Large clinical cohort undergoing simultaneous single nucleotide and copy number variant analysis reveals broad mutation spectrum and high diagnostic yield for neuromuscular disorders

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Disclosures & Acknowledgements

- Dr. Aradhya is medical director and full-time employee of Invitae, a clinical genetic information company in San Francisco, California.

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Outline

Genetic testing in a large unselected cohort of individuals with a range of neuromuscular disorders provides insight into:

- Diagnostic yield with simultaneous sequencing and CNV analysis
- Mutation spectrum and patterns
- Reclassification of variants of uncertain significance (VUS)
- Differential diagnosis
Introduction

- Neuromuscular disorders are highly genetically heterogeneous

- Molecular genetic testing used to:
  - confirm clinical diagnoses
  - identify subtype
  - inform management and prognosis

- Small studies performed previously, showing yield of 19 – 60% (HSP, neuromuscular panel)

- Routine analysis of intragenic copy number variation (CNV) by NGS is now possible, enabling evaluation of its contribution to neuromuscular disorders, aside from classic disorders like DMD, SMA, CMT1A.
Neurology panels

- Last decade has seen rapid adoption of **NGS panels for diagnosing neurological genetic disorders**. Cost of testing has dropped considerably

- Neuromuscular disorders, neurodegenerative disorders, and late-onset movement disorders are covered comprehensively

- Panels with **SNVs, CNVs, indels, rearrangements detected simultaneously** in a single assay with a 10-21 day turn-around time

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Neurology panels

- Various neuromuscular disorders tested in a comprehensive panel.

- Genetic testing for neuromuscular disorders available for
  - Muscular dystrophies
  - Myopathies
  - Myasthenic syndromes
  - Spinal muscular atrophy
  - Myotonia congenita
  - Dystonia

- Testing is available for neuropathies and related disorders
  - Charcot-Marie-Tooth disease
  - HSAN
  - Motor neuropathies
  - Small-fiber neuropathies
  - Hereditary spastic paraplegia
Gene panel-based testing for neuromuscular disorders

- **Gene Panel Design**
  - Phenotype-specific gene panels were curated based on the:
    - Strength of evidence supporting the association between a gene and a disorder
    - To also address differential diagnoses

- **Next-generation sequencing (NGS) hybridization capture assay**
  - Targeted custom-designed multi-gene panels (not exome-based)
  - Simultaneous identification of single-nucleotide variants (SNVs), short and long indels, exon-level deletions/duplications (CNVs), mosaic variants, and structural arrangements disrupting coding sequences
  - High depth of coverage (avg 350x), NGS solutions for *SMN1* and other difficult genes

- **Patient cohort analysis**
  - Unbiased cohort of >25,000 patients suspected to have a neuromuscular disorder
  - Clinical variant interpretation using Sherloc, based on ACMG guidelines
Retrospective analysis

- Diagnostic genetic **testing for 25,356 individuals** in this cohort
  - Aged <1-96y, mean 43y
  - 45% female

- Definitive molecular diagnosis
  - 5,055 of 25,356 individuals received a positive results
  - Overall **diagnostic yield of 20%** (range 4 – 33%)
  - Single-gene tests
    - CMT1A 38%
    - DMD/BMD 37%
    - SMA 21%
Variant spectrum

- 33,551 variants classified as LP/P or VUS
  - 84% SNV, 6% indels, 10% CNVs (619 unique events, 3,366 in total)

- Among 7,789 LP/P variants, 39% were intragenic CNVs
  - 93% of LP/P CNVs were in SMN1, PMP22, DMD and 7% were in 77 other genes
  - 113 diagnoses

- 1,328 LP/P CNVs identified in AR conditions
  - 30 in compound heterozygous state with a non-CN variant
  - 856 present in homozygous state (mostly SMN1 deletion)

- Several recurrent mutations identified including both CNVs and SNVs
Contribution of intragenic CNVs to diagnostic yield

- Contribution of exon-level deletions and duplications to the diagnostic yield where the percentage of LP/P are sequence variants (green) or copy number variants (blue), shown by different panels

- *PMP22, DMD, and SMN1* had the highest number of CNVs, as expected

- 7% CNVs were in 77 other genes
Molecular diagnostic positive yield

- Diagnostic yield by panel shown by percentage of definitive positive molecular diagnostic results (MDx)

- >15% yield in CMT, muscular dystrophy, neuropathies, limb-girdle MD, SMA

- Of the 163 genes that provided definitive diagnostic results, PMP22 provided the largest number, followed by SMN1, DMD, MFN2, MPZ, SPAST, TTR, SCN4A and GJB1
Expanded panel analysis and differential diagnoses

- Positive results in following genes obtained from broader panel
  - *SMN1* (7%)
  - *DMD* (49%)
  - *PMP22* (62%)

- Rare genetic cases
  - 200 of 2,501 individuals received a molecular diagnosis in a gene related to, but not suspected based on, their clinical diagnosis
  - 16% of patients would have been missed with single gene analysis alone
  - 25% of males with suspected DMD had an etiology related to a different gene

- Differential diagnosis supported by NGS panels
  - 8% positive from a broad panel after initial testing was negative
Frequency and resolution of variants of uncertain significance

- 25,356 individuals carried 25,762 VUS
  - 17,321 unique variants in 266 genes
  - Reported **one or more VUS in 53%** of individuals
    - range 1-13 (mean=1.9; median=1)
  - At least 16% of individuals with one or more VUS had a co-occurring definitive diagnostic result

- Follow-up family studies were able to re-classify 2% of VUS on average per gene
  - 158 to LP/P (44%) and 198 to LB/B (56%)
  - Segregation studies provided evidence to reclassify VUS $\rightarrow$ LP/P due to de novo status in AD conditions, *trans* phase in AR conditions
  - Most de novo events observed in *RYR1*, *ACTA1*, *SPAST*, and *MPZ*
Frequency and resolution of variants of uncertain significance
Summary

- NGS-based panel testing with sequence and CNV detection provided **diagnosis for 4-33% of ~25000 patients.** Faster, less expensive, better analytic performance than exome.

- Commonly tested conditions can have a mild or uncharacteristic phenotype
  - SMA, DMD/BMD, CMT1A diagnosed through both single gene and panel testing
  - Differential diagnosis by NGS panels: **8% positive from broad panel** after initial negative result

- Intragenic CNVs are an important contributor to pathogenic variant burden
  - **39% of all positive results included a CNV.**
  - 80% of unique CNVs included a few exons. 77 genes contained LP/P intragenic CNVs
  - Confirmed 30 individuals as compound heterozygote including CNV

- VUS resolution most often in disorders with dominant inheritance. **On average, 2% of VUS per gene were resolved,** reaching more than 5% of VUS in some genes