Fanconi Anemia 101

Blanche P Alter, MD, MPH, FAAP
Clinical Genetics Branch
Division of Cancer Epidemiology and Genetics
Bethesda, MD

FA Camp, June 27, 2014
When You Hear . . . ., Think . . . ., Or . . . .
Open Minds

“I’ve never seen this before.”

should be

“I’ve never recognized this before.”
Questions

1. How many are new to FA Camp?
2. How many have been here before?
3. How many have FA and are 18 years of age or older?
4. How many think that the diagnosis of FA was initially missed by one or more physicians?
5. How many were called “VATER” initially?
My Tasks

1. FA101 (advanced FA) for those who have not heard it.
2. Update on what is new in FA research.
3. Address specific concerns of those who are new to FA.

All in 50 minutes.....(+ questions)
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Hematology</th>
<th>Leukemia</th>
<th>Solid Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi Anemia (FA)</td>
<td>Aplastic</td>
<td>AML</td>
<td>SCC</td>
</tr>
<tr>
<td>Dyskeratosis Congenita (DC)</td>
<td>Aplastic</td>
<td>AML</td>
<td>SCC</td>
</tr>
<tr>
<td>Diamond-Blackfan Anemia (DBA)</td>
<td>Pure anemia</td>
<td>AML</td>
<td>Sarcomas</td>
</tr>
<tr>
<td>Shwachman-Diamond Syndrome (SDS)</td>
<td>Neutropenia</td>
<td>AML</td>
<td>-</td>
</tr>
<tr>
<td>Severe Congenital Neutropenia (SCN)</td>
<td>Neutropenia</td>
<td>AML</td>
<td>-</td>
</tr>
<tr>
<td>Amegakaryocytic Thrombocytopenia</td>
<td>Thrombocytopenia</td>
<td>AML</td>
<td>-</td>
</tr>
<tr>
<td>Thrombocytopenia Absent Radii (TAR)</td>
<td>Thrombocytopenia</td>
<td>AML</td>
<td>-</td>
</tr>
</tbody>
</table>

These disorders are the “Inherited Bone Marrow Failure Syndromes” (IBMFS).
History: Guido Fanconi

- **Fanconi Anemia** (Fanconi pancytopenia syndrome): 1927, 3 brothers with pancytopenia and physical abnormalities, "perniziosiforme"

- **Fanconi Syndrome** (renal Fanconi syndrome): 1936, proteinuria, glucosuria, phosphaturia, aminoaciduria, citraturia, and proximal renal tubular acidosis
Fanconi Anemia: Definition

- Autosomal recessive
  - 1 X-linked recessive gene
- Physical findings
- Aplastic anemia
- Leukemia
- Solid tumors
- Chromosome instability
- DNA repair defect
- >16 genes
108/2245 literature FA cases, 1927-2012, had least 3 features.  
29 called VATER in the reports. 103/108 had R (renal) and L (limb).

Alter, Rosenberg: Molecular Syndromology, 2013

<table>
<thead>
<tr>
<th>Anomalies</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertebral anomalies</td>
<td></td>
</tr>
<tr>
<td>Anal atresia</td>
<td></td>
</tr>
<tr>
<td>Cardiac anomalies</td>
<td></td>
</tr>
<tr>
<td>Tracheo-esophageal fistula</td>
<td></td>
</tr>
<tr>
<td>Esophageal atresia (also duodenal atresia)</td>
<td></td>
</tr>
<tr>
<td>Renal structural anomalies</td>
<td></td>
</tr>
<tr>
<td>Limb anomalies, essentially radial and/or thumbs</td>
<td></td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td></td>
</tr>
</tbody>
</table>
Fanconi Anemia: Adults

Absent radius, 0

Aplastic anemia, 5

?, 6

Aplastic anemia, 16

Cancer, 30

BMT donor, 50s

All photos with permission
FA Literature: Age at Diagnosis 1927-2011

Shimamura and Alter, Blood Reviews 2010

![Graph showing frequency of age at diagnosis]

Frequency

Age, Years

6.7 yr

Shimamura and Alter, Blood Reviews 2010
FA Literature: Physical Findings, 60%

- Low Birth Weight
- Short
- Thumbs
- Skin hyperpigmented
- Gonads male
- Microcephaly
- Renal
- Eyes
- Skin café au lait
- Developmental delay
- Ears, deaf
- Radii
- Cardiopulmonary
- Legs, hips, feet
- Gastrointestinal tract
- Brain/pituitary
- Gonads female
- Short or skin only

Shimamura and Alter, Blood Reviews 2010

M:F 1.2:1
Characteristics of Persons with FA

- Physical findings described in the literature may not be found in all persons with FA
  - 11% had short stature and skin findings only
  - At least 25% of those reported had no physical findings
- Some persons without physical findings may be diagnosed at a later age
Laboratory Findings in FA

- Low blood counts (pancytopenia)
- Large red cells (macrocytosis) Non-specific
- Increased fetal hemoglobin (Hb F)
- Chromosome breakage in lymphocytes or fibroblasts cultured with a DNA crosslinker, e.g. diepoxybutane (DEB) or mitomycin C (MMC)
FA Inheritance

- People with FA:
  - Unaffected parents: one FA and one normal gene (carriers)
  - Affected offspring: one FA gene from each parent

- Children of FA:
  - Each has one FA gene (carriers); chance of FA marrying an FA carrier of the same genotype is ~1/200 or less
FA: More Accurate Carrier Frequency

- 1:300 in New York State in 1971 (Swift, 1971)
  - “Taking only the families I know, twelve definitive cases have been born in New York State in that period [1956 until 1967] among a total of 4.185x10^6 live births, providing an estimated birth incidence of 1 in 3.48x10^5. If the Normal and FA alleles follow the Hardy-Weinberg law, the expected heterozygote frequency is about 1 in 300.”

- ~1:100 in Ashkenazi Jews, Afrikaners, Spanish Gypsies, black sub-Saharan Africans

- Birth rate of FA (known to FARF) ~60% ascertainment, general birth rate, Hardy-Weinberg equation

- 1:181 in US in 2010 (birth incidence 1/130,000)

- 1:93 in Israel in 2008 (birth incidence 1/35,000)

- FA is more common than we think

Rosenberg, Tamary, Alter: AJMG 2010
Disease-Associated Mutations

A mutation is a change in the normal base pair sequence

Commonly used to define DNA sequence changes that alter protein function
16 FA Genes

*Breast cancer in carriers

Adapted from Leiden Open Variation Database, http://chromium.liacs.nl/LOVD2/FANC/home.php
## FA: Complementation Groups/Genes

<table>
<thead>
<tr>
<th>Group</th>
<th>Locus</th>
<th>cDNA</th>
<th>Exons</th>
<th>AA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16q24.3</td>
<td>5.5</td>
<td>43</td>
<td>1455</td>
<td>~70</td>
</tr>
<tr>
<td>B</td>
<td>Xp22.31</td>
<td>2.8</td>
<td>10</td>
<td>859</td>
<td>Rare</td>
</tr>
<tr>
<td>C</td>
<td>9q22.3</td>
<td>4.6</td>
<td>14</td>
<td>558</td>
<td>~10</td>
</tr>
<tr>
<td>D1/BRCA2*</td>
<td>13q12.3</td>
<td>11.4</td>
<td>27</td>
<td>3418</td>
<td>Rare</td>
</tr>
<tr>
<td>D2</td>
<td>3p25.3</td>
<td>5</td>
<td>44</td>
<td>1451</td>
<td>Rare</td>
</tr>
<tr>
<td>E</td>
<td>6p21-22</td>
<td>2.5</td>
<td>10</td>
<td>536</td>
<td>~5</td>
</tr>
<tr>
<td>F</td>
<td>11p15</td>
<td>1.3</td>
<td>1</td>
<td>374</td>
<td>Rare</td>
</tr>
<tr>
<td>G/XRCC9</td>
<td>9p13</td>
<td>2.5</td>
<td>14</td>
<td>622</td>
<td>~10</td>
</tr>
<tr>
<td>H/KIAA1794</td>
<td>15q25-26</td>
<td>4.5</td>
<td>38</td>
<td>1328</td>
<td>Rare</td>
</tr>
<tr>
<td>J/BACH1/BRIP1*</td>
<td>17q22.3</td>
<td>4.6</td>
<td>20</td>
<td>1249</td>
<td>Rare</td>
</tr>
<tr>
<td>L/PHF9/POG</td>
<td>2p15-16.1</td>
<td>1.7</td>
<td>14</td>
<td>375</td>
<td>Rare</td>
</tr>
<tr>
<td>M/Hef</td>
<td>14q21.3</td>
<td>6.5</td>
<td>22</td>
<td>2014</td>
<td>Rare</td>
</tr>
<tr>
<td>N/PALB2*</td>
<td>16p12.1</td>
<td>3.5</td>
<td>13</td>
<td>1186</td>
<td>Rare</td>
</tr>
<tr>
<td>O/RAD51C*</td>
<td>17q25.1</td>
<td>2.7</td>
<td>9</td>
<td>76</td>
<td>Rare</td>
</tr>
<tr>
<td>P/SLX4*</td>
<td>16p13.3</td>
<td>26.6</td>
<td>15</td>
<td>1834</td>
<td>Rare</td>
</tr>
<tr>
<td>Q/ERCC4/XPF</td>
<td>16p13.12</td>
<td>39.2</td>
<td>11</td>
<td>916</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*Breast cancer genes
Fanconi Anemia Core Complex
DNA damage
Oxidative stress
Cytokines
FA/BRCA DNA Repair Pathway

Adapted from
Shimamura and Alter, Blood Reviews 2010
FA in Tokyo
Who Should be Tested for FA?

- Characteristic birth defects (eg thumbs, kidneys, poor growth, etc, especially VACTERL)
- Aplastic Anemia (AA)
- Myelodysplastic Syndrome (MDS)
- Acute Myeloid Leukemia (AML)
- Decreased fertility
- Early characteristic cancer
- Siblings of persons with FA
What are the FA Tests?

- Chromosome breakage, DEB or MMC
- D2 ubiquitination (Western blot)
- BRCA2 (Western blot)
- Complementation with retroviruses
- Sequencing of candidate genes (e.g., FANCC IVS4+4 A->T)
- Sequencing of all cloned genes
- NextGen sequencing: exome, GWAS, etc

Blood lymphocytes, skin fibroblasts
Chromosomes

Shimamura and Alter, Blood Reviews, 2010, courtesy of Lisa Moreau, Dana Farber Cancer Institute
FA: D2 Ubiquitination

Shimamura et al, Blood, 2002

Also called a “Western” or “D2” “blot

Green and Kupfer, HemOnc Clin NA, 2009
Complementation Analysis, Retroviruses

- FA cells are sensitive to DEB or MMC
- Transfect retroviruses containing cloned FA genes
- Transfected cells no longer sensitive
  - Normal gene ‘complemented’ FA cells, defining the complementation group
- Transfected cells still sensitive
  - Normal gene not identified for FA cells
Retrovirus-mediated Correction of FA Cells

Retrovirus-mediated Correction of TA 0252's T-cells analyzed by flow cytometry after five days of MMC-Incubation

- S11EG
- SFA
- S11FCIEG
- S11FEIEG2
- S11FFIEG
- S11FG

FANCA
But, Welcome to the Modern Era
DNA the molecule of life

Trillions of cells
Each cell:
- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions
Gene Finding by Exome Sequencing

Exome sequencing cases → Coding variants → Exome sequencing controls → Exclude common variants → Candidate genes

Genetic variation databases
Blood Production (Hematopoiesis)

- Pluripotent Stem Cell
- Myeloid Stem Cell
- Lymphoid Stem Cell

Myeloid Stem Cell:
- Neutrophils
- Monocytes
- Eosinophils
- Basophils
- Red Cells
- Platelets

Lymphoid Stem Cell:
- T
- B
- Lymphocytes

See handout “Hematology 101”
Bone Marrow Biopsy

Normal

Aplastic
Proof of Mosaicism in FA

- Peripheral blood lymphocyte chromosome breakage test normal
- Skin fibroblast chromosome breakage test abnormal
Mosaicism from Recombination
Complications

- Aplastic Anemia
- Acute Leukemia
- Myelodysplastic Syndrome
- Solid Tumors
- Liver Tumors
Definitions

- Aplastic Anemia (AA)
  - Pancytopenia
  - Hypocellular bone marrow
- Acute Leukemia (AL)
  - Malignant proliferation of immature cells
- Myelodysplastic Syndrome (MDS)
  - Cytopenias with hypercellular bone marrow
Blood Cytopenias: Signs and Symptoms

- Thrombocytopenia
  - bruises, petechiae
- Anemia
  - fatigue, lassitude, dyspnea
- Neutropenia
  - infections
FA: When to Treat Bone Marrow

- Cytopenias
  - Hb <8 g/dL or symptoms
  - Platelets <30,000/mm³
  - WBC <500/mm³
- Leukemia
  - Blasts in blood
  - >20% blasts in marrow
- MDS
  - Morphologic + cytopenias
  - Not for clone alone
FA: Treatment for Bone Marrow

- Transplant
- Androgen
- Hematopoietic growth factors
- Gene therapy?
FA: Medical Treatment

- Oxymetholone
  - 2-5 mg/kg/day oral
- Danazol
  - ~200-400 mg/day oral
- Folic acid
  - 1 mg/day oral
FA: Supportive Care

- RBCs: for Hb <8 g/dl or symptoms
- Platelets: for platelets <10,000/mm$^3$ or symptoms
- Blood products:
  - no family member donors
  - Leukopoor, possibly irradiated
- Antibiotics:
  - as needed for infections
FA: Treatment with Transplant

- Bone marrow, cord blood, or peripheral blood stem cells
- HLA-related donor
  - when meet any treatment criteria
- Alternate donor (mismatched unrelated [MUD], partial match family member)
  - Leukemia or clinical MDS (not clone alone)
  - Refractory aplastic anemia
Stem Cell Transplant (SCT)

- How many?
  - 40-50% of people with FA

- Survival?
  - Half are more than 5-10 years beyond SCT
  - Overall 80-90% with current methods

- Does age at SCT matter?
  - Need to do at whatever age meet criteria for SCT.
  - Consider seriously to do or not do “Preemptive” SCT, because half of those with FA may never need SCT
188 leukemias and 286 solid tumors in 413/2190 patients; 47 had 2-4 cancers.
FA Risk of Adverse Events

Alter et al, BJH, 2010
Relative Risk of Cancer in FA

Data from North American Survey, Germany, Israel, and NCI
Solid tumor or leukemia preceded the diagnosis of FA in 35% of those with these events.
Diagnosis of FA before Cancer

- Aplastic anemia
- Birth defects
- Family history
Diagnosis of FA after Cancer

- Clinical suspicion based on appearance
- Family history
- FA-type cancers, atypically young, no risk factors
- Unrecognized marrow failure
- Absence of marrow involvement (e.g. somatic mosaicism)
Possible Causal Factors for Cancer in FA

- Genetics
- Stem cell transplant - GVHD, XRT
- HPV
- Immunodeficiency
- Tobacco
- Alcohol
- Dental XRays
- Oral trauma (braces)
Genetics: Cancer Populations

- **FANCD1/BRCA2:**
  - AML
  - Brain tumors
  - Wilms (kidney) tumor

- **Other FA:**
  - Head and neck squamous cell carcinomas (HNSCC)
  - Gynecologic SCC
Transplant: Head and Neck Cancer

NAS and Paris

Hypothetical cumulative incidence curves for SCC expected if the competing risks of non-SCC death could be removed. BMT would increase risk of HNSCC 4-fold, and 16 years earlier. All had GVHD.

Rosenberg et al, Blood 2005
HPV Hypotheses

1. HPV is causal in cancer in FA.
2. Unvaccinated FA patients acquire immunity to community HPV earlier than non-FA individuals.
3. FA patients have an abnormal (reduced) immune response to HPV vaccination.
HPV and FA Tumors: Background

- Kutler, JNCI 2003
  - 21 of 25 tumors were HPV16/18+ (19 were HPV16+)
  - 6/7 vulvar; 15/18 HNSCC
- Van Zeeburg, JNCI 2008
  - Two of 21 tumors were HPV16+
  - 0/16 HNSCC, 0/2 esophagus, 2/3 anogenital
- Alter, Intl J Ca 2013
  - One of 9 tumors was HPV16+
  - 0/5 HNSCC, 1/4 Gyn
HPV16/18 Antibodies, Vaccinated FA

- Prior BMT: 1, 2; 1 or 2, not 3 doses.
- Lines are geometric means of levels in healthy females.
- One male, 12 years post-BMT, had antibody to HPV16 but not 18, 1.2 yrs post-vaccination.
- People with FA achieved levels similar to healthy females.

Alter et al, Vaccine 2014
HPV Summary

1. None of 9 FA and DC HNSCC had HPV DNA.
2. One of 4 FA Gyn SCC had HPV16 DNA.
3. Antibody levels in unvaccinated patients with FA or other IBMFS resembled healthy women.
4. Antibody levels in vaccinated patients were generally in the range seen in healthy women.
5. FA patients should follow standard HPV vaccine recommendations.
What is “MDS” in IBMFS?

- **WHO 2008**
  - Refractory cytopenia of childhood (RCC)
  - Any abnormal appearance (dyspoiesis) in 2 or 3 lineages,
  - Or, >10% in at least one lineage

- **NCI 2013**
  - >10% dyspoiesis in 1-3 lineages
  - Clone alone, without dyspoieses or cytopenias, may not have a bad prognosis
FA: Adult Females

- Late onset of menses (14-16)
- Heavy periods if platelets low
- Early onset of menopause (30s)
- Decreased fertility, but possible
- Increased need for Caesarean sections (due to pre-eclampsia)
- Worsening bone marrow function during pregnancy
- Endocrine problems: cholesterol, thyroid, growth hormone, metabolic syndrome, small pituitary, osteopenia
- Cancer: AML, HNSCC, Vulva, vagina, cervix
A Marker for Ovarian Insufficiency

- Antimullerian hormone (AMH) is menstrual cycle-independent.
- It provides a simple assessment of ovarian function.
- Documentation of declining AMH may lead to early management of premature ovarian insufficiency complications e.g. infertility, osteoporosis, and menopausal symptoms.

Sklavos, Giri et al JCEM 2014
FA: Adult Males

- Short stature
- Infertility associated with low sperm count
- Endocrine problems: cholesterol, thyroid, growth hormone, metabolic syndrome, small pituitary, osteopenia
- Cancer
  - AML
  - HNSCC
FA Surveillance/Management

- Every 4-6 months (or more as needed): CBC
- Annual:
  - BM aspirate/biopsy/chromosomes
  - Liver enzymes, chemistries, lipids, thyroid; ultrasound
  - Dental
  - Head and neck exam with nasolaryngoscopy
  - Gyn exam
  - Skin exam
  - Consider esophageal endoscopy?
- HPV vaccine
Transition from Pediatric to Adult Care

- **When?**
  - Age 18
  - Age 21
  - When leave home for work or college

- **Who decides?**
  - Those with FA
  - Parents
  - Doctors

- **How?**
Inherited bone marrow failure syndromes (IMFS) are rare disorders in which there is usually some form of aplastic anemia (failure of the bone marrow to produce blood), associated with a family history of the same disorder. Some of these conditions have typical changes in physical appearance or laboratory findings which suggest a specific diagnosis. There are several well-described syndromes which can be recognized by health care experts. There are also patients who are harder to classify, but who appear to belong in this category.

Patients with these syndromes have a very high risk of development of cancer (either leukemia or certain solid tumors). At the moment we cannot predict which specific patient with an IMFS is going to develop cancer. The NCI EMFS (Consort Study will enroll North American families in which at least one member has or had an IMFS. We plan to:

- Include individuals known to have an IMFS as well as their first degree relatives (brothers, sisters, parents, and children);
- Collect clinical information from study participants and their physicians;
- Perform detailed physical examinations, x-rays and routine laboratory tests on those who are interested in traveling to the NH to be seen in person by our team;
- Attempt (on a research basis) identification of the specific genetic mutation that is associated with each family's disease;
- Screen participants for early cancers related to the specific cancers that occur in each syndrome;
- Perform detailed research laboratory studies on blood and tumors collected from study participants, in an effort to understand the process by which cancers develop;
- Monitor study participants in an ongoing fashion to determine the rates of which complications develop related to each disease, and to identify those complications more precisely;
- Provide suggestions to study participants and their physicians regarding how to best take care of family members who are affected with a particular IMFS, and offer genetic counseling, and an opportunity to learn the results of mutation testing, for those persons who decide that this information will be of use to them.

The Principal Investigator responsible for this study is Blanche P. Alter, MD, MPH. For further information regarding her credentials and experience, please see: http://dcpw.cancer.gov/graphics/Alter.html.

Our overall goal is to reach a better understanding of how cancers develop in persons with IMFS, so that we may improve the health care which can be offered to persons with these disorders.

Clinical Genetics Branch: Neelam Giri, Sharon Savage
Westat: Lisa Leathwood, Maureen Risch, Ann Carr