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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Declaration of Conflict of Interest

Type	Company
Employment full time / part time	Invitae
Ownership interest (stock, stock-options, patent or intellectual property)	Invitae

Background

- Molecular genetic testing used to
  - confirm clinical diagnoses
  - identify subtype
  - inform management and prognosis
- Corroborated in several small studies
- Analysis of intragenic copy number variation (CNV) is now possible, enabling evaluation of its contribution to neuromuscular disorders.

Objective

To examine a large unselected cohort of individuals with a range of neuromuscular disorders and report the:

- Diagnostic yield with simultaneous sequencing and CNV analysis
- Mutation spectrum and properties
- Reclassification of variants of uncertain significance (VUS)

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Methods

- Gene Panel Design
  - Phenotype-specific gene panels were curated based on the:
    - strength of evidence supporting the association between a gene and a disorder
    - differential diagnosis
- Next-generation sequencing (NGS)
  - Non-exome based NGS panels
  - Simultaneous identification of single-nucleotide variants (SNV), short and long indels, exon-level CNVs, and structural arrangements disrupting coding sequences, including SMN1
- Subjects and Reporting
  - Unbiased cohort of patients suspected to have a neuromuscular disorder
  - Analysis and reporting of variants according to validated SHERLOC

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Results

Results

- Diagnostic genetic testing for 25,356 individuals
  - 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%
  - Single gene tests
    - CMT1A 38%
    - DMD/BMD 37%
    - SMA 21%

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Results

- Classification of variants
  - 33,551 variants classified as LP/P or VUS
    - 84% SNV
    - 6% indels
    - 10% CNVs – most in *SMN1, PMP22, DMD*
  - 7% of clinically-significant CNVs were in 77 other genes
    - 113 diagnoses
    - all of which were intragenic
  - 1,328 LP/P CNVs identified in AR conditions
    - 30 in compound heterozygous state with a non-CNV variant
    - 856 present in homozygous state

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Results

- Testing patterns
  - Positive results 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 the etiology of a different gene
    - 19% related to neuromuscular disorder not muscular dystrophy

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Results

- 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
  - Follow-up family studies were able to re-classify 2%
    - 158 to LP/P and 198 to LB/B
    - Most commonly by demonstrating *de novo* inheritance

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Discussion

Discussion – diagnostic yield

- NGS-based panel testing with simultaneous sequence and CNV detection can provide a diagnosis for 4-33% of affected patients
  - faster, less expensive with better coverage than exome as a first line test

- Clinically well-recognized conditions can have a mild or uncharacteristic phenotype
  - SMA, DMD/BMD, CMT1A were all diagnosed through single gene testing AND broader panels

- Differential diagnosis supported by NGS panels
  - 8% positive from a broad panel after initial testing was negative
  - 133 suspected to have DMD/BMD had a molecular diagnosis in a gene unrelated to muscular dystrophy

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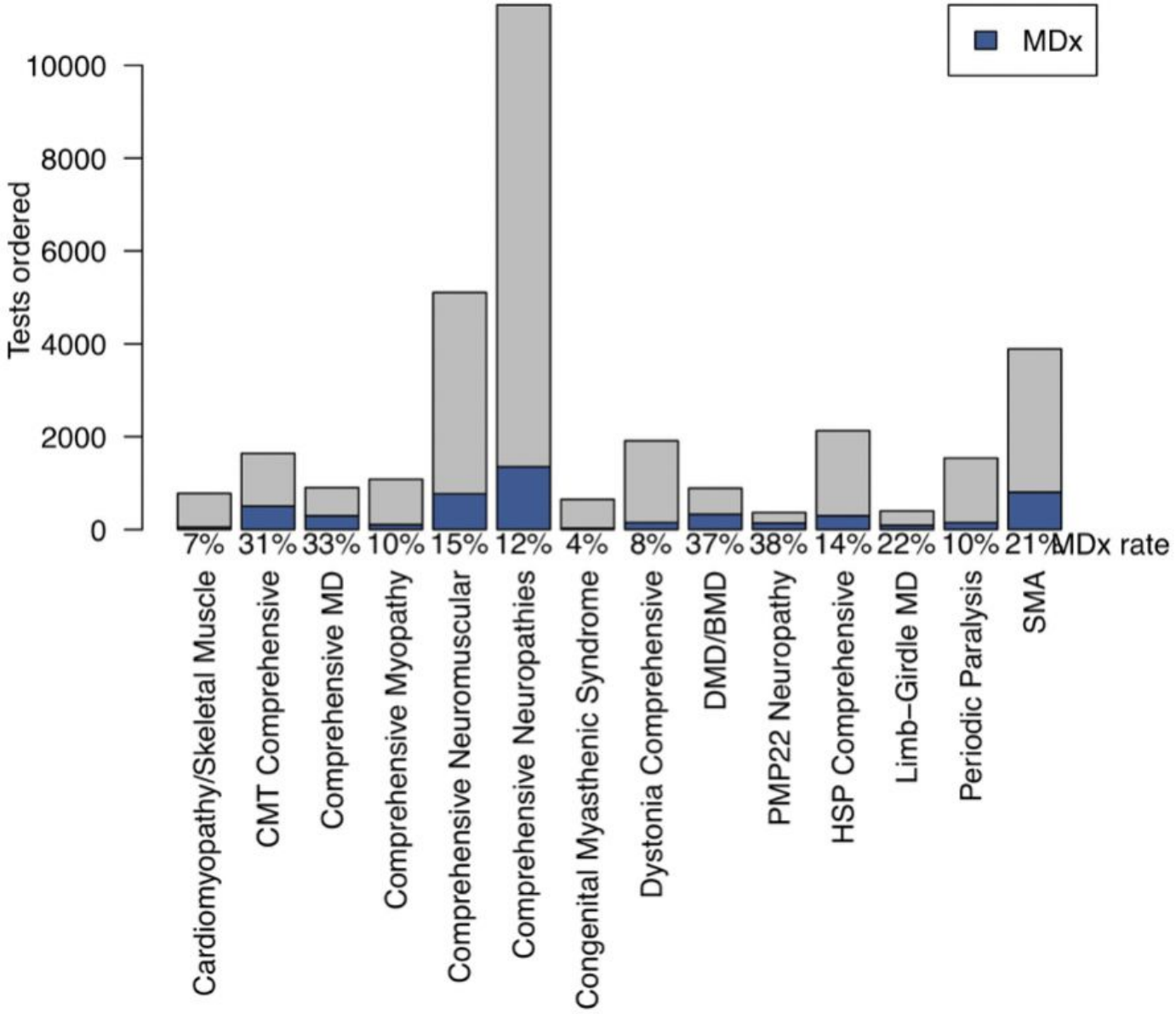

Diagnostic yield by panel shown by percentage of definitive positive results

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Discussion – importance of CNV detection

- 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 non-common genes contained LP/P intragenic CNVs
  - Confirmed 30 individuals as compound heterozygote including CNV

- 113 individuals identified who would otherwise have been invisible using traditional sequencing methods or exome (typically without intragenic CNV)
  - cost and time savings

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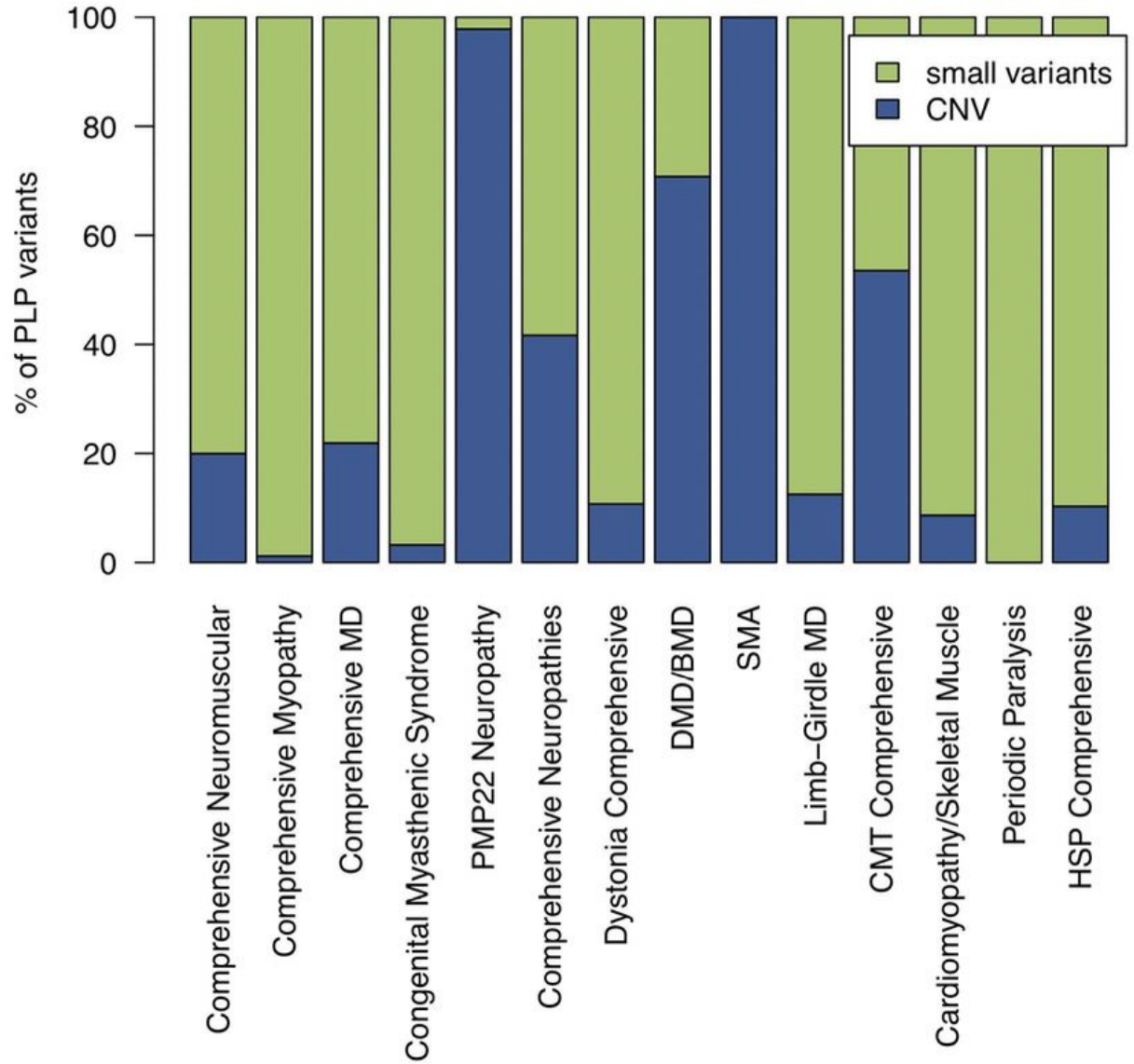

Contribution of CNVs to diagnostic yield where the percentage of LPV/PV are sequence (green) or CNV (blue) based on panel.

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Discussion – reclassification of VUS

- Complexities of interpretation of variants in the long list of genes associated with neuromuscular disorders
- Most VUS identified as a single heterozygous allele in AR conditions
- VUS in genes associated with AD conditions with high penetrance were more likely to be reclassified as LP/P
- Segregation studies provided useful evidence for pathogenicity in 48% of VUS
  - *de novo* status in AD conditions
  - *trans* phase in AR conditions

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Thank you

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