SciMedCentral

Journal of Neurological Disorders & Stroke

Editorial

New Developments in Molecular Diagnosis of Neuromuscular Disorders

Vaidehi Jobanputra* and Ali Naini

Department of Pathology, Columbia University, New York, NY, USA

Corresponding author
Vaidhehi Jobanputra, Department of Pathology, Columbia University, New York, NY, USA, E-mail: vj2004@columbia.edu

Submitted: 16 July 2013

Accepted: 16 July 2013

Published: 20 July 2013

Copyright

© 2013 Jobanputra and Naini

OPEN ACCESS

EDITORIAL

Neuromuscular disorders (NMDs) are a group of genetic disorders that affect the peripheral nervous system and muscle, consequently leading to a significant disability in children as well as in adults. NMDs include more than 200 monogenic disorders with a total incidence exceeding 1 in 3,000 [1]. Some of the more extreme diseases in the spectrum include amyotrophic lateral sclerosis (ALS), congenital muscular dystrophies and myopathies, Duchenne Muscular Dystrophy (DMD) and spinal muscular atrophy (SMA).

Over the past decade, tremendous research and clinical efforts have expanded our understanding of the molecular basis of NMDs. However, the cause (s) remains unknown in the majority of patients, despite clinical and histological evidence. Genetic and phenotypic heterogeneity, lack of segregation data in sporadic cases and non-specific clinical features are the challenges in detecting the genetic variants underlying the specific phenotypes in individual families. The aforementioned situations provide the molecular diagnoses to guide clinical management of the disease. In addition, some of the genes implicated in NMDs are the largest human genes, such as TTN, NEB, RYR and DMD as well as the different types of mutations (sequence variants including point mutations and small indels, deletions of entire genes and deletion duplication of one or more exons), that are present in these genes, require a variety of molecular techniques to detect them, which may not be feasible in every diagnostic laboratory. Finally, the presence of unknown genes implicated in NMDs adds to the complexity of molecular diagnosis.

The most common method of genetic diagnosis in these disorders is based on a gene-by-gene approach, starting from the most relevant one in a particular family or a proband. Historically, sanger sequencing and deletion duplication analysis of single genes were the most common methods but, these techniques were low throughput and inefficient in comprehensive molecular diagnosis in a majority of cases because of the varied availability of these tests in individual diagnostic laboratories.

In the last few years, the next generation sequencing (NGS) technology has become an effective strategy for massively parallel analysis of a large number of genes and has led to the successful identification of several Mendelian disease genes [2]. The first application of whole genome sequencing in neuromuscular diseases was sequencing of a patient with a recessive form of Charcot–Marie–Tooth neuropathy [3]. Since then, several recent studies have validated an integrated approach to the diagnosis of NMDs using the panel sequencing of genes, including deletion duplication analysis [4], a subset of NMDs such as congenital muscular dystrophies [5,6] or exome sequencing in congenital myopathies [7].

Vasli et al. [4] used targeted enrichment for 267 known NMD genes followed by NGS in patients affected by different NMDs with or without known mutations. In this study, more than 97% of the targeted exons of known NMD genes were fully covered. DNA multiplexing and blind variant ranking retrieved different mutation types for diseases with different segregations.

Valencia et al. [8] used a highly multiplexed PCR-based target enrichment method (RainDance) in conjunction with NGS, to perform mutation testing in 12 congenital muscular dystrophy (CMD) genes and compared the results with Sanger sequencing. The RainDance NGS panel showed great consistency in coverage depth, on-target efficiency, versatility of mutation detection, and genotype concordance with Sanger sequencing, demonstrating the test's appropriateness for clinical use. Compared to single tests, a higher diagnostic yield was observed by panel implementation. The panel's limitation was the amplification failure of select gene-specific exons, which required Sanger sequencing for test completion.

Bohm et al. [7] used an integrated approach, combining exome sequencing, clinical and histological investigations to identify the causative mutation and diagnose clinically different neonatal or adult-onset congenital myopathies. This study showed that this integrated approach is a fast, efficient, and reliable molecular diagnosis tool for congenital myopathies. The limitations of exome sequencing include inability to detect intronic mutations and to generate a large list of variants of uncertain significance. The validation of the disease-causing mutations therefore requires the synergistic combination of the exome sequencing data with clinical and histological analyses. Exome sequencing and histology can be performed in parallel and the results need to be evaluated by specialized diagnostic centers.

Clinical and molecular pathology laboratories are fast moving

towards the routine use of panel sequencing or whole exome sequencing, which are more efficient diagnostic approaches. The limitations of the current massively parallel sequencing strategies include (1) inability to detect mutations that are due to repeat expansion, (2) incomplete coverage of the capture libraries, (3) higher false positive rates, (4) a large list of generated variants, which requires distinction between non-pathogenic variations without clinical significance versus disease-causing mutations and (5) incidental findings from exome sequencing. However, the technology is changing fast with improvements in target capture, sequencing approaches to increase coverage, bioinformatics algorithms and software tools for variant calling and filtering. The cost of these tests is less than conventional testing of a tired approach of testing one gene at a time, especially for larger genes.

The impact of massively parallel sequencing for genetic diagnosis of NMDs is summarized by Vasli et al. [8]. Rapid developments in NGS has a huge potential for clinical laboratory testing of NMDs. Identification of the pathogenic mutation and the accurate diagnosis will improve clinical management and genetic counseling in patients with NMDs and will also pave the path forward for clinical trials.

REFERENCES

1. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. Neuromuscul Disord 1991; 1: 19-29.

2. Ng SB, Nickerson DA, Bamshad MJ, Shendure J. Massively parallel sequencing and rare disease. Hum Mol Genet 2010; 19: R119-124.

3. Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, et al. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med 2010; 362: 1181-1191.

4. Vasli N, Böhm J, Le Gras S, Muller J, Pizot C, Jost B, et al. Next generation sequencing for molecular diagnosis of neuromuscular diseases. Acta Neuropathol 2012; 124: 273-283.

5. Valencia CA, Rhodenizer D, Bhide S, Chin E, Littlejohn MR, Keong LM, et al. Assessment of target enrichment platforms using massively parallel sequencing for the mutation detection for congenital muscular dystrophy. J Mol Diagn. 2012; 14: 233-46.

6. Valencia CA, Ankala A, Rhodenizer D, Bhide S, Littlejohn MR, Keong LM, et al. Comprehensive mutation analysis for congenital muscular dystrophy: a clinical PCR-based enrichment and next-generation sequencing panel. PLoS One 2013; 8: e53083.

7. Böhm J, Vasli N, Malfatti E, Le Gras S, Feger C, Jost B, et al. An integrated diagnosis strategy for congenital myopathies. PLoS One 2013; 8: e67527.

8. Vasli N, Laporte J. Impacts of massively parallel sequencing for genetic diagnosis of neuromuscular disorders. Acta Neuropathol 2013; 125: 173-185.

Cite this article

Jobanputra V, Naini A (2013) New Developments in Molecular Diagnosis of Neuromuscular Disorders. J Neurol Disord Stroke 1(1): 1003.