Genetic results in a cohort of 489 patients with inherited myopathies

Kimberly Gall[1], Julie Hathaway[1], Lotta Koskinen[2], Kirsi Alakurtti[2], Åsa Hagström[2], Mònica Segura Castell[2], Heli Kuisma[2], Eija H Seppälä[2], Janica Djupsjöbacka[2], Mikko Muona[2], Tuuli Pietilä[2], Pertteli Salmenperä[2], Inka Saarinen[2], Ray Veeraraghavan[1], Samuel Myllykangas[2], Juha Koskenvuo[2]

[1]Blueprint Genetics Inc, Marlborough, USA; [2]Blueprint Genetics, Espoo, Finland

P622

Introduction

- Inherited myopathies (IMs) are a broad group of conditions with both clinical and genetic heterogeneity.
- Some can be readily diagnosed clinically; however, other diagnoses are elusive even after muscle biopsy.
- A positive genetic test can provide information about prognosis, quantifies risk to other family members, and can be necessary for participation in relevant clinical trials or to access precision medicine therapies.
- With the advent of next-generation sequencing (NGS), we can simultaneously sequence multiple genes, thus improving the clinical utility of genetic testing for individuals with IMs.
- Given this, we investigated the yield of genetic testing in a cohort of patients with suspected IMs to determine the clinical utility of simultaneously testing both nuclear and mitochondrial genes in this patient population.

Methods

- Clinical reports of consecutively tested patients who underwent Comprehensive Muscular Dystrophy/Myopathy Panel testing at Blueprint Genetics (a CLIA-certified diagnostic laboratory) were examined.
- All patients were tested for the indication of IMs. Testing included sequence and copy number variant (CNV) analyses of NGS data from a validated whole or clinical exome assay performed on blood, saliva or extracted DNA samples.
- Target regions included coding exons (+/-20 bp from the intron/exon boundary) of up to 161 genes associated with IMs and up to 125 noncoding variants in these genes catalogued as disease-associated by HGMD and/or ClinVar.
- CNV analysis was performed bioinformatically from NGS data using two variant-calling algorithms including a proprietary method specific for exon-level deletions.
- Over half (272/489) of individuals tested had the mitochondrial genome (mtDNA) included in their panel test. Variant interpretation was performed in accordance with ACMG/AMP guidelines.
- A positive result in a nuclear gene was defined as a pathogenic (P) or likely pathogenic (LP) variant(s) consistent with the patient's reported phenotype and associated disease inheritance; a positive result in a mitochondrial gene was defined as a P/LP variant with >5% heteroplasmy consistent with the patient's reported phenotype.
- Chi-square ($\chi 2$) analysis was used to determine statistical significance ($P<0.05$).

Demographic	Total cohort, (%)	Cohort with positive results, (%)	Positive rate, (%)
Female	207/489 (42.3%)	39/119 (32.8%)	39/207 (18.8%)
Male	282/489 (57.7%)	80/119 (67.2%)	80/282 (28.4%)
Pediatric (0-18yrs)	221/489 (45.2%)	75/119 (63%)	75/221 (33.9%)
Adult (19-83yrs)	268/489 (54.8%)	44/119 (37%)	44/268 (16.4%)

Table 1. Patient demographics

Pediatric	
Gene	**Percentage of positive results**
DMD	42.7%
COL6A1, LAMA2, LMNA	5.3% each
DYSF, NEB (NEB), RYR1	4% each
COL6A2, CAPN3, TTN, ACTA1, SELENON, SGCA, TPM2, MTM1	2.7% each

Table 2. Positive results by most common genes in the pediatric population. Genes highlighted in pink are complicated by >90% or >98% sequencing homology. Genes highlighted in blue were unique to the pediatric population with positive results.

Adult	
Gene	**Percentage of positive results**
PABPN1	15.9%
DMD	14.6%
ANO5	9.1%
COL6A2, TCAP	6.8% each
CAPN3, TTN, FKRP, CAV3, LDB3	4.5% each

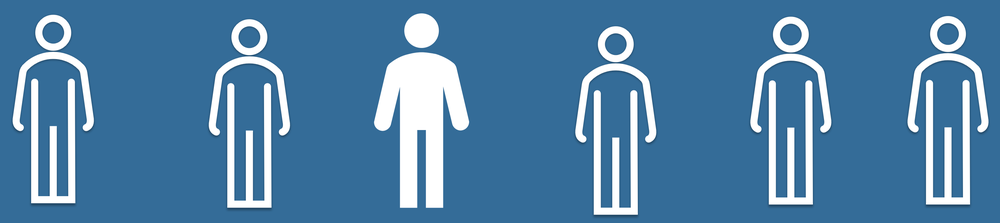
Table 3. Positive results by most common genes in the adult population. Genes highlighted in pink are complicated by >90% or >98% sequencing homology. Genes highlighted in blue were unique to the adult population with positive results.

Blueprint Genetics

Conflict of interest statement: All authors are employed by Blueprint Genetics.

Results

- A total of 489 patient reports were reviewed. The median age at time of testing was 24 years (range: neonatal to 83 years). Children (0-18 years) made up 45.2% (221/489) of those tested. The cohort was 57.7% male (282/489). (Table 1)
- A positive result was reported for 24.3% (119/489) of patients, with children being significantly more likely to receive a positive result (33.9%, 75/221) than adults (16.4%, 44/268) (χ^2=12.18, $P<.05$). (Table 2, Table 3)
- LP/P variants in *DMD* accounted for almost a third of all diagnoses (31.9%, 38/119) in the total cohort; individuals tested in childhood were significantly more likely to have a positive result in *DMD* (χ^2=19.89, $p<.01$).
- LP/P CNVs accounted for 21.0% (25/119) of the positive results; 84.0% (21/25) were identified in *DMD*. Three (12.5%, 3/24) CNVs were less than 500 bps in size.
- Noncoding variants and variants in mitochondrial genes were responsible for 1.7% (2/119) of positive results.



Approximately 1 in 6 adults undergoing multi-gene panel testing for muscular dystrophy or myopathy received a positive result.

Approximately 1 in 3 children undergoing multi-gene panel testing for muscular dystrophy or myopathy received a positive result.

Conclusions

- This study demonstrates the clinical utility of multi-gene panel testing that includes both nuclear and mitochondrial genes, as almost 25% of individuals with suspected IMs in this cohort received a positive result. Individuals tested in childhood were significantly more likely to have a positive result than those tested in adulthood.
- Variants in *DMD* accounted for almost a third of positive results; individuals tested in childhood who received a positive result were significantly more likely to have a *DMD* variant than those tested in adulthood.
- CNVs accounted for over one-fifth of the positive results, with the majority being in *DMD*.
- These results have significant implications given the recent FDA approval of gene therapy for IMs such as Duchenne muscular dystrophy.
- Noncoding variants, mitochondrial variants, and small (<500 bp) CNVs should be considered for inclusion in testing of individuals with muscular dystrophy or myopathy, as these were responsible for 1 in 25 positive results.