Navigating PIDD and genetic testing: Advantages and limitations

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Disclosures

• Scientific Advisory Board membership
  • ADMA biologics, Gigagen
• Consultancies
  • CSL, Cytovia, Enzyvant, Editas, Grifols, Sigilon, Sobi, Takeda, Teva
• Editor/Author
  • Up to date
Primary Immunodeficiency / Inborn Errors of Immunity
Genetic inability of the immune system to provide an advantage over the environment

- 2021: >450 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
Utility of the 10 Warning Signs

Clinical Predictors of Primary Immunodeficiency Diseases in Children

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- 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%
# The IUIS classification

<table>
<thead>
<tr>
<th>IUIS table #</th>
<th>PIDD classification</th>
<th>#Genes/Pls 2017</th>
<th>#Genes/Pls 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunodeficiencies affecting cellular and humoral immunity</td>
<td>49</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>Combined immunodeficiencies with associated or syndromic features</td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Predominantly antibody deficiencies</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>Diseases of immune dysregulation</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>Congenital defects of phagocyte number, function or both</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>Defects in intrinsic and innate immunity</td>
<td>52</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td>Autoinflammatory disorders</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>Complement deficiencies</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>9</td>
<td>Bone Marrow Failure Syndromes</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>Phenocopies of PID</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Total = 416 (64 new genes)
2021 IUIS update

<table>
<thead>
<tr>
<th>IUIS Classification Groups</th>
<th>Primary Immunodeficiency Disease Category</th>
<th>Number of Genetic Defects</th>
<th>New Genetic Defects</th>
<th>Number of Diseases</th>
<th>New Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Cellular and humoral immunodeficiencies</td>
<td>60</td>
<td>9</td>
<td>52</td>
<td>8</td>
</tr>
<tr>
<td>II</td>
<td>Syndromic combined immunodeficiencies</td>
<td>65</td>
<td>15</td>
<td>61</td>
<td>13</td>
</tr>
<tr>
<td>III</td>
<td>Antibody deficiencies</td>
<td>43</td>
<td>13</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>IV</td>
<td>Immune dysregulatory diseases</td>
<td>47</td>
<td>9</td>
<td>46</td>
<td>9</td>
</tr>
<tr>
<td>V</td>
<td>Phagocytic diseases</td>
<td>42</td>
<td>4</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>VI</td>
<td>Innate immunodeficiencies</td>
<td>71</td>
<td>20</td>
<td>59</td>
<td>17</td>
</tr>
<tr>
<td>VII</td>
<td>Autoinflammatory diseases</td>
<td>49</td>
<td>16</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>VIII</td>
<td>Complement deficiencies</td>
<td>36</td>
<td>2</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>IX</td>
<td>Diseases due to bone marrow failure</td>
<td>43</td>
<td></td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Phenocopies of PIDs</td>
<td>?</td>
<td>1</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>

Growth of PIDs (IEIs) is exponential

![Graph showing Growth of PIDs (IEIs) is exponential](image)
Growth of PIDs (IEIs) is exponential

Open access: https://link.springer.com/article/10.1007%2Fs10875-019-00737-x

Immunodeficiency Disorders

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Education Gaps

Immunodeficiencies are no longer considered rare conditions. Although susceptibility to infections has become well-recognized as a sign of most primary immunodeficiencies, some children will present with noninfectious manifestations that remain underappreciated and warrant evaluation by an immunologic specialist. Providers must also consider common secondary causes of immunodeficiency in children.
TABLE 5. Pulmonary Manifestations of PIDs

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>ASSOCIATED PID GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial lung disease</td>
<td>COPA, ITCH, STAT3, TMEM173, TNFAIP3, XIAP</td>
</tr>
<tr>
<td>Pulmonary alveolar proteinosis</td>
<td>CD40LG, CSF2RA, GATA2, SLC7A7</td>
</tr>
<tr>
<td>Capillaritis and hemorrhage</td>
<td>COPA</td>
</tr>
<tr>
<td>Eosinophilic pneumonia</td>
<td>NSMCE3</td>
</tr>
</tbody>
</table>

PID = primary immunodeficiency.
New day in PID/IEI

No Mutation

Variant

Uncertain

Benign

Likely

Pathogenic
PI Prevalence

Population Prevalence of Diagnosed Primary Immunodeficiency Diseases in the United States

J. M. Boyle - R. H. Buckley

• GIVE US YOUR 5 SICKEST

Only random digit dialing telephone survey of PIDD

US prevalence 1:1200 persons
1349 children enrolled at birth
WGS performed

**Genotype first approach**
- 100s of variants (29% of children)
- 1 PID

**Phenotype first approach**
(retrospective)
29 had a “10 warning signs” sign
3 had potentially relevant “non causative variants”

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**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)
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
Diagnostic yield summary (evaluation of 45 studies)

NGS (panel)

Total n = 1,196 (16 studies)

Low consanguinity evaluations

DOI: 10.1080/1744666X.2020.1814145

Similar outcomes in most phenotypic diagnoses – Hyper IgE syndrome

NGS for HIES

HIES

NIH HIES score =34.0 avg

6-73 range
Unbiased WES results for 280 families with PIDD
An case study example of otherwise difficult to diagnose patients

5% have more than one confirmed disease causing gene
“blended” phenotype
Getting an answer: yield by clinical diagnosis

Getting an answer: Diagnostic Implications and Impact
Same outcomes in resource constrained settings

Clinical Utility of Whole Exome Sequencing and Targeted Panels for the Identification of Inborn Errors of Immunity in a Resource-Constrained Setting

N=78
NGS=26
WES=52
NGS+WES=2

Diagnosis made

Management changed

Change in management  No change

Pretest probability at work:
As experts you know who to test...

Immunologic Research
https://doi.org/10.1002/s122026-020-09131-x

ORIGINAL ARTICLE

Jeffrey's insights: Jeffrey Modell Foundation's global genetic sequencing pilot program to identify specific primary immunodeficiency defects to optimize disease management and treatment

Jessica Quinn¹ · Vicki Modell¹ · Jennifer Holle² · Rebecca Truty² · Swaroop Aradhya² · Britt Johnson² · Jordan Orange¹ · Fred Modell¹
Pretest probability at work

Table 1  Clinical relevance of genetic test results

<table>
<thead>
<tr>
<th>Confirmed or likely molecular diagnosis</th>
<th>Carrier status</th>
<th>Heterozygous results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 heterozygous variant in AD gene</td>
<td>1 heterozygous allele in AR gene</td>
<td>1 heterozygous allele in AD inheritance</td>
</tr>
<tr>
<td>1 heterozygous variant in XL gene</td>
<td>1 heterozygous allele in AR gene</td>
<td>1 heterozygous allele in XL gene in female</td>
</tr>
<tr>
<td>2 heterozygous variants in AR genes</td>
<td>1 heterozygous allele in XL gene in female</td>
<td>1 heterozygous allele in AR gene</td>
</tr>
<tr>
<td>1 heterozygous P/LP allele and 1 VUS in AR genes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=158

Benefits of diagnosing and treating PIDD

Global report on primary immunodeficiencies: 2018 update from the Jeffrey Modell Centers Network on disease classification, regional trends, treatment modalities, and physician reported outcomes

Vicki Modell¹ · Jordan S. Orange¹ · Jessica Quinn¹ · Fred Modell¹
Benefits of diagnosing and treating PIDD

Table 14  Costs of the most frequent conditions affecting patients with PI pre- and post-diagnosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pre-Dx average no. of episodes</th>
<th>Pre-Dx cost per episode</th>
<th>Pre-Dx annual cost</th>
<th>Post-Dx average no. of episodes</th>
<th>Post-Dx cost per episode</th>
<th>Post-Dx annual cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent otitis media</td>
<td>4.2</td>
<td>$528</td>
<td>$2,217</td>
<td>1.6</td>
<td>$528</td>
<td>$845</td>
</tr>
<tr>
<td>Serious sinus and upper respiratory infections</td>
<td>4.6</td>
<td>$1,125</td>
<td>$5,175</td>
<td>2.1</td>
<td>$1,125</td>
<td>$2,562</td>
</tr>
<tr>
<td>Viral infections</td>
<td>3.7</td>
<td>$1,275</td>
<td>$4,717</td>
<td>1.4</td>
<td>$1,275</td>
<td>$1,785</td>
</tr>
<tr>
<td>Acute bronchitis</td>
<td>3.1</td>
<td>$1,700</td>
<td>$5,270</td>
<td>0.8</td>
<td>$1,700</td>
<td>$1,360</td>
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<tr>
<td>Bacterial pneumonia</td>
<td>2.8</td>
<td>$3,552</td>
<td>$9,645</td>
<td>0.6</td>
<td>$3,552</td>
<td>$2,131</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease and bronchiectasis</td>
<td>4.3</td>
<td>$3,165</td>
<td>$13,609</td>
<td>1.4</td>
<td>$3,165</td>
<td>$4,311</td>
</tr>
<tr>
<td>Hospitalization days</td>
<td>19.8</td>
<td>$2,480</td>
<td>$49,104</td>
<td>3.1</td>
<td>$2,480</td>
<td>$7,688</td>
</tr>
<tr>
<td>Physician/ER visits</td>
<td>70.8</td>
<td>$180</td>
<td>$12,744</td>
<td>11.7</td>
<td>$180</td>
<td>$2,106</td>
</tr>
<tr>
<td>Days on antibiotics</td>
<td>166.2</td>
<td>$10</td>
<td>$1,662</td>
<td>72.8</td>
<td>$10</td>
<td>$728</td>
</tr>
<tr>
<td>School/work days missed</td>
<td>33.9</td>
<td>$195</td>
<td>$6,010</td>
<td>8.9</td>
<td>$195</td>
<td>$1,735</td>
</tr>
<tr>
<td>Total per patient without IgG</td>
<td></td>
<td></td>
<td>$111,053</td>
<td></td>
<td></td>
<td>$25,171</td>
</tr>
</tbody>
</table>

$85,882

•DOI: 10.1007/s12026-018-8996-5

AAAIA Work Group Report

American Academy of Allergy Asthma & Immunology

Diagnostic interpretation of genetic studies in patients with primary immunodeficiency diseases:
A working group report of the Primary Immunodeficiency Diseases Committee of the American Academy of Allergy, Asthma & Immunology

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Troy R. Torgerson, MD, PhD,a,b and Jolan E. Walter, MD, PhD,a,b,c Houston, Tex; San Francisco, Calif; Salt Lake City, Utah; Boston, Mass; Great Neck and Rochester, NY; Washington, DC; Atlanta, Ga; Rochester, Minn; Charlottesville, Va; St Petersburg, Fla; Durham, NC; Kansas City, Mo; Philadelphia, Pa; and Seattle, Wash
List of tables

I. Genetic terms and definitions
II. Comparisons between genetic testing methods
III. PIDD genes that might require extra genetic testing considerations
IV. Evidence and criteria for determination of variant pathogenicity
V. Population databases
VI. Resources for evaluating immunologic plausibility
VII. Prediction algorithm resources for variant interpretation

Summary Statements 1-5 (interpretation)

1. If the variant allele frequency in the general population is significantly greater than the prevalence of the PIDD, it is unlikely to represent the molecular cause for the condition. (is it common)

2. Population- and disease-specific databases should be used to provide evidence for or against pathogenicity for specific variants, with recognition of the limitations of these databases. (is it found in immunodeficiency)

3. Absence of a variant from population databases or a minor allele frequency of less than the expected carrier frequency for a recessive condition provides moderate evidence for pathogenicity of the variant. (is it rare)

4. Functional validation should be used, when possible, to establish the pathogenicity of variants and their causal relationships with PIDDs. (does it impair the protein/pathway)

5. Immunologic plausibility should be considered in determining variant pathogenicity and requires the expertise of a clinical immunologist. (does it make sense)
Summary Statements 6-10 (interpretation)

6. Pathogenic variants should **cosegregate** with an identified immunologic defect according to Mendelian patterns of inheritance. *(if you have gene you should have disease)*

7. Incomplete phenotypic penetrance can be considered when variant cosegregation with disease deviates from Mendelian expectations, but other potential genetic diagnoses must first be excluded. *(be extra careful with creative explanations)*

8. **De novo variants** should be examined closely for potential pathogenicity. *(look deeply into things that are new in your patient)*

9. Biallelic pathogenic variants should be present in patients with **autosomal recessive** conditions. *(if a disease needs both copies of a gene make sure both copies are abnormal)*

10. **Digenic inheritance** assertions remain hypothetical and should not be used to declare a genetic explanation in the absence of substantial functional evidence for pathogenicity. *(two genes can combine to cause one disease but only in theory right now)*

Summary Statements 11-14 (interpretation)

11. Variants that result in **loss of gene product expression** carry very strong potential for pathogenicity and should be considered further. *(pay attention to knockouts)*

12. A number of **computational tools** have been developed to assist with predicting the potential for variants to alter the function of resulting gene products, but this determination remains imprecise. *(bioinformatic tools help sometimes)*

13. Although the presence of a probable genetic explanation might reduce the likelihood that other genetic changes are pathogenic, the presence of a **dual molecular diagnosis** must not be excluded. *(two things can happen – blended)*

14. A variant in a **gene strongly associated with the immunodeficient phenotype** in the patient should be viewed with increased suspicion for pathogenicity. *(if the shoe fits... think extra)*
**Pathogenic designation options**

(A) 1 very strong plus at least
   1 strong, or
   2 moderate, or
   1 moderate and 1 supporting, or
   2 supporting

(B) 2 strong

(C) 1 strong plus at least
   3 moderate, or
   2 moderate and 2 supporting, or
   1 moderate and 4 supporting pathogenic criteria.

---

**TABLE IV: Evidence and criteria for determination of variant pathogenicity**

<table>
<thead>
<tr>
<th>Type of criteria</th>
<th>Benign evidence</th>
<th>Pathogenic evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
<td>Supporting</td>
</tr>
<tr>
<td>Collected data</td>
<td>MAF exceeds disease prevalence</td>
<td>Reproducible source suggests variant is benign</td>
</tr>
<tr>
<td>Function and biological data</td>
<td>Functional studies demonstrate no deleterious effect</td>
<td>Missense in gene with many pathogenic missense variants Likely functional effect in immunologically plausible gene candidate*</td>
</tr>
<tr>
<td>Allelic distribution data</td>
<td>Non-segregation with inappropriate segregation with disease</td>
<td>In cis with a pathogenic variant in the same gene</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variant-based computational data</td>
<td>Computational evidence argues against effect on gene product</td>
<td>Computational evidence supports a deleterious effect on gene product</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Alternate cause detected</td>
<td>Phenotype or family history highly specific for gene</td>
</tr>
</tbody>
</table>
Likely Pathogenic designation

(A) 1 very strong and 1 moderate;
(B) 1 strong and 1 moderate;
(C) 1 strong and 2 supporting;
(D) 3 moderate;
(E) 2 moderate and 2 supporting
(F) 1 moderate and 4 supporting pathogenic criteria.
Real world case studies
advantages and limitations

Case study 1
2 year old with THI (IgG = 170) normal IgM
Persistant diarrhea after rotavirus vaccine
Very low NK cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>Mode of Inheritance</th>
<th>Variant</th>
<th>Zygosity</th>
<th>Inherited From</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40LG</td>
<td>Hyper IgM Syndrome Type 1</td>
<td>X-Linked</td>
<td>≤3684+T+C</td>
<td>Hemizygous</td>
<td>Unknown</td>
<td>Variant of Uncertain Significance</td>
</tr>
</tbody>
</table>

**INTERPRETATION**

This individual is hemizygous for a variant of uncertain significance in the CD40LG gene. This result does not establish a molecular diagnosis in this individual.

- Not observed at a significant frequency in large population cohorts (Lek et al., 2016)
- Observed in hemizygous state in this patient and not observed in hemizygous state in controls
- In silico analysis supports that this variant does not alter splicing
- Has not been previously published as pathogenic or benign to our knowledge

We interpret this as a Variant of Uncertain Significance.
Case study 1
2 year old with THI – VUS unlikely to explain
Now with normal IgA/M and switched B cells

Case study 2
Adolescent with fatigue and frequent infections
Elevated IgG, normal titers, Low NK cells

One Pathogenic variant identified in MEFV. MEFV is associated with autosomal recessive familial Mediterranean fever. Single pathogenic variants may contribute to risk of recurrent fevers.

Additional Variant(s) of Uncertain Significance identified.

<table>
<thead>
<tr>
<th>GENE</th>
<th>VARIANT</th>
<th>ZYGOSITY</th>
<th>VARIANT CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEFV</td>
<td>c.2080A&gt;C (p.Met694Val)</td>
<td>heterozygous</td>
<td>PATHOGENIC</td>
</tr>
<tr>
<td>ANK2F1</td>
<td>c.3990C&gt;C (p.Glu697As)</td>
<td>heterozygous</td>
<td>Uncertain Significance</td>
</tr>
<tr>
<td>DUOX2</td>
<td>c.1825C&gt;T (p.Pro609Ser)</td>
<td>heterozygous</td>
<td>Uncertain Significance</td>
</tr>
</tbody>
</table>

Started on colchicine – life transformed...
Beyond Simple Genetics
Multilocus Variation – a frontier in PID

Conclusions – genetic testing for PIDD
advantages and limitations....“Podium to practice”

1. Genetic testing for PIDD is more accessible and realistic than ever
2. Can offer a definitive diagnosis and end a diagnostic journey (~25%)
3. If you have a suspicion for a PIDD – a genetic test is warranted –
your pretest probability based upon a clinical diagnosis of PIDD
4. WES+CNV has a slightly higher yield than NGS panel (but not by all
that much)
5. Genetic variant navigation is multifaceted and consider using the
recent AAAAI document as a guide and the AAAAI worksheet
6. Many real-world challenges still exist and while genetic test results
can be definitive there are new and relevant gray zones to navigate
and should be anticipated and transmitted to patients
Question 1

A 24 year old with a 10 year history of CVID has also had several years of diarrhea, arthralgias, and skin rashes. She is being treated according to CVID guidelines but is challenged by her intestinal and joint problems. She has never had genetic testing performed and you want to see if she might have PIDD that could be treated differently. Which test should you order?

A. A NGS PIDD panel  
B. Exome sequencing (ES)  
C. ES + copy number variation (CNV)  
D. Whole genome sequencing (WGS)  
E. CTLA4 gene testing by Sanger sequencing

Question 2

You order an NGS PIDD panel from a reputable clinical genetics laboratory for an adult patient with very low IgG and low numbers of B cells. The results identify a “variant of uncertain significance” in the IKZF1 (IKAROS), CD19, BTK and COPA genes. No gene variants were listed as pathogenic. Your next best step would be to:

A. Order exome sequencing (ES) with copy number variation (CNV)  
B. Perform flow cytometry for CD19  
C. Consult a clinical geneticist  
D. Perform whole genome sequencing (WGS)  
E. Use the AAAAI guidelines to stratify the variants identified