Genetic screening and diagnosis of primary immune deficiency

Anthony J. Infante, MD, PhD
Division of Immunology & Infectious Diseases
Disclosures

• Consultant
  – TDSHS newborn screening program

• Site PI for clinical trials
  – Grifols SCIG phase III trial
  – Baxalta HyQvia monitoring

• No associations with commercial diagnostic entities
Changing landscape of primary immune deficiency (PID)

• Over 100 individual, genetically well-defined, immunologically-mediated syndromes
  – Many represented by only a handful of cases
  – Most revealed by familial clustering

• New categories
  – Immune dysregulation syndromes
  – Autoinflammatory syndromes
  – Innate immunity defects

• Novel genetics
  – Dominant negative mutations
  – Gain-of-function (GOF) mutations
Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency

Waleed Al-Herz1,2, Aziz Bousfiha3, Jean-Laurent Casanova4,5, Talal Chatila6, Mary Ellen Conley 4, Charlotte Cunningham-Rundles7, Amos Etzioni 8, Jose Luis Franco9, H. Bobby Gaspar 10*, Steven M. Holland 11, Christoph Klein12, Shigeaki Nonoyama13, Hans D. Ochs14, Erik Oksenhendler 15,16, Capucine Picard5,17, Jennifer M. Puck 18, Kate Sullivan19 and Mimi L. K.Tang20,21,22

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Who walks in the door?

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<table>
<thead>
<tr>
<th>Categories</th>
<th>Global</th>
<th>U.S.A.</th>
<th>International</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined T and B-cell Immunodeficiencies</td>
<td>3,163</td>
<td>608</td>
<td>2,555</td>
</tr>
<tr>
<td>Other Well Defined Immunodeficiency Syndromes</td>
<td>9,427</td>
<td>3,413</td>
<td>6,014</td>
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<tr>
<td>Diseases of Immune Dysregulation</td>
<td>1,553</td>
<td>282</td>
<td>1,271</td>
</tr>
<tr>
<td>Congenital Defects of Phagocyte Numbers and Function</td>
<td>3,189</td>
<td>461</td>
<td>2,728</td>
</tr>
<tr>
<td>Predominantly Antibody Deficiencies</td>
<td>31,162</td>
<td>8,388</td>
<td>22,774</td>
</tr>
<tr>
<td>Defects in Innate Immunity</td>
<td>328</td>
<td>118</td>
<td>210</td>
</tr>
<tr>
<td>Autoinflammatory Disorders</td>
<td>3,600</td>
<td>352</td>
<td>3,248</td>
</tr>
<tr>
<td>Complement Deficiencies</td>
<td>3,652</td>
<td>564</td>
<td>3,088</td>
</tr>
<tr>
<td>Other Immunodeficiencies</td>
<td>4,290</td>
<td>1,416</td>
<td>2,874</td>
</tr>
</tbody>
</table>

**Total**

- **Global**: 60,364
- **U.S.A.**: 15,602
- **International**: 44,762
Most outcomes are good

• Antibody deficiencies
  – 50% of cases
  – Upper and lower respiratory infections
  – Treatable with IGRT

• SCIDS
  – 25% of cases
  – Almost all diagnosed by newborn screen
  – 80-90% curable with HSCT
Meeting the Diagnostic Challenge

- Awareness, outreach, and referral
- More sophisticated immunophenotyping and functional analysis
- Genetic diagnosis
  - Candidate gene approaches
  - Newborn screening
  - Genomic analysis
Case 1

• 7 mo. old boy, recently emigrated from India, parents contracted to work at large local business
• Presented to ER with fever, deltoid abscess, axillary lymphadenopathy
• Returned to ER with inability to bear weight on left leg
<table>
<thead>
<tr>
<th>Laboratory</th>
<th></th>
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<tbody>
<tr>
<td>CBC</td>
<td>ALC: 216 cells/mm³</td>
</tr>
<tr>
<td>Lymphocyte subsets</td>
<td>CD3: 6 cells/mm³; CD4: 2; CD8: 0; CD19: 1, CD16/56: 189</td>
</tr>
<tr>
<td>HIV1/2</td>
<td>Negative</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>IgA undetectable, IgM undetectable, IgG 140 mg/dl</td>
</tr>
<tr>
<td>CSF</td>
<td>83 WBCs/mm³, 50% PMNs, 42% MNCs, 2% L; protein 48 mg/dl; glucose 49 mg/dl</td>
</tr>
<tr>
<td>Microbiology</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td>No bacterial growth at 48 hrs.; later positive for AFB identified as M. bovis/BCG</td>
</tr>
<tr>
<td><strong>CSF</strong></td>
<td>Negative bacterial meningitis screen and gram stain; negative fungal smear and culture; negative PCR for HSV-1 and 2, CMV</td>
</tr>
<tr>
<td><strong>Stool</strong></td>
<td>Enterovirus isolated; identified as iVDPV1</td>
</tr>
<tr>
<td><strong>Lymph node FNA</strong></td>
<td>AFB stain positive; identified as M. bovis/BCG</td>
</tr>
</tbody>
</table>
A Phenotypic Approach for IUIS PID Classification and Diagnosis: Guidelines for Clinicians at the Bedside

Journal of Clinical Immunology
August 2013, Volume 33, Issue 6, pp 1078–1087
Algorithmic approach
Case 1

- Genetic diagnosis of RAG-1 deficient SCIDS
- Vaccine-associated paralytic polio (VAPP)
- BGG-osis
- Family history of consanguinity and early infant death
- After meeting with several consultants and ethics committee, parents decided to withdraw life support
Vaccine-Associated Paralytic Poliomyelitis and BCG-osis in an Immigrant Child with Severe Combined Immune Deficiency Syndrome — Texas, 2013

Robert Trimble, MD¹, Jane Atkins, MD², Troy C. Quigg, DO³, Cara C. Burns, PhD⁴, Gregory S. Wallace, MD⁴, Mary Thomas, MBBS⁵, Anil T. Mangla, PhD⁵, Anthony J. Infante, MD, PhD¹ (Author affiliations at end of text)
SCIDS treatment is highly effective if done early in life

Possible screening approaches

• Absolute lymphocyte count
• Lymphocyte subsets by flow cytometry
  – Low or absent T cells (typically <300)
  – Especially low naïve T cells (CD45RA)
• Genetic analysis for candidate gene mutations
  – 14 genes at last count
• Absent or very low TREC
What are TREC{s?}
Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States

Antonia Kwan, PhD, MRCPCH1,2; Roshini S. Abraham, PhD3; Robert Currier, PhD4; Amy Brower, PhD5; Karen Andruszewski, BS6; Jordan K. Abbott, MD7; Mei Baker, MD8,9; Mark Ballow, MD10; Louis E. Bartoshesky, MD11; Francisco A. Bonilla, MD, PhD12,13; Charles Brokopp, DrPH14; Edward Brooks, MD15; Michele Caggana, ScD16; Jocelyn Celestin, MD17; Joseph A. Church, MD18,19; Anne Marie Comeau, PhD20,31; Jordan K. Abbott, MD7; Morten J. Cowan, MD1,2; Charlotte Cunningham-Rundles, MD22; Trivikram Dasu, PhD23; Nina Dave, MD24; Karen Andruszewski, BS6; Jordan K. Abbott, MD7; Morten J. Cowan, MD1,2; Charlotte Cunningham-Rundles, MD22; Trivikram Dasu, PhD23; Nina Dave, MD24; Maria T. De La Morena, MD25; Ulrich Duffner, MD26; Chin-To Fong, MD27; Lisa Forbes, MD28,29; Debra Freedenberg, MD30; Erwin W. Gelfand, MD7; Jaime E. Hale, BS20; I. Celine Hanson, MD28,29; Beverly N. Hay, MD31; Diana Hu, MD32; Anthony Infante, MD, PhD15; Daisy Johnson, BSN30; Neena Kapoor, MD18,19; Denise M. Kay, PhD16; Donald B. Kohn, MD33; Rachel Lee, PhD30; Heather Lehman, MD10; Zhili Lin, PhD34; Fred Lorey, PhD4; Aly Abdel-Mageed, MD, MBA26; Adrienne Manning, BS35; Sean McGhee, MD36,37; Theodore B. Moore, MD33; Stanley J. Naides, MD38; Luigi D. Notarangelo, MD12,13; Jordan S. Orange, MD28,29; Sung-Yun Pai, MD12,13; Matthew Porteus, MD, PhD36,37; Ray Rodriguez, MD, JD, MPH, MBA24; Neil Romberg, MD39; John Routes, MD40; Mary Ruehle, MS41; Arye Rubenstein, MD42; Carlos A. Saavedra-Matiz, MD16; Ginger Scott, RN30; Patricia M. Scott, MT43; Elizabeth Secord, MD41; Christine Seroogy, MD44; William T. Shearer, MD, PhD28,29; Subhadra Siegel, MD45; Stacy K. Silvers, MD46; E. Richard Stiehm, MD33; Robert W. Sugerman, MD46; John L. Sullivan, MD31; Susan Tanksley, PhD30; Millard L. Tierce IV, DO41; James Verbsky, MD, PhD40; Beth Vogel, MS16; Rosalyn Walker, MD24; Kelly Walkovich, MD21; Jolan E. Walter, MD, PhD47,48; Richard L. Wasserman, MD, PhD46; Michael S. Watson, MS, PhD5; Geoffrey A. Weinberg, MD27; Leonard B. Weiner, MD49; Heather Wood, MS6; Anne B. Yates, MD24; Jennifer M. Puck, MD1,2

Results

• Infants born from January 2008 through July 2013 were included. Representatives from 10 states plus the Navajo Area Indian Health Service contributed data from 3,030,083 newborns screened with a TREC test.

• Screening detected 52 cases of typical SCID, leaky SCID, and Omenn syndrome, affecting 1 in 58,000 infants (95%CI, 1/46,000-1/80,000)

• Survival of SCID-affected infants through their diagnosis and immune reconstitution was 87%(45/52), 92%(45/49) for infants who received transplantation, enzyme replacement, and/or gene therapy
Case 2

• 10 month old boy transferred for neuro rehab
• PMH
  – *S. pneumo* meningitis age 4 mos., recovered
  – *S. pneumo* meningitis age 9 mos., devastated
• Immunizations up-to-date
• Hypogammaglobulinemia noted
III. Predominantly antibody deficiencies

Recurrent bacterial infections eg: Otitis, pneumonia, sinusitis, diarrhoea, sepsis

Serum Immunoglobulin Assays: IgG, IgA, IgM

- **IgG, IgA and/or IgM ↓**
  - Exclude 2° causes: drugs [Hx], myeloma [bone marrow], lymphoma / thymoma [CT]. Ig loss (not hypo-IgM) in urine, GI, or skin

- **IgG and IgA↓ and normal or increased IgM**
  - Healthy infant, no increased bacterial infections. Normalisation at 36-60 months
  - Transient hypogammaglobulinemia of infancy

- **IgA↓**
  - Specific antibody responses
    - (anti-PPS antibodies and Tet/Dip/hib +/- reimmunisation)
  - Healthy infant, no increased bacterial infections. Normalisation at 36-60 months
  - Transient hypogammaglobulinemia of infancy

- **Normal IgA, IgG, IgM**
  - 1 IgG subclasses 1, 2, 3 levels (measure at least two)
  - 2 Specific antibody responses (anti-PPS antibodies and Tet/Dip/hib)

- **CD19+ absent**
  - X-Linked Agammaglobulinaemia (BTK)
    - Rare AR
    - Agammaglobulinaemia: deficiencies of µ, heavy chain (KHM), IgG1 (CD79a), IgG2 (CD79b), IgG3 (CD22), BLNK (CD19), CD19*, CD21*, CD22, CD40, CD40L, P55 subunit of PI3K (PIK3R1)

- **CD19+ > 1%**
  - Common Variable Immunodeficiency Disorders (CVID)
    - Very rare AR disorders:
      - ICOS*, CD19*, CD21*, CD22, LRBA
    - Less common AR hyper IgM disorders, with lymphoid hyperplasia:
      - AID-def (AICDA), UNG-def (UNG), Others (unknown genes)
      - XL, CD40L (CD40LG)
      - Or
      - AR, CD40* (CD40)

- **IgA with Specific Ab deficiency**
  - Doubtful clinically significant
  - Check specific antibody responses
  - Doubtful clinical significance
  - Specific Ab deficiency

- **No**
  - Selective IgA

- **Yes**
  - IgG1 & IgG2 are Low
  - Only IgG1 is Low
  - Only IgG2 is Low

- **Check IgG again!**
Case 2: definitive diagnosis

- Very low B cells in blood
- BTK mutation detected
- Dx: X-linked agammaglobulinemia (XLA)
Delay in diagnosis common in XLA
“Severe bacterial infections (pneumonia, sepsis, meningitis, osteomyelitis) ... may occur in a normal child, a second occurrence should alert the physician to possible immunodeficiency.” Conley & Stiehm, Immunologic Disorders in Infants & Children, 4th edition, 1996.

Should this advice be modified in the era of highly effective bacterial vaccines for *H. influenzae, S. pneumoniae, N. meningitidis*?
Effects of vaccination

HIB

PCV
A Devastating Outcome in Undiagnosed X-Linked Agammaglobulinemia– A Call for Earlier Screening

Karen E. Bruner, MD¹, Anthony J. Infante, MD, PhD²

¹ Wilford Hall Ambulatory Surgical Center, Joint Base San Antonio, ²University of Texas Health Sciences Center San Antonio

Trainee poster award, 2nd place, ACAI, 2014
When to screen for XLA?

• After a single episode of invasive bacterial infection?
• Newborn screening?
XLA in children hospitalized for community acquired pneumonia

- 254 subjects (131 males) median age 4.5 years
- Hospitalized for CAP
- Screened for hypogammaglobulinemia and vaccine responses
- 2 boys found to have genetically confirmed XLA
- “several” other children with “humoral immunity abnormalities”
- Proposal: screen all boys <5 yo with CAP

NBS for XLA?

- Avoid rare but devastating outcomes
- Prevent bronchiectasis
- Reduce hospitalizations for CAP?
Multiplex RT-PCR for TRECs and KRECs

- 2560 freshly collected, anonymous DBS/Guthrie cards
- 28 stored cards from patients later diagnosed with assorted immune deficiencies
- RT-PCR for TRECs, KRECs and β-actin
- Results expressed as copies per μL of blood

CONCLUSION: screening for XLA is feasible and can be combined with SCIDS screening in a cost-effective manner
Assessment of newborn screening for PID

- Statewide newborn screening has been successfully implemented for SCIDS
- Expected results of early SCIDS detection appear to be realized
- XLA can have adverse consequences when diagnosis is delayed
- Newborn screening for XLA is feasible and may be warranted
Novel syndromes/genetics

- Loss-of-function mutations
  - Traditional way of thinking about primary immune deficiency
- Dominant negative (interfering) mutations
- Gain-of-function mutations
  - Novel syndromes often involving autoimmunity and lymphoproliferation
- Examples: STAT3 mutations
LOF

(a) Null loss-of-function mutation ($m$)
STAT3

• “Signal transducer and activator of transcription”
STAT3 phenotypes

• STAT3 LOF mutations
  – Autosomal dominant hyper-IgE syndrome
  – Lung and skin infections
  – Skeletal and tooth abnormalities

• STAT3 GOF mutations
  – Autoimmunity-diabetes, enteropathy, ITP, et al.
  – Lymphadenopathy
AD-HIES/Job’s-Buckley/STAT3 LOF
Mechanism of dominant negative mutations

Figure 8–67. Molecular Biology of the Cell, 4th Edition.
# STAT3 GOF mutation family

## Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset, Current age</th>
<th>STAT3 variant</th>
<th>Hematologic</th>
<th>Endocrine</th>
<th>GI</th>
<th>Other</th>
<th>Lymphoproliferation</th>
<th>Post-natal short</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 9</td>
<td>&lt;1y, 28y</td>
<td>p.A703T</td>
<td>AIHA, AITP, AIN</td>
<td>No</td>
<td>Small bowel thickening</td>
<td>LIP, atopic dermatitis, alopecia</td>
<td>Yes</td>
<td>HSM</td>
</tr>
<tr>
<td>Patient 10</td>
<td>15y, Dec 28y</td>
<td>p.A703T</td>
<td>AIHA, AIN</td>
<td>No</td>
<td>No</td>
<td>LIP</td>
<td>Yes</td>
<td>HSM</td>
</tr>
<tr>
<td>Patient 11</td>
<td>12y, F 24y</td>
<td>p.A703T</td>
<td>AITP, AIN</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>HSM</td>
</tr>
</tbody>
</table>

Mutation analysis by WES  
Milner, et al., Blood 2014
GOF mutation
Anti-IL-6R therapy in STAT3 GOF

Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations
**Clinical manifestations:**

**STAT3 LOF = AD-HIES**
- Mucocutaneous infections (S. aureus and C. albicans)
- Pneumonia (S. aureus and S. pneumoniae), pneumatoceles
- Dermatitis
- Connective tissue abnormalities

**Clinical manifestations:**

**STAT3 GOF**
- ALPS-like
- IPEX-like
- STAT5b-deficiency-like
- Various organ autoimmunity
- Repeated infections
- Immune deficiency: hypo-IgG, reduced switched memory B cells

**Loss of function**
- ↑ IgE
- ↓ Th17
- ↓ T follicular helper
- ↓ B-cell maturation and function

**Gain of function**
- ↑ IL-6 signaling
- ↑ SOCS3
- ↓ pSTAT5
- ↓ pSTAT1
- ↓ Tregs
Summary:
Changing landscape of PID

- Improved outcomes through newborn screening
- Novel syndromes with novel genetic mechanisms
- Powerful genetic and genomic tools