PROPEL 101: Molecular mechanisms

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Recommendations for approaching the reading materials

• For the Supplemental material:
  • Not about memorizing/learning ALL the different mutations, etc.
  • Meant to help appreciate the scope of just how many ways genetic variation, acting through RNA and protein functionally, can contribute to disease
  • Pick a couple sections that sound most interesting and do a deep dive on those. But don’t worry about absorbing the details of all of it

• For the journal club
  • Primary goal to translate the 6 major mechanism stages we’re about to discuss to describe the specific approach/details of the paper
Why do we perform scientific research?

• In your previous two sessions, you’ve learned about some tools we have at our disposal to perform research.
• But why do we do it?
What is disease?

Tanisawa et al., 2016

One risk variant

Many risk variants throughout genome

Contribution of environmental factors

Contribution of genetic factors (Heritability)

Environmental factors

(Lifestyle, Exercise, Diet, Drinking, Smoking)

Socioeconomic status

Genetic factors

Complex diseases/traits

- Type 2 diabetes
- Cardiovascular disease
- Cancer
- Height
- Body weight

Accidental injury

- Traffic accident
- Disaster

Monogenic diseases

- Muscular dystrophy
- Werner syndrome
What is a “molecular mechanism”?
How to establish a convincing molecular mechanism

• *The journal club paper follows this construct, so if you can isolate the different stages of their analysis and associate them with each of these steps, it will make understanding and presenting it a little easier*
1. Choose your disease/phenotype of interest
2. Choose the genetic variation (DNA) you have reason to believe might be involved

Point Mutation
AKA: Single nucleotide polymorphism (SNP)
AKA: Single nucleotide variant (SNV)

Insertion Mutation

Deletion Mutation

Deletion
Duplication
Inversion
Insertion
Translocation

https://learn.genetics.utah.edu/content/genetics/mutate/
Strategies for identifying genetic variation that may be relevant to disease phenotype
Relating genetic variation to "function" is one of the biggest and most active fields in current genetics research. 

https://www.genome.gov/Funded-Programs-Projects/Impact-of-Genomic-Variation-on-Function-Consortium
3. Consider how your favorite variant may impact transcription (gene expression)

Ramsuran et al., 2018, Frontiers in Immunology
upstream ORFs (uORFs) and translation initiation

Open reading frame (ORF):

Any genomic region that has the correct upstream sequence motifs to be recognized by ribosomes and translated.
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Dodbele and Wilusz, EMBO Journal, 2020
What is haploinsufficiency and how does it impact transcription?
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Matharu et al., 2018, Science
What is haploinsufficiency and how does it impact transcription?

Matharu et al., 2018, Science
4. Consider how your favorite variant may impact translation (protein expression)

<table>
<thead>
<tr>
<th>DNA level</th>
<th>mRNA level</th>
<th>protein level</th>
<th>Silent</th>
<th>Nonsense</th>
<th>Missense</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTC</td>
<td>AAG</td>
<td>Lys</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TTT</td>
<td>AAA</td>
<td>Lys</td>
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</tr>
<tr>
<td>ATC</td>
<td>UAG</td>
<td>STOP</td>
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<td></td>
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</tr>
<tr>
<td>TCC</td>
<td>AGG</td>
<td>Arg</td>
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<tr>
<td>TGC</td>
<td>ACG</td>
<td>Thr</td>
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</tr>
</tbody>
</table>

Point mutations

- **Silent**: No change in protein sequence
- **Nonsense**: Stop codon
- **Missense**: Change in amino acid

Conservative vs. non-conservative effects

https://www.ebi.ac.uk/training/online/courses/human-genetic-variation-introduction/what-is-genetic-variation/what-effect-do-variants-in-coding-regions-have/
Protein structure == protein function

https://learn.genetics.utah.edu/content/genetics/mutate/
Frameshift mutations can change the coding sequence (CDS) that gets translated.
Frameshift mutations can change the coding sequence (CDS) that gets translated.
5. Come up with a hypothesis for how you think your favorite variant may be impacting your disease/phenotype of interest.
Wild-type
- Complex formed
- Normal cell physiology

Gain-of-function
- Hyperactivation of signalling pathways
- Formation of protein aggregates

Loss-of-function
- Complex not formed
- Complex function not performed

Dominant Negative
- Toxic protein
- Non-functional protein complex

Segalat, 2007, Orph. J. Rare Dis.
Gonzaga-Jauregui et al., 2021, Gen Rare Dis.
dominant negative vs haploinsufficiency

6. Design *in silico*/*in vitro*/*in vivo* experiments to test
The signatures of function are passed on through our DNA

- **H. sapiens**
- **S. cerevisiae**
- **M. tuberculosis**
- **E. coli**

Protein amino acid sequence
Why are the mechanisms we build from our understanding of basic biology so important for how we think about disease?
Multiple sclerosis has been considered a disease of the immune system since the 1970s.

Lin et al., 2012, Practical Neurology

Our interpretation of disease mechanism shapes clinical treatment

"Various clinical and experimental findings suggest a pathogenic role of antibodies in multiple sclerosis (MS). Yet, whether antibodies contribute to the pathogenesis or progression of MS is still a subject of intense debate."
- den Dunnen, et al., 2021, Neural Regen Res.

Melamed and Lee, 2020, Front. Imm.
International Multiple Sclerosis Genetics Consortium, 2019

Still just a disease of the immune system?

>200 genome wide risk loci for multiple sclerosis

Genomic region containing primary immune risk genes
We are all just figuring this out as we go, based on what is known at any given moment... which is constantly changing as we learn more.

So state your assumptions and don’t take things too seriously.
Thanks for your interest!

• Feel free to email me: kristen.wade@ucsf.edu