Limb-Girdle Muscular Dystrophy and other Myopathy Patients Diagnostic Yield in Large Cohort of more than 6000 patients

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INTRODUCTION

Limb-girdle muscular dystrophies (LGMD) are a group of heterogeneous genetic disorders involving predominantly proximal muscle weakness. Disease diagnosis and specific subtype identification is complicated by the clinical and genetic heterogeneity with overlap among the subtypes, which can lengthen the diagnostic odyssey and overall cost. Definitive molecular diagnosis helps accurate identification of specific LGMD subtypes or other muscular dystrophy (MD) subtypes to drive clinical trial enrollment. Delays in obtaining definitive molecular diagnosis are observed for many patients with MD due to the multiple tests require to cover the complex genetic variant spectrum and cost burden. Traditional molecular testing strategies include initial Sanger sequencing or next-generation sequencing (NGS) for sequence variant analysis, and follow-up copy number variation (CNV) analysis by microarray and additional reflex testing depending on the variant spectrum of the suspected MD subtype. To expand our understanding of the genetic spectrum underlying LGMDs and other overlapping MDs, we implemented a comprehensive NGS-based multi-gene panel to detect both sequence variants including coding and known deep intronic variants and CNVs in one assay.

MATERIALS AND METHODS

A custom Agilent SureSelect targeted sequence capture was used to enrich all 66 genes included in the panel, followed by NGS on an Illumina NovaSeq 6000 with 150-base pair paired-end reads. Sequence variants were assessed by our proprietary analysis and interpretation pipeline, Ordered Data Interpretation Network (ODIN). Variants were called at a minimum coverage of 8× and an alternate allele frequency of 20% or higher. Variants were evaluated by their frequency, as reported in public databases (gnomAD, dbSNP, EVS, and Leiden LOVD). Variants having a frequency greater than expected, given the prevalence of disease, were considered benign. Variant interpretations and classifications were performed using the American College of Medical Genetics (ACMG) standards and guidelines for the interpretation of sequence variants.

Copy number variants (CNV) analysis was assessed using Biodiscovery's NxClinical software (BioDiscovery, El Segundo, CA). Male and female reference sets were created from healthy controls using NGS data with the help of BAM Multiscale Reference (MSR) Builder module. CNVs were detected using the Hidden Markov Model-based fast adaptive states segmentation technique algorithm. CNV analysis was designed to detect deletions and duplications of three exons or more; in some instances, due to the size of the exons or other factors, not all CNVs were analyzed.

RESULTS

In a large cohort of 6473 patients, we identified genetic variants in different genes related to MDs and demonstrated how integrating CNV analysis into routine NGS-based panel testing is instrumental in identifying rare LGMDs and other overlapping MD subtypes. Molecular diagnosis was established in 19.6% (1266) of 6473 cases examined. Copy number variants were identified in 95 of 1266 cases. Different sizes of copy number variants including single exon, multi-exon, and whole genes were successfully identified in different MD genes. Major genes including *CAPN3* (5.4%, 68/1266), *DYSF* (4.0%, 51/1266), *GAA* (3.7%, 47/1266), *ANO5* (3.6%, 45/1266), and *FKRP* (2.7%, 34/1266) contributing to LGMD were identified. Genes of other overlapping MD subtypes identified included, *PABPN1* (10.5%, 133/1266), *VCP* (2.2%, 28/1266), *MYOT* (1.2% 15/1266), *LDB3* (1.0%, 13/1266), *COL6A1* (1.5%, 19/1266), *FLNC* (1.1%, 14/1266), and *DNAJB6* (0.8%, 10/1266).

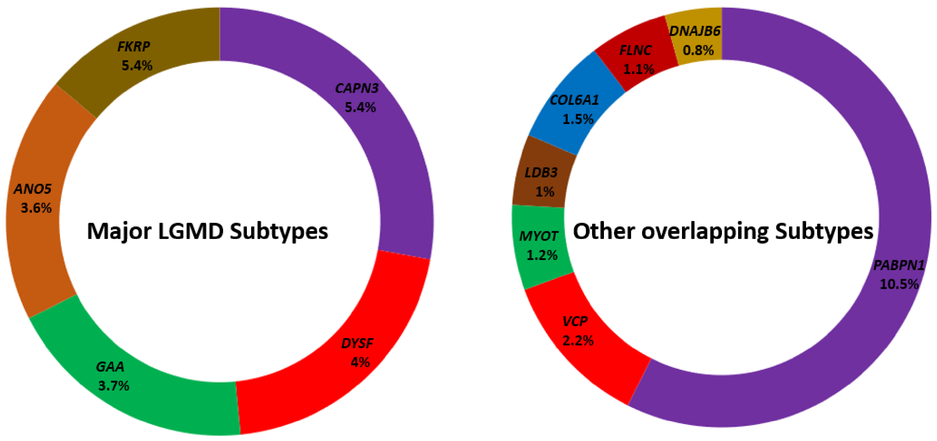

Figure 1. Major LGMD subtypes and other overlapping muscular dystrophy subtypes identified.

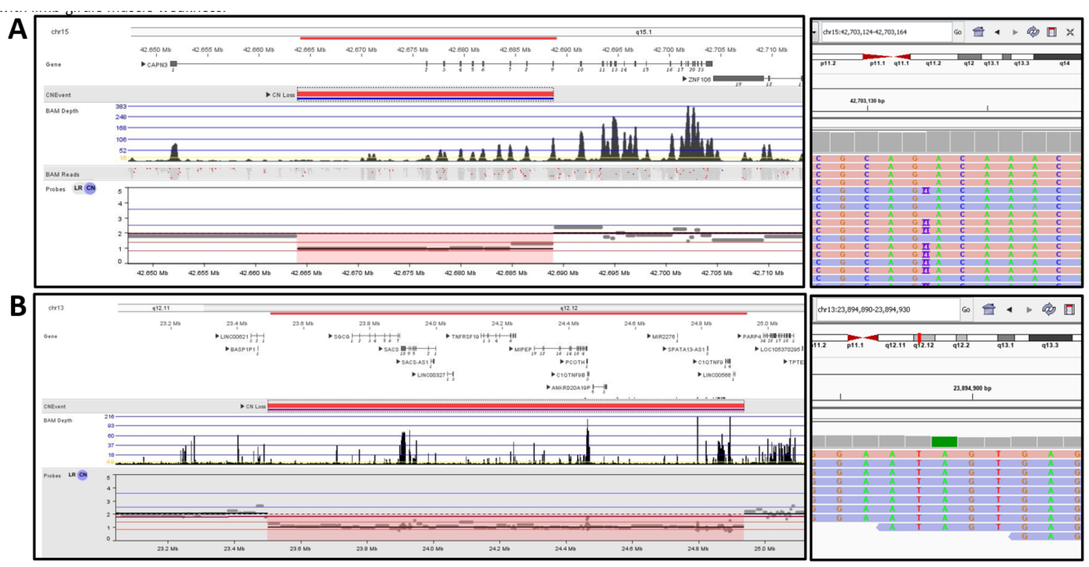

Figure 2. (A) Deletion of exons 2 to 9 and sequence variant c.2318_2321dup in exon 22 were identified in the *CAPN3* gene in a 18 year old male with LGMD (B) Entire *SGCG* gene deletion and sequence variant c.702G>A (p.Met234Ile) in exon 7 of the *SGCG* gene were identified in a 38 year old male with muscular dystrophy.

CONCLUSION

- To the best of our knowledge, this is the first large-scale report of diagnostic muscular dystrophy (MD) panel testing using a single NGS assay to detect all variant types.
- This comprehensive NGS panel study greatly helped to identify LGMD subtypes as well as other myopathies with clinical features overlapping with LGMD subtypes.
- Increased prevalence was observed for genetically confirmed Oculopharyngeal Muscular Dystrophy (OPMD), *VCP* related inclusion body myopathy, and other dominant overlapping MDs.
- This has the potential to attract more research and development into these rare disorders, hopefully culminating in more targeted therapies and improved patient outcomes.