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NGS- testing using an expanded gene panel on Neuromuscular patients in Norway – results and limitations

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Introduction

Neuromuscular disorders (NMDs) contain a broad group of clinically heterogeneous disorders. They often display overlapping clinical signs, making clinical diagnostics challenging. Some of the more frequent diseases such as Limb-Girdle muscular dystrophy, congenital myopathies and Charcot-Marie-Tooth disease are getting increasingly heterogeneous as new genetic associations are made and different genetic testing strategies evolve. Over a two-year period, we have offered an expanded gene panel based on Next-Generation Sequencing (NGS) to patients with NMDs. We performed NGS-analysis on 256 genes and subsequently increased the analysis to include 328 genes, after feedback from referring doctors. Our results are based on analysis of 202 patients where previous standard genetic and clinical testing did not reveal a specific diagnosis. These patients were pre-screened based on clinical information as shown in figure 1.

Methods

Patients were tested through a diagnostic service at our department and followed the testing scheme as illustrated in figure 1. NGS was performed using Illumina TruSight One Sequencing panel (4813 genes) and run on an Illumina NextSeq 500 Desktop Sequencer. Analyses were done using Illumina BaseSpace BWA Enrichment Workflow and annotation, filtration and variant curation were done using Cartagenia Bench NGS (Agilent Technologies) and Alamut Visual (Interactive Biosoftware).

Conclusion

Our gene panel resulted in a high diagnostic rate of approximately 40%, comparable to whole exom sequencing studies (WES). We recommend using a broad gene panel for NMDs as phenotypic overlap is seen between the major disease groups and subgroups, and many private mutations are found. In cases with detection of one pathogenic variant in recessive disorders, coverage of problematic regions, i.e. exon 1, should be checked manually and/or Sanger sequenced, as well as checked for possible larger del/dup. Good clinical description is the key to successful diagnoses of NMD patients. A collaborative effort between lab and clinicians to facilitate correct biological and clinical interpretation of sequence variants, was instrumental to our work.

Results

Among 202 patients, we received a possible diagnosis in 86 patients, approximately 40 %, as presented in figure 1. In 53 patients the genetic variants were interpreted to be possibly pathogenic/pathogenic (class 4 and 5) and a diagnosis was set. In 33 of the patients, we informed of variants interpreted to be of uncertain clinical significance (VUS) as they had not previously been reported but found in genes relevant to their clinical symptoms. VUS in genes in which potential treatments are available, e.g. myasthenic syndrome, were also reported. Many of the patients presented atypical phenotypes compared to previous reports, but for many of the genes involved, only a few patients had been previously reported. Also, since we had limited clinical information on the patients at time of testing, surprising genetic results were obtained in some patients.

Detection of larger rearrangements is a limitation of the method, as several diseases have been found associated with repetitive elements in some of these genes. Low coverage of problematic regions in genes, i.e. exon 1, is a common problem in NGS analysis and should in some cases prompt additional Sanger sequencing. An example in our study occurred in one patient with suspected LGMD where one pathogenic sequence variant in the *SGCB*-gene was detected in the NGS analysis, and subsequent Sanger sequencing of exon 1 revealed an additional pathogenic variant in exon 1 previously known in many families.

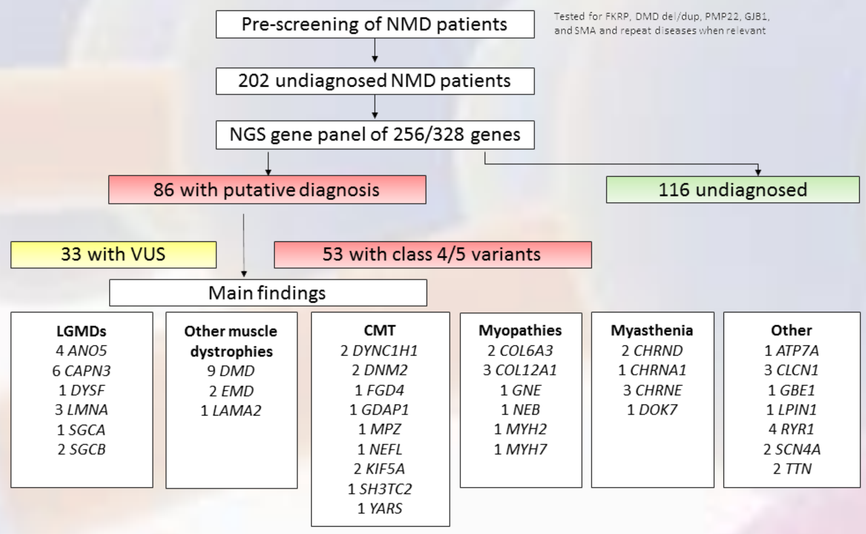

Figure 1: Flow chart of diagnostic process and the breakdown of the main genetic findings

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