Molecular diagnosis using NGS germline panels facilitates diagnosis and improves therapy of rare inherited hematological disorders

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Hematology Oncology  
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The molecular hematology laboratory

- Perform genetic diagnosis of:
  - Thalassemia, hemoglobinopathies
  - Rare anemias, erythrocytosis, rare iron metabolism disorders
  - Hemophagocytic lymphohistiocytosis (HLH)
  - Autoimmune lymphoproliferative syndrome (ALPS)
  - Inherited bone marrow failure syndromes (IBMFS)
  - Inherited predisposition to myelodysplastic syndromes (MDS)
Agenda

- Clinical presentation of inherited predisposition to MDS/AML
- The advantage of molecular diagnosis
- The technique NGS panels, challenges of interpretation of sequence variants
  - Case reports- accurate diagnosis leads to optimal therapy
- Overall results in our laboratory-3.5 years experience
MDS

- WHO definition: clonal hematopoietic disorder characterized by
  - Ineffective hematopoiesis
  - Cytopenias, single- or multilineage dysplasia
  - Increased propensity to evolve to AML

- Primary MDS typically presents in older adults, with median age at disease onset of 72-75 y in the context of somatic mutations acquired with age

- MDS presenting in children and younger adults (<40y) is more frequently associated with germline genetic predisposition
Inherited predisposition to MDS/AML

- Physical stigmata and family history if present may provide important clues to diagnose

- Cytopenias and BMF alone are common presentations and thus diagnosis may be difficult

- Each individual genetic disorder is rare, as a group they account for at least 4%-15% of patients with MDS

Keel et al Haematologica 101:1343-50, 2016
Inherited predisposition to MDS/AML

- Primary inherited predisposition to MDS/AML
- Secondary associated with 'classical' inherited bone marrow failure syndromes (IBMFS)
Primary inherited predisposition to MDS/AML
MDS predisposition genes

- GATA2
- SAMD9/SAMD9L
- RUNX1
- ETV6
- ANKRD26
- DDX41

- TP53
- CEBPA
- SRP72
- ERCC6L2
- MYSM1
- Etc..
Spectrum of manifestation of germ line mutations in GATA-2

**MonoMAC**
- Monocytopenia, non-TB mycobacteria infections

**Emberger syndrome**
- MDS, lymphedema, warts

**DCML**
- Dendritic cell, monocyte, and B and natural killer lymphoid deficiency vulnerability to viral infections

**Congenital neutropenia**
- Recurrent infections, warts, progression to MDS/AML

**BM Failure**

**GATA2-transcription factor critical for hematopoiesis & lymphatic development**
Monosomy 7 as the most common cytogenetic aberration in GATA2 MDS

- MDS germline GATA2 mutations were found in 37% of patients with monosomy 7
- Stratified by age, 72% of adolescents with monosomy 7 were carriers of GATA2 mutation
- In children 7% of MDS due to GATA2 mutation
- Progression to MDS/AML, usually associated with monosomy 7 in 30-50% of patients

SAMD9/SAMD9L germline MDS syndrome

• Heterozygote gain of function mutations in \textit{SAMD9} and its paralogue \textit{SAMD9L} result in cytopenias and high risk of developing MDS with monosomy 7

• First diagnosed (2016) were two distinct clinical presentation
  – \textit{MIRAGE}-Myelodyplasia, Infection, Restriction of growth, Adreanal insufficiency, Genital phenotype and Enteropathy (SAMD9L mutations)
  – \textit{Atxia}-pancytopenia syndrome-Cerbellar atxia, cyopenias, predisposition to marrow failure and AML (SAMD9)

• In children 17% of MDS due to mutations in SAMD9/9L
Adaptation mechanisms to escape from germline SAMD9/9L mutations

Pancytopenia

MDS

Revenant clone

Transient monosomy 7

Clonal evolution

Self correction (UPD7q)

De7q

Monosomy 7

Mut

WT

Δmut

mut

WT

WT

WT

Types of SAMD9/9L mutations:
mut: missense germline (gain-of-function)
Δmut: acquired protein truncating (loss-of-function)
MDS predisposition genes

- GATA2
- SAMD9/SAMD9L
  - RUNX1
  - ETV6
  - ANKRD26
- DDX41
- TP53
- CEBPA
- SRP72
- ERCC6L2
- MYSM1
- Etc..

Familial MDS associated with thrombocytopenia
Familial MDS associated with thrombocytopenia

- Autosomal dominant mutations in \textit{RUNX1}, \textit{ETV6}, \textit{ANKRD26}

- Mild to moderate thrombocytopenia

- Normal-sized platelets (normal MPV)
- In some patient additional functional platelet defects leading to excessive bleeding

- BM may be hypocellular with dysmegakaryopoiesis

- An increased risk of developing MDS, AML, or lymphoid malignancies
Inherited predisposition to MDS and thrombocytopenia

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>% of inherited thrombocytopenia</th>
<th>% developing MDS/AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital thrombocytopenia</td>
<td>ANKRD26</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>ETV-related thrombocytopenia</td>
<td>ETV6</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>FPD/AML</td>
<td>Runx1</td>
<td>3</td>
<td>40</td>
</tr>
</tbody>
</table>

Noris and Pecci Hematology :385-399, 2017
Inherited predisposition to MDS/AML

- Primary inherited predisposition to MDS/AML

- Secondary predisposition to MDS/AML 'classical' inherited bone marrow failure syndromes (IBMFS)
Relative frequencies of the classical IBMFS among 270 Israeli patients
Fanconi anemia (FA)

- Mainly autosomal recessive genetic disorder

- Characterized by
  - Congenital anomalies
  - Bone marrow failure (BMF)
  - Cancer predisposition-myelodysplastic syndrome (MDS) progressing to acute myeloblastic leukemia (AML), solid tumors

- Caused by inability to repair DNA interstrand cross-links (ICLs)
Typical FA anomalies
Fanconi anemia

- A significant subset (25%-30%) of patients lack these physical findings

- Hematologic abnormalities are variable and include cytopenias, red cell macrocytosis, hypocellular marrow with dysplasia

- First manifestation can be MDS or AML

- The cumulative incidence for MDS-40% by age 50, AML 20% by age 40
Repair pathway in normal and FA cells

ICL - interstrands cross links
NHEJ - Non-homologous end joining

Bessler et al 2015
Chromosomal Fragility Test-DEB
Dyskeratosis Congenita (DC)

Proteins involved in telomere maintenance

DC phenotypic triad
Dyskeratosis congenita

- Patients often present without overt syndromic features
- Hematologic complications including BMF, MDS, and AML
- Each may be the sole and first manifestation of the disorder
- The cumulative incidence of MDS in DC has been estimated to be 2% by age 50 years
Flow-FISH lymphocyte telomere length

Gold standard to diagnosis of DC ‘equivalent’ to chromosome breakage of FA

Short telomeres <1st percentile
# Additional IBMFS with predisposition to MDS/AML

<table>
<thead>
<tr>
<th>IBMFS</th>
<th>MDS/AML</th>
</tr>
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<tbody>
<tr>
<td>Shwachman Diamond syndrome (SDS)</td>
<td>Cumulative incidence MDS/AML-18.8% by age of 20</td>
</tr>
<tr>
<td></td>
<td>36% by age of 30</td>
</tr>
<tr>
<td>Diamond Blackfan anemia (DBA)</td>
<td>Cumulative incidence AML-2% by age 45</td>
</tr>
<tr>
<td>Severe congenital neutropenia (SCN)</td>
<td>Overall hazard of MDS/AML is 2.3%/year after 10 years receiving G-CSF</td>
</tr>
</tbody>
</table>
Germ line mutations in patients referred due to persistent cytopenias

173 families, 36.9% >18 years of age at presentation
### 'Classical' IBMFS- known genes involved

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No of genes mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fanconi anemia (FA)</td>
<td>23</td>
</tr>
<tr>
<td>Dyskeratosis congenita (DC)</td>
<td>13</td>
</tr>
<tr>
<td>Diamond Blackfan-Anemia (DBA)</td>
<td>21</td>
</tr>
<tr>
<td>Severe congenital neutropenia (SCN)</td>
<td>23</td>
</tr>
<tr>
<td>Shwachman-Diamond Syndrome (SDS)</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83</strong></td>
</tr>
</tbody>
</table>
Molecular diagnosis for inherited predisposition to MDS/AML

• Optimal therapy
  • Allows surveillance to detect early signs of disease progression
  • Stem cell transplantation (SCT) before progression to leukemia
    • Proper conditioning—reduced in FA and DC
    • Avoid choosing a sibling donor carrying the same germline mutations as the proband
• Family counseling
Sanger sequencing

A gene-by-gene sequencing (Sanger sequencing)

• Advantages
  – Accurate
  – Relatively cheap

• Disadvantages
  – No definitive diagnosis reached
  – Labor intensive Fanconi anemia FANCA 46 exons
Whole exome sequencing

~20,000 genes
Targeted genomic capture & next generation sequencing
Advantages of panel targeted NGS

• Avoid gene-by-gene sequencing (hundred genes in a panel)
• High depth of coverage across all genes of interest
• Interpretation is easier relative to WES
• New genes of interest can be incorporated as they are reported
• Cost effective~ 800USD
Workflow for next-generation sequencing (NGS)

MDS-Skin fibroblasts
Bioinformatics interpretation of sequence variants

- **Population database** - frequencies of variants in large populations. Polymorphism variant with frequency >1%

- **Assessment of the variants** compared to Disease Data Bases - variants found in patients with disease

- **Computational (In Silico) Predictive Programs** - predict whether variants are likely to be deleterious on the basis of evolutionary conservation or predicted structural effects
Stratification of sequence variants

- Pathogenic
- Likely pathogenic
- Variant of unknown significant (VOUS)
- Likely benign
- Benign

Standards and Guidelines for the Interpretation of Sequence Variants American College of Medical Genetics Genet Med. 17(5): 405-424, 2015
The grey-zone: variant of uncertain significances

A variant of unknown significant (VOUS) with today's knowledge may become a significant mutation in the future.
Evaluation of mutation pathogenicity

- Prediction programs may be helpful but must be interpreted with caution

- Strong evidence for pathogenicity
  - Known mutation associated with clinical disorder with supportive *in vitro* studies

- Demonstration that the variant tracks with the clinical disorder in multiple families, or in several affected individuals within the family

- Functional test (chromosomal breakage, telomere length) support the genetic studies
Final report

• Bioinformatics evaluation is done independently by 3 experienced laboratory technicians

• Combined laboratory and clinicians meeting

• The presence of each clinical significant variant is verified by Sanger sequencing

• Referral for genetic counseling
Examples how NGS panels usage improve diagnosis and clinical managements-
predisposition to MDS/AML
Case report 1

- 18 year-old young man
- Known to our service since he was 7 months old, presented with thrombocytopenia 50-70X10^9/L
- No bleeding tendency
- BM-numerous megakaryocytes
- Serological work up was negative: EBV, CMV, HIV, serum immunoglobulins, Coombs, ANA, serum complement, LAC
Case report 1

• Considered to have ITP

• At age of 12 macrocytosis MCV 97.5, HbF-1%

• BM biopsy hypoplastic marrow (20-40% cellularity)

• Chromosomal breakage (DEB), telomere length-WNL

• Germline panel mutation in ETV6 c.1103T>G
Case report 1- treatment

- Thrombocytopenia with genetic predisposition to MDS

- Prospective clinical surveillance: CBC every 3-6 months, marrow surveillance once a year including morphology, cytogenetics, MDS FISH panel and somatic NGS panel

- SCT prior to AML
11 year old boy  
Presented with pancytopenia  
O/E WNL  

WBC-2.78X10⁹/L, ANC-0.2X10⁹/L  
Hb-8.8gr%, reticulocytes-0.9%  
PLT-24X10⁹/L  
BM biopsy hypoplastic, 20% cellularity  

Started on ATG, Cyclosporine A and elthrombopeg
Case report 2

- 11 year old boy
  Presented with pancytopenia
  O/E WNL

- WBC-2.78X10⁹/L, ANC-0.2X10⁹/L
  Hb-8.8gr%, reticulocytes-0.9%,
  PLT-24X10⁹/L
  BM biopsy hypoplastic,
  20% cellularity

- Started on ATG, Cyclosporine A
  and elthrombopag
Case 2-Therapy

• SCT unrelated matched donor

• Father CBC and BM at diagnosis, education about signs and symptoms of leukemia, CBC every 6 months

• Surveillance for children: CBC every 6 months, marrow surveillance once a year

• SCT before AML develops
Overall results of IBMF/MDS panel analysis 2016-6.2019

Schneider Children’s Medical Center of Israel
Molecular diagnosis of patients referred with cytopenias (2016-6.2019) (N=134)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No of patients</th>
<th>% of all pts referred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited predisposition to MDS by NGS panel</td>
<td>37</td>
<td>27.6</td>
</tr>
<tr>
<td>IBMFs diagnosed by Sanger sequencing</td>
<td>12</td>
<td>8.9</td>
</tr>
<tr>
<td>Congenital thrombocytopenia by NGS</td>
<td>9</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58</strong></td>
<td><strong>43.3</strong></td>
</tr>
</tbody>
</table>
Primary predisposition to MDS diagnosed by BMF/MDS NGS panel (N=18)

<table>
<thead>
<tr>
<th>Disease</th>
<th>No of Patients</th>
<th>Gene mutated (No of pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited predisposition to MDS</td>
<td>10</td>
<td>SAMD9L (5), ERCC6L2 (3), GATA2 (1), MYSM1 (1)</td>
</tr>
<tr>
<td>Familial thrombocytopenia and MDS</td>
<td>8</td>
<td>ANKRD26 (5), ETV6 (2), RUNX1 (1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>18</strong></td>
<td></td>
</tr>
</tbody>
</table>
IBMFS diagnosed by BMF/MDS NGS panel (N=19)

<table>
<thead>
<tr>
<th>Disease</th>
<th>No of patients</th>
<th>Gene mutated (No of pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>7</td>
<td>FANCA (4), FANCE (1), FANCB (1), FANCS (1)</td>
</tr>
<tr>
<td>DBA</td>
<td>5</td>
<td>RPL15 (1), RPS10 (1), RPS7 (1), RPS26 (1), ADA2 (1)</td>
</tr>
<tr>
<td>SCN</td>
<td>2</td>
<td>CSFR3 (1), JAGN1 (1)</td>
</tr>
<tr>
<td>DC</td>
<td>5</td>
<td>TERC (1), TERT (2), TIN2 (1), WRAP 53 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>
## Congenital thrombocytopenia Non-IBMFS/MDS (N=9)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene mutated (No of pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH9 related platelet disorder</td>
<td>MYH9 (4)</td>
</tr>
<tr>
<td>Bernard-Soulier syndrome</td>
<td>GP1BA (1)</td>
</tr>
<tr>
<td>Congenital macrothrombocytopenia</td>
<td>ACTN1 (2)</td>
</tr>
<tr>
<td>Gray PLT Sy</td>
<td>NBEAL2 (1)</td>
</tr>
<tr>
<td>Glanzmann thrombasthenia</td>
<td>ITGB3 (1)</td>
</tr>
</tbody>
</table>
Summary and conclusions

- Predisposition to MDS/AML is either primary or secondary to 'classical 'inherited BMF syndromes

- Most often clinical presentation is characterized by cytopenia and accurate clinical diagnosis is impossible

- Molecular diagnosis is essential

- Number of possible causative mutated genes is constantly increasing and usually requires NGS multi-gene analysis
Summary and conclusions (cont’d)

- Using combination of Sanger sequencing and BMF/MDS NGS panel we identified the cause of cytopenia in 59 (43%) of 134 patients
- 49 (36.5%) patients had inherited predisposition to MDS/AML
- 37 (27.6%) patients diagnosed achieved only by use of BMF/MDS NGS panel
- In our experience the custom made germline IBMF/MDS NGS panel is an effective tool for diagnosis and proper management of patients with inherited MDS/AML
November 2019

New clinic: consultation for patients (children & adult) with inherited bone marrow failure syndromes

Pediatric Hematology Oncology Division

Clinic secretary: Sara Hazek

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