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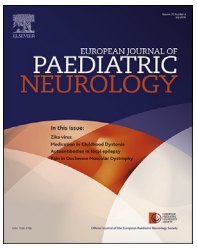

Review article

The relevance of gene panels in movement disorders diagnosis: A lab perspective

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A B S T R A C T

Next-Generation Sequencing (NGS) is a group of new methods that allow sequencing a variable number of known genes (targeted resequencing) or even the whole human genome (whole genome sequencing-WGS) and have contributed to an exponential genetic knowledge growth, especially in rare diseases, in the past few years. Since 2015, in the Molecular Neurogenetics Unit of Neurological Institute "Carlo Besta", some gene panels have become available to screen all the known genes associated with Movement Disorders (MD) in children and adults as a diagnostic package. Over 221 patients analyzed (part of the Telethon Network of Genetic Biobanks – TNGB), pathogenic variants were found in 25 (11.31%), allowing a definitive genetic diagnosis. Among them, we found mutations in 10/114 patients with dystonia (8.8%); 10/59 patients with Parkinson's disease (16.9%); 1/25 patients with Neurodegeneration with Brain Iron Accumulation (NBIA) (4%) and 4/23 patients with neurotransmitter and biopterin metabolism synthesis defect (17.4%). Our results are in line with those published in literature; targeted resequencing does not replace Sanger sequencing totally, but its usage needs to be discussed with clinicians taking into account both the patient's clinical picture and radiological and neurophysiological data.

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1. Introduction

Over the past decade, the increasing diffusion of Next-Generation Sequencing (NGS) technologies has contributed to an exponential genetic knowledge growth,[1,2] especially in the field of rare diseases.[3]

NGS refers to a group of novel methods that allow sequencing a variable number of known genes (targeted resequencing) or even the whole human genome (whole genome sequencing-WGS).

The enormous spreading of these new techniques is due to the high number of bases sequenced simultaneously with a very low cost as compared to technologies previously used in genetics laboratories (Sanger sequencing method or "first-generation" sequencing).[4]

With targeted resequencing, a subset of genes or target regions (gene panel) are sequenced. It is possible to find commercial panels for the screening of genes involved in the pathogenesis of a specific group of diseases or to design panels including only specific genes of interest (custom panel).

Clinical exome is a more extended approach that allows sequencing of all genes (about 4000 genes) associated with any known mendelian diseases.

Whole exome sequencing (WES) is a method that allows sequencing the coding regions of all human genes (about 20,000).

Application of WES for diagnostic purposes has still significant limitations due to the costs and time and computer tools that are needed for data analysis and data storage.

Targeted resequencing is a good strategy to screen panels of selected genes thanks to low costs and use of fair resource.[5,6] For these reasons, targeted resequencing is especially suitable for diagnostic purposes.

Since 2015, in the Molecular Neurogenetics Unit of Carlo Besta Neurological Institute, some customized gene panels have become available to screen most of the known genes associated with Movement Disorders (MD) in children and adults as a diagnostic package.

The term "Movement Disorders" refers to a heterogeneous group of neurological conditions characterized by the production of abnormal voluntary movements or involuntary movements. Some of these conditions have a genetic basis, whereas a large number are considered multifactorial diseases, with a genetic component that does not follow classical mendelian rules. Virtually, all rare movement disorders, especially those manifesting in infancy and childhood, are due to mutations in known or still undiscovered genes.

In the biobank of our institute, part of the Telethon Network of Genetic Biobanks (TNGB), a large number of DNAs from patients with movement disorders are available.

2. Materials and methods

The Molecular Neurogenetics Unit of "Carlo Besta" Neurological Institute is a third-level diagnostic facility including different laboratories that perform genetic analyses requested by clinicians from all Italy as well as research projects in different areas of medical sciences.

In this study, we included DNA samples of 221 patients with genetically undiagnosed movement disorders available in our "Cell lines and DNA Bank of Paediatric Movement Disorders and Neurodegenerative Diseases" of the TNGB, that have been analyzed over the last three years according to the referring clinicians' requests. All patients were referred by neurologists specialized in Movement Disorders and gave informed consent for genetic studies. Parental consent was obtained for under-18 subjects.

At the time of genetic testing 72 cases were under the age of 18 years and 149 were aged over-18. Pediatric patients were tested for dystonia-, neurotransmitters/biopterin and NBIA-related genes (51, 15 and 6 cases, respectively). No patients under the age of 18 were tested for PD-related genes.

Secondary causes of movement disorders, such as acquired basal ganglia lesions, perinatal asphyxia, cerebral infections were ruled out as part of the diagnostic workup. Patients' DNA was tested by targeted resequencing using customized gene panels (Nextera Rapid Capture Custom Enrichment), including 65 genes associated with movement disorders, selected on the basis of a systematic literature review. The panel was designed by Illumina Design Studio tool. The region of interest is the CDS (Coding sequence) with ± 20 bp intronic flanking region to include splicing mutations, whereas the untranslated region (UTR) is sequenced only in genes with mutations in UTR described as pathological. In detail, the panel contains 20 genes associated with Parkinson's disease, 10 genes associated with Neurodegeneration with Brain Iron Accumulation syndromes (NBIA), 29 genes associated with dystonia, 11 genes associated with neurotransmitter and biopterin synthesis defects (Table 1). Some genes are present in more than a group because of their involvement in two or more diseases (for example, *PLA2G6* can be responsible for a type of NBIA or in patients with Parkinsonism).

Only for dystonic patients' cohort and for NBIA subgroup we had previously screened with Sanger sequencing some genes, that are the most frequently mutated, to exclude any mutations: *DYT1* (common GAG deletion) and *PANK2* and *PLA2G6* (for more information about primers sequences and protocols please contact: disturbimovimento@istituto-besta.it).

Enrichment-based library preparation begins with Nextera tagmentation, which converts input genomic DNA (50 ng) into adapter-tagged libraries (~250 of nucleotides).

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