNATIONAL FOUNDATION

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

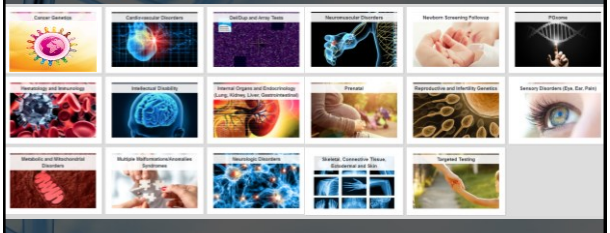
BONE MARROW FAILURE DISORDERS

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?

GENETIC TESTING IS USED FOR MANY DISORDERS



TYPES OF GENETIC TESTING

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

Other DNA Analyses
- Paternity
- Forensic
- Ancestry

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

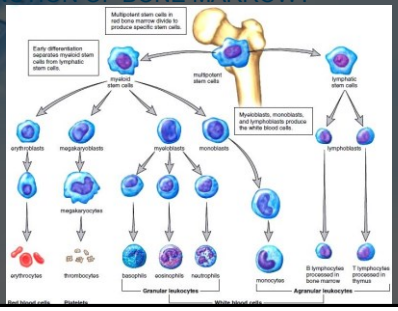
Cytogenetics – Chromosomal Analysis
Genetics – really just one tool available for diagnosis

WHAT IS THE FUNCTION OF BONE MARROW?

Hematopoiesis

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

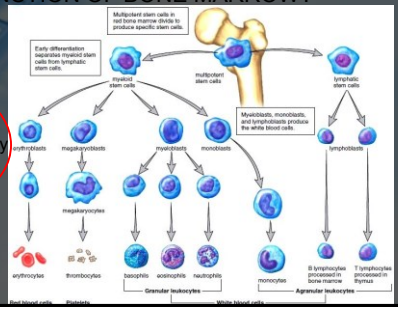
3 main blood cells
1. Red Blood Cells
2. Platelets (thrombocytes)
3. White Blood Cells



WHAT IS THE FUNCTION OF BONE MARROW?

Hematopoiesis

Key: Healthy bone marrow makes healthy blood cells



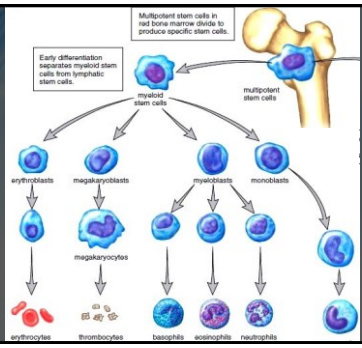
Key: BMF is insufficient hematopoiesis

Aplastic Anemia (AA)
– failure to produce progenitor and mature blood cells

Paroxysmal Nocturnal Hemoglobinuria (PNH)
– destruction of RBC

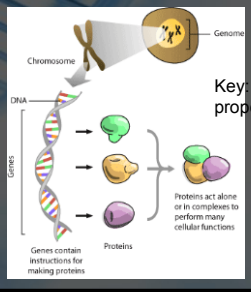
Myelodysplastic Syndrome (MDS)
– production of ineffective progenitor cells

Myeloproliferative Neoplasms (MPN)
Acute Myeloid Leukemia (AML)
– over production of progenitor cells



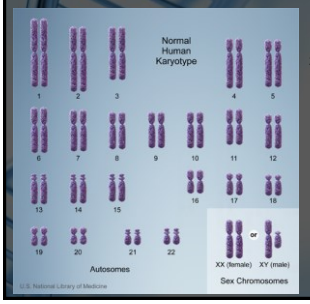
So how are genes related to bone marrow failure?

GENES ENCODE PROTEINS; PROTEINS MAKE OUR BODIES WORK



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

DNA STRUCTURE



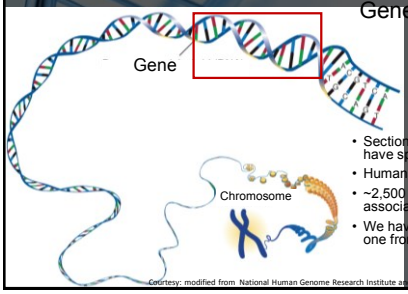
Genes are packaged in Chromosomes

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

1 pair sex chromosomes

DNA STRUCTURE

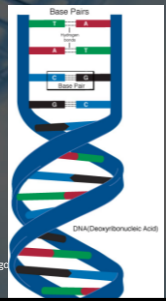


Genes

- 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

Courtesy: modified from National Human Genome Research Institute and the Smithsonian National Museum of Natural History

DNA STRUCTURE



http://ghr.nlm.nih.gc

Genes are organized sequences of nucleotides (base pairs)

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

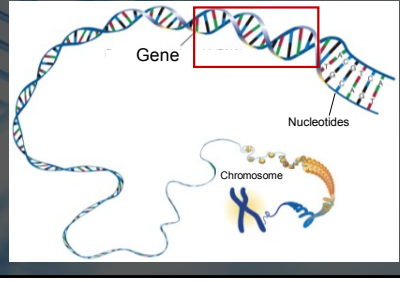
Courtesy: National Human Genome Research Institute and the Smithsonian National Museum of Natural History

DNA IS A RECIPE BOOK FOR OUR BODIES

All DNA in our Cells
↓
Chromosomes
↓
Genes
↓
Nucleotides

Book
↓
Chapters
↓
Sentences
↓
Letters

TYPES OF MUTATIONS



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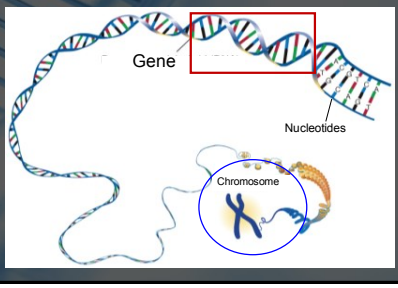
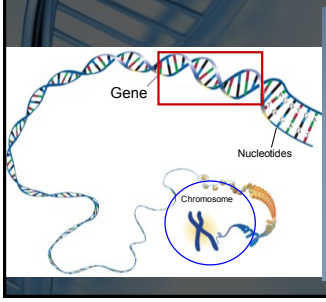
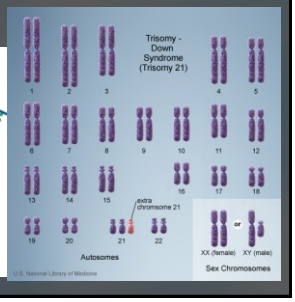


TYPES OF MUTATIONS - CHROMOSOMES





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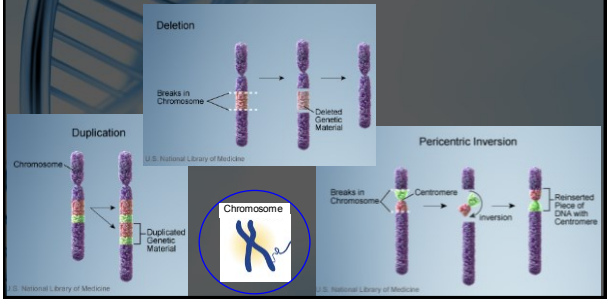

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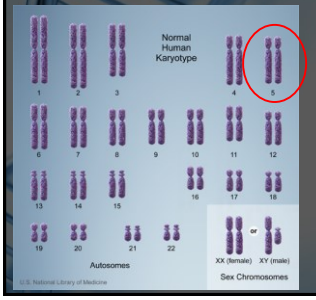
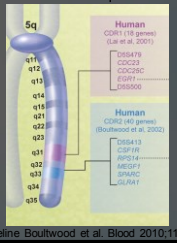
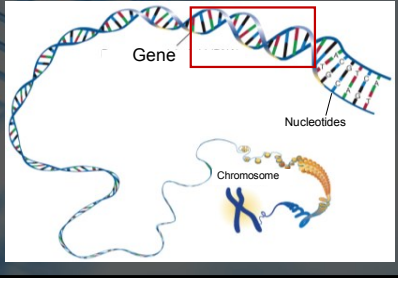


5q minus syndrome
- deletion of part of chromosome 5 associated with acquired MDS



Jacqueline Boultwood et al. Blood 2010;116:5803-5811

TYPES OF MUTATIONS - NUCLEOTIDES



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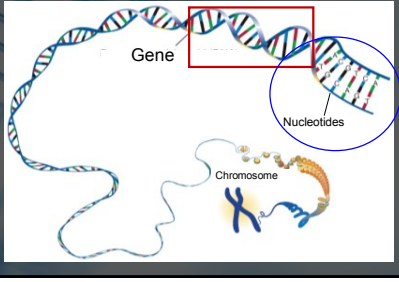

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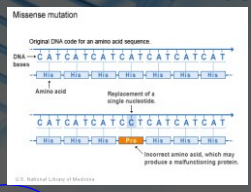
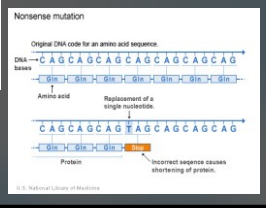
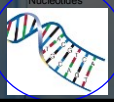




Nucleotides



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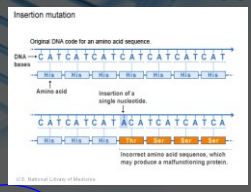
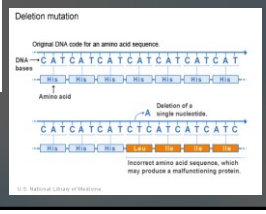


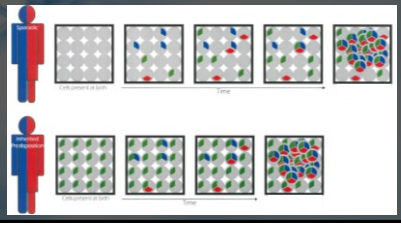


Nucleotides

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.



QUICK SUMMARY

How are genes associated with bone marrow failure?

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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?

APLASTIC ANEMIA (AA)

Aplastic Anemia – failure to produce enough progenitor and mature blood cells

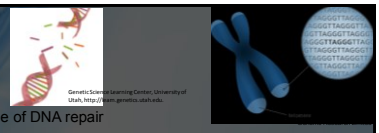
Acquired – events lead to immune response attacking blood progenitor cells, the cause in most cases is unknown

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 syndroms (SDS)
- Dyskeratosis congenita (DKC)
- Diamond – Blackfan anemia (DBA)

APLASTIC ANEMIA



Hereditary

- 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).

FANCONI ANEMIA RESEARCH FUND
Advancing Research. Supporting Families.

APLASTIC ANEMIA

FA - Importance of Early Diagnosis

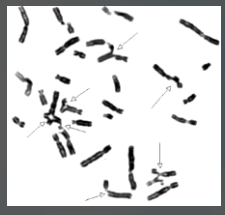
- Prevent inappropriate management of hematologic disease (cytopenias, aplastic anemia, leukemia)
- Permits consideration of Bone Marrow Transplant (BMT), androgens, hematopoietic growth factors, and surgical intervention for solid tumors
- BMT can cure the blood phenotype, but patients are still at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

APLASTIC ANEMIA

FA - Importance of Early Diagnosis

Diagnosing FA:

- Distinct clinical characteristics of FA
- Chromosomal breakage is a hallmark of FA
  + DEB
- Distinct genes associated with FA



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

Genes

FANCA	FANCF	FANCN (PALB2)	FANCT (UBE2T)
FANCB	FANCG	FANCO (RAD51C)*	FANCU (XRCC2)
FANCC	FANCI	FANCP (SLX4)	FANCV (MAD2L2/REV7)
FANCD1 (BRCA2)	FANCJ (BRIP1)	FANCQ (ERCC4)**	
FANCD2	FANCL	FANCR (RAD51)***	
FANCE	FANCM	FANCS (BRCA1)	

*Mutation in RAD51C, is associated with a Fanconi anemia-like syndrome (OMIM: 613390)
**Specific mutations in ERCC4 (XPF) are associated with xeroderma pigmentosum (OMIM: 278760) and XFE progeroid syndrome (OMIM: 610965)
***A dominant mutation in RAD51 is associated with a Fanconi anemia-like syndrome

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

APLASTIC ANEMIA

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

Genes

FANCA	FANCF	FANCN (PALB2)	FANCT (UBE2T)
FANCB	FANCG	FANCO (RAD51C)*	FANCU (XRCC2)
FANCC	FANCI	FANCP (SLX4)	FANCV (MAD2L2/REV7)
FANCD1 (BRCA2)	FANCJ (BRIP1)	FANCQ (ERCC4)**	
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APLASTIC ANEMIA

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*

APLASTIC ANEMIA

CASE STUDY #1 – FA

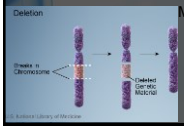
Found 2 mutations in the *FANCA* gene

Nucleotides

Mutation 1: Deletion of 4 nucleotides

AGTTTCAGTTCCTCATGTTCAGATTGTTCTCAGAGG

AGTT____TCCTCATGTTCAGATTGTTCTCAGAGG



Mutation 2: Missing a large part of Chromosome 16 and *FANCA*

The piece that is missing contained part of the *FANCA* gene

APLASTIC ANEMIA

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
- Patients 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

- Confirmed patient has FA and not acquired AA due to autoimmune response, so would not necessarily treat this FA patient with Immunosuppressive Therapy
- Know 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

APLASTIC ANEMIA

Same process can be applied to the other hereditary forms of AA

- Shwachman – Diamond syndroms (SDS) – *SBDS*
- Dyskeratosis congenita (DKC) ≥ 11 genes
- Diamond – Blackfan anemia (DBA) ≥ 18 genes
- Severe congenital neutropenia (SCN) ≥ 24 genes
- Congenital amegakaryoctic thrombocytopenia (CAMT) - *MPL*
- *GATA2* gene deficiency

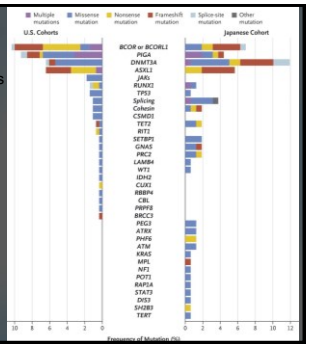
Key: Hereditary BMF disorders often have distinct clinical features and we know many of the genes associated with many of these disorders and can tailor treatment

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

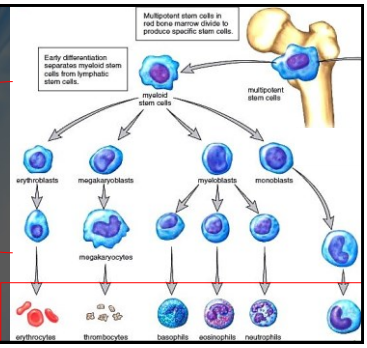
Yoshizato et al. NEJM 2015



REMEMBER: BMF is insufficient Hematopoiesis

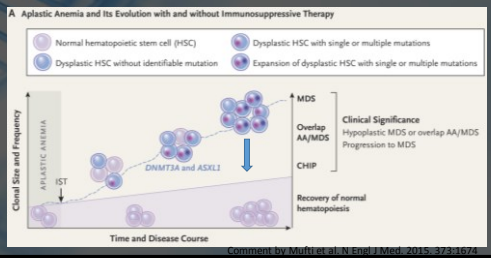
Mutations in the progenitor cells…

…result in insufficient mature cells.



APLASTIC ANEMIA

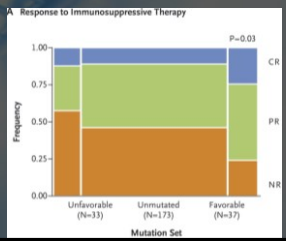
Model of Disease Progression



Comment by Mufti et al. N Engl J Med. 2015; 373:1674

APLASTIC ANEMIA

- The types of mutations and prevalence can predict response to immunosuppressive therapy (IST) and course of disease to inform treatment and care.



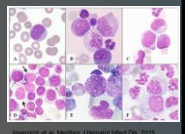
Yoshizato et al. NEJM 2015

MYELODYSPLASTIC 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.



Ilmenlozzi et al. Mediterr J Hematol Infect Dis. 2015 7:e2015035

- 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

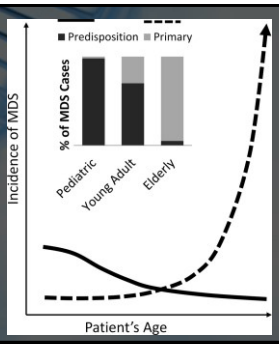
Hereditary MDS

- Like AA, there are specific genes and disorders associated with MDS
- Clinical presentation and genetics can help determine prognosis and care

Syndrome	Gene	Inheritance	Heme Malignancy	Other Associated Abnormalities
Familial platelet disorder with propensity to myeloid malignancies	*RUNX1*	AD	MDS/AML/T-cell ALL	Thrombocytopenia, bleeding propensity, aspirin-like platelet dysfunction
Thrombocytopenia 2	*ANKRD26*	AD	MDS/AML	Thrombocytopenia, bleeding propensity
Familial AML with mutated DDX41	*DDX41*	AD	MDS/AML, CMML	None
Thrombocytopenia 5	*ETV6*	AD	MDS/AML, CMML, B-cell ALL, multiple myeloma	Aplastic anemia
Familial MDS/AML with mutated GATA2	*GATA2*	AD	MDS/AML/CMML	Neutropenia, monocytopenia, MonoMAC syndrome, Emberger syndrome
Familial aplastic anemia with SRP72 mutation	*SRP72*	AD	MDS	Aplastic anemia
Familial AML with mutated CEBPA	*CEBPA*	AD	AML	None
Fanconi anemia	Complementation Groups	AR, X-linked	MDS, AML	Pancytopenia, macrocytic anemia, congenital malformations
Telomeropathies (dyskeratosis congenita)	*TERC, TERT*, others	AD, AR	MDS/AML	Macrocytosis, aplastic anemia, oral leukoplakia, dysplastic nails, lacy skin rash

Bannon and DiNardo, 2016

MDS



Babushok and Bessler, 2015

MDS

CASE STUDY #2 – MDS

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

Nucleotides

Mutation in the *RUNX1* gene predicted to alter splicing

TTTCAAGGTACTGGCCCG

TTTCAAGTTACTGGCCCG

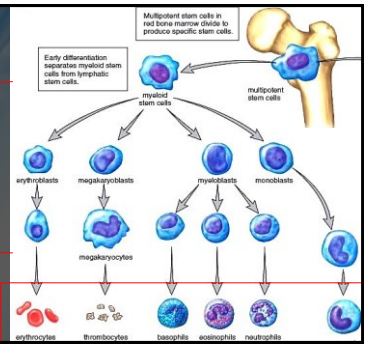
UTILITY OF TESTING FOR GENETIC CAUSES OF MDS

- Confirmed patient has a mutations 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 patients family history? And should other family members be tested?

REMEMBER: BMF is insufficient hematopoiesis

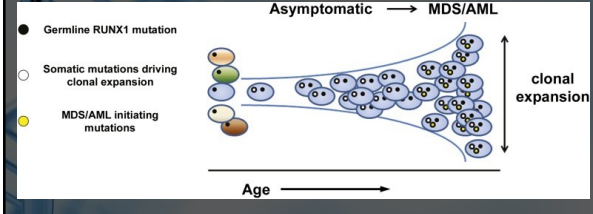
Mutations in the progenitor cells…

…result in insufficient mature cells.



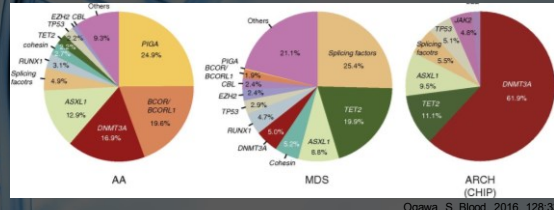
MDS

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



Churpek et al. Blood. 2015 126:2484

MUTATIONS IN MDS



Ogawa, S. Blood. 2016. 128:337

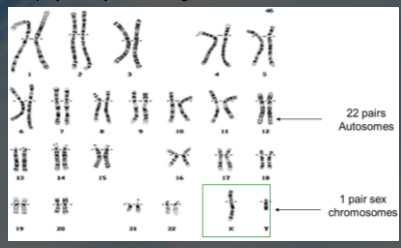
Key: MDS progresses in a similar manner as AA, but different combinations of mutations arise over time

MDS

Chromosomal analysis also plays a key role in diagnosis of MDS:

5q, +8, -Y, del(20q)

Karyotype



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

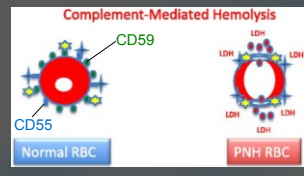
PARAOXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

PNH – destruction 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)



Charles J. Parker Hematology 2016;2016:208-216

PNH

- Major Clinical Manifestations – bone marrow failure, hemolytic anemia, and thrombophilia
- Again, a clonal disease – clonal expansion and clone size determine the clinical characteristics and severity of the disease
- Once suspected, diagnosis is straightforward by flow cytometry, but not always suspected since clinical manifestations vary among patients

Key: Genetic analysis of clonal cells can help differentiate PNH diagnosis from other possible causes of AA

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

Babushok and Bessler, 2015

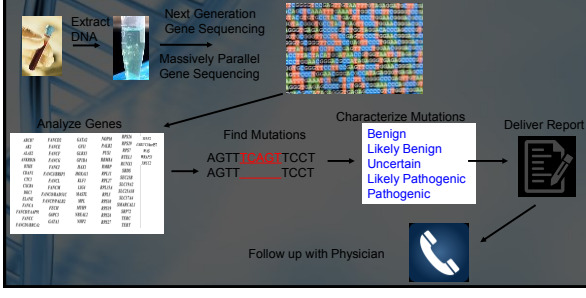
GENETIC TESTING APPROACH FOR BMF SYNDROMES

A recent study on inherited BMF syndromes analyzed 72 genes in 158 patients

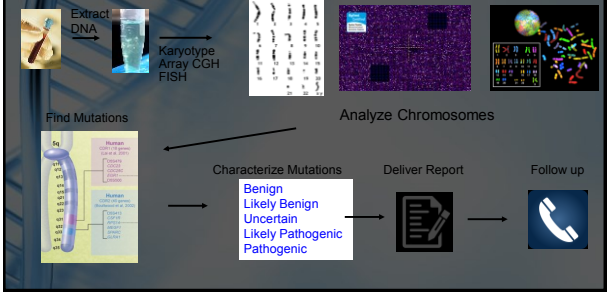
ABCB7	*FANCD2*	*GATA2*	*NOP10*	*RPS26*	*TINF2*
AK2	*FANCE*	*GFI1*	*PALB2*	*RPS29*	*USB1/C16orf57*
ALAS2	*FANCF*	*GLRX5*	*PUS1*	*RPS7*	*WAS*
ANKRD26	*FANCG*	*GP1BA*	*RBM8A*	*RTEL1*	*WRAP53*
BTHS	*FANCI*	*HAX1*	*RMRP*	*RUNX1*	*XRCC2*
CDAN1	*FANCJ/BRIP1*	*HOXA11*	*RPL11*	*SBDS*	
CTC1	*FANCL*	*KLF1*	*RPL27*	*SEC23B*	
CXCR4	*FANCM*	*LIG4*	*RPL35A*	*SLC19A2*	
DKC1	*FANCO/RAD51C*	*MASTL*	*RPL5*	*SLC25A38*	
ELANE	*FANCP/PALB2*	*MPL*	*RPS10*	*SLC37A4*	
FANCA	*FECH*	*MYH9*	*RPS19*	*SMARCAL1*	
FANCB/FAAP95	*G6PC3*	*NBEAL2*	*RPS24*	*SRP72*	
FANCC	*GATA1*	*NHP2*	*RPS27*	*TERC*	
FANCD1/BRCA2				*TERT*	

Ghemlas et al. J Med Genet. 2015 52:575

GENETIC TESTING PROCESS – ADVANCED TECHNOLOGY

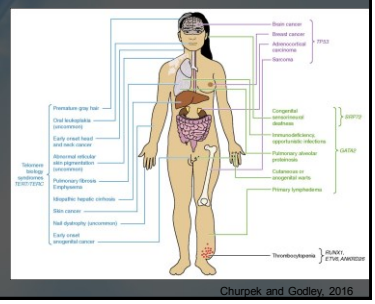


GENETIC TESTING PROCESS – ADVANCED TECHNOLOGY



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?



Churpek and Godley, 2016

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

Babushok et al., 2016

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 INTERNATIONAL FOUNDATION
http://www.aamds.org/

BE THE MATCH®
https://bethematch.org/

Genetics Home Reference — Your Guide to Understanding Genetic Conditions
https://ghr.nlm.nih.gov/

OMIM
https://www.ncbi.nlm.nih.gov/omim

FANCONI ANEMIA RESEARCH FUND
Advancing Research. Supporting Families.
http://www.fanconi.org/

Global Genes
https://globalgenes.org/

PREVENTION GENETICS
https://www.preventiongenetics.com/