Genetics and genomics of Fanconi Anemia

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Presentation outline

• Phenotype of Fanconi Anemia
• Molecular pathogenesis
• Clinical genetic testing
• Monitoring for BMF and MDS/AML
• Case example

Fanconi Anemia (FA)

• Rare autosomal recessive bone marrow failure (BMF) syndrome
  – 2% of cases are X-linked recessive
• Prevalence is 1/160,000
• Characterized by:
  – Physical abnormalities
  – BMF
  – Increased risk for malignancy
Physical abnormalities associated with FA

- Found in >70% of patients
- Growth retardation or short stature
- Abnormal skin pigmentation
- Skeletal malformations of the limbs (mostly abnormal thumbs)
- Microcephaly
- Ophthalmic and genitourinary tract anomalies

Growth retardation and abnormal skin pigmentation

Skeletal malformations of the limbs
Genitourinary tract anomalies
• Horseshoe kidney

Bone marrow failure
• Progressive BMF with pancytopenia typically presents in first decade
• Thrombocytopenia or leukopenia precede anemia
• Pancytopenia worsens over time
• Risk of developing any hematological abnormality is 90% by 40 years

Increased risk for malignancy
• Increased risk of developing myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)
• Relative risk for AML is increased 500-fold
• 13% of FA patients will develop AML by 50 years
• Increased risk of developing solid tumors of the head and neck, skin, gastrointestinal and genitourinary tract
  – Squamous cell carcinoma
• FA patients hypersensitive to radiation and alkylating agents
  – Makes therapy tricky
Molecular pathogenesis of FA

- Locus heterogeneity
  - ~20 genes known to cause FA
- FA pathway maintains genome stability
- Major role in responding to replication stress by facilitating the resolution of DNA lesions
- FA proteins involved in lesion recognition and repair cascade

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Complementation group</th>
<th>% of FA with pathogenic variants in this gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>AR</td>
<td>D1</td>
<td>~3%</td>
</tr>
<tr>
<td>BRIP1</td>
<td>AR</td>
<td>J</td>
<td>~2%</td>
</tr>
<tr>
<td>FANCA</td>
<td>AR</td>
<td>A</td>
<td>80%-10%</td>
</tr>
<tr>
<td>FANCB</td>
<td>XLR</td>
<td>B</td>
<td>~2%</td>
</tr>
<tr>
<td>FANCC</td>
<td>AR</td>
<td>C</td>
<td>~14%</td>
</tr>
<tr>
<td>FANCD2</td>
<td>AR</td>
<td>D2</td>
<td>~3%</td>
</tr>
<tr>
<td>FANCE</td>
<td>AR</td>
<td>E</td>
<td>~3%</td>
</tr>
<tr>
<td>FANCF</td>
<td>AR</td>
<td>F</td>
<td>~2%</td>
</tr>
<tr>
<td>FANCG</td>
<td>AR</td>
<td>G</td>
<td>~10%</td>
</tr>
<tr>
<td>FANCI</td>
<td>AR</td>
<td>I</td>
<td>~1%</td>
</tr>
</tbody>
</table>

AR-autosomal recessive, AD-autosomal dominant, XLR- X-linked recessive
Adapted from Gene Reviews: Fanconi Anemia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance</th>
<th>Complementation group</th>
<th># individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC4</td>
<td>AR</td>
<td>G</td>
<td>2</td>
</tr>
<tr>
<td>FANCL</td>
<td>AR</td>
<td>L</td>
<td>5 families</td>
</tr>
<tr>
<td>FANCM</td>
<td>AR</td>
<td>M</td>
<td>1</td>
</tr>
<tr>
<td>MAD2L2</td>
<td>AR</td>
<td>V</td>
<td>1</td>
</tr>
<tr>
<td>PALB2</td>
<td>AR</td>
<td>N</td>
<td>9</td>
</tr>
<tr>
<td>RAD51</td>
<td>AD</td>
<td>R</td>
<td>2</td>
</tr>
<tr>
<td>RAD51C</td>
<td>AR</td>
<td>Q</td>
<td>1</td>
</tr>
<tr>
<td>SLX4</td>
<td>AR</td>
<td>P</td>
<td>4</td>
</tr>
<tr>
<td>UBE2T</td>
<td>AR</td>
<td>T</td>
<td>1</td>
</tr>
<tr>
<td>XRCC3</td>
<td>AR</td>
<td>U</td>
<td>1</td>
</tr>
</tbody>
</table>

AR-autosomal recessive, AD-autosomal dominant, XLR- X-linked recessive
Adapted from Gene Reviews: Fanconi Anemia
Clinical diagnosis of FA

- FA should be suspected in individuals with the following findings:
  - Physical features
    - Short stature, limb anomalies, etc.
  - Laboratory findings
    - Macrocytosis, cytopenia
  - Pathology findings
    - BMF, aplastic anemia, MDS/AML

Establishing the diagnosis: Clinical genetic testing for FA

- Chromosomal breakage studies
- Molecular Genetic Testing
  - Targeted mutation analysis
  - Sequence analysis (NGS/Sanger)
  - Deletion/duplication testing
- Other testing methods
  - Determination of complementation groups
  - Cell cycle arrest
Chromosomal breakage studies for FA

- T-lymphocytes are cultured in the presence of clastogens
  - Diepoxybutane (DEB) (Gold standard)
  - Mitomycin C (MMC)
  - Creates intra- and interstrand crosslinks
  - Forces cell to try to resolve the breaks
- FA cells have increased chromosome breakage compared to concurrent normal control

<table>
<thead>
<tr>
<th>MMC induced breakage compared to controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing amounts of MMC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Cells</th>
<th>MMC</th>
<th>50nM</th>
<th>150nM</th>
<th>300nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>25</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>35</td>
<td>45</td>
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<tr>
<td>4</td>
<td>30</td>
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<td>60</td>
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<tr>
<td>5</td>
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<td>55</td>
<td>65</td>
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<tr>
<td>6</td>
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<td>50</td>
<td>60</td>
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</tr>
<tr>
<td>7</td>
<td>45</td>
<td>55</td>
<td>65</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>65</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
</tr>
</tbody>
</table>
Chromosome breakage methodology

• Set-up concurrent cultures for FA patient and control

<table>
<thead>
<tr>
<th>FA patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (untreated) culture</td>
<td>Baseline (untreated) culture</td>
</tr>
<tr>
<td>DEB</td>
<td>DEB</td>
</tr>
<tr>
<td>MMC</td>
<td>MMC</td>
</tr>
</tbody>
</table>

• Add MMC to cultures at set-up
• Add DEB to cultures on day 3
• Harvest cultures on day 4

Scoring FA aberrations

• Score breakage in 25 metaphases from the FA and control baseline cultures
• Score breakage in 50 metaphases from the FA and control DEB and MMC cultures

<table>
<thead>
<tr>
<th>Control</th>
<th>Number of cells</th>
<th>FA</th>
<th>Number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>25</td>
<td>Baseline</td>
<td>25</td>
</tr>
<tr>
<td>DEB</td>
<td>50</td>
<td>DEB</td>
<td>50</td>
</tr>
<tr>
<td>MMC</td>
<td>50</td>
<td>MMC</td>
<td>50</td>
</tr>
</tbody>
</table>

ISCN Chromatid Aberrations

• Chromatid gap-chtg
• Chromatid break-chtb
• Chromatid exchange-chte
• Triradial-tr
• Quadriradial-qr
• Complex-cx
How to record FA aberrations

- Chromatid gap
  - 1 break
- Chromatid break
  - 1 break
- Triradial
  - 2 breaks
- Quadriradial
  - 2 breaks

More than 10 breaks in a cell is termed "too many to count" (TMTC) and is scored as 10.

Nonconvincing aberrations

- Should not be scored:
  - Gap not as wide as a chromatid
  - Acrocentric associations
  - Two overlapping chromosomes

Technical analysis

Breaks per cell = total breakage events / total cells counted

Tech 1

<table>
<thead>
<tr>
<th>Breaks per cell</th>
<th>Total Breaks</th>
<th>Total Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
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</tbody>
</table>

Tech 2

<table>
<thead>
<tr>
<th>Breaks per cell</th>
<th>Total Breaks</th>
<th>Total Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Reporting results

- Each laboratory will have a range for average breaks per cell in each culture (baseline, DEB, MMC)
- Example:

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>DEB</th>
<th>MMC</th>
</tr>
</thead>
<tbody>
<tr>
<td># cells counted</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Normal range</td>
<td>0-0.20</td>
<td>0-0.25</td>
<td>0-0.70</td>
</tr>
<tr>
<td>Fanconi range</td>
<td>0.25</td>
<td>0.40-7</td>
<td>&gt;1.00</td>
</tr>
</tbody>
</table>

- Result is consistent with FA if MMC and/or DEB cultures are positive

FA breakage on skin fibroblasts

- Used if peripheral blood analysis has:
  - Previously failed
  - Low WBC cell count
  - Negative or equivocal, but still suspect FA
    - Somatic mosaicism (10-20% of patients)

Back to the testing algorithm
Mutation analysis for FA

- Typically performed after positive breakage studies
- Identifying mutations is valuable because:
  - Recurrence risk
  - Test at-risk family members
  - Prenatal testing
  - Genotype/phenotype correlation
  - Future research possibilities

Mutation analysis for FA

- Multi-gene panels (NGS) or targeted mutation analysis (Sanger)
- NGS panel contains most common FA genes
  - May include “FA-like” genes
  - Rare or recently identified FA genes may not be included

<table>
<thead>
<tr>
<th>BRCA2 (FANCD1)</th>
<th>FANCJ</th>
<th>FANCI</th>
<th>PALB2 (FANCN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRIP1 (FANCI)</td>
<td>FANCD2</td>
<td>FANCC</td>
<td>RAD51C</td>
</tr>
<tr>
<td>FANCA</td>
<td>FANC</td>
<td>FANCL</td>
<td>SLX4 (FANCP)</td>
</tr>
<tr>
<td>FANCB</td>
<td>FANCF</td>
<td>FANCM</td>
<td></td>
</tr>
</tbody>
</table>

Back to the testing algorithm
Deletion/duplication analysis for FA

- Reflexed after positive breakage but negative or equivocal NGS testing
  - No mutations or one mutation identified by NGS
    - Homozygous deletion or compound heterozygous for a mutation and a del/dup
      - Performed by aCGH or MLPA
  - Del/dup pathogenic variants reported in:
    - FANCA (40%)
    - FANCB
    - FANCD2
    - FANCF
    - FANCI

Example of del/dup for FANCA gene

Exons:1-3
arr[GChR37] 16q24.3(89880335_89928824)x0

Back to the testing algorithm
Complementation studies for FA

- Complementation studies
  - Historically, a breeding or cell culture experiment to establish the relationship between two recessive mutations after crossing the parents or donor cells.

- Complementation group
  - Able to sub-classify cell lines from patients without knowing what the defective genes or DNA mutations are.

Complementation studies

Complementation group A

\[ \text{gene 1} \quad \text{gene 2} \]

\[ \text{X} \]

Complementation group B

\[ \text{gene 1} \quad \text{gene 2} \]

\[ \text{gene 1} \quad \text{gene 2} \]

\[ \text{X} \]

Normal phenotype

Complementation studies for FA

- Currently, a cell culture experiment that utilizes retroviral gene transfer to restore the phenotype.

Grumpy FA patient cell line with unknown complementation group
Complementation studies for FA

- Currently, a cell culture experiment that utilizes retroviral gene transfer to restore the phenotype

Cells uptake the virus and cDNA

Retrovirus harboring wild-type cDNA for FANCC

cDNA incorporates into genome and begins expression of wild-type FANCC and restores the phenotype

Cell-cycle arrest in FA

- FA cells have increased cell-cycle arrest at the G2/M phase after treatment with crosslinking agents
- Can compare G2/M arrest in control cells and FA cells by flow cytometry
Monitoring for bone marrow dysfunction

- Bone marrow evaluation and blood counts
  - Morphological and cytogenetic analysis
  - Monitor for cytopenias
  - Identify early signs of MDS/AML
  - Critical for treatment strategies
  - Hematopoietic stem cell transplant is the only curative therapy
- Timing is critical

Cytogenetic analysis of FA cells

- Common cytogenetic abnormalities in FA cells as compared to de novo AML

<table>
<thead>
<tr>
<th>Chromosome abnormality</th>
<th>FA AML (%)</th>
<th>De novo AML (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+1q</td>
<td>22</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-7</td>
<td>17</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7q-</td>
<td>11</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>+3q</td>
<td>11</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

No risk of MDS-AML based on 1q gain

- P-value < 0.001 for chromosome 1q gain
Increased risk of MDS-AML based on 3q gain

Cancer Genetics and Cytogenetics (2010) 203:108-186; P<.001

Increased risk of MDS-AML based on monosomy 7


Fluorescence in situ hybridization (FISH)

FISH analysis
- Evaluate common FA cytogenetic abnormalities
- Correlate with hematopathological findings
- Clonal abnormality not observed
  - Continue annual evaluation
- Clonal abnormality observed
  - Monitor BM for MDS/AML
FA FISH examples

3 copies of 1q25
3 copies of BCL6 (3q27)
1 copy of 7q

Beware! FA FISH analysis can be normal on a direct FISH, but abnormal metaphases can be observed from culture.

G-banded analysis example

Gain of chromosome 1q

Gain of chromosomes 1q, 3q, and 21q, loss of chromosomes 7q, 13q and 20q

Bold aberrations associated with risk of MDS/AML.
FA case example

- 19 yr old Female patient with FA
- Abnormal breakage studies were performed at 4 years of age
  - Consistent with a diagnosis of FA
- Blood counts routinely followed throughout her life
  - Were abnormal but recently stabilized to normal range
- Bone marrow biopsy performed
  - Breakage studies sent

Breakage studies at diagnosis consistent with FA

<table>
<thead>
<tr>
<th>CHROMOSOME STUDIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMBER OF CELLS COUNTED: 500</td>
</tr>
</tbody>
</table>

CHROMOSOME BREAKAGE ANALYSIS

Chromosome breakage analysis was performed with the following results:

MEAN LUNGLOMOSOME BREAK/FULL

| PATIENT   | 0.02 |
| FACTORY    | 0.05 |
| FA RANGE   | 0.02 - 0.90 |

BREAKAGE: 0.02

INTERPRETATION: Baseline and diagnostic (500) induced chromosome breakage are highly elevated and are consistent with the diagnosis of Fanconi anemia.

Current baseline breakage analysis

<table>
<thead>
<tr>
<th>BREAKS PER CELL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
</tr>
</tbody>
</table>

BASELINE
Current DEB breakage analysis

Breaks per cell = 25/50 = 0.50

Normal

Current MMC breakage analysis

Breaks per cell = 21/50 = 0.42

Baseline DEB MMC

Control: 0.02 0.04 0.14
FA Patient: 0 0.50 0.42
Normal Range: 0-0.16 0-0.26 0.04-0.70
FA Range: 0-0.24 0.40-6.22 1.00-18.34

Results

- Baseline breakage: Within normal limits
- DEB breakage: Abnormal
- MMC breakage: Inconclusive

What happened?
Genotypic reversion

- Phenomenon in which spontaneous correction of the mutation occurs
  - Recombination
- Repopulate the peripheral blood
  - Can normalize cell counts
- Fibroblast breakage studies may be necessary

MMC induced breakage in a FA mosaic

Conclusion

- FA is a BMF syndrome characterized by physical and hematological abnormalities
- Extreme locus heterogeneity
- Chromosome breakage test is a first-tier diagnostic test
  - Followed by molecular studies
- FISH and G-banded karyotype analysis is used to monitor BM for clonal changes
- Be aware of genetic reversion the lymphocytes
Acknowledgements

• Teresa Smolarek, Ph.D.
• Lauren Walters-Sen, Ph.D.
• Corrine O’Brien, B.S.
• Pamela Long, Ph.D.

Muffins with Mom!

References


• Fanconi Anemia: Guidelines for Diagnosis and Management

• Schwab, RA et al., The Fanconi Anemia Pathway Maintains Genome Stability by Coordinating Replication and Transcription. Mol Cell. 2015:60(3):351-361


Questions?