Primary Immunodeficiency Diseases in children and adults
Primary Immune Deficiency Diseases (PID)
• Background
• Approach to diagnosis of PID
• Genetic diagnosis of PID
• Lack of focus is good thing
• Looks like duck but not a duck
Primary Immunodeficiency diseases

• Inherited diseases of immune system

• Affect different components of the immune system

• Clinically heterogeneous
Why do we need to diagnose PIDs?

• Collective Prevalence of high as 1 in 10000 suggesting a very high burden of disease
• Often missed causing significant morbidity and mortality
• Multiple family members may get affected leading to financial burden on the family and society
• Early diagnosis and adequate management can lead to significant reduction in morbidity and mortality
<table>
<thead>
<tr>
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<td>Group VIII</td>
<td>Complement deficiencies.</td>
</tr>
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<td>Group IX</td>
<td>Phenocopies of PID</td>
</tr>
</tbody>
</table>
International Union of Immunological Societies (IUIS):
- 354 distinct disorders
- 344 different gene defects listed (Feb2017)
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- Autoimmunity
- Autoinflammation

Inborn errors of immunity

- Recurrent infections
- Malignancies
PID in children

• PID presenting early neonatal period
  • LAD-I
  • Omenn syndrome
  • Di George Syndrome
  • SCN
  • Reticular dysgenesis
PID in children

• PID presenting within first 6 months of life
  • SCID
  • CID
  • Di George Syndrome
  • Diseases of immune dysregulation
  • CGD
  • HIGM: CD40-CD40L deficiency
  • MSMD
  • HLH
PID in children

- PID presenting between 6 months-1 year
  - XLA
  - Other agammaglobulinemia
  - Wiskott–Aldrich syndrome
  - DiGeorge syndrome
  - Chronic mucocutaneous candidiasis
  - Hypogammaglobulinaemia
  - Phagocytic defect - CGD
PID in children

• PID presenting after the age of 5 year
  • AT, other DNA repair disorder
  • Common variable immunodeficiency
  • Specific antibody deficiency
  • Complement disorder
  • Milder forms of PID
Primary Immunodeficiency (PI) causes children and adults to have infections that come back frequently or are unusually hard to cure. 1:500 persons are affected by one of the known Primary Immunodeficiencies. If you or someone you know is affected by two or more of the following Warning Signs, speak to a physician about the possible presence of an underlying Primary Immunodeficiency.

1. Four 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 fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections including septicemia.
10. A family history of PI.

Presented as a public service by:
PID in adults

- Antibody deficiencies:
  - Common Variable Immune Deficiency: CVID
  - Specific antibody deficiencies
  - IgG subclass deficiency
- Autoimmune lymphoproliferative syndrome
- Hypomorphemic forms of many severe PID
- Phenocopies of PID
**10 Warning Signs of Primary Immunodeficiency**

Primary Immunodeficiency (PI) causes children and adults to have infections that come back frequently or are unusually hard to cure. 1:500 persons are affected by one of the known Primary Immunodeficiencies. If you or someone you know is affected by two or more of the following Warning Signs, speak to a physician about the possible presence of an underlying Primary Immunodeficiency.

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<td><strong>1.</strong></td>
<td>Two or more new ear infections within 1 year.</td>
</tr>
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<td><strong>2.</strong></td>
<td>Two or more new sinus infections within 1 year, in the absence of allergy.</td>
</tr>
<tr>
<td><strong>3.</strong></td>
<td>One pneumonia per year for more than 1 year.</td>
</tr>
<tr>
<td><strong>4.</strong></td>
<td>Chronic diarrhea with weight loss.</td>
</tr>
<tr>
<td><strong>5.</strong></td>
<td>Recurrent viral infections (colds, herpes, warts, condyloma).</td>
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<td><strong>6.</strong></td>
<td>Recurrent need for intravenous antibiotics to clear infections.</td>
</tr>
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<td><strong>7.</strong></td>
<td>Recurrent, deep abscesses of the skin or internal organs.</td>
</tr>
<tr>
<td><strong>8.</strong></td>
<td>Persistent thrush or fungal infection on skin or elsewhere.</td>
</tr>
<tr>
<td><strong>9.</strong></td>
<td>Infection with normally harmless tuberculosis-like bacteria.</td>
</tr>
<tr>
<td><strong>10.</strong></td>
<td>A family history of PI.</td>
</tr>
</tbody>
</table>
Clinical clues:

• Age of presentation
• Pattern of infections: organism and the site involved
• Associated clinical manifestations
  • Autoimmunity and autoinflammation
  • Endocrine manifestations
  • Malignancies
  • Skeletal abnormalities
  • Skin manifestations
• Family history:
  • Early sibling death due to similar illness
  • Consanguinity
  • X-linked pattern
Approach diagnosis of PID

Clinical presentation

Molecular diagnosis

Initial immunological evaluation
Complete blood count:

- **Absolute counts:**
  - ANC
    - Neutropenia: ANC<500 (Severe congenital neutropenia/cyclic neutropenia)
    - Neutrophilia: Leukocyte Adhesion Deficiencies (LAD)
  - ALC
    - Lymphopenia: Combined Immunodeficiency (CID)
    - Lymphocytosis: Autoimmune lymphoproliferative Disorders
  - AEC
    - Eosinophilia: Hyper IgE syndromes/ Omenn’s syndrome
  - AMC
    - Monocytopenia in GATA2 deficiency

- **Platelet count with MPV:**
  - Wiskott Aldrich Syndrome (WAS)
Immunological evaluation:

• Total number of cells

• Function of cells:
  • T cell proliferation
  • Antibody production
  • DHR assay

• Assays for specific diagnosis:
  • CD18 expression
  • Perforin expression
Initial evaluation:

- Serum immunoglobulin levels
- Lymphocyte subset analysis
- NBT test
- Complement levels
The 2015 IUIS Phenotypic Classification for Primary Immunodeficiencies

Aziz Bousfiha¹ · Leila Jeddane¹ · Waleed Al-Herz²,³ · Fatima Ailal¹ · Jean-Laurent Casanova⁴,⁵,⁶,⁷,⁸ · Talal Chatila⁹ · Mary Ellen Conley⁴ · Charlotte Cunningham-Rundles¹⁰ · Amos Etzioni¹¹ · Jose Luis Franco¹² · H. Bobby Gaspar¹³ · Steven M. Holland¹⁴ · Christoph Klein¹⁵ · Shigeaki Nonoyama¹⁶ · Hans D. Ochs¹⁷ · Eric Oksenhendler¹⁸,¹⁹ · Capucine Picard⁵,²⁰ · Jennifer M. Puck²¹ · Kathleen E. Sullivan²² · Mimi L. K. Tang²³,²⁴,²⁵
Spectrum of infections: CID

**Common Microorganisms**
- Viruses
  - CMV
  - Vaccinia
  - Adenovirus, HSV,
  - Measles
- Pyogenic bacteria
- Mycobacteria: BCGiosis
- Fungi
  - Candida, Aspergillus, PCP
- Protozoa
  - Cryptosporidium

**Less common Microorganisms**
- Bacteria
  - Campylobacter
  - Mycobacteria
  - Listeria

**Early onset (<6 months)**
- Failure to thrive
- Oral candidiasis
- Protracted diarrhea
- Skin rash
- Respiratory Tract
- Systemic viral infections
- Gastroenteritis
- Opportunistic infections
- GVHD
- Hepatosplenomegaly (Omenn syndrome)
1. Evaluation of defects with altered T lymphocyte population

• Severe Combined Immunodeficiency (SCID) disorder characterized by CD3+ T cell lymphopenia. Further classification based on B and NK cell numbers/function.

- T-SCID
- B+
  - NK- • X-SCID
    - JAK3 defect
  - NK+ • IL-7 Ra
- B-
  - NK+ • RAG1/2
    - Artemis
  - NK- • ADA
    - PNP
Interpretation of lymphocyte subset analysis

- Check % as well as absolute counts
- The normal ranges vary significantly depending on the age of the patient
- In infants under 4 months of age, a CD4 count of <1000/mm³ is generally associated with impaired cellular immunity, whereas it is <500/mm³ in children over 2 years of age and in adults.
- Immunosuppressive therapies like steroids also significantly alter the values of T and B cell subsets and should be interpreted carefully.
- Do not rely on single observation, repeat counts or compare with the previous counts if available
Immunophenotyping T Lymphocytes

CD3+/CD4+ T helper 29-52%

CD3+/γδ Cells

CD8+ T cytotoxic cells 13.5-22%

CD3+ /γδ cells

CD3+/αβ Cells

Naïve Helper T cells CD45RO-/CD45RA+/CD27+ (49-71%)

Memory Helper T cells CD45RO+/CD45RA-/CD27+ (24-42%)

Memory Effector Helper T cells CD45RO+/CD45RA-/CD27- (2.1-7.4%)

Th17 (IL-17)

Effector T cells CD45RO-/CD45RA-/CD27- (0.1-2.2%)

Regulatory T cells CD25+/CD127dim/ FoxP3+/CD4+ (2.2-4.1%) -IPEX -APECED

Naïve Cytotoxic T cells CD45RO-/CD45RA+/CD27+ (48.6-87.5%)

Memory Cytotoxic T cells CD45RO+/CD45RA-/CD27+ (9.8-37%)

Memory Effector Helper T cells CD45RO+/CD45RA-/CD27- (0.2-6.9%)

Effector T cells CD45RO-/CD45RA-/CD27- (0.8-14%)

CD3+/TCR αβ+/ CD4-/CD8- DNT% (ALPS)

Memory Effector T cells CD45RO+/CD45RA-/CD27- (9.8-37%)
Measurement of Naïve T cell subsets (CD45RA+ CD62L+ T cells) Recent Thymic emigrants (RTE) and Memory T cells (CD45RO+ T cells)

Healthy control

Patient

CD45RO+ T cells in a scid child suggestive of maternal engraftment Absent naïve T cells
T+ Severe combined Immunodeficiency

- Activation markers on T cells: **HLA-DR**
- TCR- V beta repertoire analysis
- T cell proliferation response to various stimuli

- T+ B+ NK+: Omen’s syndrome
  - Elevated HLA-DR expression on CD3+ T cells
  - Restricted repertoire of T cells
- CD8+B+NK+: CD4 lymphopenia : MHC class II deficiency
  - Lack of HLA-DR expression on B cells, Monocytes
- CD4+B+ NK-: ZAP70 kinase deficiency
  - Defective CD4+Th cell proliferation
Spectrum of infections: Antibody deficiency

Onset typically between 4 months-1 year

**Common Microorganisms**
- Pyogenic bacteria
  - Staphylococci
  - Streptococci
  - Hemophilus

**Less common Microorganisms**
- Enteroviruses
  - Polio
  - ECHO
  - Salmonella
  - Campylobacter
  - Mycoplasma

- Respiratory tract
  - Otitis media, mastoiditis
  - Chronic sinusitis
  - Broncho and lobar pneumonia
  - Bronchiectasis
  - Pulmonary infiltrates (granulomas)

- GI tract
  - Giardia
  - Nodular hyperplasia, ileitis, colitis

- Skin infections
- Sepsis/Meningitis
- Skeletal
  - Arthritis (bacterial, mycoplasma, noninfectious)
Evaluation of Predominantly antibody deficiency

• B cell function:
  • IgG, A, M and E
  • Specific antibody responses against both protein and polysaccharide vaccines
  • Ig subclass estimation

• B cell numbers: CD19 or CD20

• B-cell immunophenotyping, and other modalities of measuring B-cell function
Immunoglobulin levels

• IgG, IgA, IgM, IgE
  – The assay results should be evaluated in the context of the tested patient’s age and clinical findings
  – Compare with age related normal ranges
  – Child < 6 months of age has circulating maternal IgG.

• Blood transfusion and immunoglobulin infusion will alter the levels and hence this history needs to be taken.

• Persistence of hypogammaglobulinemia is required for at least 3 months needs to be documented for diagnosis of CVID

• Hypergammaglobulinemia can be the result of HIV-1, CGD, and ALPS
Immunoglobulin levels:

• Always rule out secondary causes of hypogammaglobulinemia
  – Drugs: Steroids, Rituximab, other chemotherapy
  – Myeloma and Lymphoma
  – Loss of Ig (usually IgM is normal) in urine, GI, skin
B cell numbers:

- Check % as well as absolute counts
- Significant variation depending on the age and hence must be compared with age matched controls
- Always interpret along with T cell and NK cell numbers
- Check history of drugs:
  - Rituximab
  - Steroids
B cell immunophenotyping

- CD19+=B cells expressing CD19 as a percent of total lymphocytes
- CD19+ CD21-=CD21 low ("immature") B cells
- CD19+ CD21+=mature B cells
- CD19+ CD27+=total memory B cells
- CD19+ CD27+ IgD+ IgM+=marginal zone or non-switched memory B cells
- CD19+ CD27+ IgD- IgM+=IgM-only memory B cells
- CD19+ CD27+ IgD- IgM-=class-switched memory B cells
- CD19+ IgM+=IgM B cells
- CD19+ CD38+ IgM+=transitional B cells
- CD19+ CD38+ IgM-=plasmablasts
- CD19+ CD20+=B cells co-expressing both CD19 and CD20 as a percent of total lymphocytes
• Hepato-splenomegaly/lymphadenopathy
• Granulomas

Deep seated abscesses, Fungal Infection Gram negative septicemia

Phagocytic defects CGD
Chronic Granulomatous disease:

- Normal
- Patient
- Carrier

- p22phox FITC
- 7D5 PE

- p22 phox defect
- X-linked gp91phox defect
Interesting cases
Case

• 18 year old male studying engineering:
  – h/o abdominal tuberculosis two times
  – h/o histoplasmosis 4 years back
  – h/o infective diarrhea frequent episodes since last 5 years
  – h/o failure to thrive
  – h/o BCGiosis at 4 months of age
• CBC:
  • Lymphopenia ALC 1200/mm3

• Immunoglobulin levels: Normal

• NBT and DHR normal

• Lymphocyte subset analysis:
  – Absolute CD4 counts: 250
  – Other subsets within normal range
  – Memory and naïve T cells within normal range

• Sample sent for NGS
  – IL12R B1 deficiency
Mendelian Susceptibility to Mycobacterial Diseases (MSMD)

Defect in IFNγ/IL12-23 Axis loop

Clinical Presentation
- BCGiosis
- Recurrent TB
- Infections by non-mycobacterial TB
- Multifocal TB
- Salmonella Infections
- Fungal infections

Diagnosis
Flow cytometric evaluation of IFNγ/IL12-23 Axis

Defective molecules in this circuit, including IFN-R1, IFN-R2, IL-12p40, IL-12R-1, STAT-1, NEMO, IKBA

IL12Rβ1 and pSTAT4 expression in Normal

IL12Rβ1 and pSTAT4 expression in patient
Autoimmune Lymphoproliferative syndrome (ALPS)

Clinical presentation
- Chronic nonmalignant lymphadenopathy
- Splenomegaly
- Autoimmunity

Diagnosis of ALPS
Double Negative T cells (CD3+TCRab+CD4-CD8- % greater than or equal to 1.5% of total lymphocytes or 2.5% of T lymphocytes, in the setting of normal or elevated lymphocyte counts
Familial Hemophagocytic Lymphohistiocytosis (FHL) with late onset

- 36 year old Male born with non consanguineous marriage and no significant family history
- He had prolonged fever for more than a 3 months with cytopenia and hepatosplenomegaly.

<table>
<thead>
<tr>
<th>HLH criteria</th>
<th>Yes</th>
<th>3/4</th>
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<td>Fever</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Cytopenia</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hemophagocytosis</td>
<td>yes</td>
<td>3/4</td>
</tr>
<tr>
<td>Ferritin (&gt;500mg/ml)</td>
<td>1,00,000ng/ml</td>
<td></td>
</tr>
<tr>
<td>sCD25 levels (1886-13474pg/ml)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>NK cell function</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (&gt;265mg/dl)</td>
<td>365mg/dl</td>
<td>2/2</td>
</tr>
<tr>
<td>Fibrinogen (&lt;150mg%)</td>
<td>65mg%</td>
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**PRF1 gene mutation- 694C>T  Arg232Cys**
- Treated with HLH protocol 2004 including cyclosporine and dexamethasone. He responded well.
- On tapering the protocol, he again developed fever and hence, the protocol was resumed.
- Maintenance therapy and then was then off steroids for more than 6 months and leading routine life.
- He again had a relapse and inspite of prompt supportive treatment, **patient succumbed** to the disease due to multiple organ failure and septic shock.
Autoantibody to interferon-gamma in Adult Lymphopenia

- Adult-onset immunodeficiency a syndrome associated with disseminated infections.
- Cell-mediated immune deficiency in HIV-negative, adult-onset immunodeficient patients linked to the presence of autoantibody to interferon-gamma (IFN-γ).
Genetic Diagnosis of PID

Genetics of PID

• >354 distinct disorders with 344 different gene

• Inheritance model:
  • Autosomal Recessive (AR),
  • Autosomal Dominant (AD)
  • X-Linked (XL)
Inheritance Model: Autosomal Recessive (AR)

- Parents are carriers.
- Risk of having an affected child is \( \frac{1}{4} \) or 25% percent chance.
- Risk of having an affected child is increased in consanguineous marriages.

Some PID following AR inheritance |
<table>
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<td>Severe Combined Immunodeficiency</td>
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<td>Chronic Granulomatous disease</td>
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<tr>
<td>Leukocyte Adhesion Defect</td>
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<tr>
<td>Familial Hemophagocytic lymphohisticytosis</td>
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</table>
Leukocyte Adhesion defect (LAD-I)

Different mutations observed in ITGB2 gene

- Missense
- Non-sense
- Intronic Splice site
- Frameshift Deletion
- Frameshift Insertion

Photo from Dr. Mukesh Desai
## X-Linked Inheritance

- Mutation is in a gene on the X chromosome.
- Females and males manifest the condition differently. Females with single copy mutation are usually carriers; males with one mutated copy are affected.
- Females carrier has 1 in 2 or 50% risk of having an affected boy and the same chance of having a carrier daughter.
- Family pedigree shows strong history of male children affected.
- Skewed X-inactivation can result in carrier females displaying symptoms of the condition as a larger proportion of their normal X chromosomes are inactivated.
<table>
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<th>Gene</th>
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<tr>
<td>X-linked agammaglobulinemia (XLA; Bruton’s disease)</td>
<td>BTK</td>
</tr>
<tr>
<td>X-linked severe combined immunodeficiency (X-SCID)</td>
<td>IL2RG</td>
</tr>
<tr>
<td>X-linked hyper IgM syndrome (CD40 ligand deficiency)</td>
<td>CD40L</td>
</tr>
<tr>
<td>X-linked lymphoproliferative disease (XLP)</td>
<td>SH2DIA</td>
</tr>
<tr>
<td>X-linked inhibitor of apoptosis (XIAP) deficiency</td>
<td>XIAP</td>
</tr>
<tr>
<td>X-linked chronic granulomatous disease (X-CGD)</td>
<td>CYBB</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome (WAS)</td>
<td>WASP</td>
</tr>
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X-linked agammaglobulinemia (XLA; Bruton’s disease)

Laboratory Findings

- All Ig low with absent B cells

XLA?

- Analysis of Bruton Tyrosine Kinase on Monocytes

- Mother shows a classical carrier pattern of BTK expression
Autosomal Dominant Inheritance model

- Affected individual has one affected copy and normal functioning copy
- One copy of mutation sufficient to cause the condition
- 1 in 2 or 50% risk of passing the mutation on to offspring who will then also be affected.
- Same mutation – degree of disease phenotype differs (variable expressivity)
- Same mutation – may not result in disease phenotype in all individuals (incomplete penetrance).

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<th>Some PIDs that follow AD inheritance</th>
<th>Gene</th>
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<tr>
<td>Hyper IgE</td>
<td>STAT3</td>
</tr>
<tr>
<td>Hereditary neutropenia</td>
<td>ELA2</td>
</tr>
<tr>
<td>Di George Syndrome</td>
<td>deletions on chromosome 22q11.2</td>
</tr>
<tr>
<td>Mendelian susceptibility to mycobacterial diseases</td>
<td>Partial IFNγR1 deficiency STAT1</td>
</tr>
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**Hyper IgE Syndrome, due to mutations in STAT3 (Jobs syndrome)**

Typical features in AD

HYPER IgE:

- Eczema,
- Repeated staphylococcal skin abscesses,
- Staph pneumonia with pneumatocele formation
- Skeletal abnormalities and fractures

### Laboratory finding of Increased IgE levels

<table>
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<tr>
<th>Immunoglobulin</th>
<th>Values</th>
<th>Normal Range</th>
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<tr>
<td>IgG</td>
<td>22.9 g/L</td>
<td>3.5 to 16.2g/L</td>
</tr>
<tr>
<td>IgA</td>
<td>0.8 g/L</td>
<td>0.17 to 3.18 g/L</td>
</tr>
<tr>
<td>IgM</td>
<td>1.42g/L</td>
<td>0.30 to 2.65 g/L</td>
</tr>
<tr>
<td>IgE</td>
<td>50800 IU/mL</td>
<td>3.0 - 423 IU/mL</td>
</tr>
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</table>
One gene, multiple phenotypes

**STAT1 gene defect**

**AD STAT1 deficiency:**
Loss of function mutation
susceptibility to mycobacterial and salmonella infections

**AD STAT1 deficiency:**
Gain-of-function mutation
chronic mucocutaneous candidiasis

**Complete/ Partial AR STAT1 deficiency:**
Loss-of-function mutation
susceptibility to severe viral and mycobacterial disease
Different genes $\rightarrow$ similar phenotype

**Chronic Mucocutaneous Candidiasis**

- IL-17RA, IL-17F, STAT1, DECTIN1, CARD9, STAT3, DOCK8, AIRE

**Mendelian Susceptibility to Mycobacterial Diseases**

- IFNGR1, IFNGR2, STAT1, IL12B, IL12RB1, NEMO, CYBB, IRF8, ISG15, TYK2
Clinical, Immunological, and Molecular Findings in Four Cases of B Cell Expansion With NF-κB and T Cell Anergy Disease for the First Time From India

Maya Gupta¹, Jahnavi Aluri¹, Mukesh Desai², Madhukar Lokeshwar³, Prasad Taur², Michael Lenardo⁴, Jenna Bergerson⁴, Aparna Dalvi¹, Snehal Mhatre¹, Manasi Kulkarni¹, Priyanka Kambli¹ and Manisha Madkaikar¹*
What strategy do we use for molecular diagnosis?

- Sanger sequencing/ targeted gene analysis

- NGS:
  - PID panel
  - Clinical exome
  - Whole exome
  - Whole genome
No shoe that fits all

- Diseases for Sanger sequencing/ specific gene analysis is preferred:
  - LAD-I
  - Perforin deficiency
  - X-HIGM
  - XLA
  - CGD
Lack of focus is good thing

- 10 months old male child
- 2\textsuperscript{nd} by order born of 3\textsuperscript{rd} degree consanguineous marriage,
- Symptomatic since day 7 of life with fever and loose stools and respiratory distress
- Elder female sibling death at 6 months of age due to respiratory illness.
- Elevated absolute lymphocyte count (ALC23023/cu mm)
- Lymphocyte subset analysis elevated CD8
- Sample sent for NGS analysis
• Targeted NGS analysis by Med genom e laboratory revealed previously reported homozygous mutation in exon 8 at c.847C>T; p.Arg283Ter of ZAP -70 gene

• 90% of CD8 positive cells were HLA-DR + and naïve CD8T cell % was very low
Limitations of Sanger sequencing

• **One-by one analysis of single genes or candidate genes.**

  PID diagnosis complicated due to genetic heterogeneity and a clinical overlap among various PID categories. In some cases, many genes are involved in the pathogenesis of a specific PID form. For eg: SCID can result from a defect in any of the 15-20 genetic defects.

• **Cost and time consuming to identify the molecular etiology.**

• **The immunophenotype pattern may not always correlate with the genetic etiology.**

  May cases remain unresolved due to lack of phenotype-genotype correlation. Screening the candidate gene does not give the molecular answer.

• **Does not detect large deletions or duplications**
Molecular Diagnosis Strategy (n=57)

FIRST LINE APPROACH: Sanger Sequencing - *IL2RG, ADA, RAG1, RAG2, IL7RA*

SECOND LINE APPROACH: Targeted NGS - PID Panel

Uncharacterized cases - Whole exome Sequencing

- **25/57 (47%)** cases molecularly characterized
- **32 cases** uncharacterized
- **24/32 cases** molecularly characterized
- **8** uncharacterized

In Process
Spectrum of genetic defects in our cohort (n=57)

<table>
<thead>
<tr>
<th>SCID gene defect</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2RG deficiency</td>
<td>18.6</td>
</tr>
<tr>
<td>JAK3 deficiency</td>
<td>15.2</td>
</tr>
<tr>
<td>RAG1/2 deficiency</td>
<td>20</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>8.5</td>
</tr>
<tr>
<td>MHC class II Def</td>
<td>10.1</td>
</tr>
<tr>
<td>ZAP70 Deficiency</td>
<td>3.4</td>
</tr>
<tr>
<td>PNP deficiency</td>
<td>1.7</td>
</tr>
<tr>
<td>Reticular dysgenesis</td>
<td>1.7</td>
</tr>
<tr>
<td>PRKDC defect</td>
<td>1.7</td>
</tr>
<tr>
<td>DCLER1C defect</td>
<td>3.4</td>
</tr>
<tr>
<td>IL7RA deficiency</td>
<td>1.7</td>
</tr>
<tr>
<td>Uncharacterized</td>
<td>14</td>
</tr>
</tbody>
</table>
Next generation sequencing

- High throughput, massively parallel technology involving simultaneous sequencing of a large number of template DNA or cDNA fragments in parallel.
- DNA sequencing can be performed on the entire genome or targeted to specific regions.
- Rapid, cost-efficient, accurate, and high-throughput sequencing of millions of DNA fragments in a reasonably short time.
Strategy for NGS for PID

Patients affected with clinical phenotypes highly suggestive of a PID, Targeted Sequencing restricted only to specific genes or to specific regions of interest (specific panels or clinical exome)

Whole exome sequencing

Whole genome sequencing
What looks like a duck but not a duck

Journal of Clinical Immunology
https://doi.org/10.1007/s10875-018-0567-y

ORIGINAL ARTICLE

Approach to Molecular Diagnosis of Chronic Granulomatous Disease (CGD): an Experience from a Large Cohort of 90 Indian Patients

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Received: 18 August 2018 / Accepted: 4 November 2018
# Springer Science+Business Media, LLC, part of Springer Nature 2018
### Distribution of PID cases

<table>
<thead>
<tr>
<th>IUIS 2017 Classification of PID</th>
<th>Diagnosed Cases Till Date</th>
<th>Diagnosed Cases 2017-2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>I. IMMUNODEFICIENCY AFFECTING CELLULAR &amp; HUMORAL IMMUNITY</td>
<td>147</td>
<td>20</td>
</tr>
<tr>
<td>II. CID WITH ASSOCIATED OR SYNDROMIC FEATURES</td>
<td>59</td>
<td>7.8</td>
</tr>
<tr>
<td>III. PREDOMINANTLY ANTIBODY DEFICIENCY</td>
<td>136</td>
<td>18</td>
</tr>
<tr>
<td>IV. DISEASE OF IMMUNE DYSREGULATION</td>
<td>151</td>
<td>20</td>
</tr>
<tr>
<td>V. CONGENITAL DEFECTS OF PHAGOCYTE NO. FUNCTION</td>
<td>234</td>
<td>31</td>
</tr>
<tr>
<td>VI. DEFECT IN INTRINSIC AND INNATE IMMUNITY</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>VII. AUTOINFLAMMATORY</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>VIII. COMPLEMENT DEFICIENCY</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>IX. PHENOCOPIES OF PID</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>753</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3:** Distribution of Primary Immunodeficiency Disorders diagnosed a) over last 11 years and b) during the period of Jan 2017 – Dec 2018
Approach diagnosis of PID

- Clinical presentation
  - Comprehensive Immune evaluation
  - Molecular diagnosis
  - Initial immunological evaluation
## Acknowledgment

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- Maya Gupta
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- Madhura
- Priyanka Kambli
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- Dr. Reetika
- Ramesh Kawale
- Nitin
- Atish Jadhav

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- Dr. Mukesh Desai - Mumbai
- Dr. Nitin Shah - Mumbai
- Dr. Prithesh Nagar - Hyderabad
- Dr. Antony Terrance - Coimbatore
- Dr. Revathi Raj - Chennai
- Dr. Sirisarani - Hyderabad
- Dr. Indumathi - Bangalore
- Dr. Aditya Gupta - Haryana
- Dr. Sunil Bhat - Bangalore
- Dr. Nita Radhakrishnan - Delhi
- Dr. Biju George - Vellore
- Dr. Janani Sankar - Chennai
- Dr. Sheela Nampoothiri - Cochin
- Dr. Ganesh - Chennai
- Dr. Indu Khosla - Mumbai
- Dr. Supriya Dutta - Mumbai
- Dr. Nitin Shah - Mumbai
- Dr. Mishra - Mumbai
- Dr. Sarath Balaji, Chennai
- Dr. Harsha Prasad - Mangalore
- Dr. Geetha - Calicut

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• Fellowship in Genetic Diagnostics
  • 6 months
  • For Post-graduate degree (MD/MS/DNB) in Pediatrics, Medicine or Obstetrics & Gynaecology, Pathology, Microbiology, Biochemistry, Laboratory Hematology, Laboratory Medicine, Anatomy, Physiology, Dermatology, Hematology, Radiotherapy, Endocrinology, Ophthalmology, Oncology or any other clinical / paraclinical specialty/Superspecialty and holding regular position in Government medical college/hospital.
“Life is like riding a bicycle. To keep your balance you must keep moving.”

Albert Einstein