SVENSKA GRUPPEN AML

Familial Leukaemia – what genetics is teaching us

Jude Fitzgibbon

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What’s in Store

- Run through the current landscape of familial leukaemia.
- Genetic testing
- The transcription factor \textit{CEBPA}
- Identify new loci
- New ideas emerging from the study of inherited leukaemia.
Acute Myeloid Leukaemia (AML)

- Haematopoietic Stem Cell Disorder
- Median age: 68 years
- Incidence: 3/100,000 in Europe
- 5-year overall survival: 42%
Familial AML/MDS

- > 95% of all AML cases are sporadic.
- <= 5% of the cases where two or more affected individuals are found within the same family.
- New WHO Entity 2016 provisional diagnostic category for heritable myeloid malignancies
Clinical challenges in Familial AML

1. Recognition of inherited forms of these diseases is difficult;
   - Patients may be unaware of their predisposition.
   - Wide variation in the age of onset and disease phenotype.
   - Absence of customized diagnostics.

2. Variable penetrance of the disease mutations: symptomatic and asymptomatic carriers.

Roadmap to improve diagnosis, treatment and management

- Identification of new familial cases, Individual at risk
- Tissue bank (Data collection)
- Molecular diagnostics, 13 predisposition loci
- New disease genes and functional validation

NOVEL GUIDELINES AND IMPROVED MANAGEMENT
Familial MDS/AML can be divided into three groups:

• Examples where approved testing exists.
• Emerging from basic research and requiring validation.
• Without an identified genetic basis.

(Little is known regarding secondary genetic mutations)
<table>
<thead>
<tr>
<th>GENE</th>
<th>AUTHORS</th>
<th>YEAR</th>
</tr>
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<tbody>
<tr>
<td>RUNX1</td>
<td>Song et al. Boston, Massachusetts, USA</td>
<td>1999</td>
</tr>
<tr>
<td>TERC</td>
<td>Vulliamy et al. London, UK</td>
<td>2001</td>
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<td>CEBPA</td>
<td>Smith et al. London, UK</td>
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<td>TERT</td>
<td>Hiroki Yamaguchi et al. Atlanta, USA</td>
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<td>GATA2</td>
<td>Hahn et al. Adelaide, Australia</td>
<td>2011</td>
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<td>ANKRD26</td>
<td>Noris et al. Pavia, Italy</td>
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<td>SRP72</td>
<td>Kirwan et al. London, UK</td>
<td>2012</td>
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<td>ACD</td>
<td>Guo et al. Philadelphia, USA</td>
<td>2014</td>
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<tr>
<td>ATG2B/GSKIP</td>
<td>Saliba et al. Villejuif, France</td>
<td>2015</td>
</tr>
<tr>
<td>DDX41</td>
<td>Polprasert et al. Cleveland, USA</td>
<td>2015</td>
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<tr>
<td>SAMD9</td>
<td>Narumi et al. Tokyo, Japan</td>
<td>2016</td>
</tr>
<tr>
<td>SAMD9L</td>
<td>Tesi et al. Stockholm, Sweden</td>
<td>2017</td>
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</table>
Current Mutational Landscape – 13 genes and counting

Myeloid malignancies only
AML: *CEBPA*
MDS/AML: *DDX41*
MPNs/AML: *ATG2B/GSKIP*

Cytopenias and/or platelet dysfunction
FPD/AML: *RUNX1*
GATA2 deficiency
Thrombocytopenia 2: *ANKRD26*
Thrombocytopenia 5: *ETV6*

Bone marrow failure syndromes
Telomere syndromes: *TERT, TERC, ACD*
Aplastic anemia/MDS: *SRP72*

Other syndromes
Cytopenia, immunodeficiency, MDS, and neurological symptoms: *SAMD9L*
MIRAGE syndrome: *SAMD9*

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Diagram showing the timeline from 1999 to 2018 with genes and syndromes categorized as follows:
- Lymphoid: 1999
- Myeloid: 2018

Gene colors and categories correspond to the text descriptions above.
Targeted sequencing panel for familial MDS/AML

Index cases were tested for mutation in 11 known disease genes.

- ACD
- ANKRD26
- ATG2B
- CEBPA
- DDX41
- ETV6
- GATA2
- RUNX1
- SRP72
- TERC
- TERT

West Midlands Regional Genetics Laboratories

Birmingham Women's NHS Foundation Trust

Birmingham, United Kingdom
Our Interest in Familial *CEBPA*

**BRIEF REPORT**

*Mutation of CEBPA in Familial Acute Myeloid Leukemia*

Matthew L. Smith, M.B., B.S., Jamie D. Cavenagh, M.D., T. Andrew Lister, M.D., and Jude Fitzgibbon, Ph.D.

*The New England Journal of Medicine*

*Smith et al.*, NEJM 2004
International collaboration on Familial *CEBPA*-AML: 25 patients – 11 pedigrees

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Year</th>
<th>Location</th>
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<tbody>
<tr>
<td>Smith</td>
<td><em>NEJM</em>, 2004</td>
<td>UK</td>
<td></td>
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<td>Sellick</td>
<td><em>Leukemia</em>, 2005</td>
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<td>Pabst et al</td>
<td><em>JCO</em>, 2008</td>
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<td>Nanri</td>
<td><em>GCC</em>, 2010</td>
<td>Japan</td>
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<td>Taskesen</td>
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<td>Savic A</td>
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<td>Debeljak</td>
<td><em>Haematologica</em>, 2013</td>
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</table>
Distribution of CEBPA mutations

Germline mutations

Acquired mutations

Key
- In-frame insertion or deletion
- Frameshift mutation
- Missense mutation

ATG (p42)

ATG (p30)

TAD1

TAD2

DBD

Leucine zipper

K313
Timeline of clinical events in all 25 Familial CEBPA AMLs

- AML + chemo
- AML + auto (syngeneic *)
- AML + allo
- Alive
- Dead
- WES
Comparison Familial versus MRC CEBPA series

A) Overall survival

B) Post-relapse survival

Tawana et al., Blood 2015
Patients are cured of initial disease but are predisposed to new leukemic episodes

New *CEBPA* C-terminal mutations at relapse

<table>
<thead>
<tr>
<th>Pedigree/individual</th>
<th>Diagnosis</th>
<th>‘Relapse’</th>
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<tr>
<td>B/II.1</td>
<td>p.R306ins3bp</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>13 mths</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 yrs</td>
<td></td>
</tr>
<tr>
<td>E/II.1</td>
<td>p.Q305ins18bp</td>
<td>p.Q312dup</td>
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<tr>
<td></td>
<td>6.6 yrs</td>
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Familial *CEBPA* reminiscent of dm*CEBPA*

**Familial cases**

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<tr>
<th>Karyotype</th>
<th>CEL-N</th>
<th>CEL-C</th>
<th>CEL-TAD</th>
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<tr>
<td>GATA2</td>
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<td></td>
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</tr>
<tr>
<td>WT1</td>
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<td>SMClα</td>
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<td>SMCl3</td>
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<td>CSF3R</td>
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<td>NRAS</td>
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<td>PTPN11</td>
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<td>NPM1</td>
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<td>DNMT3A</td>
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<td>IDH1</td>
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<td></td>
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<tr>
<td>DXH37</td>
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<tr>
<td>Total mutations</td>
<td>4</td>
<td>3</td>
<td>3</td>
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</table>

**TCGA SAMPLES**

- Normal karyotype
- Monosomy 7
- Recurrent mutation
- Identical mutation
- Non-recurrent mutation
- Complex karyotype

Tawana *et al.*, Blood 2015
Collective experience

• **Chemotherapy alone** can be used to treat patients without adverse disease features.

• High frequency of **late ‘chemosensitive’ relapses**, where intensive consolidation, preferably with allogeneic transplantation, may well prove beneficial in preventing further disease events.

• **Perform germ-line testing in sporadic CEBPA AML of <40 yrs at diagnosis.**

• **Counselling** of affected individuals and asymptomatic carriers requires full knowledge and understanding of the implications of inherited CEBPA mutations and, above all, recognition and consideration for an individual’s choice.

• Where possible, we advocate **screening of all potential sibling donors**, preparing for both treatment escalation in affected cases and identifying asymptomatic mutation carriers for counselling and surveillance.
Donor cell leukaemia in *DDX41* families

**FAMILY 1**

- **DDX41 c.3G>A**
- Berger *et al.*, 2017

**FAMILY 2**

- Kobayashi *et al.*, 2017

Germline *DDX41* mutation
- Patient 49 years
  - p.F498fs (53.1%)
  - p.R525H (7.9%)

Somatic *DDX41* mutation
- Donor 56 years
  - p.F498fs (47.3%)
  - p.R525H (0.4%)

MDS/AML
- AML 74 years (nt)
- MDS 79 years (nt)

Deceased (nt) not tested mutational analysis
NA not available clinical data
Lesson from familial leukaemia

- Genes not mutated in sporadic AML - Novel mechanism
- Under appreciated mechanisms of mutation
- Clustering of mutations within pedigrees - Host genetics
- A clever way of making a monosomy
- Disease Latency and penetrance - Protection factor
Our cohort of MDS/AML families – new Loci (Vulliamy, Dokal & Fitzgibbon)

82 MDS/AML families

Known Genes Sequencing Panel
West Midlands Regional Genetics Laboratories
Birmingham Women's NHS Foundation Trust

46 uncharacterised families

41 characterised families

WES

<table>
<thead>
<tr>
<th>MUTATED GENE</th>
<th>NUMBER OF FAMILIES</th>
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<tbody>
<tr>
<td>CEBPA</td>
<td>7</td>
</tr>
<tr>
<td>DDX41</td>
<td>5</td>
</tr>
<tr>
<td>GATA2</td>
<td>7</td>
</tr>
<tr>
<td>RUNX1</td>
<td>12</td>
</tr>
<tr>
<td>SRP72</td>
<td>2</td>
</tr>
<tr>
<td>TERC</td>
<td>3</td>
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<td>TERT</td>
<td>4</td>
</tr>
<tr>
<td>ETV6</td>
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Challenges in identifying a familial MDS/AML loci

PEDIGREE 1

Coronary artery disease
AML

MDS
RUNX1
p.D96H

AML

3

2

TCP
RUNX1
p.D96H

TCP
RUNX1
p.D96H

MDS
RUNX1
p.D96H

WES

57 Germline variants

RUNX1
p.D96H

family
Challenges in identifying a familial MDS/AML loci

FAMILIES WITHOUT A CLEAR CANDIDATE

UNCHARACTERISED PEDIGREES

WES data integration → Fixed criteria of filtering → Putative candidate genes → Functional validation
Challenges in identifying a familial MDS/AML loci

HOW TO FIX CRITERIA OF FILTERING?

1. Variant frequency (e.g. ExAC <0.0001)

2. Predicted to be damaging (Polyphen, MutationTaster, SIFT, Provean scores)

3. In at least 2 families (?)

4. Others: family phenotype, protein function, type of mutation, mutated in sporadic AML, other scores (PhyloP, GERP, and CADD)
Some putative candidate genes

<table>
<thead>
<tr>
<th>GENE</th>
<th>VARIANT FREQUENCY (ExAC)</th>
<th>PREDICTED TO BE DAMAGING</th>
<th>IN AT LEAST 2 FAMILIES</th>
<th>PROTEIN FUNCTION</th>
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<tbody>
<tr>
<td>ADA</td>
<td>0</td>
<td>YES</td>
<td>YES (2 families)</td>
<td>Adenosine deaminase (SCID related)</td>
</tr>
<tr>
<td>DHX34</td>
<td>&lt; 0.0001</td>
<td>YES</td>
<td>YES (4 families)</td>
<td>DExH-box helicase (involved in NMD)</td>
</tr>
<tr>
<td>PDCD4</td>
<td>0</td>
<td>YES</td>
<td>YES (2 families)</td>
<td>Potential TSG (downregulated in AML)</td>
</tr>
<tr>
<td>PRR23B</td>
<td>0</td>
<td>Not all variants</td>
<td>YES (3 families)</td>
<td>Proline Rich 23B (regulated by GATA2)</td>
</tr>
<tr>
<td>VPS13C</td>
<td>&lt; 0.0001</td>
<td>Not all variants</td>
<td>YES (5 families)</td>
<td>Vacuolar Protein (mitochondrial function)</td>
</tr>
</tbody>
</table>
Lessons from familial leukaemia

• Genes not mutated in sporadic AML - Novel mechanism
• **Under appreciated mechanisms of mutation**
• Clustering of 2\textsuperscript{nd} mutations within pedigrees - Host genetics
• A clever way of making a monosomy
• Disease Latency and penetrance - Protection factor
Atypical mutations: intragenic deletion in *RUNX1* – 20% of pedigrees

**Deletion breakpoints**

- **chr21:36389492-37056053** – 66,6561 bp deletion

**CGH Array**

1. DNA labeling
2. Comparative hybridisation
3. Scan of fluorescence signals
4. Normalisation, data analysis
5. Interpretation

**RUNX1 – Chr 21- 1.21Mb**

**Deletion breakpoints** chr21:36389492-37056053 – 66,6561 bp deletion
Germ-line GATA2 – synonymous mutations

Major classes of germline mutations in cases GATA2 deficiency:

1. Truncating (stop-gain, frameshift, splice-site);
2. Missense within zinc fingers;
3. Noncoding substitutions in the conserved EBOX-GATA-ETS regulatory region (intron 4);
4. In frame indels and whole gene deletions.
5. Silent (synonymous) exonic substitutions – Splice defects

2449 Systematic Assessment of GATA2 Genetic Variation Reveals the Presence of Novel Disease-Causing Synonymous Exonic Mutations

Bone Marrow Failure
Program: Oral and Poster Abstracts
Session: 508. Bone Marrow Failure: Poster II

Sunday, December 10, 2017, 6:00 PM-8:00 PM
Bldg A, Lvl 1, Hall A2 (Georgia World Congress Center)

Emilia J. Kozyra, MSc1,2, Victor Pastor Loyola, MSc3,4, Claudia Wehr5,6, Sushree Sangita Sahoo, M.Sc.3,7,8, Rebecca Voss9, Enikő Amina Szvetnik10, Shinuke Hirabayashi, MD11, Albert Catala12, Henrik Hasle, MD, PhD13,14, Marry M. Van den Heuvel-Elbrink, MD15,16, Krisztíán Kallay, MD17, Riccardo Masetti, MD, PhD18, Barbara De Moerloose, MD, PhD19, Markus Schmugge, MD20, Owen Smith, MD21, Marek Ussowicz22, Jan Stary23, Ester Mejštrikova24, Ramuné Pasaulytė25, Irith Baumann, Dr. med.26, Gudrun Göhring, MD27, Brigitte Schlegelberger, Prof. Dr.27, Ulrich Salzer6,28, Michael Lübbert29, Eirini Trompouki, PhD30, Charlotte M. Niemeyer, MD11 and Marcin W. Wlodarski, MD, PhD32
Lessons from familial leukaemia

• Genes not mutated in sporadic AML - Novel mechanism
• Under appreciated mechanisms of mutation
• **Clustering of 2\textsuperscript{nd} mutations within pedigrees** - Host genetics
• A clever way of making a monosomy
• Disease Latency and penetrance - Protection factor
Clustering of same secondary mutations in pedigrees - GATA2

Acquired GATA2 mutations in 3 relatives with germline CEBPA mutations

**CEBPA III.2:**
- P23fs (germ-line)
- K313dup (40%)

**GATA2:**
- N317I (30%)

**CEBPA II.1:**
- P23fs (germ-line)
- K302_K313dup (38%)

**GATA2:**
- L321F (31%)

**CEBPA II.5:**
- P23fs (germ-line)
- Q305_K313dup (38%)

**GATA2:**
- L321F (15%)
- R330Q (23%)
Disease Latency - *RUNX1*

A

1. Affected
2. Asymptomatic carrier

B

- **RUNX1 p.R201***
- **SH2B3**
- **12q aUPD**
- **+21q, -2q**
- **JAK2**
- **9p aUPD**
- **CDC27**
- **RBBP8**
- **-7, -9q**
- **CDC27**
- **JAK2**
- **U2AF2**

- Last follow-up
- Death
- Stem cell transplantation
- Diagnosis of MDS/AML
- Relapse

Tawana et al.
Lesson from familial leukaemia

- Genes not mutated in sporadic AML - Novel mechanism
- Under appreciated mechanisms of mutation
- Clustering of mutations within pedigrees - Host genetics
- **A clever way of making a monosomy**
- Disease Latency and penetrance - Protection factor
1. Heterozygous germline gain-of-function (GoF) mutations in *SAMD9L* are associated with cytopenia, immunodeficiency, MDS and neurological disease (e.g. ataxia-pancytopenia).

2. These GoF mutations cause decreased cell proliferation compared to WT protein and in turn, lead to loss of *SAMD9L*-mutant allele and monosomy 7.

So there is a section to lose the mutation (loss of Chromosome 7) to increase proliferation.
Familial MDS and Transient Monosomy 7 as a Sole Clinical Manifestation of *SAMD9L*-related Disease

Non-random loss of mutant *SAMD9L* allele can be achieved by:
1. Complete (Monosomy 7)
2. Partial (deletion 7q)
3. UPD 7q
4. Somatic truncating *SAMD9L* mutations

**Constitutional *SAMD9L* mutations cause familial myelodysplastic syndrome and transient monosomy 7**

Reduced penetrance in *GATA2*-mutated MDS/AML - p.T354M mutations

Bodor et al., *Haematologica* 2012
1. **ASXL1** mutation (p.Gly646TrpfsTer12) as a secondary genetic event in **GATA2** symptomatic carriers

**ASXL1** mutations are insufficient to promote onset of MDS.
2. *GATA2* monoallelic expression discriminates between symptomatic and asymptomatic carriers and correlates with clinical parameters
3. A CpG-SNP provided a means of distinguishing between alleles.

- **GATA2 locus**
  - **ENHANCER 2 (-77kb)**
  - **PROMOTER 1**
  - **PROMOTER 2**
  - **ENHANCER 1 (+9.5kb)**
  - **p.Thr354Met**

- **Genomic DNA**
  - **III.7_asymptomatic**
  - **IV.10_symptomatic**

- **cDNA**
  - **rs1806462**
  - **c.1061C>T**
  - **A allelic with T**
  - **mutant GATA2 allele → CpG lost**
  - **WT GATA2 allele → CpG gained**

- **promoter 2 SNP**
  - **CCC [C/A] GAG**
4. Epigenetic reprogramming regulates monoallelic *GATA2* expression

1. DNA methylation as a mechanism of silencing the WT allele.
4. Epigenetic reprogramming regulates monoallelic \textit{GATA2} expression

1. DNA methylation as a mechanism of silencing the WT allele.

2. Enhanced H3K4me3 promoter deposition on the mutant allele.

Mutually exclusive
Collaboration is Key

How to improve diagnosis, treatment and management?

- Identification of new familial cases, Individual at risk
- New disease genes and functional validation
- Molecular diagnostics, 14 predisposition loci
- Tissue bank (Data collection)
- Novel guidelines and improved management

Collaborations
• Recognition of familial myeloid neoplasia in adults
• Practical considerations for diagnosis and management of patients and carriers
• **RUNX1** deficiency (familial platelet disorder with predisposition to myeloid leukemia, FPDMM)
• **GATA2** deficiency and related myeloid neoplasms
• Familial **CEBPA**-mutated acute myeloid leukemia
• **DDX41**-related myeloid neoplasia
• **ETV6** in hematopoiesis and leukemia predisposition
• Classical inherited bone marrow failure syndromes with high risk for myelodysplastic syndrome and acute myelogenous leukemia
• Cancer predisposition syndromes associated with myeloid malignancy
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Joanne Mason
Csaba Bodor

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Charlotte Niemeyer (Germany)
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Lucy Godley (USA)

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