5 points to know:
Genetic testing in A/I clinical practice

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Disclosures

- Author/Section Editor, Wolters-Kluwer (Uptodate)
- Research Funding
  - NHLBI, NIAID
  - Jeffrey Modell Foundation

1. Know the Indications

Allergic conditions

- Mastocytosis: *KIT* variants
- Hypereosinophilia: *JAK1*, *JAK2*, *FIP1L1-PDGFRA*
- Primary atopic diseases: *STAT4, CARD11, CARD14*, *ERBIN, STAT5B, IL6ST*

1. Know the Indications

Workup for Inborn Errors of Immunity (IEI)

- Sequencing is indicated for any patient with suspected IEI
- Genetic diagnosis is potentially definitive for most forms of IEI
  - SCID/CID
  - Primary immunodysregulatory disorders
  - Autoinflammatory disorders
  - HLH-associated conditions
  - Syndromes with associated immunodeficiency

When to go hunting for gene defects?

- Early onset disease
- Sentinel infections
- Multi-system autoimmunity
- Severe atopy
- Families with multiple affected members

2. Know the tests

- **Next-gen sequencing**
  - Gene panel sequencing
  - Whole exome
  - Whole genome

- **Chromosomal microarrays**
  - For analysis of large deletions/duplications (e.g.: 22q11.2)

- **Specialty/targeted sequencing**
  - MLPA(for specific microdeletions)
  - Targeted sanger sequencing for familial mutations

Next-gen sequencing

- **Whole genome sequencing (WGS)**: ideal for novel gene discovery
    - Primary limitation is cost / time required in data analysis
    - Total: 3.2 billion bp

- **Whole exome sequencing (WES)**: Hybrid capture technology
    - Reduces time / cost of sequencing at the expense of eliminating noncoding sequences.
    - Total: ~30 million bp (1% of genome)
        - Still covers ~85% of disease-causing mutations

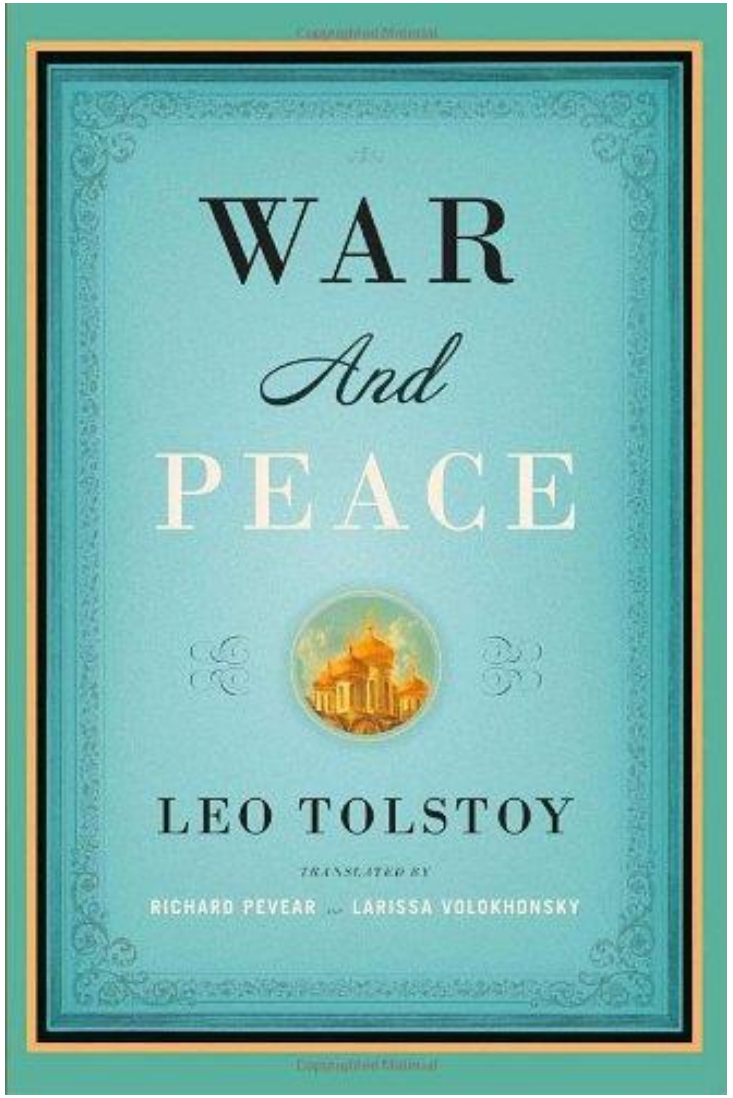
Next-gen sequencing

- **Gene panel sequencing: Phenotype-driven panels of genes**
  - **Pros:**
    - Often results quickly, with standardized analysis pathways
  - **Cons**
    - Gene lists are quickly outdated
    - Methods and hit rate vary widely by company
      - A negative test result generally means nothing.

Company	*Panel*	*# of genes*
Invitae	PID panel	474
Blueprint	PID panel	336
Fulgent	PID panel	592

WGS vs. WES Sequencing

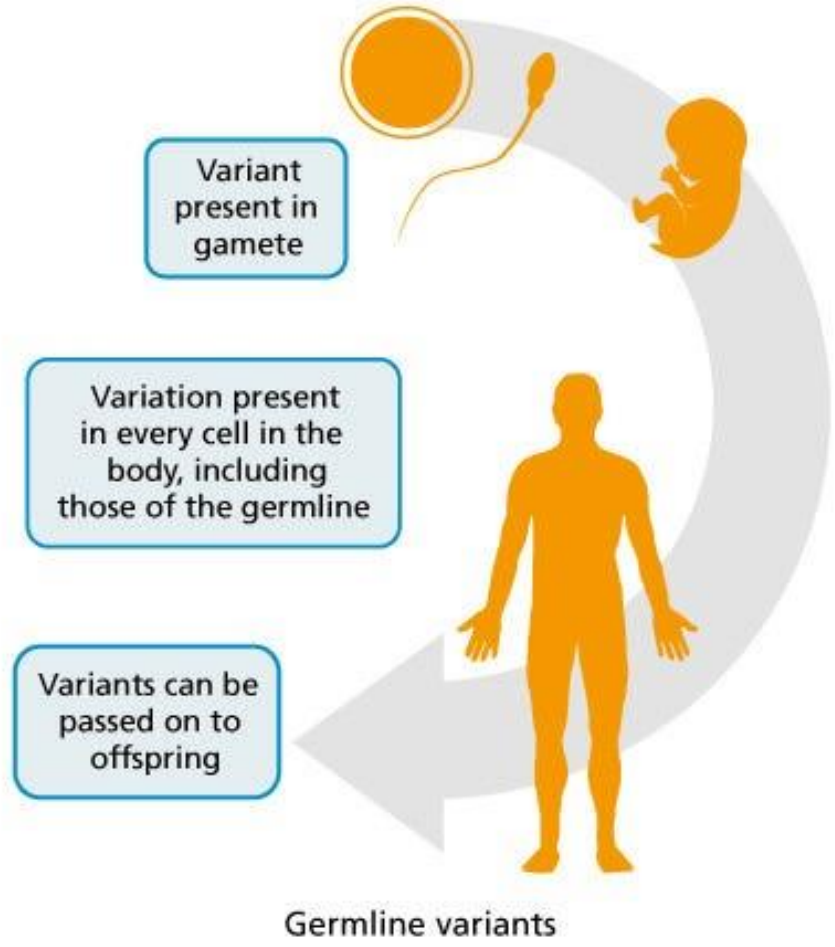
- WGS: 1000 X
- WES: 10 X

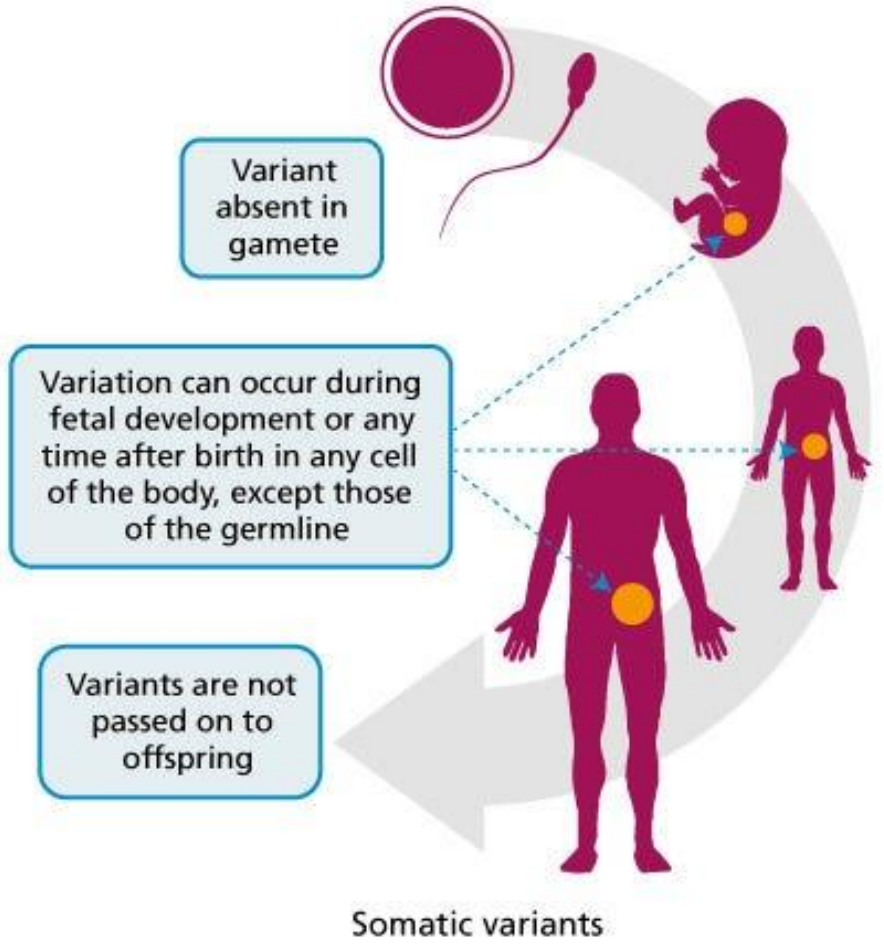


Limitations of standard NGS sequencing

- Regions with pseudogenes
  - *IKBKG* (NEMO), *NCF1* (p47 CGD)
- Large structural variations
  - *UNC13D* (FHLH, common inversion in N. europe)
- Somatic mosaicism
  - Rare somatic variants (<15% MAF) may be missed

IEI due to somatic mosaicism





Described somatic mutations in IEI:

NLRP3
NLRC4
TMEM173
UBA1
FAS
KRAS
NRAS
NOD2
STAT5b
JAK1
TLR8

What to send?

- Panels are a fine start, but negative results not definitive

- WES/WGS are most powerful approach
  - Trios are key: Always sequence parents too for variant interpretation

- Send a microarray +/- karyotype for anyone with syndromic features
  - WES and panels may not catch large CNVs or structural variants

3. Know your resources

- Online references:
  - Clinvar: https://www.ncbi.nlm.nih.gov/clinvar/
  - Gnomad: https://gnomad.broadinstitute.org
  - ClinGen Immunology CDWG: https://www.clinicalgenome.org/working-groups/clinical-domain/immunology/
- Professional societies
  - Clinical Immunology Society listserv
  - AAAAI PID committee
- Clinical sequencing companies
  - Geneticists will usually be willing to discuss cases and variants

4. Know the payor system (and how to bypass them)

- Clinical sequencing usually requires prior authorization
  - Utilize consensus recommendations (CIS, AAAAI)
  - Template letters may be used to support PA's.
  - Peer to peer calls: emphasize that 1) genetic sequencing is diagnostic for many forms of IEI, and 2) Genetic diagnosis can change therapy

- Sponsored clinical sequencing
  - Jeffrey Modell Foundation
  - Pharming pharmaceuticals (APDS)
  - X4 Pharmaceutical (WHIM syndrome)

5. Know the Impact

- **Risk assessment**:
  - Risk of HLH
    - XLP, XIAP, STAT1, STAT3, LRBA, CTLA4
  - Risk of malignancies
    - APDS, MAGT1, CD27, LRBA
  - Other co-morbidities (liver, lung, skin)

- **Targeted therapies**
  - JAKinibs in STAT1, STAT3, STAT4, SOCS1, and interferonopathies
  - Abatacept for LRBA, CTLA4, Treg-opathies
  - Sirolimus, PI3K inhibitors for APDS
  - HSCT in severe cases

Conclusions

- Sequencing is critical for diagnosis and care of IEI
  - Early sequencing → faster diagnosis
  - Genetic dx often changes approach to treatment

- Interpretation of VUSs can be challenging
  - Many good tools online
  - Make use of local and national expertise
    - AAAAI PID committee membership
    - CIS Listserv

Acknowledgements

National Institute of Allergy and Infectious Diseases

Children's National Hospital

Jeffrey Modell Foundation