Genetic testing should not routinely be done in the diagnosis of CVID

AAIFNC Spring Journal Club, 2019

Eric M Wohlford, MD, PhD
UCSF Immunology Fellow - PGY4
Common Variable Immunodeficiency (CVID)

- The most common severe antibody deficiency (1/25,000)
- Primarily a B cell defect
  - Impaired antibody production
- Can have T cell defects
- Variable!
  - Infections (94%)
  - Autoimmunity (20%)
  - CLD (29-40%)
  - GI disease (15-21%)
  - Lymphoma/cancer (8-15%)

Resnick et al, 2012
**CVID: Sine qua non**

- Per PAGID and ESID, 1999
  - >2 years of age
  - Markedly reduced IgG and IgA/IgM (<2 SD below mean RR)
  - No isoheamagglutinins or poor vaccine response
  - Absence of other cause of immunodeficiency state

Conley et al, 1999
Workup of CVID

- It’s all about the immunoglobulins
  - IgM (40 mg/dL), IgA (30 mg/dL), IgG (~260 mg/dL)
  - Many have no Ig detected
  - Must repeat levels - must be persistent to be real
  - Response to protein (Td) and polysaccharide (PPSV23) Ags

- Some patients have LFT abnormalities
  - A/w nodular regenerative hyperplasia

Resnick et al, 2012
Additional Workup of CVID

- Flow (sometimes)
  - Low class-switched memory B cells (IgD-, IgM-, CD27+)
- T cell proliferation studies can be abnormal

Koelsch et al, 2007
Management of CVID

- Ig replacement is the cornerstone of therapy
  - IV vs SC formulation
  - 300-400 mg/kg every 4 weeks to start
- PPx antimicrobials in pts with CLD
- PJP PPx if T cells low (CD4 < 200)
- Treatment of autoimmunity if this arises
  - Genetic testing guided therapy
- All age appropriate cancer screenings

Cunningham-Rundles, 2010

https://www.gamunex-c.com
Genetic Testing in CVID?

- Single genetic defects are associated with 2-10% of cases
- Should genetic testing routinely be done in CVID?

To answer this question, we must evaluate what is known about genetic testing in primary immunodeficiency (PID) and specifically CVID...
Genetics of CVID

Monogenic cause (estimated 2-10%)
- PIK3CD, 26.74%
- LRBA, 26.74%
- CTLA4, 6.42%
- unknown genetic cause
- modifier genes (prevalence unknown):
  - TNFRSF13B (TACI)
  - TNFRSF13C (BAFF-R)
  - MSH5, MSH2
  - MLH1, RAD50, FCGR2A, HLA-DQ/DR, ORC4L, CLEC16A, etc.

Genes contributing to CVID:
- PRKCD, 2.14%
- PLCG2, 2.14%
- NFKB2, 5.35%
- NFKB1, 1.60%
- PIK3R1, 4.81%
- VAV1, 0.53%
- RAC2, 0.53%
- BLK, 0.53%
- IKZF1 (IKAROS), 3.21%
- IRF2BP2, 0.53%
- ICOS, 3.74%
- TNFSF12 (TWEAK), 0.53%
- CD19, 3.74%
- CD81, 0.53%
- CR2 (CD21), 1.07%
- MS4A1 (CD20), 0.53%
- TNFRSF7 (CD27), 4.81%
- IL21, 0.53%
- IL21R, 3.21%

Boegart et al, 2016
Genetics of CVID

- Many candidate genes in a small minority of CVID patients
Genetics of CVID

Boegart et al, 2016
Many PIDs benefit from genetic testing

Rostrum

Now Is the Time to Use Molecular Gene Testing for the Diagnosis of Primary Immune Deficiencies

Jennifer Heimall, MD  Philadelphia, Pa

Heimall, 2019
Gene targets in CVID

- CTLA4 haploinsufficiency - abatacept
- LRBA deficiency - abatacept
- STAT1 GOF Immune dysregulation - ruxolitinib
- PIK3CD immune dysfunction - leniolisib, rapamycin

- Any monogenic CVID - HSCT?

Modified from Boegart et al, 2016
Critical to select the right hammer for the right nail

- *In silico* predictions (genetic testing without clinical guidance) predict pathologic mutations 50% of time
- Side effects of
  - Abatacept: infections (sometimes fatal), cancer, side effects of other DMARDs are more common
  - Ruxolitinib: >30% anemia, thrombocytopenia. Infections and cancers have occurred
  - Leniolisib (unknown): theoretical risk of neutropenia, hepatitis, diarrhea
  - Sirolimus (Rapamycin) >30% anemia, thrombocytopenia, fever, kidney injury
  - HSCT: <50% survival rate, failure, GVHD, infection, SOS, TMA
Primum non nocere
### Genetic testing not routinely recommended in CVID

**TABLE 1.** Selected examples of application of genetic testing to primary immunodeficiency diagnosis and management

<table>
<thead>
<tr>
<th>Primary immunodeficiency (clinical and lab features)</th>
<th>Genetic testing modality</th>
<th>Impact on treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn SCID and T-cell lymphopenia</td>
<td>TREC</td>
<td>Early diagnosis</td>
</tr>
<tr>
<td>Newborn B-SCID, agammaglobulinemia and B-cell lymphopenia</td>
<td>KREC</td>
<td>Early diagnosis</td>
</tr>
<tr>
<td>T-cell lymphopenia consistent with SCID</td>
<td>SCID gene panel, large PID gene panel or WES</td>
<td>Treatment decisions regarding use of alkylator-based conditioning, possibility for clinical gene therapy, prognosis of survival, and immune reconstitution after HSCT</td>
</tr>
<tr>
<td>Isolated T-cell lymphopenia</td>
<td>Chromosomal array (in conjunction with or following nondiagnostic SCID gene panel)</td>
<td>Determination of 22q11 deletion syndrome and consideration of thymus transplant</td>
</tr>
<tr>
<td>Combined immunodeficiency</td>
<td>Large PID gene panel or WES ±chromosomal array</td>
<td>Selection of targeted therapy to disrupted pathways and in some cases consideration for HSCT</td>
</tr>
<tr>
<td><strong>CVID with complex symptoms (autoimmunity)</strong></td>
<td>Consider WES ± chromosomal array</td>
<td>Potential for monogenic cause amenable to targeted therapy or HSCT</td>
</tr>
<tr>
<td>Innate defects</td>
<td>Large PID panel or WES ± chromosomal array</td>
<td>Prognosis, appropriate supportive care, consideration for HSCT</td>
</tr>
<tr>
<td>Agammaglobulinemia</td>
<td>Targeted sequencing</td>
<td>IVIG, prognosis</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>CGD gene panel</td>
<td>Prognosis, consideration of HSCT or gene therapy</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome or x-linked thrombocytopenia</td>
<td>Targeted sequencing</td>
<td>Prognosis, consideration of HSCT, or gene therapy</td>
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<tr>
<td>Neutropenia</td>
<td>Neutropenia gene panel</td>
<td>Prognosis, consideration of supportive care or HSCT</td>
</tr>
<tr>
<td>Recurrent fevers</td>
<td>Periodic fever syndrome gene panel or WES</td>
<td>Selection of targeted therapy to disrupted pathway</td>
</tr>
</tbody>
</table>

*HSCT, Hematopoietic stem cell transplant; IVIG, intravenous immunoglobulin; KREC, kappa recombining excision circle; PID, primary immunodeficiency; SCID, severe combined immunodeficiency; TREC, T-cell receptor excision circle; WES, whole exome sequencing.*
Genetic testing not **routinely** recommended in CVID

<table>
<thead>
<tr>
<th>TABLE I. Selecter:</th>
<th>Diagnosis and management</th>
<th>Impact on treatment</th>
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<tr>
<td>Primary immunoDefi</td>
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<td>usual diagnosis</td>
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**monogenic cause** (estimated 2-10%)

- Agammaglobulinemia
- Chronic granulomatous disease
- Wiskott-Aldrich syndrome or X-linked thrombocytopenia
- Neutropenia
- Recurrent fevers

**unknown genetic cause**

- CGD gene panel
- Targeted sequencing
- Neutropenia gene panel
- Periodic fever syndrome gene panel or WES

**Potential for monogenic cause amenable to targeted therapy or HSCT**

- Prognosis, appropriate supportive care, consideration for HSCT
- Prognosis, consideration of supportive care or HSCT

**HSCT**, Hematopoietic stem cell transplant; **IVIG**, intravenous immunoglobulin; **KREC**, kappa recombining excision circle; **PID**, primary immunodeficiency; **SCID**, severe combined immunodeficiency; **TREC**, T-cell receptor excision circle; **WES**, whole exome sequencing.

Heimall, 2019
Genetic testing in selected patients

- All CVID (≈80%)
  - 80% AI dz, atypical phenotype

- 20% of AI dz, atypical phenotype
  - 70% No causal mutation
  - 30% monogenic
  - 6% monogenic

- Unknown or nondiagnostic mutations (94%)

Maffucci et al, 2016
CVID mutations are found in healthy individuals

de Valles-Ibáñez et al, 2018
CVID patients with mutations also harbor mutations in interacting proteins

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<table>
<thead>
<tr>
<th>Patient</th>
<th>CVID gene</th>
<th>Variants</th>
<th>Interacting protein</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>L287</td>
<td>SERPINA1</td>
<td>1 het</td>
<td>CELA1</td>
<td>2 het</td>
</tr>
<tr>
<td>L286</td>
<td>MSH2</td>
<td>1 het</td>
<td>ATR</td>
<td>1 het</td>
</tr>
<tr>
<td>L285</td>
<td>MSH2</td>
<td>1 het</td>
<td>CREBBP</td>
<td>1 het</td>
</tr>
<tr>
<td>L284</td>
<td>PRKCD</td>
<td>1 het</td>
<td>CREBBP</td>
<td>1 het</td>
</tr>
<tr>
<td>L283</td>
<td>PRKCD</td>
<td>1 het</td>
<td>PDP1</td>
<td>1 het</td>
</tr>
<tr>
<td>L282</td>
<td>PRKCD</td>
<td>1 het</td>
<td>RUNX2</td>
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<td>L281</td>
<td>DOCK8</td>
<td>1 het</td>
<td>CDC42</td>
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</tr>
<tr>
<td>L278</td>
<td>STAT1</td>
<td>1 het</td>
<td>FGFR3</td>
<td>1 hom</td>
</tr>
<tr>
<td>L277</td>
<td>STAT1</td>
<td>1 het</td>
<td>FGFR4</td>
<td>1 het, 1 hom</td>
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<tr>
<td>L276</td>
<td>NFKB1</td>
<td>1 het</td>
<td>NF3R1</td>
<td>1 het</td>
</tr>
<tr>
<td>L275</td>
<td>SERPINA1</td>
<td>1 het</td>
<td>IRS3</td>
<td>1 hom</td>
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<tr>
<td>N207</td>
<td>PIK3CD</td>
<td>1 het</td>
<td>IRS2</td>
<td>1 het</td>
</tr>
<tr>
<td>N206</td>
<td>PIK3CD</td>
<td>1 het</td>
<td>TLR2</td>
<td>1 het</td>
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<tr>
<td>N205</td>
<td>MSH2</td>
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<td>ATR</td>
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<tr>
<td>N204</td>
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<td>1 hom</td>
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<td>RALY</td>
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<td>RALY</td>
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<td>CDC42</td>
<td>1 hom</td>
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<tr>
<td>N213</td>
<td>PLCG2</td>
<td>1 het</td>
<td>FLT1</td>
<td>1 hom</td>
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<tr>
<td>N216</td>
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<td>RALY</td>
<td>1 hom</td>
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<tr>
<td>N227</td>
<td>NOD2</td>
<td>1 het</td>
<td>ERBB2/P</td>
<td>1 hom</td>
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<tr>
<td>N227</td>
<td>PIK3CD</td>
<td>1 het</td>
<td>IRS2</td>
<td>1 hom</td>
</tr>
<tr>
<td>N227</td>
<td>PIK3CD</td>
<td>1 het</td>
<td>RALY</td>
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<tr>
<td>N229</td>
<td>SERPINA1</td>
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<td>CELA1</td>
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<tr>
<td>N229</td>
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<td>RALY</td>
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<tr>
<td>N233</td>
<td>CR2</td>
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<td>PHD1</td>
<td>2 het</td>
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<td>N234</td>
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<td>1 het</td>
<td>NBN</td>
<td>1 hom</td>
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<tr>
<td>N234</td>
<td>NFKB1</td>
<td>1 het</td>
<td>NCOR2</td>
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</tbody>
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de Valles-Ibáñez et al, 2018
Limitations of genetics of CVID

- CVID associated with autoimmunity represents a minority of cases
  - Of those, a tiny fraction have a single genetic cause
- Patients with CVID who undergo WGS have multiple mutations (mean = 9.4 mutations a/w CVID per patient)
- Monozygotic twins discordant for CVID have shown differential methylation of CVID genes
  - CVID is also environmentally modulated
- Family and population studies show CVID-associated mutations exist in healthy individuals

Boegart et al, 2016
Now Is the Time to Use Molecular Gene Testing for the Diagnosis of Primary Immune Deficiencies

Eric M Wohlford, MD, PhD, unpublished observations, San Francisco, Ca
Should you **routinely** send a test that will not change your management?
Should you **routinely** send a test that will not change your management?

**NO**
What's the harm in testing?

- The cost of sending genetic testing is high
- Poor resource utilization
- Vast majority of patients have no mutation or multiple mutations
- Knowledge can harm
  - Individual patients may make decisions based on clinically questionable data
  - Family members may fear for future generations without cause
  - Insurance companies may further harm patients with genetic discrimination
Testing is costly

- Invitae: $1250 USD
- UC Davis Genome Center: $1000 USD
- Stanford Medicine: $1600 USD
- UCSF: $1800 USD
- Blueprint Genetcs: $2500 USD

Many companies’ rates are not published online and not included here.
What's the harm in testing?

Genetic Tests Can Hurt Your Chances Of Getting Some Types Of Insurance

August 7, 2018 - 9:00 AM ET

MICHELLE ANDREWS
What's the harm in testing?
What's the harm in testing?
Conclusions

- CVID is a complex disease
  - There is no single genetic basis for the vast majority of disease
- CVID is a clinical diagnosis with clear laboratory diagnostic criteria
  - Genetic testing is not a criterion for diagnosis
- Genetic testing does not change management in the vast majority of CVID
- Genetic testing causes undue harm without clear benefit
  - Genetic discrimination
  - Personal psychological harm
  - Future harm to families of affected patients
When all you have is a hammer...
And when you have genetic testing...
References


Rebuttal
Should you **routinely** send a test that will not change your management?

**NO**
A lot of sequencing for a tiny number of patients

Boegart et al, 2016
What's the harm in testing?

Learning one's genetic risk changes physiology independent of actual genetic risk


What's the harm in testing?

Dx Sequence DNA for actual genetic risk

Maximal treadmill effort

Randomization/results reporting

Maximal treadmill effort

Meal -> blood GLP-1 and ghrelin level

Randomization/results reporting

Meal -> blood GLP-1 and ghrelin level

Turnwald et al, 2019
What's the harm in testing?

Does learning of one's genetic risk for disease alter one's actual risk by making people more likely to exhibit the expected phenotype?

“Merely receiving genetic risk information changed individuals’ cardiorespiratory physiology, perceived exertion and running endurance during exercise, and changed satiety physiology and perceived fullness after food consumption in a self-fulfilling manner. Effects of perceived genetic risk on outcomes were sometimes greater than the effects associated with actual genetic risk…”

Turnwald et al, 2019
More money more problems
Genetic testing in all patients?

- All CVID: ~80%
  - AI dz, atypical phenotype: ~20%

- Maffucci et al, 2016: No
  - Unknown or nondiagnostic mutations: 94%
  - Monogenic: 30%
  - No causal mutation: 70%

NO
Genetic testing in select patients

- All CVID: ~80%
- AI dz, atypical phenotype: ~20%
- No causal mutation: 70%
- monogenic: 30%

Jackpot

Maffucci et al, 2016
Sometimes the needles are obvious
Conclusions

- CVID is **variable**
  - no single genetic basis for the vast majority
- CVID is a **clinical diagnosis**
  - Genetic testing is **not** a criterion
- Genetic testing **does not change management** for the vast majority
- Genetic testing can **harm**
  - Genetic **discrimination**
  - Psychological and physiological **harm**
- Costs are high **without clear benefit**