Fanconi Anemia 101

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When You Hear …., Think …., Or ….
Some Things are Clear; Some are Not
Hummingbirds in Cuba

Bee, 1.5 inch

Emerald, 4 inch
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?
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
FA Gene?

All photos with permission
### FA Literature Cases with VACTERL-H

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

108/2245 literature FA cases, 1927-2012, had least 3 features. 29 called VATER in the reports. 103/108 had R (renal) and/or L (limb).

Alter, Rosenberg: Molecular Syndromology, 2013
FA Literature: Age at Diagnosis
1927-2011

Shimamura and Alter, Blood Reviews 2010
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
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

M:F 1.2:1

Shimamura and Alter, Blood Reviews 2010
Laboratory Findings in FA

- Low blood counts (pancytopenia)
- Large red cells (macrocytosis)
- 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: Hands

Right

Left
Hb and MCV

WBC and Platelets

Alter, J Hand Surg, 1992
FA: Surgery

- Recommendations to surgeons and anesthesiologists:
  - Hb, platelets
  - MCV - increased early in marrow failure
  - *Trends* may precede abnormal values
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, Afrikaaners, 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
FA Inheritance

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

- Children of persons with FA:
  - Each child will have one FA gene (carriers)
Autosomal Recessive Inheritance

Affected

Carriers

Normal
Autosomal Recessive Inheritance

Child of affected and unaffected
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
# FA: Complementation Groups/Genes

<table>
<thead>
<tr>
<th>Group</th>
<th>Locus</th>
<th>cDNA</th>
<th>Exons</th>
<th>AA</th>
<th>%</th>
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<tr>
<td>A</td>
<td>16q24.3</td>
<td>5.5</td>
<td>43</td>
<td>1455</td>
<td>~70</td>
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<tr>
<td>B</td>
<td>Xp22.31</td>
<td>2.8</td>
<td>10</td>
<td>859</td>
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<tr>
<td>C</td>
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<td>4.6</td>
<td>14</td>
<td>558</td>
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<td>D1/BRCA2*</td>
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<td>10</td>
<td>536</td>
<td>~5</td>
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<td>1</td>
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<td>I/KIAA1794</td>
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<td>20</td>
<td>1249</td>
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<tr>
<td>L/PHF9/POG</td>
<td>2p15-16.1</td>
<td>1.7</td>
<td>14</td>
<td>375</td>
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<tr>
<td>M/Hef</td>
<td>14q21.3</td>
<td>6.5</td>
<td>22</td>
<td>2014</td>
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<tr>
<td>N/PALB2*</td>
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<td>13</td>
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<tr>
<td>O/RAD51C*</td>
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<td>76</td>
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<tr>
<td>P/SLX4*</td>
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<tr>
<td>Q/ERCC4/XPF</td>
<td>16p13.12</td>
<td>39.2</td>
<td>11</td>
<td>916</td>
<td>Rare</td>
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*Breast cancer genes
16 FA Genes

Adapted from Leiden Open Variation Database, http://chromium.liacs.nl/LOVD2/FANC/home.php
FA/BRCA DNA Repair Pathway

DNA damage
Oxidative stress
Cytokines

Fanconi Anemia
Core Complex

ubiquitin

UBE2T
USP1

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

- Characteristic birth defects (e.g., 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 cell lines
- Complementation with retroviruses
- Sequencing of candidate genes (e.g., \textit{FANCC} IVS4+4 A\textgreater{}T)
- Sequencing of all cloned genes
- NextGen sequencing: exome, GWAS, etc

\textit{Blood lymphocytes, skin fibroblasts}
Chromosome Breakage
FA: D2 Ubiquitination

Shimamura et al, Blood, 2002

Green and Kupfer, HemOnc Clin NA, 2009
Complementation Analysis, Cloned FANC Genes

- FA cells are sensitive to DEB or MMC
- Introduce specific cloned FA genes
- Cells no longer sensitive
  - Normal gene ‘complemented’ patient cells, defining the complementation group
- Cells still sensitive
  - Normal gene not identified for patient 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

Cell survival [%]

c (MMC) [nM]

S11EG
SFA
S11FCIEG
S11FEIEG2
S11FFIEG
S11FG

FANCA
Exome Sequencing and Filtering

- Exome sequencing cases
- Coding variants
- Exome sequencing controls
- Exclude common variants
- Candidate genes
- Genetic variation databases

http://www.nature.com/ng/journal/v42/n1/images/ng0110-13-F1.jpg
Blood Production (Hematopoiesis)

- Pluripotent Stem Cell
  - Lymphoid Stem Cell
    - T Lymphocytes
    - B Lymphocytes
  - Myeloid Stem Cell
    - Neutrophils
    - Monocytes
    - Eosinophils
    - Basophils
    - Red Cells
    - Platelets
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
Gastroenterology

- **Anatomical**
  - Esophageal atresia, tracheoesophageal fistula, duodenal atresia, imperforate anus

- **GI Symptoms**
  - Reflux, gastric emptying delay, poor appetite

- **Liver disease**
  - Androgens: abnormal liver function, peliosis, adenomas, hepatomas,
  - Transfusions: iron overload

- **Nutrition**
  - Enteral supplements via NG or NJ tubes or gastrostomies
Endocrine, %

- Osteopenia/porosis, % of adults: 92
- Puberty abnormal: 73
- Abnormal lipids: 65
- Short +/- GHD: 55
- Glucose/insulin: 51
- Hypothyroid: 37
- Hypothyrroid: 39
- Obese: 27
- Metabolic syndrome: 21
- Any: 17
- Midline brain: 14
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
Aplastic Anemia: 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: 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
FA: Medical Treatment

- Oxymetholone
  - 2-5 mg/kg/day oral
- Danazol
  - ~200-400 mg/day oral
- Folic acid
  - 1 mg/day oral
FA: Treatment with G-CSF

- 5 μg/kg/day subcutaneous
- Decrease dose and/or give on alternate days
- Keep absolute neutrophil count >1000/mm³
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
188 leukemias and 286 solid tumors in 413/2190 patients; 47 had 2-4 cancers.
FA Risk of Adverse Events

**Annual Risk**
- BMF
- AML
- ST

**Cumulative Incidence**
- BMF
- AML
- ST

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

Data from North American Survey, Germany, Israel, and NCI
FA: Adult Females

- Late onset of menses (14-16)
- Heavy periods if platelets low
- Early onset of menopause (30s)
- Decreased fertility
- Increased need for Caesarean sections
- Worsening bone marrow function during pregnancy
- Osteoporosis
- Cancer
  - AML
  - HNSCC
  - Vulva, vagina, cervix
- HPV Vaccine
FA: Adult Males

- Short stature
- Infertility
- Endocrine problems: cholesterol, thyroid, growth hormone, metabolic syndrome, small pituitary, osteopenia
- Cancer
  - AML
  - HNSCC
- HPV vaccine
FA Surveillance/Management

- Every 4-6 months (or more as needed): CBC
- Annual:
  - BM aspirate/biopsy/chromosomes
  - Liver enzymes, chemistries, lipids, thyroid
  - Liver ultrasound
  - Dental
  - Head and neck with laryngoscopy
  - Gyn exam
  - Skin exam
  - Consider esophageal endoscopy?
- HPV vaccine
Hypotheses re: the Role of HPV

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.
1. None of 9 FA and DC HNSCC tumors 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 Survival before and after 2000

Overall Survival

\[ p < 0.001 \]

\(~80\%\) survival > age 18
Field Trip

Even the car rental companies want to be helpful for FA research.
Inherited bone marrow failure syndromes (IBMFS) 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 in 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 IBMFS is going to develop cancer. The NCI EMFS (Contact) Study will enroll North American families in which at least one member has had an IBMFS. We plan to:

1. **Include individuals known to have an IBMFS as well as their first degree relatives (brothers, sisters, parents, and children).**
2. Collect clinical information from study participants and their physicians.
3. 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.
4. Attempt (on a research basis) identification of the specific genetic mutation that is associated with each family’s disorder.
5. Screen participants for early changes related to the specific cancer that occur in each syndrome.
6. Perform detailed research laboratory studies on blood and tumors collected from study participants, in an effort to understand the process by which cancers develop.
7. Monitor study participants in an ongoing fashion to determine the rate at which complications develop related to each disease, and to identify those complications more precisely.
8. Provide suggestions to study participants and their physicians regarding how to best take care of family members who are affected with a particular IBMFS, and
9. 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://cancer.gov/aboutnci/publications/Alter.html](http://cancer.gov/aboutnci/publications/Alter.html).

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