Haematological Insights from Down Syndrome

Irene Roberts
Professor of Paediatric Haematology
University of Oxford
The link between Down syndrome and blood cell development

Cumulative risk of a child with DS developing leukaemia: 2.1% by age 5 years

Commonest chromosomal abnormality in humans
National DS Cytogenetic Register: 700-800 live births (1.1/1,000)
# Increased susceptibility to leukaemia in Down syndrome

<table>
<thead>
<tr>
<th>Condition</th>
<th>Standardised incidence ratio (SIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cancers</td>
<td>1.2</td>
</tr>
<tr>
<td>All leukaemias</td>
<td>17.63</td>
</tr>
<tr>
<td><strong>Acute lymphoblastic (ALL)</strong></td>
<td></td>
</tr>
<tr>
<td>age 1-4 yrs</td>
<td>24.36</td>
</tr>
<tr>
<td>age 5-29 yrs</td>
<td>40.7</td>
</tr>
<tr>
<td><strong>Acute myeloid (AML)</strong></td>
<td></td>
</tr>
<tr>
<td>age 0-4 yrs</td>
<td>20.28</td>
</tr>
<tr>
<td>age 5-29 yrs</td>
<td>153.9</td>
</tr>
<tr>
<td><strong>Solid tumours</strong></td>
<td></td>
</tr>
<tr>
<td>age 0-29 yrs</td>
<td>0.44</td>
</tr>
<tr>
<td>age 30-&gt;60 yrs</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Both lymphoid and myeloid leukaemias are more common

Young children are especially susceptible

Solid tumours are less frequent

Hasle et al, Lancet, 2000
Why are haematopoietic cells uniquely susceptible to malignant transformation by trisomy 21?
Leukaemia in children with Down syndrome: clues from the clinical features

- **Acute lymphoblastic leukaemia:**
  - only occurs after age 1
  - always affects \textbf{B cells} (never T cell leukaemia)
Leukaemia in children with Down syndrome: clues from the clinical features

• **Acute lymphoblastic leukaemia:**
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• **Acute myeloid leukaemia (ML-DS):**
  - originates in fetal life and presents before age 5y
  - the leukaemic cells are megakaryoblastic and have mutations in the GATA1 gene
  - is preceded by a neonatal preleukaemia called TAM (Transient Abnormal Myelopoiesis)
Acquired N-terminal mutations in the *GATA1* gene uniquely transform fetal blood cells with trisomy 21

![Diagram of GATA1 gene and protein](image)

- Acquired mutations in exons 2/3 of the *GATA1* gene encode a truncated (short) protein, GATA1s, which is present in all cases of TAM and ML-DS

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- \textit{GATA1} mutations are already present at birth.
- Both TAM and ML-DS are unique to children with Down syndrome.

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- *GATA1* mutations are already present at birth.
- Both TAM and ML-DS are unique to children with Down syndrome.
- N-terminal *GATA1* mutations are not leukaemogenic in the absence of trisomy 21.

*Wechsler et al, Nature Gen 2002*

*Hollanda et al, Nat Gen 2006*
TAM and ML-DS: a model of leukaemogenesis

Fetal blood cells

+21

?10%

GATA1 mutation

Birth

+21 GATA1s

~20%

Additional genetic/epigenetic events

ML-DS

+21 GATA1s
TAM and ML-DS: a model of leukaemogenesis

1. How does trisomy 21 perturb the growth and development of fetal blood cells?
TAM and ML-DS: a model of leukaemogenesis

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2. What is the effect of trisomy 21 on post-natal blood cell development?

What are the clinical, haematological and molecular features of TAM?
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Perturbation of fetal haematopoiesis by trisomy 21

HAEMOPOIESIS (BLOOD PRODUCTION) IN FETAL LIFE

The types of cell in fetal blood are the same as those after birth, although their frequency and function are adapted to cope with the needs of the developing fetus.
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Blood cells are derived from haematopoietic stem cells (HSC) which differentiate into specialised progenitor cells committed to the myeloid (eg CMP) or lymphoid lineage (eg CLP).
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Abnormal fetal blood cell production starts before birth in Down syndrome

- Haematopoietic stem cells (HSC) are increased

Roy et al, 2012
Abnormal fetal blood cell production starts before birth in Down syndrome

- Haematopoietic stem cells (HSC) are increased
- B lymphoid cells are decreased

Roy et al, 2012
Abnormal fetal blood cell production starts before birth in Down syndrome

Normal

Trisomy 21

- Haematopoietic stem cells (HSC) are increased
- B lymphoid cells are decreased
- Megakaryocyte-erythroid precursors are increased

Trisomy 21 perturbs fetal haematopoiesis in the absence of *GATA1* mutations
Gene expression profiling of Trisomy 21 fetal CD34+ cells

Increased expression of erythroid genes

Reduced expression of B lymphoid genes

Increased expression of genes on Hsa21 chromosome 21 'signature'

Binbin Liu
TAM and ML-DS: a model of leukaemogenesis

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The Oxford-Imperial DS Cohort Study

- Started in 2007, prospective study
- Neonatal network in S England and Scotland (18 centres)
- Target recruitment: 400 babies with DS
- FBC and film in first week of life
- GATA1 mutation analysis (PCR, NGS)

Follow up:
- TAM: every 3m to age 2, then 6-12m to age 5
- No TAM (GATA1 mutation): annually to age 5

Helen Richmond, Neha Bhatnagar, Kelly Perkins, Joana Bonnici, Paresh Vyas
The Oxford-Imperial DS Cohort Study

The aims of the study

• To precisely describe the characteristics of blood cells in newborn babies with Down syndrome

• To identify the clinical, haematological and molecular features of TAM and use these to develop diagnostic and treatment algorithms to improve outcome

• To understand why trisomy 21 can cause leukaemia
Oxford-Imperial DS Neonatal Cohort Study: definition of TAM

- WHO: *increased* peripheral blood blasts in a neonate with DS
- Retrospective studies: variable clinical and haematological definitions
- OIDSCS: peripheral blood blasts >10% and a *GATA1* mutation detected by PCR (direct sequencing/dHPLC) in a DS neonate

Roberts et al, 2013
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### BLAST CELLS IN NORMAL CONTROLS

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Term cord blood</td>
<td>0-8%</td>
<td>0%</td>
</tr>
<tr>
<td>Healthy term and preterm</td>
<td>0-4%</td>
<td>0%</td>
</tr>
<tr>
<td>Sick preterm neonates</td>
<td>0-8%</td>
<td>1%</td>
</tr>
</tbody>
</table>
Oxford-Imperial DS Neonatal Cohort Study: \textit{GATA1} analysis

Total no of babies 
\( n = 200 \)

17
\textit{GATA1} mutation by direct seq/dHPLC

183
No \textit{GATA1} mutation detected by direct seq/dHPLC

8.5%
TAM

91.5%
No TAM

Roberts et al, 2013
Abnormal erythropoiesis in neonates with Down syndrome

Dyserythropoiesis is seen in almost all neonates with DS suggesting that trisomy 21 itself alters erythropoiesis

Roberts et al, 2013
Abnormal platelet production in neonates with DS

Thrombocytopenia is common in neonates with Down syndrome. Trisomy 21 perturbs megakaryopoiesis and platelet production.

Roberts et al, 2013
Abnormal leucocytes in neonates with Down syndrome

Trisomy 21 causes abnormal neutrophil, monocyte and basophil development

Roberts et al, 2013
Trisomy 21 causes trilineage perturbation of haematopoiesis which persists after birth
What are the clinical, haematological and molecular features of TAM?
Increased blast cells in DS neonates with and without TAM

Blast cells are seen on blood films of ~98% of all neonates with Down syndrome and are not specific to TAM

Roberts et al, 2013
Increased blast cells in DS neonates with and without TAM

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What is the significance of circulating blasts in neonates with DS where GATA1 mutations are not detectable by direct sequencing or dHPLC?
Detection of small mutant GATA1 clones in blood cells from DS neonates using next generation sequencing (NGS)

Using NGS, mutant GATA1 clones can be detected in ~30% of neonates with Down syndrome

Neonates with large clones (~1/3) have clinical and/or haematological signs of TAM; large clones can also be detected by direct sequencing/dHPLC

Small mutant GATA1 clones (~2/3) cannot be detected by direct sequencing/dHPLC and are clinically and haematologically 'silent'

Roberts et al, 2013
Oxford-Imperial DS Neonatal Cohort Study: GATA1 analysis

Total no of babies recruited, n=368

GATA1 mutation by seq/dHPLC

CLINICAL TAM

9%

SILENT TAM

20%

No GATA1 mutation detected by seq/dHPLC

NGS of GATA1 exon 2 n=126

28

22%

No GATA1 mutation by NGS

TAM EXCLUDED

33

335

126

209

98
Clinical features of DS neonates with and without TAM

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>TAM (n=33)</th>
<th>No GATA1 mut&lt;sup&gt;n&lt;/sup&gt; by NGS (n=98)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm (&lt;37 wks)</td>
<td>10 (30%)</td>
<td>16 (16.3%)</td>
<td>0.094</td>
</tr>
<tr>
<td>IUGR</td>
<td>9 (27.3%)</td>
<td>13 (13.3%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Sepsis</td>
<td>2 (6.3%)</td>
<td>5 (5.1%)</td>
<td>0.663</td>
</tr>
<tr>
<td>Jaundice</td>
<td>23 (69%)</td>
<td>54 (55.1%)</td>
<td>0.149</td>
</tr>
<tr>
<td>Bleeding</td>
<td>5 (15.2%)</td>
<td>4 (4.1%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>12 (36%)</td>
<td>3 (3.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (6.3%)</td>
<td>1 (1.0%)</td>
<td>0.069</td>
</tr>
<tr>
<td>Effusions, incl ascites</td>
<td>3 (9.1%)</td>
<td>1 (1.0%)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Congenital Heart Dis</td>
<td>18 (56%)</td>
<td>48 (49%)</td>
<td>0.721</td>
</tr>
<tr>
<td>GI anomaly</td>
<td>6 (18.2%)</td>
<td>8 (8.2%)</td>
<td>0.053</td>
</tr>
</tbody>
</table>

No clinical features are specific for TAM
Haematological features of DS neonates with & without TAM

In TAM:
• Thrombocytopenia is NOT more common

p=0.7358

No TAM  n=98
TAM       n=33
Haematological features of DS neonates with & without TAM

In TAM:
- Thrombocytopenia is NOT more common
- Anaemia is very uncommon

<table>
<thead>
<tr>
<th></th>
<th>No TAM</th>
<th>TAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>98</td>
<td>33</td>
</tr>
</tbody>
</table>

Hb (g/dl)

Platelets (x10^9/L)

\[ p=0.7358 \]

\[ p<0.0001 \]
Haematological features of DS neonates with & without TAM

In TAM:
- Thrombocytopenia is NOT more common
- Anaemia is very uncommon
- WBC and blasts are increased

No TAM n=98
TAM n=33
Haematological features of DS neonates with & without TAM

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- The % blasts is lower in babies with IUGR

No TAM n=98
TAM n=33

p=0.7358

p<0.0001

p<0.0001

p<0.0001

p=0.0002
Haematological features of DS neonates with & without TAM

In TAM:
• Thrombocytopenia is NOT more common
• Anaemia is very uncommon
• WBC and blasts are increased
• The % blasts is lower in babies with IUGR

No haematological features are specific for TAM
The only useful clue is % blasts so a blood film is essential!
Algorithm for diagnosis and monitoring of mutant GATA1 clones in DS neonates

Confirm DS by karyotyping
FBC and film
Save FBC sample for GATA1 analysis

BLASTS >10%
High chance of 'classical TAM'

GATA1 exon 2/3 mutation analysis by DHPLC/ direct sequencing

GATA1 mutation detected by Ss/DHPLC

'CLASSICAL TAM'
Monitoring of FBC/film until age 5
Serial GATA1 mutation analysis

GATA1 mutation NOT detected by Ss/DHPLC

BLASTS <10%
Low chance of 'classical TAM'

GATA1 exon 2/3 mutation analysis by NGS

GATA1 mutation detected by NGS

'SILENT TAM'
Monitoring of FBC/film until age 5
Serial GATA1 mutation analysis

No GATA1 mutation by NGS

Blasts >10%
Further studies

Blasts <10%
Further GATA1 analysis unnecessary
Monitoring mutant *GATA1* clones to predict development of ML-DS

- Permanent remission of TAM - ~80%
- Remission of TAM but persistent mutant *GATA1* clone, ML-DS by age 5y
- Silent TAM, persistent mutant *GATA1* clone, ML-DS by age 5y

Silent TAM may transform to ML-DS
TAM and ML-DS: a model of leukaemogenesis

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Experimental approach:
Whole genome sequencing of cases of TAM and ML-DS

In TAM only mutations of the GATA1 gene are found

Yoshida et al, 2013
What are the molecular events that cause TAM to transform to ML-DS?

In ML-DS up to 12 additional mutations are found and ~50% affect the cohesin complex.

Yoshida et al, 2013
What is the role of cohesin mutations in leukaemic transformation?

**Cohesin is essential for:**
- chromosome segregation
- DNA repair
- formation of DNA loops crucial for regulating gene expression
1. Trisomy 21 causes extensive, cell-intrinsic changes in fetal megakaryocyte/erythroid and B lymphoid development at the HSC and progenitor level prior to acquisition of GATA1 mutations.

2. Trisomy 21 causes trilineage perturbation of neonatal haematopoiesis. Acquired, preleukaemic GATA1 mutations occur in ~30% of all DS neonates; most mutations are clinically & haematologically silent.

In most cases mutations of the cohesin complex, or associated genes, are responsible for leukaemic transformation of trisomy 21 haematopoietic cells with persisting acquired GATA1 mutations.
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What are the genes on chromosome 21 that perturb the behaviour of blood cells?

- 473 genes (215 protein coding and 94 non-coding RNAs)
  - Genes implicated in haematological malignancies include CSTB, DYRK1A, ERG, ETS2, OLIG2, RUNX1, TIAM
  - Other genes relevant to haematopoiesis include AIRE, BACH1, CBG, DNMT3L, GABPA, IFNAR1, IFNR2, IFNG2, RCAN1, SOD1, SON

Binbin Liu, Natalina Elliott, Andi Roy