Targeted gene panel use in 2249 neuromuscular patients: the Australasian referral center experience

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Methods
We designed two iterations of a comprehensive targeted gene panel for neuromuscular disorders. Version 1 included 336 genes, which was increased to 464 genes in Version 2. Both panels used TargetSeq probe-based hybridization for target enrichment followed by Ion Torrent sequencing. Targeted high-coverage sequencing and analysis was performed on 2249 neurology patients from Australia and New Zealand (1054 Version 1, 1195 Version 2) from 2012 to 2015. No selection criteria were used other than referral from a suitable medical specialist (e.g., neurologist or clinical geneticist). Patients were classified into 15 clinical categories based on the clinical diagnosis from the referring clinician. Results: Six hundred and sixty-five patients received a genetic diagnosis (30%). Diagnosed patients were significantly younger than undiagnosed patients (26.4 and 32.5 years, respectively; \( P = 4.6326 \times 10^{-9} \)). The diagnostic success varied markedly between disease categories. Pathogenic variants in 10 genes explained 38% of the disease burden. Unexpected phenotypic expansions were discovered in multiple cases. Triage of unsolved cases for research exome testing led to the discovery of six new disease genes. Interpretation: A comprehensive targeted diagnostic panel was an effective method for neuromuscular disease diagnosis within the context of an Australasian referral center. Use of smaller disease-specific panels would have precluded diagnosis in many patients and increased cost. Analysis through a centralized laboratory facilitated detection of recurrent, but under-recognized pathogenic variants.

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**Introduction**

Neuromuscular diseases impair voluntary movement by affecting structure or function of the motor unit. Over 500 genes are associated with Mendelian neuromuscular diseases. Genetic diagnosis informs prognosis, allows carrier- and familial-screening, and enables evidence-based treatment. In 2012, a collaboration between the Neurogenetic Diseases Group (Harry Perkins Institute of Medical Research), the Department of Diagnostic Genomics (PathWest), and the Lotterywest State Bioinformatics Facility Genomics (LSBFG) designed a comprehensive panel that included all known neuromuscular disease genes that were amenable to sequencing with NGS. The panel became widely ordered by clinicians across Australia and New Zealand, and effectively became the de facto standard of care for Australasia. The panel was also used for investigating patients with apparently novel neuromuscular phenotypes on a research basis. Here we present findings from the analysis of >2200 patients (both diagnostic and research) on these comprehensive neuromuscular disease gene panels.

**Materials and Methods**

**Panel design**

**Version 1**

The panel included genes from the 2012 Neuromuscular Disorders Gene Table with pathogenic variants detectable by NGS (n = 245), unpublished candidate disease genes (n = 23), and cardiomyopathy disease genes (n = 59). Cardiomyopathy genes were included due to the overlap of skeletal muscle diseases with associated cardiomyopathy and to avoid needing two panels. Overall, 336 genes were targeted. Unpublished candidate disease genes were selected based on discussion with world experts.

**Version 2**

In 2014, 193 new disease genes were added from the 2014 Neuromuscular Disorders Gene Table. Candidate disease genes with no diagnostic yield on Version 1 were removed. Cardiac genes that were not relevant for neuromuscular diseases were removed. There were 464 genes on Version 2. The clinical disease groupings and their associated genes for Version 1 and Version 2 are provided in Table S1.

**Sequencing**

Ion Torrent Proton sequencing (Life Technologies) was performed at LSBFG using NEB NeXT Ultra (New England Biosciences) and Ion Xpress Barcode Adapters with T-overhangs, and Ion TargetSeq Custom Enrichment Kits (Life Technologies). Positive control samples were selected from an archived cohort at Diagnostic Genomics. For Version 1, 32 controls were sequenced: 20 patients with known small-scale variants (e.g., single nucleotide variants) and 12 patients with known copy number variants (CNVs). This was partly to determine the optimal number of samples per chip. Target coverage analysis was performed with BedTools. For Version 2, a verification rather than full validation was performed as sequencing was already optimized. Additional probes were added to provide deeper sequencing of known low-coverage areas from Version 1 (Table S2). Therefore, running a number of positive control samples during the first sequencing runs was not required. Instead, an identical control DNA sample was sequenced on every run for Version 2 as a benchmarking sample.

**Variant calling, annotation, analysis, and validation**

Base calling, mapping, and variant calling were performed using Torrent Suite 3.6.2 or 4.2 with high stringency settings (GRCh37). In the diagnostic setting, Cartagenia BenchLab software (Agilent Technologies) was used for annotation and analysis. Research samples were annotated using ANNOVAR, as previously described. In the diagnostic setting, bioinformatic filters were used as a first pass to restrict analysis to a subpanel of genes associated with each clinical phenotype. If no pathogenic variant was identified, the entire panel was analyzed. Variants with a minor allele frequency of >2% were bioinformatically filtered from analysis.

For this study, a CNV was defined as ≥50 nucleotides, whereas a “small indel” was <50 nucleotides. For quantification of variant subtypes, “truncating” included frameshift, start loss, and stop gain variants. Splicing variants were allocated into a separate category because their effects are difficult to predict without functional data.

Due to the high sequencing read-depth obtained with the panel, it was possible to identify CNVs ranging from one exon to entire genes by calculating large deviations in read depth. For each sequencing run, the read depth per exon was normalized at 1. If read depth per exon...
exceeded two standard deviations from the normalized read depth, it was flagged as a possible CNV (i.e., close to 0.5 for heterozygous deletion or 1.5 for heterozygous duplication). Validation of this method was performed on 12 positive control samples (Table S3). CNVs were confirmed using appropriate Sanger-based sequencing approaches or multiplex ligation-dependent probe amplification (MLPA) depending on size. The diagnostic laboratory adopted the American College of Medical Genetics (ACMG) guidelines to classify variants when they became available in 2015. Variants were only reported as pathogenic if they were classified as class 4 (likely pathogenic) or class 5 (pathogenic). Variants of class 3 or lower were not reported as pathogenic. Segregation studies were rarely feasible due to the absence of familial DNA, with the exception of de novo variants in pediatric patients. The research laboratory was able to interpret variants on a research-only basis, as it was not accredited by the National Association of Testing Authorities, Australia (NATA). If a likely class 4 or class 5 variant was found, interpretation was referred to Diagnostic Genomics for validation in the NATA-accredited laboratory. Sanger sequencing was used to validate putative missense/small indels.

Cohort

Many patients analyzed on Version 1 were already known to Diagnostic Genomics (DNA samples stored from as far back as 1989), as opposed to truly “prospective” samples. Diagnostic panel requests were accepted from neurologists, clinical geneticists, pediatricians, and other appropriate specialists. Research panel requests were undertaken on a collaborative basis with the Neuromuscular Diseases Group. If multiple members of the same family were sequenced, only the proband was included in the present analysis. The clinical and pedigree information provided by the requesting clinician was highly variable, ranging from meticulous to single words (e.g., “weakness”). Age of onset was typically unavailable. We instead used patient age at first sample submission to Diagnostic Genomics (“patient age at referral” or “PAR”) as a proxy. Details of prior single gene testing were almost always unavailable, so this was excluded from the study.

Clinical correlation

We measured which disease groups and genes had the best concordance between the original clinical diagnosis and the genetic diagnosis (“clinical correlation”; e.g., a patient clinically determined to have LGMD has a causative variant in a LGMD associated gene). Clinical categories with <5 patients, and genes with <5 reports issued were excluded from this analysis to prevent skewed analysis due to small sample size.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics (IBM Corp. Released 2015. IBM SPSS Statistics for Macintosh, Version 23.0. Armonk, NY: IBM Corp). The chi-square test of homogeneity was used to assess inter-version differences between diagnostic success and clinical correlation. We calculated the mean difference in age between solved and unsolved patients using a Welch t-test. Correlation between PAR and diagnostic success was assessed using Pearson’s product-moment correlation. Due to the low number of patients aged 70 years or older, these data points were collapsed into a single bin of “≥70 years.” A scatter plot of diagnostic success (Fig. 1) was generated using GraphPad Prism version 8.0.0 for Macintosh (GraphPad Software, San Diego, CA, www.graphpad.com).

Results

Panel validation

The average sequencing depth was 309-fold (>96% target regions ≥20-fold depth). Positive controls on Version 1 achieved a 90.0% success rate (18/20) for small-scale variants (Table S3). In the two unsuccessful controls, one variant was in a poorly coverage region, and the other in a homopolymer region, which in our experience sequence poorly on the Proton platform. Version 1 CNV controls achieved a 91.6% success rate (11/12). The failed sample had a PAFAH1B1 exon 3 deletion, which was called as deletion of exons 2–4. The optimal number of samples per chip was 16. For Version 2, improved coverage of known gaps from Version 1 was evident from analysis of the benchmarking sample from each run. Each chip still used 16 samples per run.

Turnaround time and cost

The typical turnaround time for sample processing, sequencing, analysis, and report issuing was 12 weeks. However, this timeframe was highly variable, depending on the need for further clinical information or functional testing. In urgent prenatal or perinatal cases where a genetic diagnosis could immediately inform care, a 2-week turnaround time could be achieved. Sample cost was AUD1000 (USD730 based on December 31st 2015 exchange rate) per patient, which included expert analysis and diagnostic reporting.
Cohort description

Overall, 2249 patients were analyzed (56% (1261) male; 42% (937) female; 2% (51) sex not specified; 1054 Version 1; 1195 Version 2). Patient number per clinical disease grouping is shown in Table 1. The mean PAR was 30.6 years (range 0–91). The mean PAR was significantly lower in the solved population compared to unsolved (solved mean 26.4 SD ± 21.4 years; unsolved mean 32.5 SD ± 24.0 years; mean difference 6.1 SD ± 1.0, 95% years, CI 4.1–8.2, \(P = 4.6326\times10^{-9}\)). There was a moderately negative correlation between PAR and diagnostic success \((r = -0.54, P = 3.4512\times10^{-164})\) with PAR explaining 30% of the variation in diagnostic success (see Fig. 1). Only 2/25 patients received a genetic diagnosis past the age of 80 years (8% success). The oldest was 83 years, with a peripheral nerve disorder caused by a BSCL2 pathogenic variant.

Diagnostic outcomes and trends

In total, 665 patients received a genetic diagnosis (overall diagnostic rate 30%). On Version 1, 32% of patients received a genetic diagnosis, which decreased to 28% on Version 2 (proportion decrease 0.04, \(P = 0.004\)). However, if genes only present on Version 2 were excluded, the diagnostic rate decreased to 26%. Missense pathogenic variants were the most frequently reported in our cohort (57%), followed by truncating (27%), splicing (8%), small indels (3%), and CNVs (4%). The diagnostic success rate for anterior horn cell (AHC) disease increased from 8% to 33% between Versions 1 and 2 (proportion increase 0.252, \(P = 0.006\)). The diagnostic success for ataxia decreased from 41% to 18% (proportion decrease 0.223, \(P = 0.01\)). The diagnostic success rate of research samples sharply decreased from 26% to 9% (\(P = 0.0006\)) on Version 2. Success of diagnostic samples also decreased from...
34% to 28% (P = 0.002). Channelopathies had the highest overall diagnostic success rate at 42%. Metabolic neurological disorders had the lowest at 10% (Table 1). Information for all solved and unsolved patients can be found in Tables S4 and S5, respectively.

The top 10 most frequently reported genes accounted for 38% of diagnoses (Table 2). For many disease categories, a small number of genes were responsible for the majority of disease burden (Table 3). Diagnoses were made in 35% (136/390) of all genes (i.e., combination of Version 1 and Version 2). Of the new genes added to Version 2, 30/193 had diagnostic reports issued (16% of the new additions; 22% of total reported genes).

Most pathogenic variants were seen on only one allele (60%), 19% on two alleles, and 21% three or more alleles. The four most common pathogenic variants comprised 8.7% of all disease alleles, namely: 

- **SPG7**: c.1529C>T, p.Ala510Val (n = 35 alleles; 3.8% of disease alleles), followed by
- **FKRP**: c.826C>A, p.Leu276Ile (n = 26 alleles; 2.8% of disease alleles), ANO5: c.191dupA, p.Asn64fs and
- **SH3TC2**: c.2860C>T, p.Arg954* (both n = 10 alleles; 1.1% of disease alleles).

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**Table 1.** Summary table of diagnostic outcomes.

| Clinical disease category     | Percentage diagnosed | Inheritance pattern percentage in solved patients (V1 and V2) | Percentage clinical correlation in solved patients (V1 and V2) |
|------------------------------|----------------------|-------------------------------------------------------------|-------------------------------------------------------------|
|                              | NSES V1      | NSES V2 | Autosomal dominant | Autosomal recessive | X-linked (dominant or recessive) |                              |
| Anterior horn cell n = 82    | 8% (3/37)*   | 33% (15/45)* | 39% | 61% | 0% | 39% |
| Ataxia n = 108               | 41% (14/34)* | 18% (14/74)* | 36% | 64% | 0% | 57% |
| Channelopathy n = 104        | 36% (20/56)  | 50% (24/48)  | 64% | 36% | 0% | 81% |
| Congenital myasthenic syndrome n = 37 | 24% (5/21) | 44% (7/16) | 18% | 82% | 0% | 64% |
| Congenital myopathy n = 145  | 43% (34/80)  | 32% (21/65)  | 55% | 42% | 4% | 95% |
| Distal arthrogryposis n = 116| 29% (18/63)  | 28% (15/53)  | 59% | 41% | 0% | 72% |
| Distal myopathy n = 45       | 35% (9/26)   | 18% (4/22)   | 62% | 38% | 0% | 54% |
| Dystonia n = 45              | N/A         | 26% (11/43)  | 73% | 18% | 9% | 91% |
| Hereditary spastic paraplegia n = 225 | 36% (36/99) | 29% (37/126) | 62% | 36% | 3% | 95% |
| Metabolic muscular n = 222   | 24% (32/137) | 17% (14/85)  | 35% | 59% | 7% | 80% |
| Metabolic neurological n = 20 | 0.0% (0/1)  | 10% (2/19)   | 50% | 50% | 0% | N/A |
| Miscellaneous n = 57         | 46% (11/24)  | 21% (7/33)   | 50% | 39% | 11% | N/A |
| Mitochondrial n = 22         | 17% (1/6)    | 25% (4/16)   | 40% | 60% | 0% | 60% |
| Muscular dystrophy n = 656   | 35% (114/323)| 34% (112/333)| 36% | 47% | 17% | 93% |
| Peripheral nerve disorder n = 347 | 27% (38/142) | 23% (47/205) | 64% | 26% | 11% | 82% |
| Overall                      | 32%*        | 28%*        | 46% | 45% | 9% | 74% |

Summary table of diagnostic success across several key variables, split by clinical disease category. Groups annotated with an asterisk (*) denote a statistically significant change. N/A, not applicable.

**Table 2.** Most frequently reported disease genes.

| Gene   | Count | Percent |
|--------|-------|---------|
| RYR1   | 49    | 7.3%    |
| DMD    | 39    | 5.8%    |
| SPG7   | 32    | 4.8%    |
| SPAST  | 25    | 3.7%    |
| TTN    | 23    | 3.4%    |
| CLCN1  | 22    | 3.3%    |
| CAPN3  | 20    | 3.0%    |
| COL6A1 | 16    | 2.4%    |
| FKRP   | 16    | 2.4%    |
| NEB    | 15    | 2.2%    |

List of the 10 genes that caused disease in the highest percentage of patients. These 10 genes harbored 38% of the pathogenic diagnostic reports in our cohort.

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Table 4. Clinical correlation percentage in genes with 5+ reports issued.

| Clinical correlation (%) | Gene(s) |
|-------------------------|---------|
| 91–100%                 | ACTA1, CAPN3, CLCN1*, COL6A1*, COL6A2*, COL6A3*, DMD, DYSF, GJB1*, KIF5A*, MTM1*, NBE*, MPM2*, PYGM*, REEP1*, SCNA4, SGCB*, SH3TC2*, SPAST, TTN |
| 81–90%                  | LMNA, MPZ, FKRP, LAMA2, RYR1, TRPV4, SPG7 |
| 71–80%                  | GMPB, KIF1A, MYH7, POMT1, ANOS, SGCA, TPM2 |
| 61–70%                  | MNF2, IGHMBP2 |
| 51–60                   | CACNA1A, CPT2, SACS |
| <50%                    | SEPN1, DYNC1H1† |

Clinical correlation percentages for genes with five or more diagnostic reports issued.

*Indicates 100%.
†Indicates 0%.

The disease groupings with the highest clinical correlation were hereditary spastic paraplegia (HSP) 95%, muscular dystrophy (93%), dystonia, and congenital myopathy (both 91%). The category with the lowest correlation was AHC disease, with 50%. Mean clinical correlation was 74% (Table 1). Fourteen genes had 100% clinical correlation (Table 4).

Often, patients were “diagnosed by sequencing” where the genetic diagnosis clarified the clinical diagnosis. Pathogenic variants in SACS were found in four patients diagnosed with a nerve disorder, one diagnosed with distal arthrogryposis, and one diagnosed with muscular dystrophy. An infant diagnosed with a neurogenic disorder had a pathogenic variant in ACTA1. Two patients (aged 51 and 67 years) diagnosed with muscular dystrophy had a pathogenic ACTA1 variant. Three patients diagnosed with AHC disease had pathogenic variants in RYR1, and two had pathogenic variants in FKRP. Pathogenic SOD1 variants were found in two patients diagnosed with neurogenic disorders, and one diagnosed with muscular dystrophy. Multiple phenotypic expansions were identified; some have been published.5,11–14

Discussion

Disease features and laboratory findings are often non-specific in neuromuscular diseases, particularly early or late in disease progression.15,16 A comprehensive gene panel is especially useful for heterogeneous diseases as it facilitates diagnosis despite uncertain disease etiology or atypical presentation, and can partly compensate for variable levels of clinical acumen/assessment and/or limited clinical information.10,17–19 However, detailed clinical information should guide genetic diagnosis and reduce turnaround time. Genetic testing does not replace clinical workup.

Neuromuscular diseases are also highly genetically heterogeneous. A comprehensive panel facilitates phenotypic expansions by allowing analysis of genes that would not have been chosen as candidates based on the clinical presentation. Our cohort provided several published examples.5,11–14 This highlights that in many instances the definitive diagnosis relies on finding the causative pathogenic variant(s).16

Use of a single panel processed through one reference center (Diagnostic Genomics) for Australasia provided a central data collection point, which facilitated the curation of variants, improving variant interpretation. Weekly interdisciplinary meetings between the clinical, scientific, neuropathological, and research teams were vital for maximizing diagnostic success and triage of unsolved patients to research projects, as reported by others.10,20,21 This process facilitated the discovery of six new disease genes.22–27

Use of a large panel, rather than exome sequencing, provided the optimal balance between cost and diagnostic success. Panel sequencing costs less than exome sequencing and has only 4–11% lower success rates than exomes restricted to known genes.18,21,28 Although panel data cannot be immediately interrogated for new disease genes, we update the panel approximately every 18 months. The panel also allowed higher read-depth per sample, compared to exome sequencing at the time. The panel provided 309-fold read-depth per sample, with 96% of targets covered to >20-fold depth. In contrast, our previous experience with SOLiD 5500XL29 sequencing offered only 66-fold read-depth and 84% coverage at 20-fold or greater depth. The higher read-depth facilitated CNV detection, and detection of variants within the triplicated regions of TTN and NEB without need for an additional Comparative Genomic Hybridisation array.30,31 Within our study, 4% of genetically diagnosed probands harbored a CNV. Although megabase scale CNVs may have been detected by aCGH, the others are likely too small to be detected. Thus the panel is better equipped to detect CNVs, without the extra cost of running a second test. NGS-based testing reduces time, cost, and invasive testing compared to the traditional diagnostic work-up.21,32 We and others recommend a “sequence early” approach, as rapid diagnosis offers the greatest opportunity to influence patient outcomes.32,33

The diagnostic success of NGS is linked to the cohort inclusion criteria stringency; lower stringency leads to lower diagnostic success.19 We did not implement strict inclusion criteria. The success rate from our “catch-all” cohort is in keeping with similar studies.10,18 Likely reflecting the experience of other diagnostic centers.15

Diagnostic success was negatively correlated with PAR. Other cohorts have displayed enrichment of diagnoses in patients <18 years.31,34,35 The higher diagnostic success in patients <18 years, especially infants in intensive care,
suggests that later-onset diseases may be more likely to have a polygenic and/or nongenetic etiology (e.g., diabetes causing a peripheral neuropathy).15,36,37

There was a statistically significant decline in diagnostic success on Version 2. Many of the cases analyzed on Version 1 were archived, unsolved samples from Diagnostic Genomics. These samples likely boosted the Version 1 success rate, as they were from well-phenotyped patients. Therefore, the diagnostic success rate on Version 2 may better reflect the outcome for a truly prospective cohort. Research cases showed a sharp decrease in diagnostic success on Version 2. We suspect this was due to researchers using the panel to exclude known disease genes in patients suspected to have a novel disease gene, based on clinical presentation. In contrast, the diagnostic success rate for AHC diseases increased ~400% (8% to 33%, n = 3/37 and n = 15/45, P = 0.006) from Version 1 to Version 2. The addition of new genes on Version 2 resulted in the diagnosis of only one patient who was clinically described to have AHC disease (SLC52A2). Over half of the AHC genetic diagnoses were in genes not typically associated with AHC disease (i.e., DNM2, FRKP, LAMA2, RYR1, SPAST, SPG11, SYNE1). We suspect the dramatic increase in diagnostic success was due to the recognition that other conditions can mimic AHC diseases.

The overall clinical correlation was 74%, similar to a recent study.10 The stated clinical diagnosis and the genetic diagnosis were only considered to match if the genotype/phenotype correlation was known at the time of analysis. Disease groupings with low clinical correlation may be especially challenging to diagnose clinically, or the full phenotypic spectrum is yet to be fully realized (e.g., AHC diseases). Conversely, diseases with high clinical correlation (e.g., HSP) may show more specific features that make them easier to diagnose clinically. Experience of the specific disease and the clinical acumen of the referring clinicians also likely contribute to clinical correlation.10

The 10 most frequently reported genes were responsible for 38% of diagnoses. These included some of the largest genes (i.e., DMD, NEB, RYR1, TTN), which were unfeasible to sequence pre-NGS due to their size.37 Now, their contribution to neuromuscular disease burden and their full phenotypic spectra are being uncovered. For example, TTN was a top contributor to congenital myopathy, equal second with ACTA1 and NEB (14%). This aligns with recent findings describing “congenital titinopathy” as a common form of congenital myopathy.38 Autosomal recessive ataxia of Charlevoix-Saguenay (ARSACS) was relatively common in our cohort (n = 10). ARSACS was initially thought to be primarily a French-Canadian disease, but has been reported in other populations,39 and now in Australia.

The percentage of AD pathogenic variants in our cohort (46.3%) is similar to a recently reported Chinese cohort (53%).18 In contrast, a Saudi population enriched for consanguinity showed 24% of AD pathogenic variants.18 The relatively high level of AD pathogenic variants in the Australian and Chinese cohorts likely reflects the low level of consanguinity in these populations. Over 60% of the pathogenic variants reported from the panels were nonrecurrent. Recurring variants were known to be common in people of European descent, reflecting the European ancestry of many Australians.30–32 The SPG7 p.Ala510Val pathogenic variant was by far the most common in our cohort, comprising 4% of all disease alleles.30

In conclusion, targeted neuromuscular disease panels in an Australasian referral center resulted in a genetic diagnosis of 665 patients. The causative variant(s) were often unexpected based on the clinical details provided; thus we believe smaller disease-specific panels would have been less effective. We identified several novel variants and genotype–phenotype expansions. The panels enabled the research team to focus on genetically undiagnosed patients that were likely to harbor variants in novel disease genes. A key advantage of a centralized referral center is the ability to better classify VUS based on their aggregate data. Collaboration between the diagnostic facility and clinical team can be vital for reaching a definitive genetic diagnosis. We recommend a centralized approach for genetic diagnosis of neuromuscular diseases and the use of comprehensive targeted gene panels.

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Conflict of Interest

The authors report no competing interests.
References

1. Bonne G, Rivier F, Hamroun D. The 2019 version of the gene table of neuromuscular disorders (nuclear genome). Neuromuscul Disord 2018;28:1031–1063.

2. Kaplan J-C. The 2012 version of the gene table of monogenic neuromuscular disorders. Neuromuscul Disord 2011;21:833–861. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960896611013666

3. Kaplan J-C, Hamroun D. The 2014 version of the gene table of monogenic neuromuscular disorders (nuclear genome). Neuromuscul Disord 2013;23:1081–1111.

4. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:1–7.

5. Cabrera-Serrano M, Ghaoui R, Ravenscroft G, et al. Expanding the phenotype of GMPPB mutations. Brain 2015;138:836–844.

6. Alkan C, Coe BP, Eichler EE. Genome structural variation discovery and genotyping. Nat Rev Genet 2011;12:363–376. Available from http://www.nature.com/articles/nrg2958

7. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–5467. [cited 2016 Sep 26 ] Available from: http://www.ncbi.nlm.nih.gov/published/271968.

8. Eijk-Van Os PGc, Schouten JP. Multiplex Ligation-dependent Probe Amplification (MLPA(R)) for the detection of copy number variation in genomic sequences. Methods Mol Biol 2011;688:97–126.

9. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–423. [cited 2017 Mar 7 ] Available from: http://www.nature.com/gim/journal/v17/n5/pdf/gim201530a.pdf

10. Hu X, Li N, Xu Y, et al. Proband-only medical exome sequencing as a cost-effective first-tier genetic diagnostic test for patients without prior molecular tests and clinical diagnosis in a developing country: the China experience. Genet Med 2018;20:1045–1053.

11. Ravenscroft G, Di Donato N, Hahn G, et al. Recurrent de novo BICD2 mutation associated with arthrogryposis multiplex congenita and bilateral perisylvian polymicrogyria. Neuromuscul Disord 2016;26:744–748. [cited 2016 Dec 13 ] Available from: http://www.ncbi.nlm.nih.gov/published/27751653

12. Scalco RS, Gardiner AR, Pitceathly RDS, et al. CAV3 mutations causing exercise intolerance, myalgia and rhabdomyolysis: expanding the phenotypic spectrum of cavelinopathies. Neuromuscul Disord 2016;26:504–510. Available from: https://doi.org/10.1016/j.nmd.2016.05.006

13. Beeacroft SJ, McLean CA, Delatycki MB, et al. Expanding the phenotypic spectrum associated with mutations of DYNC1H1. Neuromuscul. Disord 2017;27:607–615. Available from: http://www.sciencedirect.com/science/article/pii/S0960896617301608

14. Zaharieva IT, Thor MG, Oates EC, et al. Loss-of-function mutations in SCN4A cause severe foetal hypokinesia or “classical” congenital myopathy. Brain 2016;139(Pt 3):674–691. Available from http://www.ncbi.nlm.nih.gov/pubmed/2670687

15. Haskel GT, Adams MG, Fan Z, et al. Diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. Neurol Genet 2018;4:e212. Available from: http://ng.neurology.org/lookup/doi/10.1212/NXG.000000000000212

16. Tian X, Liang WC, Feng Y, et al. Expanding genotype/phenotype of neuromuscular diseases by comprehensive target capture/NGS. Neurol Genet 2015;1:e14.

17. Antoniadi T, Buxton C, Dennis G, et al. Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. BMC Med Genet 2015;16:84. Available from:http://bmcmedgene.com/articles/10.1186/s12881-015-0224-8

18. Abdulwahab A, Abouelhoda M, Abouthuraya R, et al. Comprehensive gene panels provide advantages over clinical exome sequencing for Mendelian diseases. Genome Biol 2015;16:226. Available from: http://genenomically.org/2015/16/1/226

19. Montaut S, Tranchant C, Drouot N, et al. Assessment of a targeted gene panel for identification of genes associated with movement disorders. JAMA Neurol 2018;75:1234–1245. Available from: http://archneru.jamanetwork.com/article.aspx?doi=10.1001/jamaneurol.2018.1478

20. Reed ES, Papandreou A, Drury S, et al. Advantages and pitfalls of an extended gene panel for investigating complex neurometabolic phenotypes. Brain 2016;139:2844–2854.

21. Schofield D, Alam K, Douglas L, et al. Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. npj Genomic Med 2017;2:4. Available from: http://www.nature.com/articles/s41525-017-0006-7

22. Lasuthova P, Rebelo AP, Ravenscroft G, et al. Mutations in ATP1A1 cause dominant Charcot-Marie-Tooth type 2. Am J Hum Genet 2018;102:505–514. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29499166%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5985288

23. Guimier A, Gordon CTT, Godard F, et al. Biallelic PPA2 mutations cause sudden unexpected cardiac arrest in infancy. Am J Hum Genet 2016;99:666–673.

24. Ravenscroft G, Zaharieva I, Bortolotti CA, et al. Bi-allelic mutations in MYL1 cause a severe congenital myopathy. Hum Mol Genet 2018;27:4263–4272. Available from: https://academic.oup.com/hmg/advance-article/doi/10.1093/hmg/ddy320/5095322
25. Varaste V, Tajsharghi H, Hossein Nejad Nedai H, et al. Ryanodine receptor type 3 (RYR3) as a novel gene associated with a myopathy with nemaline bodies. Eur J Neurol 2018;25:841–847.

26. Olié M, Engvall M, Ravenscroft G, et al. Myoglobinopathy is an adult-onset autosomal dominant myopathy with characteristic sarcoplasmic inclusions. Nat Commun 2019;10:1396. Available from: https://doi.org/10.1038/s41467-019-09111-2

27. Ravenscroft G, Nolent F, Rajagopalan S, et al. Mutations of GPR126 are responsible for severe arthrogryposis multiplex congenita. Am J Hum Genet 2015;96:955–961. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26004201

28. Pajusalu S, Kahre T, Roomere H, et al. Large gene panel sequencing in clinical diagnostics—results from 501 consecutive cases. Clin Genet 2018;93:78–83. Available from: http://doi.wiley.com/10.1111/cge.13031

29. McKernan KJ, Peckham HE, Costa GL, et al. Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding. Genome Res 2009;19:1527–1541.

30. Sagath L, Lehtokari V-L, V launched-S. Beecroft McArdle disease. Neuromuscul Disord 2017;27:997–1002.

31. Karakaya M, Storbeck M, Strathmann EA, et al. Targeted sequencing with expanded gene profile enables high diagnostic yield in non-3q-spinal muscular atrophies. Hum Mutat 2018;39:1284–1298. Available from: http://doi.wiley.com/10.1002/humu.23560

32. Stark Z, Schofield D, Martyr M, et al. Does genomic sequencing early in the diagnostic trajectory make a difference? A follow-up study of clinical outcomes and cost-effectiveness. Genet Med 2019;21:173–180. Available from: http://www.nature.com/articles/s41436-018-0006-8

33. Stark Z, Marum JE, Elliott J, et al. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genet Med 2018;20:1554–1563.

34. Nam SH, Bin Hong Y, Hyun YS, et al. Identification of genetic causes of inherited peripheral neuropathies targeted gene panel sequencing. Mol Cells 2016;39:382–388. Available from: http://www.molcells.org/journal/view.html?doi=10.14348/molcells.2016.2288

35. Bulman DE, Gillespie M, Bernard F, et al. Whole-exome sequencing is a valuable diagnostic tool for inherited peripheral neuropathies: outcomes from a cohort of 50 families. Clin Genet 2017;93:301–309.

36. Mohassel P, Landon-Cardinal O, Foley AR, et al. Anti-HMGCR myopathy may resemble limb-girdle muscular dystrophy. Neurol Neuroimmunol Neurolinflammation 2019;6:e523.

37. Ravenscroft G, Bryson-Richardson RJ, Nowak KJ, Laing NG. Recent advances in understanding congenital myopathies. F1000Research 2018;7:1921. Available from: https://doi.org/10.12688/f1000research.16422.1

38. Oates EC, Jones KJ, Donkervoort S, et al. Congenital titinopathy: comprehensive characterization and pathogenic insights. Ann Neurol 2018;83:1105–1124. Available from: http://doi.wiley.com/10.1002/ana.25241

39. Synofzik M, Soehn AS, Ghurek-Augustat J, et al. Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. Orphanet J Rare Dis 2013;8:41. [cited 2016 Nov 28] Available from: http://www.ncbi.nlm.nih.gov/pubmed/23497566

40. Carrié A, Piccolo F, Leturcq F, et al. Mutational diversity and hot spots in the alpha-sarcoglycan gene in autosomal recessive muscular dystrophy (LGMD2D). J Med Genet 1997;34:470–475.

41. Muller JS, Miländer G, Muller-Felber W, et al. Rapsyn N88K is a frequent cause of congenital myasthenic syndromes in European patients. Neurology 2003;60:1805–1810.

42. Inal-Gültekin G, Toptaş-Hekimoğlu B, Görmez Z, et al. Myophosphorylase (PYGM) mutations determined by next generation sequencing in a cohort from Turkey with McArdle disease. Neuromuscul Disord 2017;27:997–1008.

43. Taroni F, Verderio E, Dworzak F, et al. Identification of a common mutation in the carnitine palmitoyltransferase II gene in familial recurrent myoglobinuria patients. Nat Genet 1999;4:314–320.

44. van Gassen KLI, van der Heijden CDCC, de Bot ST, et al. Genotype–phenotype correlations in spastic paraplegia type 7: a study in a large Dutch cohort. Brain 2012;135:2994–3004.

45. DiVincenzo C, Elzenga CD, Medeiros AC, et al. The allelic spectrum of Charcot-Marie-Tooth disease in over 17,000 individuals with neuropathy. Mol. Genet Genomic Med 2014;2:522–529. Available from: http://doi.wiley.com/10.1002/mgg3.106

46. Witting N, Duno M, Petri H, et al. Anoctamin 5 muscular dystrophy in Denmark: prevalence, genotypes, phenotypes, cardiac findings, and muscle protein expression. J Neurol 2013;260:2084–2093. Available from: http://link.springer.com/10.1007/s00415-013-6934-y

47. Lašňová P, Mazanec R, Vondráček P, et al. High frequency of SH3TC2 mutations in Czech HMSN I patients. Clin Genet 2011;80:334–345. Available from: http://doi.wiley.com/10.1111/j.1399-0004.2011.01640.x

48. Hicks D, Sarkozy A, Muelas N, et al. A founder mutation in Anoctamin 5 is a major cause of limb girdle muscular dystrophy. Brain 2011;134:171–182. Available from: http://link.springer.com/10.1007/s00415-013-6934-y

49. Walter MC, Petersen JA, Stucka R, et al. FKRP (826C>G) frequently causes limb-girdle muscular dystrophy in...
German patients. J Med Genet 2004;41:e50 LP-e50. Available from: http://jmg.bmj.com/content/41/4/e50.abstract

50. Roxburgh RH, Marquis-Nicholson R, Ashton F, et al. The p.Ala510Val mutation in the SPG7 (paraplegin) gene is the most common mutation causing adult onset neurogenetic disease in patients of British ancestry. J Neurol 2013;260:1286–1294.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Definitions of the clinical disease groupings, and their associated genes on both versions of the neurogenetic disease panel.

Table S2. Genomic regions with low coverage on Version 1 that had extra probes on Version 2.

Table S3. Genetic information on the patients samples used for validation on Version 1.

Table S4. Full genetic and clinical data for all patients with a genetic diagnosis. The “stated disease grouping” category refers to the clinical diagnosis provided by the clinician at referral for genetic testing. DCM, dilated cardiomyopathy.

Table S5. Clinical data for all patients that did not receive a genetic diagnosis. The “stated disease grouping” category refers to the clinical diagnosis provided by the clinician at referral for genetic testing.

Table S6. Total number of reports issued per gene on either version of the panel, excluding genes with no reports issued.