1st National Neurogenetic Variant Interpretation Course

Lecture by:

Sali Farhan, PhD, FCCMG (Assistant Professor, Clinical Molecular Geneticist)
Allison Dilliott, PhD (Post-Doctoral Scholar)
Alana Mistry, MSc, Nellie Fotopoulou, PhD, and Kayla Horowitz, MSc
(Genetic Counsellors at The Neuro)
Meet Our Genetic Counselling Team

Alana Mistry, MSc, CGC
Clinical Genetic Counsellor

Kayla Horowitz, MSc, GC
Clinical/Research Genetic Counsellor

Nellie Fotopoulos, PhD, CCGC
Clinical/Research Genetic Counsellor
Meet Our Genome Analysis Team

- Sali Farhan, PhD, FCCMG
  Assistant Professor and Clinical Molecular Geneticist

- Allison Dilliott, PhD
  Post-Doctoral Scholar

- Isabela Bucco, Genome Analyst
- Mia Ginsberg, Genome Analyst
Outline - Learning Objectives

Part I:

1. Understand the role of genetic counsellors in neurology.
2. Recognize when an underlying genetic cause should be considered.
3. Become familiar with the process of gene panel selection and lab report interpretation.

Part II:

4. From DNA to data - the process of generating meaningful results.
5. Review ACMG criteria for variant classification and VUS interpretation.
6. Introduction to frequently used variant interpretation resources.
What is Genetic Counselling?

“Genetic counselling is the process of helping people understand and adapt to the medical, psychological, and familial implications of genetic contributions to disease”

Genetic Counsellors can work in…

- Oncology
- Ophthalmology
- Pediatrics
- Neurology
- Research
- Prenatal care & family planning
- Cardiology
- Personalized medicine
- Lab
- Education
- and more…
Clinical GC Role

- Psychosocial counselling
- Family history, mode of inheritance, risk counselling
- Partnership with healthcare team
- Education, support, advocacy
- Expertise in gene panel selection
- Review potential results and implications
- Family communication

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Why take a family history?

Eliciting the family history can allow for recognition of inheritance patterns and the opportunity for diagnosis, counselling, potential targeted prevention and treatment, and the identification of at-risk relatives.
When to Consider a Referral to Neurogenetics

- Two or more family members with the same disease on same side of the family
  - ++ suspicion for rare diseases in multiple relatives (i.e. ALS)
- Vertical transmission of disease in a family
- Earlier age of onset than expected in the general population
- A hereditary condition reported in a close relative (i.e. Huntington’s)
Factors that complicate the interpretation of a pedigree

- Incomplete or reduced penetrance
- Variable expressivity
- Small families
- Premature death of relatives
- *De novo* ("new") mutation
- Recessive disorders
- Genetic anticipation
- Complex sociocultural factors
Inheritance pattern - Autosomal Dominant (AD)

- Affected
- Unaffected

Children are at 50% risk.
Inheritance pattern - Autosomal Recessive (AR)

- Carriers are typically unaffected
- Often no family history seen
- Consanguinity is a key risk factor

Children are at 25% risk
Genetic Anticipation

Age of Onset

Manifestations

Subclinical symptoms
Adult onset
Juvenile onset
Congenital onset
## Common Neurogenetic Indications

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genes</th>
<th>Type of Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADASIL</td>
<td>NOTCH3</td>
<td>Sequence (~95%)</td>
</tr>
<tr>
<td>Alzheimer’s</td>
<td>APP, PSEN1, PSEN2; APOe4</td>
<td>Sequence; risk allele; late-onset</td>
</tr>
<tr>
<td>Frontotemporal Dementia (FTD)</td>
<td>C9orf72, GRN, MAPT</td>
<td>&gt;60 G_4C_2 rpt expansion; sequence</td>
</tr>
<tr>
<td>Parkinson’s disease (AD or AR)</td>
<td>GBA, LRRK2, SNCA, PARK2, PARK7, PINK1, VPS35, etc.</td>
<td>Sequence</td>
</tr>
<tr>
<td>OPMD</td>
<td>PABPN1</td>
<td>11 to 18 GCN rpt exp (99%)</td>
</tr>
<tr>
<td>FSHD</td>
<td>D4Z4</td>
<td>&lt;9 rpt contraction</td>
</tr>
<tr>
<td>Huntington’s</td>
<td>HTT</td>
<td>&gt;40 CAG rpt expansion</td>
</tr>
<tr>
<td>ALS (AD, AR)</td>
<td>C9orf72, SOD1, FUS, ATXN2, etc.</td>
<td>&gt;60 G_4C_2 rpt expansion; sequence; risk allele</td>
</tr>
<tr>
<td>SCA (&gt;60 types) (AD, AR, X-linked)</td>
<td>ATN1, ATXN1, ATXN2, ATXN3, CACNA1A, etc.</td>
<td>CAG rpt exp; # pathogenic rpts depend on gene</td>
</tr>
<tr>
<td>HSP (AD, AR, X-linked)</td>
<td>SPAST, ATL1, SPG31, KIF1A, REEP1 etc.</td>
<td>Sequence (~80%)</td>
</tr>
<tr>
<td>CMT (AD, AR, X-linked)</td>
<td>PMP22</td>
<td>Duplication (80%)</td>
</tr>
<tr>
<td>Myotonic Dystrophy Type 1, 2</td>
<td>DMPK; CNBP</td>
<td>&gt;100 CTG rpt exp; 75-11K CCTG rpt exp</td>
</tr>
</tbody>
</table>
Sporadic vs. Familial ALS

“Familial”/Hereditary ALS

Family with reduced penetrance

Isolated case with an identified gene variant

“Sporadic” ALS
MNI Criteria for Genetic Testing in Alzheimer’s Disease

For patients with a diagnosis of Alzheimer’s:

- Offer testing to all early-onset cases (diagnosed before age 65) with or without family history and those diagnosed older than 65 with at least 1 close relative with early-onset diagnosis.

- For those diagnosed after age 65 without a family history of early-onset, genetic testing is generally not offered but clinical judgement can be used in cases of many relatives with late-onset diagnosis.

Genetic testing for symptomatic patients only. If clinical trials or treatments become available, then asymptomatic patients with a significant family history could be tested. Can quote empiric lifetime risk based on family history.
Recommended Components of HD Predictive Testing Process:

1. **Telephone Contact**
2. **Visit 1**
   - Genetic Counseling
   - Sign Informed Consent Document
   - Mental Health Assessment
   - Neurological Exam
   - Draw Blood
3. **Visit 2**
   - Disclosure of Results in Person
   - Arrange Post-result Follow-up
4. **Follow-Up**
   - Prearranged phone call or in-person visit

“Predictive patient”: an asymptomatic at-risk individual for an incurable neurodegenerative disease (i.e. Huntington’s, genetic ALS/FTD, CADASIL, etc.)

Please **always** refer these cases to Genetics for pre-test counselling!
Typical Genetic Testing Process

Historically, almost all genetic testing ordered in Medical Genetics

Now, mainstreaming has become more common in neurology and other specialties
Typical Genetic Testing Process

- Referral from MD or self-referred to Genetics
- Lab selection and patient blood draw
- Genetic counselling or MD appointment

in-province vs out-of-province vs out-of-country testing options
Typical Genetic Testing Process

Referral from MD or self-referred to Genetics

Lab selection and patient blood draw

Genetic counselling or MD appointment

Result disclosure and family letter if indicated

If +, referral from MD to Genetics

in-province vs out-of-province vs out-of-country testing options
Type of results

**Pathogenic/Likely Pathogenic Variant identified**
- Can confirm diagnosis
- Testing available for all relatives

**Variant of uncertain significance identified**
- Result is inconclusive
- Recontact clinic in 1-2 years

**Negative** (benign or likely benign changes identified)
- Based on current knowledge
- May or may not lead to more testing
Example Report: Positive

<table>
<thead>
<tr>
<th>PATIENT INFORMATION</th>
<th>SPECIMEN INFORMATION</th>
<th>PROVIDER INFORMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAST, First ID:</td>
<td>Type:</td>
<td>Physician</td>
</tr>
<tr>
<td>DOB: Month, Day, Year</td>
<td>Collected: Month, Day, Year</td>
<td>Genetic Counselor</td>
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<tr>
<td>Sex:</td>
<td>Received: Month, Day, Year</td>
<td>Institution</td>
</tr>
<tr>
<td></td>
<td>PG ID: ####-####-####</td>
<td></td>
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</tbody>
</table>

MOLECULAR GENETICS REPORT:
Huntington Disease Testing via the HTT Repeat Expansion Test

SUMMARY OF RESULTS

POSITIVE: Heterozygous for 42 CAG Repeats
(One Full Penetrance Allele)

RESULTS AND INTERPRETATIONS: This patient is heterozygous for one HTT normal allele with 17 CAG repeats and one expanded HTT allele with 42 CAG repeats. An expanded HTT allele of this size is consistent with this individual developing Huntington disease with variable age of onset (see for example Jama et al. 2013. PubMed ID: 23414820; Bean & Bayrak-Toydemir. 2014. PubMed ID: 25356969).

These results should be interpreted in the context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on the following pages.

NOTES: Genetic counseling is recommended. Testing for the HTT CAG repeat expansion is available for at-risk, adult family members.
Example Report: Negative

MOLECULAR GENETICS REPORT:
Huntington Disease Testing via the \textit{HTT} Repeat Expansion Test

RESULTS AND INTERPRETATIONS: This patient is heterozygous for two normal \textit{HTT} alleles, one with 17 CAG repeats and one with 18 CAG repeats.

These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on the following pages.
### Molecular Genetics Report: Glycogen Storage Disease and Disorders of Glucose Metabolism Panel

**Example Report: Variant of Uncertain Significance (VUS)**

**SUMMARY OF RESULTS**

**Heterozygous for a Variant of Uncertain Significance in PHKG2**

<table>
<thead>
<tr>
<th>Gene, Transcript</th>
<th>Mode of Inheritance, Gene OMIM</th>
<th>DNA Variations, Predicted Effects, Zygosity</th>
<th>ClinVar ID</th>
<th>Highest Allele Frequency in a gnomAD Population</th>
<th>In Silico Missense Predictions</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHKG2, NM_000294.2</td>
<td>AR, 172471</td>
<td>c.1187T&gt;A, p.Ile396Lys, Heterozygous</td>
<td>Not listed in ClinVar</td>
<td>Not Present</td>
<td>Conflicting</td>
<td>UNCERTAIN</td>
</tr>
</tbody>
</table>

**Mode of Inheritance:** Autosomal Dominant=AD, Autosomal Recessive=AR, X-Linked=XL

**ClinVar ID:** Variant accession (www.ncbi.nlm.nih.gov/clinvar)

**GnomAD:** Allele Frequency registered in a large population database (gnomad.broadinstitute.org). Value listed is the highest allele frequency reported within one of seven population categories recognized in gnomAD v.2.0 (The "Other" population is excluded).

**Missense Predictions:** Summarized output (Damaging, Conflicting, or Tolerated) via PolyPhen-2, SIFT, MutationTaster, and FATHMM (PMID: 26556599).

**RESULTS AND INTERPRETATIONS:** This patient is heterozygous in the PHKG2 gene for a sequence variant designated c.1187T>A, which is predicted to result in the amino acid substitution p.Ile396Lys. To our knowledge, this variant has not been reported in literature. Although it is possible that the PHKG2 c.1187T>A (p.Ile396Lys) variant could be pathogenic, at this time its clinical significance is uncertain due to the absence of conclusive functional and genetic evidence.

This patient is also apparently negative for copy number variants (CNVs) within the genomic regions of this test.

These results should be interpreted in context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other information for this test on the following pages.

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What to do with VUS?

ACMG guidelines:

VUS should **not** be used in clinical decision-making. All clinical decisions should be based on personal and family history alone.
Part I: Q&A
Part II

1. From DNA to data - the process of generating meaningful results

2. Review ACMG criteria for variant classification and VUS interpretation

3. Introduction to frequently used variant interpretation resources
Example of supplementary information in a clinical lab report:

GENES ANALYZED: AIP, ALK, ANKRD26, APC, ARMC5, ATM, AXIN2, BAP1, BARD1, BLM, BMP1R1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CHEK2, DDX41, Dicer1, DIS3L2, EPCAM, ETV6, EXT1, EXT2, FANCC, FH, FLCN, GALNT12, GATA2, GPC3, GREM1, HOXB13, HRAS, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MLH3, MRE11, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PDGFRa, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PRKAR1B, PTCH1, PTCH2, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, REST, RET, RUNX1, SAMD9L, SDHA, SDHAF2, SDHB, SDHc, SDHd, SMAD4, SMARCA4, SMARCbl, SMARCc1, SRP72, STK11, SUFU, TERc, TERt, TMEM127, TP53, TRIP13, TSC1, TSC2, VHL, WT1, XRCC2

SUMMARY STATISTICS:

<table>
<thead>
<tr>
<th>Pipeline</th>
<th>Version</th>
<th>Average NGS Coverage</th>
<th>Fraction Bases Covered with NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium</td>
<td>1.1.0</td>
<td>275x</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

Minimum NGS coverage is ≥20x for all exons and ≥10x of flanking DNA, and ≥10x from 11-20bp of flanking DNA.

- Test limitations
- Test methods
- Sensitivity (true positive) and specificity (true negative) metrics of test
- Recommendations

Marshall et al., 2020. PMID: 33110627
Genetic testing methodology

• Genetic testing typically encompasses next-generation sequencing:
  • Genome
  • Exome
  • Targeted gene panels

• Most tests are built on an exome backbone, but are often offered as disease-specific panels that sequence pre-determined gene sets
Genetic testing methodology

**FASTQ:** Raw sequencing data

**BAM:** Alignment to the human genome “reference sequence”

**VCF:** Variant calls

<table>
<thead>
<tr>
<th>#CHROM</th>
<th>POS</th>
<th>REF</th>
<th>ALT</th>
<th>QUAL</th>
<th>FILTER</th>
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<tbody>
<tr>
<td>22</td>
<td>6,352,492</td>
<td>T</td>
<td>G</td>
<td>52</td>
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<tr>
<td>22</td>
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<td>G</td>
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<td>q10</td>
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<tr>
<td>22</td>
<td>6,352,501</td>
<td>T</td>
<td>A</td>
<td>29</td>
<td>PASS</td>
</tr>
</tbody>
</table>
Protein truncating variants

- c.126G>C  
  p.Trp42Ter

- c.119_120insG  
  p.Ile40fs

Missense variants

- c.A118C  
  p.Ile40Leu
Additional variation detected from genome sequencing

<table>
<thead>
<tr>
<th>Variant type</th>
<th>Gene(s) (if applicable)</th>
<th>Disorder(s)</th>
<th>References (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNVs and small insertions and deletions* (1–50 base pairs)</td>
<td>N/A</td>
<td>Heritable disease</td>
<td>Zook et al.37 Eberle et al.58</td>
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<tr>
<td>Copy number variation* (deletions and duplications)</td>
<td>N/A</td>
<td>Heritable disease including known microdeletion/duplication syndromes</td>
<td>Gross et al.33 Stavropoulos et al.27 Lindstrand et al.49</td>
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<tr>
<td>Mitochondrial variation* (SNVs, deletions, duplications, and heteroplasmy of at least 5%)</td>
<td>N/A</td>
<td>Known mitochondrial disorders</td>
<td>Duan et al.56</td>
</tr>
<tr>
<td>Structural variantsb</td>
<td>N/A</td>
<td>Heritable disease including those caused by translocations, inversions, and other genomic rearrangements</td>
<td>Lindstrand et al.49</td>
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<tr>
<td>Repeat expansionsc</td>
<td>FMR1</td>
<td>Fragile X and related disorders</td>
<td>Dolzhenko et al.49</td>
</tr>
<tr>
<td></td>
<td>HTT</td>
<td>Huntington disease</td>
<td></td>
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<tr>
<td></td>
<td>SCA1</td>
<td>Spinocerebellar ataxia 1</td>
<td></td>
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<tr>
<td></td>
<td>DMPK</td>
<td>Myotonic dystrophy 1</td>
<td></td>
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<tr>
<td></td>
<td>C9orf72</td>
<td>Amyotrophic lateral sclerosis</td>
<td></td>
</tr>
<tr>
<td>Selected pseudogenesc</td>
<td>SMN1 and SMN2</td>
<td>Spinal muscular atrophy</td>
<td>Chen et al.37</td>
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<tr>
<td></td>
<td>CYP21A2</td>
<td>21-Hydroxylase deficiency</td>
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<tr>
<td></td>
<td>CYP2D6</td>
<td>Codeine sensitivity</td>
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<td></td>
<td>HBA1 and HBA2</td>
<td>Alpha thalassemia</td>
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<td></td>
<td>PMS2</td>
<td>Colorectal cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PKD1</td>
<td>Polycystic kidney disease 1</td>
<td></td>
</tr>
</tbody>
</table>

*Recommended minimum variant types for clinical validation of WGS. Copy number variation is defined here as unbalanced changes (deletions and duplications) that are at the resolution of chromosomal microarray analysis.

bSome initiative groups have clinically validated. Structural variants are defined here as any genomic alteration >50 base pairs, including balanced and unbalanced changes.

cExamples of targeted loci that could be validated and reported as part of a clinical WGS test.
Variant Annotations

Gene-disease associations
  • Online Mendelian in Man (OMIM®)
  • ClinGen gene curations

General population databases - minor allele frequencies
  • GnomAD - WES > 125,000 samples; WGS > 76,000 samples

*In silico* prediction tools
  • Apply biochemical properties and conservation to predict variant pathogenicity

ClinVar - variant-level previous disease associations
Gene-disease associations

OMIM®

*147450
Table of Contents
Title
Gene-Phenotype Relationships
Text
Description
Cloning and Expression
Mapping
Gene Function
Molecular Genetics
History
Animal Model
Allucic Variants
Table View
See Also
References
Contributors
Creation Date
Edit History

*147450

SUPEROXIDE DISMUTASE 1; SOD1

Alternative titles: symbols
SUPEROXIDE DISMUTASE, CYTOSOLIC
SUPEROXIDE DISMUTASE, SOLUBLE
SOD, SOLUBLE
SUPEROXIDE DISMUTASE, COPPER-ZINC
INDOPHENOL OXIDASE A; IPOA

HGNC Approved Gene Symbol: SOD1

Cytogenetic location: 21q22.11
Genomic coordinates (GRCh38): 21:3,639,693-31,608,931

Gene-Phenotype Relationships

Location Phenotype
21q22.11

Phenotype NIM number Inheritance Phenotype mapping key
Amyotrophic lateral sclerosis 1 17450
Synaptic degeneration and oculomotor apraxia, progressive 610930

ClinGen Expert Panels

Amyotrophic Lateral Sclerosis Spectrum Disorders
Expert Panel

Search in table

Showing 1 to 23 of 23 rows

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>MOI</th>
<th>SOP</th>
<th>Contribution</th>
<th>Classification</th>
<th>Last Eval.</th>
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<td>AD</td>
<td>SOP</td>
<td>Primary</td>
<td>Limited</td>
<td>05/08/2017</td>
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<td>AD</td>
<td>SOP</td>
<td>Primary</td>
<td>Limited</td>
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<td>CCNF</td>
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<td>SOP</td>
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</table>

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General population databases

SOD1 (gnomAD v2.1.1)

GnomAD

- WES > 125,000 samples; WGS > 76,000 samples
- Disease exclusion cohorts available, e.g. non-neurological
- Multiple ancestral populations, although largely European
In silico prediction tools

PolyPhen-2
- Score that incorporates biochemical/physical properties

SIFT
- Score that incorporates amino acid conservation information

REVEL
- Score that predicts the pathogenicity of missense variants based on a combination of scores from 13 individual tools
Genomic variants are typically classified on a five-point scale to indicate the likelihood that the particular variant is associated with disease.

Pathogenic variants in disease genes related to phenotype (or symptoms) means that a cause of the patient’s symptoms has been identified.

Clinically, both pathogenic and likely pathogenic variants are treated the same—as if they are likely disease causing.

Variants of Uncertain significance (VUS) have an uncertain relationship to disease.

It is not recommended that Variants of Uncertain Significance be used for clinical decision making.

International efforts are underway to reclassify VUS variants as benign or pathogenic.

Finding a VUS is common among large-scale tests like gene panels, whole exome, and whole genome sequencing.
Evidence Framework:

“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”

Richards et al., 2015. PMID: 25741868

<table>
<thead>
<tr>
<th>Population data</th>
<th>Benign</th>
<th>Supporting</th>
<th>Pathogenic</th>
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</thead>
<tbody>
<tr>
<td>MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2</td>
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</table>

<table>
<thead>
<tr>
<th>Computational and predictive data</th>
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<tbody>
<tr>
<td>Multiple lines of computational evidence suggest no impact on gene /gene product BP4</td>
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<tr>
<td>Missense in gene where only truncating cause disease BP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silent variant with non predicted splice impact BP7</td>
<td></td>
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</tr>
<tr>
<td>In-frame indola in repeat without known function BP3</td>
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<table>
<thead>
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<th>Functional data</th>
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<tr>
<td>Well-established functional studies show no deleterious effect BS3</td>
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</table>

<table>
<thead>
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<th>Segregation data</th>
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<tbody>
<tr>
<td>Nons segregation with disease RS4</td>
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<table>
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<th>De novo data</th>
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<tbody>
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<td>De novo (without paternity &amp; maternity confirmed) PM6</td>
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<table>
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<tbody>
<tr>
<td>Observed in trans with a dominant variant BP2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed in cis with a pathogenic variant BP2</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Other database</th>
<th></th>
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<tbody>
<tr>
<td>Reputable source without shared data = benign BP6</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Other data</th>
<th></th>
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</tr>
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<tbody>
<tr>
<td>Patient's phenotype or PH highly specific for gene PP4</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

PMID: 25741868
Rules for Combining Criteria to Classify Sequence Variants
Richards et al., 2015. PMID: 25741868

---

### Pathogenic

1. **Very Strong (PV51)** \( \text{AND} \)
   - \( \geq 1 \) Strong (FS1–FS4) \( \text{OR} \)
   - \( \geq 2 \) Moderate (PM1–PM6) \( \text{OR} \)
   - 1 Moderate (PM1–PM6) and 1 Supporting (PP1–PP5) \( \text{OR} \)
   - \( \geq 2 \) Supporting (PP1–PP5)

2. \( \geq 2 \) Strong (FS1–FS4) \( \text{OR} \)

3. 1 Strong (FS1–FS4) \( \text{AND} \)
   - \( \geq 3 \) Moderate (PM1–PM6) \( \text{OR} \)
   - 2 Moderate (PM1–PM6) \( \text{AND} \) \( \geq 2 \) Supporting (PP1–PP5) \( \text{OR} \)
   - 1 Moderate (PM1–PM6) \( \text{AND} \) \( \geq 4 \) Supporting (PP1–PP5)

### Likely Pathogenic

1. **Very Strong (PV51)** \( \text{AND} \) 1 Moderate (PM1–PM6) \( \text{OR} \)

2. 1 Strong (FS1–FS4) \( \text{AND} \) 1–2 Moderate (PM1–PM6) \( \text{OR} \)

3. 1 Strong (FS1–FS4) \( \text{AND} \) \( \geq 2 \) Supporting (PP1–PP5) \( \text{OR} \)

4. \( \geq 3 \) Moderate (PM1–PM6) \( \text{OR} \)

5. 2 Moderate (PM1–PM6) \( \text{AND} \) \( \geq 2 \) Supporting (PP1–PP5) \( \text{OR} \)

6. 1 Moderate (PM1–PM6) \( \text{AND} \) \( \geq 4 \) Supporting (PP1–PP5)

### Benign

1. Stand-Alone (BA1) \( \text{OR} \)

2. \( \geq 2 \) Strong (BS1–BS4)

### Likely Benign

1. 1 Strong (BS1–BS4) and 1 Supporting (BP1–BP7) \( \text{OR} \)

2. \( \geq 2 \) Supporting (BP1–BP7)

*Variants should be classified as Uncertain Significance if other criteria are unmet or the criteria for benign and pathogenic are contradictory.*
Variant level previous disease associations

**ClinVar**

- Public archive of relationships among human variations and phenotypes, with supporting evidence
- Submissions have differing levels of complexity

<table>
<thead>
<tr>
<th>Variation Location</th>
<th>Gene(s)</th>
<th>Protein change</th>
<th>Condition(s)</th>
<th>Clinical significance (Last reviewed)</th>
<th>Review status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_000454.5(SOD1)c.50G&gt;C (p.Gly17Ala)</td>
<td>SOD1</td>
<td>G17A</td>
<td>Amyotrophic lateral sclerosis type 1, not provided</td>
<td>Pathogenic/Likely pathogenic (Aug 27, 2021)</td>
<td>criteria provided, multiple submitters, no conflicts</td>
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<tr>
<td>NM_000454.5(SOD1)c.56T&gt;C (p.Ile19Thr)</td>
<td>SOD1</td>
<td>I19T</td>
<td>Amyotrophic lateral sclerosis type 1</td>
<td>Uncertain significance (Sep 1, 2021)</td>
<td>criteria provided, single submitter</td>
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<tr>
<td>NM_000454.5(SOD1)c.59A&gt;G (p.Asn20Ser)</td>
<td>SOD1</td>
<td>N20S</td>
<td>not specified, Amyotrophic lateral sclerosis type 1</td>
<td>Conflicting interpretations of pathogenicity (Dec 17, 2021)</td>
<td>criteria provided, conflicting interpretations</td>
</tr>
</tbody>
</table>
Conventional analytical approach:
1) genotype-driven
2) phenotype-driven
Conventional analytical approach:

- What gene(s) does the variant impact?
- Is the variant expected to impact the function of the gene(s) and if so, does it cause a human phenotype?
- How well does the variant or gene’s disease association match that of the patient?
- Has this particular variant (or this variant type, e.g. LOF variants) been shown to cause a phenotype?
- Is the variant returnable as a secondary or incidental finding?

Marshall et al., 2020. PMID: 33110627
Limitations of variant interpretations:

***Our variant interpretations are only as good as the resources available***

- Not all ancestries are adequately represented
- Not all types of variation are captured (somatic, mosaic, deep intronic); and even if captured, we do not fully know how to interpret them
- Not all genes are characterized
- Tissue and development-specific expression
<table>
<thead>
<tr>
<th>Population</th>
<th>overall</th>
<th>controls</th>
<th>non-cancer</th>
<th>non-neuro</th>
<th>non-TOPMed</th>
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<tbody>
<tr>
<td></td>
<td>exomes</td>
<td>genomes</td>
<td>exomes</td>
<td>genomes</td>
<td>exomes</td>
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<td>African/African American</td>
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<td>Latino/Admixed American</td>
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<td>424</td>
<td>8,556</td>
<td>123</td>
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<td>Ashkenazi Jewish</td>
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<tr>
<td>East Asian</td>
<td>9,197</td>
<td>780</td>
<td>4,523</td>
<td>458</td>
<td>8,846</td>
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<tr>
<td>European (Finnish)</td>
<td>10,824</td>
<td>1,738</td>
<td>6,697</td>
<td>581</td>
<td>10,816</td>
</tr>
<tr>
<td>European (non-Finnish)</td>
<td>56,885</td>
<td>7,718</td>
<td>21,384</td>
<td>2,782</td>
<td>51,377</td>
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<tr>
<td>South Asian</td>
<td>15,308</td>
<td>*</td>
<td>7,845</td>
<td>*</td>
<td>15,263</td>
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<tr>
<td>Other</td>
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<tr>
<td>XX</td>
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<td>XY</td>
<td>67,961</td>
<td>8,741</td>
<td>29,059</td>
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<td>64,629</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>125,748</strong></td>
<td><strong>15,708</strong></td>
<td><strong>54,704</strong></td>
<td><strong>5,442</strong></td>
<td><strong>118,479</strong></td>
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<table>
<thead>
<tr>
<th>Population</th>
<th>overall</th>
<th>controls/biobanks</th>
<th>non-cancer</th>
<th>non-neuro</th>
<th>non-TOPMed</th>
<th>non-v2</th>
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<tbody>
<tr>
<td></td>
<td>exomes</td>
<td>genomes</td>
<td>exomes</td>
<td>genomes</td>
<td>exomes</td>
<td>genomes</td>
</tr>
<tr>
<td>African/African American</td>
<td>20,744</td>
<td>4,654</td>
<td>20,583</td>
<td>18,253</td>
<td>12,431</td>
<td>14,377</td>
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<tr>
<td>Amish</td>
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<td>10</td>
<td>456</td>
<td>431</td>
<td>56</td>
<td>455</td>
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<tr>
<td>Latino/Admixed American</td>
<td>76,477</td>
<td>2,345</td>
<td>75,533</td>
<td>7,424</td>
<td>6,460</td>
<td>6,878</td>
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<tr>
<td>Ashkenazi Jewish</td>
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<td>1,651</td>
<td>1,694</td>
<td>499</td>
<td>1,538</td>
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<tr>
<td>East Asian</td>
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<td>1,215</td>
<td>2,486</td>
<td>2,604</td>
<td>1,883</td>
<td>1,414</td>
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<tr>
<td>European (Finnish)</td>
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<td>2,750</td>
<td>5,316</td>
<td>3,485</td>
<td>5,270</td>
<td>3,662</td>
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<td>Middle Eastern</td>
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<td>123</td>
<td>152</td>
<td>155</td>
<td>135</td>
<td>154</td>
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<tr>
<td>European (non-Finnish)</td>
<td>34,029</td>
<td>3,427</td>
<td>32,411</td>
<td>31,966</td>
<td>10,533</td>
<td>25,988</td>
</tr>
<tr>
<td>South Asian</td>
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<td>1,588</td>
<td>2,403</td>
<td>2,418</td>
<td>2,405</td>
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<td>Other</td>
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<td>395</td>
<td>1,012</td>
<td>1,002</td>
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<tr>
<td>XX</td>
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<td>6,717</td>
<td>38,060</td>
<td>35,271</td>
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<tr>
<td>XY</td>
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<td>35,963</td>
<td>32,171</td>
<td>23,995</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>76,156</strong></td>
<td><strong>16,465</strong></td>
<td><strong>74,023</strong></td>
<td><strong>67,442</strong></td>
<td><strong>40,433</strong></td>
<td><strong>57,344</strong></td>
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</table>

…more likely to see ‘rare’ variants in patients from an under-represented population
Neurogenetic VUS Rounds at the Neuro

1 hour virtual bi-weekly meeting with our team to review and internally classify VUS identified in our patients

All Canadian neurologists and genetic counsellors are welcome to submit cases and participate in our meetings!

If interested, please contact:

sali.farhan@mcgill.ca
alana.mistry@muhc.mcgill.ca
Final Q&A
Additional resources

- Clinical variant interpretation: [https://www.youtube.com/watch?v=VqrEtABxvhY&t=3s](https://www.youtube.com/watch?v=VqrEtABxvhY&t=3s)
- gnomAD tips: [https://www.youtube.com/watch?v=Bh8AKkl-DhY](https://www.youtube.com/watch?v=Bh8AKkl-DhY)
- Variant classification: [https://www.youtube.com/watch?v=xVA-jg_AqSg](https://www.youtube.com/watch?v=xVA-jg_AqSg); [https://www.youtube.com/watch?v=wTBxyT8a2PI](https://www.youtube.com/watch?v=wTBxyT8a2PI)
- General genome analysis: [https://www.youtube.com/watch?v=yeUETPbZgGw](https://www.youtube.com/watch?v=yeUETPbZgGw)
- ClinGen Resource videos, refreshers, etc: [https://www.youtube.com/channel/UCsn4nEVUTpVQz70rClgMMsQ](https://www.youtube.com/channel/UCsn4nEVUTpVQz70rClgMMsQ); in silico tools: [https://www.youtube.com/watch?v=MJtgahM5Zak](https://www.youtube.com/watch?v=MJtgahM5Zak)