Development of a Next-Generation Sequencing (NGS) Gene Panel for Lysosomal Storage Diseases

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Molecular genetic testing has not been used extensively as the primary diagnostic test for LSDs but this may change with the advent of rapid, reliable and affordable high-throughput DNA sequencing, the so called next generation sequencing.

The aim of this work was to develop a next-generation sequencing (NGS)-based workflow for the identification of variations in exons and their intronic flanking regions in genes involved in lysosomal function. Our NGS-based workflow was designed using an Agilent SureSelect Target Enrichment protocol followed by sequencing by an Illumina MiSeq platform. The complete gene list includes 96 genes encoding for lysosomal proteins, lysosomal regulators and non-lysosomal proteins involved in lysosomal biogenesis. All genes encoding non-lysosomal proteins may play a role in lysosomal function and in LSDs.

As proof of sensitivity and specificity, a set of 12 samples from patients affected by several types of LSDs was tested. We analyzed samples from Mucolipidosis II alpha/beta, Mucopolysaccharidosis type VI, Gaucher Disease, Fabry disease, Pycnodysostosis, Krabbe disease, GM2-gangliosidosis, and Neuronal Ceroid Lipofuscinosis. In 5 of them causative mutations had been previously identified, while in 4 only the pathology was known and in the remaining 3, even the specific disease was unknown. Using our protocol we were able to detect all the known mutations and characterize the mutations in the patients where the pathology was identified. Currently, we are still analyzing the unknown cases to identify the putative pathogenic variations.

This gene panel seems therefore to be a valid tool for the genetic analysis of lysosomal gene mutations. In addition, it provides a comprehensive view on the role that variants in 96 genes involved in the lysosomal function plays in health and disease.