Genomics and Genetic Evaluation of Immunodeficiencies

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Disclosure

No conflicts of interest relative to this presentation

Learning Objectives

- Identify the basic elements of a gene structure, DNA replication, RNA transcription and protein translation
- Recognize the different types of DNA mutations, inheritance, penetrance and expressivity patterns
- Define different methods and approaches for DNA variant (mutation) analysis
- Distinguish the advantages and the limitations of next generation sequencing (NGS) in establishing the genetic basis of primary immunodeficiencies
Mutation/Polymorphism/Variant

A mutation is defined as a permanent change in the nucleotide sequence, while a polymorphism is defined as a variant with a frequency above 1%. However, the terms “mutation” and “polymorphisms”, which have been used widely, often lead to confusion due to incorrect assumptions of pathogenic and benign effects respectively. Thus, it is recommended that both terms be replaced by the term “variant” with the following modifiers: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance, (4) likely benign, or (5) benign. While these modifiers may not address all human phenotypes, they comprise a five-tier system of classification for variants relevant to Mendelian disease as addressed in this guidance. It is recommended that all assertions of pathogenicity (including "likely pathogenic") be reported with respect to a condition and inheritance pattern (e.g. c.1521_1523delCTT (p.Phe508del)) pathogenic, cystic fibrosis, autosomal recessive).

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. PMID: 25741868
Synonymous Nonsynonymous Null

Genetic Variation Analysis
Nomenclature

Wild Type Sequence | one two big zoo
Missense | one two big zoo
Nonsense | one two
Frame shift | one two big zoo
Insertion (in frame) | one two big zoo
Deletion (in frame) | one two big zoo
Duplication (in frame) | one two big zoo

Alternative (Muller’s morphs) | Description
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Amorph | Complete loss of function
Hypomorph | Partial loss of function
Hypervariables | Dominant gain of function
Neomorph | Novel function
Antimorph | Dominant negative function

Single base substitutions/point mutations represent ~70% of disease causing changes.
Genetic Lab Report

Typically report single nucleotide substitutions using the five tier system:

- Variant pathogenic - prior report of disease association
- Variant likely to be pathogenic, likely to be benign or benign based on "in silico" evaluation (computer programs that predict the impact of the change, the conservation of the involved nucleotide, etc)
- Variant of unknown significance (VUS) which is the most difficult to utilize clinically since it leaves the potential of a causal relationship to disease unknown

Inheritance Patterns

Autosomal Recessive

In nonconsanguinous families diseased individuals typically have compound heterozygous variants while in consanguinous families disease is usually associated with homozygous variants

Autosomal Dominant

One allele with the disease causing variant results in disease (i.e. there is one abnormal & one normal allele)

X-linked Recessive

Disease is only seen in males unless there is altered X chromosome inactivation or an X0 female (Turner syndrome)

X-linked Dominant
Inheritance Patterns

Germline mutations
Whole organism carries the mutation

Gonadal mosaicism
Only gonads carry the mutation

Penetrance and Expressivity

Penetrance
All-or-None phenomenon
“Black/White”

Expressivity
Severity of a phenotype
“Shades of gray”

Genetic Analysis: Sanger Method

- Gene specific testing using a chain termination method
- PCR to amplify desired genomic region
- Second PCR with normal (deoxy) plus modified (dideoxy) labeled nucleotides that terminate the PCR extension
- Generates different sized fragments of DNA
- Gel electrophoresis applied to separate these fragments for analysis
Genetic Analysis: Sanger Method

Control
Carrier
Patient

X-linked SCID due to a single nucleotide substitution

Next Generation Sequencing (NGS)/Massively Parallel Sequencing

Targeted: select panel of genes (vary in number based on the target objective)

Exome: \( \sim 3 \times 10^7 \) coding bp (\( \sim 1\% \) of genome)
\( \sim 20,000 \) protein-coding genes predicted to detect \( \sim 85\% \) of disease causing mutations

Genome: \( \sim 3 \times 10^9 \) coding & noncoding bp

Genetic Analysis

Next-Gen/Massively Parallel sequencing

[Diagram of genetic analysis process]
Classification by Phenotype or Syndrome

- Clinical: Severe Combined Immunodeficiency, velo-cardiofacial, Cartilage-hair hypoplasia, ...
- Immunologic: Agammaglobulinemia with no B cells, Hyper IgM, Hyper IgE, ...
- ID susceptibility: Susceptibility to Mycobacterial Diseases, to Herpes simplex virus, CMC, ...

...multiple gene defects could be associated with each phenotype/syndrome (genetic heterogeneity)

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**Mutation Analysis in PIDs**

**Rationale/Cost calculation**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene syndrome</th>
<th>Total arrangements</th>
<th>Ranges sequencing for all target genes</th>
<th>Targeted NGS (cost per target PID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOCA deficiency</td>
<td>DOCA deficiency</td>
<td>62</td>
<td>$600</td>
<td>$500</td>
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<tr>
<td>Immunodeficiency with hyper IGE syndrome</td>
<td>Immunodeficiency with hyper IGE syndrome</td>
<td>62</td>
<td>$600</td>
<td>$500</td>
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<tr>
<td>ID susceptibility to Mycobacterial Diseases</td>
<td>ID susceptibility to Mycobacterial Diseases</td>
<td>62</td>
<td>$600</td>
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</tbody>
</table>

**Validation**

| True positive | 52 | True negative | 96/12 |
| False positives | 1 | False positives | 2 |
| Accuracy (sensitivity) | 98.11% | Accuracy (specificity) | 98.8.11% |

- 120 PID pts tested
- 173 gene targeted capture
- 18 pts diagnosed
- 15% efficacy

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**Targeted NGS: a cost-effective approach to molecular diagnosis of PIDs**

- 50 CVID patients tested
- 269 gene targeted analysis
- 15 pts diagnosed
- 30% efficacy (excluding TACI mutations) in a set of selected CVID patients using targeted analysis

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**Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency**

- 50 CVID patients tested
- WES: 269 gene targeted analysis
- 15 pts diagnosed
- 22/50 = 30% efficacy (excluding TACI mutations) in a set of selected CVID patients using targeted analysis
Rapid molecular diagnostics of severe primary immunodeficiency determined by using targeted next-generation sequencing

20 SCID+ pts tested
46 gene targeted capture
14 pts diagnosed
70% efficacy

Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders

- 278 PID pts tested
- WES + in silico CNV analysis
- 110 pts likely diagnosed
40% efficacy

Genetic Variation Analysis in PIDs
Next-Gen/Massively Parallel Sequencing

Diagnostic Success Rate

- Type of study: WGS > WES > Targeted
- Type of phenotype: SCID > ... Autoinflammatory
- Type of family: 2 or more affected > singleton
- Type of mutation: Exons > UTR, intron,...

but NO SINGLE MOLECULAR TOOL will solve all the diagnostic challenges, more importantly,
DOES NOT REPLACE KNOWLEDGE OR COMMON SENSE
Case Study Applying NGS

I have a very interesting case of a girl with HIM who has been tested for the known genes - all are normal. We do have a research protocol to evaluate patients with unknown PIDs, you will need to get informed consent and then forward a sample.

Case Study: 6yo F, HIM syndrome

• History per referring MD:
  • Denied consanguinity, healthy at birth, fully vaccinated (well tolerated)
  • <1 yo: ear/respiratory infections
  • 3 yo: bronchiectasis (Pseudomonas)
  • 4 yo: LN, HSM, cytopenias (Pit, RBC, lymphocytes)
    – IgM 1966, IgG 26, IgA 40; Poor antibody response (Tet, Pneumo, Rubella, isohemagglutinins)
    – Bone marrow biopsy, no malignancy
    – Lymph node biopsy, many CD138 IgM+ (rare CD138 IgG+)
    – Mildly reduced mitogen proliferations (antigens NL)
    – Genetic testing for CD40, CD40L, ALPS was NI. HIV neg.
    – IVIG and Bactrim prophylaxis.
Case Study: 6yo F, HIM syndrome
Targeted NGS, 250 PID-causing genes

~400 gDNA changes!

Case Study: 6yo F, HIM syndrome
Targeted NGS, 250 PID-assoc genes

Exonic  Harmful  Rare

~400 changes → 9 potentially deleterious changes but none of them explain the pt’s HIM phenotype!

Case Study: 6yo F, HIM syndrome

TARGETED NGS diagnostic approach was not conclusive, could we arrange to see the patient at NIH?
Case Study: 6yo F, HIM syndrome

- Physical Examination
  - Short stature for age
  - Tachypnea, crackles
  - Abd: HSM, G tube
  - Dental caries,
  - Ankle bony outgrowth

Case Study: 6yo F, HIM syndrome

Targeted NGS, review of ATM data

<table>
<thead>
<tr>
<th>Reads</th>
<th>% Coverage</th>
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<tr>
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Not 100% covered during the targeted NGS
Case Study: 6yo F, HIM syndrome

Diagnosis: ataxia telangiectasia, increased IgM is seen in ~30% of ATM and absence of neurologic findings is seen very rarely but reported

Lesson From the 6 yo “HIM” Patient

• Careful physical exam and medical history is absolutely crucial in interpreting genomic data
• Phenotypic variability is a reality and must be taken into account when thinking about genomic evaluation
• NGS is a powerful technique but as with any lab test it is not infallible, careful data review may be necessary since interpretation criteria may exclude potentially relevant genes

Start with a Clinical Phenotype

• Evaluate the genotype
  – Test genomic DNA
  – Assess copy number (deletions/insertions)
  – Possible additional testing using cDNA
• Characterize the protein when possible: present vs decreased vs absent, if present is it altered in size (immuniblot)
• Undertake ex vivo functional testing (if possible) to validate the role of the defect in affecting immune function
Why Evaluate Genomic Changes in PIDs

- Provides opportunity to screen for at risk patients and provide family counseling
- Allows screening potential family donors
- Identifies potential therapeutic targets
- Increases understanding of a specific disorders, genetic mechanisms (penetrance, expressivity)
- Enhances opportunity to define potential contributions of environmental factors (epigenetics) on human disease

Thank You