Personalized approach to PID

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Professor of Pediatrics
Columbia University
DISCLOSURES

• Consultant, Speaker; Honorarium
  • Shire

• Consultant; Consulting fee
  • CSL Behring

• Advisory Board Member; Honorarium
  • ADMA

• Author; Royalty
  • UptoDate
LEARNING OBJECTIVES

• Describe the newest classification and approach to primary immunodeficiency

• Define the utility and use of genetic characterization of primary immunodeficiency

• Illustrate the benefit of obtaining a specific PI diagnosis
Primary Immunodeficiency

Genetic inability of the immune system to provide an advantage over the environment

• 2018: >350 diseases
• Uniform newborn screening for SCID
• Banner successes in gene therapy and promise for genetic “surgery”
• Mechanisms informing novel therapies for cancer and autoimmunity (i.e. tofacitinib)
• Insightful and unexpected biology
• Opportunities for precision medicine
Hallmarks of Primary Immunodeficiency

• Susceptibility to the external environment
  • Recurrent Infection
  • Severe Infection
  • Unusual Infection

• Susceptibility to the internal environment
  • Cancer
  • Autoinflammation
  • Autoimmunity
10 Warning Signs of Primary Immunodeficiency

Primary Immunodeficiency (PI) causes children and young adults to have infections that come back frequently or are unusually hard to cure. In America alone, up to 1/2 million people suffer from one of the 100 known Primary Immunodeficiency diseases. If you or someone you know are affected by two or more of the following warning signs, speak to a physician about the possible presence of an underlying Primary Immunodeficiency.

1. Eight or more new ear infections within 1 year.
2. Two or more serious sinus infections within 1 year.
3. Two or more months on antibiotics with little effect.
4. Two or more pneumonias within 1 year.
5. Failure of an infant to gain weight or grow normally.
6. Recurrent, deep skin or organ abscesses.
7. Persistent thrush in mouth or elsewhere on skin, after age 1.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections.
10. A family history of Primary Immunodeficiency.
Utility of the 10 Warning Signs

- **Hendershot, RW et al. J All Clin Immunol 2003;111:S222**
  - At least one warning sign in 95%
  - At least 2 warning signs in 78%
  - At least 3 in 62%
  - 2 or more sinus infections in 1 yr – 68%
  - 2 + mos. on antibiotics – 54%
  - Need IV antibiotics to clear infections – 46%

- **Reda, et. al, Allergy Asthma Immunol Res 2013 5:88-95**
  - Sensitivity of any one sign 100%, Specificity of 26%
    - PPV 53%, NPV 100%
  - Two signs: sensitivity 94%, Specificity 64%
  - Three signs: sensitivity 77% specificity 86%
Population Prevalence of Diagnosed Primary Immunodeficiency Diseases in the United States

J. M. Boyle • R. H. Buckley

Only random digit dialing telephone survey of PIDD

US prevalence 1:1200 persons
Statistics, open arms and primary care practices

- Statistically if a primary care practice is 10,000 patients…
- There should have 5 with primary immunodeficiency!
- Are they aware of their PIDD patients?
- GIVE US YOUR 5 SICKEST CHILDREN…
Question 1

Which statement is most accurate regarding the 10 warning signs for primary immunodeficiency

a) They are neither sensitive or specific
b) Having two or three signs improves the specificity
c) Only sinusitis and need for IV antibiotics are valid signs
d) The negative predictive value of not having PID if a patient has no signs is poor
Even without an index of suspicion
SCID Newborn Screening

• Guthrie card punches
• PCR measure of naïve T cells
• Quantify TREC
  • T Cell Receptor Excision Circles
TREC assay (normal)


Isolate TRECs and enumerate per unit volume

TCR Locus

Thymic T cell

Periphera T cell (clonal expansion)

TCRδ TREC
**NBS TREC for SCID – current experience**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital syndrome</td>
<td>34</td>
</tr>
<tr>
<td>Secondary</td>
<td>29</td>
</tr>
<tr>
<td>Unspecified</td>
<td>26</td>
</tr>
<tr>
<td>SCID</td>
<td>9</td>
</tr>
<tr>
<td>Idiopathic T cell lymphopenia</td>
<td>3</td>
</tr>
</tbody>
</table>

---

**Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States**

Antonia Kwan, PhD, MRCPCH; Roshini S. Abraham, PhD; Robert Currier, PhD; Amy Brower, PhD; Karen Andruszewski, BS; Jordan K. Abbott, MD; Mei Baker, MD; Mark Ballow, MD; Louis E. Bartoshesky, MD; Vincent R. Bonagura, MD, Francisco A. Bonilla, MD, PhD; Charles Brokopp, DPhH; Edward Brooks, MD; Michele Caggana, ScD; Joedyn Celestin, MD; Joseph A. Church, MD; Anne Marie Corneau, PhD; James A. Connelly, MD; Morton J. Cowan, MD; Charlotte Cunningham-Rundles, MD; Trivikram Daku, PhD; Nina Dave, MD; Marla T. De La Morena, MD; Ulrich Duffner, MD; Chin-To Fong, MD; Lisa Forbes, MD; Debra Freedenberg, MD; Erwin W. Gefand, MD; Jaime E. Hale, BS; I. Celine Hansen, MD; Beverly N. Hay, MD; Diana Hu, MD; Anthony Infante, MD, PhD; Daisy Johnson, BSN; Neena Kapoor, MD; Denise M. Kay, PhD; Donald B. Kohn, MD; Rachel Lee, PhD; Heather Lehman, MD; Zhili Lin, PhD; Fred Lory, PhD; Aly Abdel-Mageed, MD, MBA; Adrienne Manning, BS; Sean McGhee, MD; Theodore B. Moore, MD; Stanley J. Naides, MD; Luigi D. Notarangelo, MD; Jordan S. Orange, MD; Sung-Yun Pai, MD; Matthew Porteus, MD, PhD; Ray Rodriguez, MD, JD, MPH, MBA; Neil Romberg, MD; John Routes, MD; Mary Ruehl, MS; Arne Rubenstein, MD; Carlos A. Saavedra-Matiz, MD; Ginger Scott, RN; Patricia M. Scott, MT; Elizabeth Secord, MD; Christine Serogy, MD; William T. Shearer, MD, PhD; Subhadra Siegel, MD; Stacy K. Silvers, MD; E. Richard Stishm, MD; Robert W. Sugerman, MD; John L. Sullivan, MD; Susan Tankersley, PhD; Millard L. Tierce IV, DO; James Verbsky, MD, PhD; Beth Vogel, MS; Rosalyn Walker, MD; Kelly Walkowicz, MD; Jolan E. Walter, MD, PhD; Richard L. Wasserman, MD, PhD; Michael S. Watson, MS, PhD; Geoffrey A. Weinberg, MD; Leonard B. Weiner, MD; Heather Wood, MS; Anne B. Yates, MD; Jennifer M. Puck, MD

**Newborn Screening for Severe Combined Immunodeficiency**

John Routes, SD, James Verbsky, SD

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NBS TREC for SCID – current experience

Disorder
- Congenital
- Secondary
- Unspecified
- SCID
- Idiopathic

Percentage
- IL2RG: 34
- IL7RA: 29
- ADA: 29
- RAG1: 26
- JAK3: 9
- Other: 9
- None: 3
- Not Tested: 3

N=42

immunodeficiency

- DiGeorge n=7
- Trisomy 21 n=21
- Other/unknown n=18
- Cytogenic Abn:n=6
- Ataxia Telan. n=4
- Trisomy 18 n=4

Intrzewski, BS; Jordan K. Abbott, MD; MD, PhD; Charles Brokopp, DPhH; MD, PhD; James A. Connelly, MD; MD; De La Morena, MD; Ulrich Duffner, MD; MD; Elaine Hanson, MD; Beverly N. Hay, MD; Donald B. Kohn, MD; Rachel Lee, PhD; MD; Sean McGhee, MD; Theodore B. Moore, MD; MS, MD, PhD; Mark M. Minn, MD; Carlos A. Saavedra-Matiz, MD; MD; Subhata Siegel, MD; John Rour

Newborn

Cardiac n=30
- Not specified n=30
- Mult. Cong. Abn. n=23
- Third Space n=15
- GI n=15
- Leukemia n=4

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True positive TREC
Pre-transplant management

- Refer to transplant center
- Relative sequestration
- Start PCP prophylaxis
- Consider fungal prophylaxis (candida)
- Start bacterial/infective prophylaxis (Ig therapy)
- Consider specific viral prophylaxis (acyclovir)
- Breast feeding OK but consider maternal CMV status
- Avoid vaccines
- Irradiate blood products
- Evaluate for and manage characteristic infections
Transplantation as the transformative standard of care in severe PID

**Year of transplant**

<table>
<thead>
<tr>
<th>Year of Transplant</th>
<th>Proportion surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000–2005</td>
<td>1.0</td>
</tr>
<tr>
<td>1995–1999</td>
<td>0.8</td>
</tr>
<tr>
<td>1968–1994</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*P = .0003*

**Donor source**

<table>
<thead>
<tr>
<th>Donor Source</th>
<th>Proportion surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGI</td>
<td>1.0</td>
</tr>
<tr>
<td>RPI</td>
<td>0.8</td>
</tr>
<tr>
<td>URD</td>
<td>0.6</td>
</tr>
<tr>
<td>MMR</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*P < .0001*

**Age at Transplant**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Proportion surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 months</td>
<td>1.0</td>
</tr>
<tr>
<td>6–11 months</td>
<td>0.8</td>
</tr>
<tr>
<td>12–238 months</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*P = .0008*
Transformative advances where transplant is sub par

Twenty-Five Years of Gene Therapy for ADA-SCID: From Bubble Babies to an Approved Drug

Francesca Ferrua\textsuperscript{1,2} and Alessandro Aiuti\textsuperscript{1,2,*}

\textsuperscript{1}San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy; \textsuperscript{2}Vita-Salute San Raffaele University, Milan, Italy.
Transformative advances where transplant is sub par.
Gene therapy for ADA-SCID (A. Aiuti)

Survival (%)

- Survival
- Intervention-Free Survival

Year

N=18

Correction of immune and metabolic defect

Ex vivo gene therapy for ADA-SCID was approved in the EU in 2016

Aiuti et al., Science 2002; NEJM, 2009
Cicalese, Ferrua et al, Blood 2016 and unpublished
2018 IUUIS update defines over 350 Primary Immunodeficiencies: an explosive field
2018 IUUIS update defines over 350 Primary Immunodeficiencies: an explosive field
## The IUIS classification

<table>
<thead>
<tr>
<th>IUIS table #</th>
<th>PIDD classification</th>
<th># Genes/PIDDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunodeficiencies affecting cellular and humoral immunity</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>Combined immunodeficiencies with associated or syndromic features</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>Predominantly antibody deficiencies</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Diseases of immune dysregulation</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Congenital defects of phagocyte number, function or both</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>Defects in intrinsic and innate immunity</td>
<td>52</td>
</tr>
<tr>
<td>7</td>
<td>Autoinflammatory disorders</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>Complement deficiencies</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>Phenocopies of PID</td>
<td>12</td>
</tr>
</tbody>
</table>

Total = 365 (350 genes)
New primary immunodeficiency diseases: context and future

Joyce E. Yu\textsuperscript{a}, Jordan S. Orange\textsuperscript{b}, and Yesim Yilmaz Demirdag\textsuperscript{a}

<table>
<thead>
<tr>
<th>IUIS classification group</th>
<th>Primary immunodeficiency disease category</th>
<th>Number of genes</th>
<th>Number of diseases\textsuperscript{a}</th>
<th>Number of new diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cellular and humoral immunodeficiencies</td>
<td>49</td>
<td>49</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>Syndromic combined immunodeficiencies</td>
<td>65</td>
<td>67</td>
<td>23</td>
</tr>
<tr>
<td>III</td>
<td>Antibody deficiencies</td>
<td>33</td>
<td>40</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>Immune dysregulatory diseases</td>
<td>40</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>V</td>
<td>Phagocytic diseases</td>
<td>39</td>
<td>39</td>
<td>8</td>
</tr>
<tr>
<td>VI</td>
<td>Innate immunodeficiencies</td>
<td>52</td>
<td>52</td>
<td>20</td>
</tr>
<tr>
<td>VII</td>
<td>Autoinflammatory diseases</td>
<td>36</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>VIII</td>
<td>Complement deficiencies</td>
<td>30</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>IX</td>
<td>Phenocopies of PIDs</td>
<td>6</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The difference between genes and diseases is because there are well-defined disorders that are likely polygenic and do not have single gene explanations.

Total = 365 (350 genes)
Genetic diagnosis of PIDD – does it matter?

- Applies a spectrum of natural history to patients
- Important anticipatory medicine to practice
- Age of atypical presentations of known diseases
- Is the atypical the typical?
- Can help justify/advocate for patient benefits
- Implications for undiagnosed family members
- Genetic counselling
- PGD
Types of genetic analyses available

- Sanger “direct” sequencing - individual genes
- “next gen” - massively parallel sequencing panels
- Whole exome sequencing
  - Varying coverage, varying analysis,
- Whole genome sequencing
- RNA sequencing
- Copy number variation (also important)
  - Karyotype, FISH, Chromosomal microarray (CMA, SNPchip)
Recent genomic diagnostic discovery = pretest probability

<table>
<thead>
<tr>
<th>Modality</th>
<th>Reference(s)</th>
<th>Details</th>
<th>Diagnostic rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>WES</td>
<td>Maffucci – 2016 Front Imunol Chinn – 2018 Blood</td>
<td>50 CVID 48 HLH 2004+</td>
<td>30% 54%</td>
</tr>
<tr>
<td>WES + CNV</td>
<td>Stray-Pedersen – 2017 JACI De Valles-Ibanez – 2018 Front Imm</td>
<td>Difficult to diagnose patients PI category rates from 10-100% CVID</td>
<td>40% 24%</td>
</tr>
<tr>
<td>WGS</td>
<td>Mousallem - 2015 JACI Belkadi – 2015 PNAS</td>
<td>Application in 6 patients WGS greater accuracy for CNV</td>
<td></td>
</tr>
<tr>
<td>WGS + RNAseq</td>
<td>Van Schouwenburg – 2015 Clin Immunol</td>
<td>Limited cases</td>
<td>Potential additive value</td>
</tr>
</tbody>
</table>
Use of Genetic Testing for Primary Immunodeficiency Patients

Jennifer R. Heimall¹ · David Hagin² · Joud Hajjar³ · Sarah E. Henrickson¹,⁴ · Hillary S. Hernandez-Trujillo⁵,⁶ · Yuval Tan⁷ · Lisa Kobrynski⁸ · Kenneth Paris⁹ · Troy R. Torgerson¹⁰ · James W. Verbsky¹¹ · Richard L. Wasserman¹² · Elena W. Y. Hsieh¹³ · Jack J. Blessing¹⁴ · Janet S. Chou¹⁵,¹⁶ · Monica G. Lawrence¹⁷ · Rebecca A. Marsh¹⁸ · Sergio D. Rosenzweig¹⁹ · Jordan S. Orange²⁰,²¹ · Roshini S. Abraham²²
CIS recommendations for genetic testing

• Clinical immunologists should be able to use genetic testing
• Genetic testing provides a definitive diagnosis and should be offered to at risk family members
• Genetic counseling should be provided by an immunologist or GC
• Choice of genetic test should be made by immunologist – there is no best “first test”
• Choice of test need by case-by-case
• Follow up genetic or functional tests may be needed
• Genetic testing is not prerequisite to initiate therapy
The Houston Project in primary immunodeficiency (2012-2018): An unbiased WES approach to otherwise difficult to diagnose PIDD

Stage 1: 485 individuals 280 families
Stage 2: 1000+ individuals
The Houston Project in primary immunodeficiency (2012-2018): An unbiased WES approach to otherwise difficult to diagnose PIDD

Stage 1: 485 individuals, 280 families

Stage 2: 1000+ individuals
Unbiased WES results for the first 280 families with PIDD
The Houston Project for PIDD...

5% have more than one confirmed disease causing gene
“blended” phenotype
Unbiased WES results for the first 280 families with PIDD

The Houston Project for PIDD...

5% have more than one confirmed disease causing gene
“blended” phenotype

- **Autosomal Recessive (43%)**
- **Autosomal Dominant (38%)**
- **X-linked Recessive (19%)**
- **Parental (73%)**
- **De novo (27%)**
Getting an answer: Diagnostic Implications

- Transient clinical immunodeficiency $n=5$
- Molecular diagnosis not yet established in the family $n=155$
- Probands with an established molecular diagnosis after WES $n=120$

Bar chart showing various conditions and their frequency.
Getting an answer: Diagnostic Implications

- Change in diagnosis only
- Change in diagnosis and management
- Change in management after confirming suspected diagnosis
- Diagnosis confirmed

n=120 families
Lessons learned from additional research analyses of unsolved clinical exome cases

Mohammad K. Eldomery\textsuperscript{1,18\dagger}, Zeynep Coban-Akdemir\textsuperscript{1\dagger}, Tamar Harel\textsuperscript{1\dagger}, Jill A. Rosenfeld\textsuperscript{1}, Tomasz Gambin\textsuperscript{1,2}, Asbjørn Stray-Pedersen\textsuperscript{3}, Sébastien Küry\textsuperscript{4}, Sandra Mercier\textsuperscript{4,5}, Davor Lessel\textsuperscript{6}, Jonas Denecke\textsuperscript{7}, Wojciech Wiszniewski\textsuperscript{1,8}, Samantha Penney\textsuperscript{1}, Pengfei Liu\textsuperscript{1,9}, Weimin Bi\textsuperscript{1,9}, Seema R. Lalani\textsuperscript{1,8}, Christian P. Schaar\textsuperscript{1,8,10}, Michael F. Wangler\textsuperscript{1,8}, Carlos A. Bacino\textsuperscript{1,8}, Richard Alan Lewis\textsuperscript{1,10}, Lorraine Potocki\textsuperscript{1,8}, Brett H. Graham\textsuperscript{1,8}, John W. Belmont\textsuperscript{1,8}, Fernando Scaglia\textsuperscript{1,8}, Jordan S. Orange\textsuperscript{11,12}, Shalini N. Jhangiani\textsuperscript{13}, Theodore Chiang\textsuperscript{13}, Harsha Doddapaneni\textsuperscript{13}, Jianhong Hu\textsuperscript{13}, Donna M. Muzny\textsuperscript{13}, Fan Xia\textsuperscript{1,9}, Arthur L. Beaudet\textsuperscript{1,9}, Eric Boerwinkle\textsuperscript{13,14}, Christine M. Eng\textsuperscript{1,9}, Sharon E. Plon\textsuperscript{1,8,11,15}, V. Reid Sutton\textsuperscript{1,8}, Richard A. Gibbs\textsuperscript{1,13,16}, Jennifer E. Posey\textsuperscript{1}, Yaping Yang\textsuperscript{1,9} and James R. Lupski\textsuperscript{1,8,11,13,17\star}
**SNV Prioritization**

- MAF <= 0.5%
- ARIC Score <= 10 (biallelic SNVs), = 0 (de novo SNVs)
- Mutation Taster/SIFT/Polyphen-2 = Deleterious
  - Phylop = Conserved

**Known gene/mutation?**
- OMIM
- Pubmed
- HGMD
- Clinvar

**Gene Function?**
- Animal model
- Tissue expression

**Related to Known Gene?**
- Gene Families and networks
- Coexpression
- Protein-protein interactions
- Similar pathway

**Other Cohorts?**
- BHCMG db
- BG db
- CNV db

**Protein Domain?**
- MutationMapper

**Filtered Variants**

**Trios**
- De novo
  - Missense, Mosaic
  - Parents

**AR-Comp. Het**
- Truncating
  - Stopgain Frameshift

**AR-Hom/XLR-Hem**
- Stopgain Frameshift Splicing Missense
Pilot Study: 74 families
Identifying candidate genes

- Mol Dx 25%
- No Mol Dx 75%

Inter-communication
Diagnosis for additional cases

Novel Genes 25.7%
Known Genes 14.9%
Candidate Genes 10.8%
No Molecular Diagnosis 48.6%
Question 2

The best genetic test to apply for a patient you suspect of having a diagnosable PID is

a) Whole exome sequencing
b) Next generation sequencing panel
c) Whole genome sequencing with copy number variation quantification
d) Targeted gene tests
e) The one approved by the patient’s insurance
Revisiting hematophagocytic lymphohistiocytosis (HLH) in the precision genomic era
HLH Diagnostic Guidelines

• Molecular diagnosis consistent with HLH, or...
• 5/8 of the following
  • Fever
  • Splenomegaly
  • Cyopenias affecting 2/3 lineages
  • Triglycerides ≥265mg/dl and/or Fibrinogen ≤150mg/dl
  • Ferritin ≥500µg/l
  • Soluble CD25 ≥2400 U/ml
  • Low or absent NK cell activity
  • Hemophagocytosis
  • No evidence of malignancy

# Historical molecular defects leading to HLH

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Protein</th>
<th>Effect of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chediak Higashi Syndrome</td>
<td>LYST</td>
<td>Lysosomal trafficking regulator</td>
<td>Inability to generate normal lytic granules.</td>
</tr>
<tr>
<td>Hermansky Pudlak syndrome 2/10</td>
<td>ADTB3A APDD1 PALD</td>
<td>AP-3b AP-3d Palladin</td>
<td>Inappropriate formation of lytic granules and movement along microtubules</td>
</tr>
<tr>
<td>Griscelli syndrome 2</td>
<td>RAB27A</td>
<td>Rab27a</td>
<td>Lytic granules move but remain with microtubules</td>
</tr>
<tr>
<td>Familial Erythrophagoctytic Lymphohistiocytosis (FHL) 2</td>
<td>PRF1</td>
<td>Perforin</td>
<td>Lytic granules are released but cannot mediate lysis</td>
</tr>
<tr>
<td>FHL3</td>
<td>UNC13D</td>
<td>Munc13-4</td>
<td>Lytic granules move to the IS but do not dock</td>
</tr>
<tr>
<td>FHL4</td>
<td>STX11</td>
<td>Syntaxin-11</td>
<td>Interferes with lytic granule fusion with the cell membrane.</td>
</tr>
<tr>
<td>FHL5</td>
<td>STXBP2</td>
<td>Munc18-2</td>
<td>Interferes with lytic granule fusion with the cell membrane.</td>
</tr>
<tr>
<td>FHL6</td>
<td>FAAP24</td>
<td>FAAP24</td>
<td>Normal NK cell function by conventional testing</td>
</tr>
</tbody>
</table>
Why does HLH occur?

- Virus
- Immunologic infection
- Infected monocyte
- NK cell/CTL
- IFN-α
- Lymphocyte
- DC
- IL-12+
- TNF+
- IFN-γ
- Monocyte
- IFN-γ
- IL-12
- TNF
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
- x
HLH in the genomic era – precision diagnosis of PID

- 122 subjects (1999 – 2016) met HLH-2004 criteria for HLH

- Median age at diagnosis: 6.1 years [2 weeks – 18.5 years]
- 51% male / 49% female
- 101 subjects received clinical or research-based genetic testing
Disease-causing familial HLH gene mutations were present in less than 20% of subjects tested.

Note: no potential digenic or polygenic single heterozygous variant combinations were statistically likely to be disease-causing.
Overall Genetic Findings
(n = 101)

Genetic explanations in 45% of families, 69% excluding cases not tested by WES

More families with PIDD and/or DIAP than fHLH
PIDD Genes Identified

CARMIL2  CASP10  CYBB  DOCK8
LRBA  MCM3AP  NCF1  PIK3CD
RAG1  RAG2  STAT1 (GOF)
STAT3 (GOF)  TTC7A  WAS  STAT2
PIDD Genes Identified

Dysregulated Immune Activation or Proliferation (DIAP)
Viral Triggering and FHL more likely under age 1
The new details matter – precise prognostics
HLH algorithm in the genomic age

1. Consider HLH
2. Test diagnostic criteria
   - HLH-2004: ≥ 5/8 positive
     - Targeted sequencing
       - Abnormal
         - Consider whole exome sequencing
       - Normal
         - Therapy required?
           - Yes
             - Whole exome sequencing
           - No
             - Observe*
3. HLH-2004: < 5/8 positive*
   - Observe*
Question 3

For patients with HLH fulfilling diagnostic criteria, the most likely molecular diagnosis will be

a) None
b) One of the historical Familial HLH genes like perforin (PRF1)
c) One of a dozen other PID genes that can also cause HLH
d) One of the DIAP genes
e) A novel genetic explanation
Beyond Simple Genetics
Multilocus Variation – a frontier in PID

Multiple Molecular Diagnoses

- Parent A: unaffected
- Parent B: unaffected
- Offspring: Affected, 2 conditions (AD de novo + AR)

Digenic Inheritance

- Parent A: unaffected
- Parent B: unaffected
- Offspring: Affected, One condition

Mutational Burden

- Parent A: unaffected
- Parent B: unaffected
- Offspring: Affected + Modifiers = Variable expressivity

Multiple diagnoses - The blend...

- Severe HPV (3) Repeated pneumonia (7), severe VZV (10), cryptococcal meningitis (11)
- Age 21
  - CD4=604, CD8=84, NK=119, CD19=134
  - Colony survival=8% (37-63%)

Chinn, et. al. Front Immunol 2017
An age of rational specific immunological therapies

Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy

Bernice Lo,1,2 Kejian Zhang,3,4 Wei Lu,1,2 Lixin Zheng,1,2 Qian Zhang,2,4 Chrysi Kanellopoulou,1,2 Yu Zhang,2,4 Zhiduo Liu,5 Jill M. Fritz,1,2 Rebecca Marsh,6 Ammar Husami,3 Diane Kissell,3 Shannon Nortman,8 Vijaya Chaturvedi,6 Hilary Haines,7 Lisa R. Young,8 Jun Mo,9 Alexandra H. Filipovich,6 Jack J. Blesing,6 Peter Mustillo,10 Michael Stephens,11 Cesar M. Rueda,12 Claire A. Chougnet,12 Kasper Hoebe,12 Joshua McElwee,13 Jason D. Hughes,15 Elif Karakoc-Aydiner,14 Helen F. Matthews,1,2 Susan Price,1,2 Helen C. Su,2,4 V. Koneti Rao,1,2 Michael J. Lenardo,1,2 Michael B. Jordan6,12;
An age of rational specific immunological therapies made possible through molecular diagnosis

**Immune deficiencies, infection, and systemic immune disorders**

Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation

**Graph A**

Pre-treatment vs. Post-treatment graph showing changes in P-STAT1 levels.

**Graph B**

Bar graph showing % MFI P-STAT1 for IFN-β and IFN-γ, normalized to Ctrl. Values: IFN-β 377%, IFN-γ 220% (for Pt) and 94% (for Ctrl), with statistical significance indicated by *** and *.

[CrossMark]
Ruxolitinib partially reverses functional natural killer cell deficiency in patients with signal transducer and activator of transcription 1 (STAT1) gain-of-function mutations

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GRAPHICAL ABSTRACT

Partial rescue of NK cell cytotoxicity in STAT1 GOF patients by ruxolitinib

A

CD56^dim NK cells positive for perforin (%)
Conclusions

• NBS is highly effective for SCID diagnosis
  • Rapid and new treatment options

• Innovation in the genetic era of immune disease
  • Precision diagnosis can be of value
  • Justification for your pretest probability

• Think about HLH in critically ill children and rethink the approach to diagnosis getting to correct treatment

• Complex genetics require an openness to new paradigms in the future

• Precision and personalized diagnosis can equate with specific therapeutics in PID