Diagnostic yield for neurological and neuromuscular disorder testing via high-depth multi-gene panel analysis with integrated sequence and copy number detection

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Abstract

Objective To evaluate diagnostic yield using systematically curated multi-gene panels with simultaneous sequence and exonic copy number variant (CNV) detection.

Methods Using high-depth next-generation sequencing (NGS), we investigated subsets of approximately 300 genes curated into ten disease-specific, multi-gene panels in 4,358 individuals diagnosed with neurological or neuromuscular disorders. Each gene in every panel was evaluated with our validated custom-built algorithms that use depth-of-coverage information and split-read detection to identify CNVs, small and large indels, and single nucleotide changes.

Results We tested 4,358 individuals with 4,632 panel tests and found a definitive diagnosis in 976 individuals (22.4%). Additional cases had results that would likely reach clinical significance with additional evidence, such as observation of de novo occurrence or compound heterozygosity in trans. Whole-gene or exonic CNVs composed 40% of all diagnostic findings, nearly two-thirds of which were identified in PMP22 or DMD. The highest observed diagnostic yields were obtained in SMA (68%) and DMD (63%) testing. The single most frequently observed pathogenic variant was gene duplication of PMP22. Diagnostic yields ranged from a low of 12% for dystonia to a high of 68% for SMA.

Conclusions Pre-curated multi-gene panels and our custom NGS-based system enable simultaneous sequence and CNV calling and, owing to high yield, are appropriate for diagnosing neurological conditions before exome sequencing.

Introduction

Owing to the heterogenous nature of many neuromuscular (NM) conditions and the inter-laboratory variability in the number of genes offered in a given panel for each NM disorder, it is often difficult to establish the clinical sensitivity of a multi-gene panel. In addition, in order to provide a molecular diagnosis, genes need to be comprehensively assessed for the spectrum of mutations that can occur. Historically, this required many types of tests: Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), exon-targeted array-CGH, and so on. This inevitably added to both the cost and time of the analysis, and historically very few studies on the genetic characterization of NM conditions employed such a comprehensive approach.

The purpose of this study was to evaluate the diagnostic yield of pre-curated gene panels with simultaneous sequence and CNV detection in patients referred for evaluation of neuromuscular diseases.

Methods

Our gene panels were established by (1) evaluating the strength of the evidence suggesting that a particular condition is caused by pathogenic variants in a particular gene, (2) evaluating whether unusual genotype/phenotype observations represent a plausible expansion of clinical phenotype associated with a gene, and (3) establishing a molecular diagnostic strategy to capture overlapping clinical presentations.[1] Lists of genes included in each panel can be found at www.invitae.com.

Germline DNA from blood was tested with neuromuscular panels. Sequence and CNV analysis were performed with validated NGS methods, allowing for concurrent analysis of sequence variants and exonic CNVs and thereby providing a method for evaluating the contribution of CNVs in inherited neuromuscular conditions. The reported CNVs were confirmed with an alternative method.

Invitae uses a proprietary, validated algorithm to detect deletions and duplications with NGS. The algorithm calls exonic deletions and duplications by calculating the statistical likelihood of each copy number state through comparison of the depth of sequence coverage at the targeted exons with the depth measured from a set of baseline samples.[2]

SMN1 and SMN2 analyses are accomplished using a validated bioinformatic approach and high-depth NGS reads derived from both SMN1 and SMN2. Combined reads are aligned to SMN1, and combined SMN1/2 copy number is determined using Invitae's read count-based copy number variant detection algorithm, CNVitae. SMN1- and SMN2-specific exon 7 copy number is resolved by counting reads with the gene determining variant in exon 7.[3]

Results

A total of 4,632 evaluations were made at Invitae for sequence and deletion/duplication analysis of genes associated with neuromuscular conditions. Ordering physicians requested 602 comprehensive neuromuscular panels, 1215 comprehensive neuropathy panels, 227 comprehensive myopathy panels, 282 muscular dystrophy panels, 117 limb-girdle muscular dystrophy panels, 897 Charcot-Marie-Tooth disease (CMT) panels, 141 dystrophinopathies (DMD gene), 662 hereditary spastic paraplegia (HSP) panels, 366 dystonia panels, and 123 for SMN1/2.

Analysis (panel)	Number of positive results (positive rate)	Positive CNV (% of positive results)	Positive SNV (% of positive results)	Number of uncertain results (VUS rate)	Most common gene (% of positive results)
Neuromuscular	148 (25%)	20(14%)	128 (86%)	352 (58%)	DMD (13%)
Neuropathy	144 (12%)	65 (45%)	79 (55%)	597 (49%)	PMP22 (46%)
Myopathy	27 (12%)	0	27 (12%)	121 (53%)	RYR1 (31%)
Muscular dystrophy	117 (51%)	22 (19%)	95 (81%)	104 (46%)	DMD (63%)
Limb-girdle muscular dystrophy	34 (33%)	5 (15%)	29 (85%)	38 (28%)	DMD (32%)
Charcot-Marie-Tooth disease	252 (28%)	118 (47%)	134 (53%)	251 (28%)	PMP22 (50%)
Dystrophinopathies (DMD gene)	84 (60%)	52 (62%)	32 (38%)	7 (8%)	n/a
Dystonia	40 (12%)	5 (12%)	35 (88%)	43 (13%)	SGCE (23%)
Hereditary spastic paraplegia	108 (16%)	16 (15%)	92 (85%)	232 (35%)	SAPST (39%)
SMA (SMN1/2 genes)	84 (68%)	83 (99%)	1 (1%)	0	n/a

Table 1. Percentage of orders for each gene panel in which likely pathogenic or pathogenic variants (positive rate), variant(s) of uncertain significance in the absence of positive findings (VUS rate) were detected. Among positive results, the percentage that are copy number variants (CNVs) and sequence variants (single nucleotide variants [SNVs]) are called out. For each panel, the gene contributing to the most positive reports is also given.

Conclusions

- A systematic approach to evaluate gene-disease associations was employed to assemble phenotype-specific, multigene panels.
- Simultaneous detection of copy number variants and sequence variants was accomplished using an NGS-based assay and custom bioinformatic tools.
- Overall diagnostic yield was 22.4%.
- Our neurology and neuromuscular panels yielded reasonable positive rates in the setting of a commercial diagnostic service.
- SMN1/SMN2 copy number analysis can be accomplished with NGS alone.
- Pathogenic CNVs account for 40% of all positive results (15% when DMD and PMP22 are excluded).
- Our validated approach delivers high-quality results while lowering cost and reducing turnaround time.

References

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