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Editorial

Next-generation sequencing technology in the diagnosis of mitochondrial disorders

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The next-generation sequencing (NGS) is the newer approach in the discovery of new disease genes and transforming the clinical diagnosis of rare inherited disorders. Since the advent of this technique, it has revolutionized the understanding of complex diseases like mitochondrial disorders at molecular level. These disorders are one of the most challenging metabolic diseases of childhood with stats of 1 in 5000 births.[1] Characterization was done through a heterogeneity of phenotypes and transmitted by inheritance modes such as sporadic, maternal, autosomal dominant, autosomal recessive, or X-linked.[2] They could target single organ or multiple organ systems with features such as myopathy, encephalopathy, seizures, lactic acidosis, sensorineural deafness, optic atrophy, diabetes mellitus, liver failure, and ataxia. In oxidative phosphorylation (OXPHOS) which occurs in mitochondria, energy metabolism is directly affected by these diseases.[3]

Mutations in the mitochondrial DNA (mtDNA) or the nuclear genome are responsible for mitochondrial diseases. The mitochondrial genome is a closed circular double-stranded molecule of approximately 16.6 kb in length. Out of 37 genes, 13 encode the main structural components of OXPHOS complexes. In addition to this, it contains 22 tRNAs and 2 rRNAs required for synthesis.[2] There is the maternal inheritance of a large proportion of mitochondrial diseases because the mt genome is inherited from the maternal line.[4] The diagnosis of these diseases is quite hard because of extreme phenotypic heterogeneity and different modes of inheritance.[5] This makes the traditional single-gene strategies difficult to diagnose. Due to clinical variability and the large number of both nuclear and mitochondrial genes in which mutations can occur parallel analysis is quite difficult. Defects in mtDNA include point mutations, single large deletion, multiple deletions, and reduced mtDNA copy number (mtDNA depletion).[6] Pathogenic variants in more than 300 genes are described to date. More than half of them have been characterized in the last decade due to advancements in NGS. These technologies serve a dual role in the discovery of various pathogenic variants as well as facilitating the diagnosis of mitochondrial diseases.[7]

Conventionally, mitochondrial diseases are diagnosed by neuroimaging, muscle biopsies, candidate gene studies, phenotyping of patient tissue, etc. Genetic testing was very rare and carried out when there are clear syndromic presentations as in the case of MELAS syndrome. Now, quite a lot of NGS technologies are successful in discovering variants and diagnosing diseases such as whole mtDNA sequencing, whole-exome sequencing (WES), targeted exome sequencing (mitoexome), whole-genome sequencing (WGS), and RNA-seq.[8] If the mtDNA mutations are present in females even at very low levels, then also it may result in the affected offspring.[9] Through NGS whole mtDNA can be sequenced and allows any variant to be allocated and provide an exact assessment of heteroplasmy levels. Many mitochondrial diagnostic centers sequence mtDNA at first to exclude mitochondrial variants. In the case of adults, the problem is most probably in mtDNA, so it is best not to perform WES/WGS first.[10]

Targeted exome sequencing is adopted mainly for traditional cytogenic and diagnosis of Mendelian disorders and it offers very good coverage for the selected gene panel.[10] Using WES, some parts of entire coding regions may remain uncovered because the target sequences captured are not uniform across the genome. Furthermore, changes in intronic regions may remain uncovered. This method reduces the number of incidental findings and variants of unknown significance. Targeted next-generation sequencing strategy helps dually in examining genes that are considered to cause disease as well as analyzing genes that are suspected to cause mitochondrial dysfunction. Hence, they could include other genes of potential importance. For example, Vamsi Mootha produced a platform for known mitochondrial proteins name MitoCarta. This platform is famous and very useful for mitochondrial scientists in the identification of genes. MitoCarta has more than 1000 proteins involved in mitochondrial functions.[11]

WES's main objective is to identify all coding variants in a genome. There were some limitations when WES was first applied, for example, the commercial whole-exome capture kits did not contain baits that target the mt genome. MitoSeek, a bioinformatics tool was developed to study off-target mitochondrial reads and it improved the WES method. Whole-exome sequencing has been successful in diagnosing patients with nuclear gene defects. A study conducted in Newcastle reported that 60% of patients received a genetic diagnosis.[7] If this trend continues, then we can adopt a "genetic first" approach avoiding skin biopsies or muscle biopsies completely. WES does not completely solve the matter as more than 40% of the cases are still without a diagnosis. This is because, WES may detect the variant, but the variant does not reside in the coding regions of the genome or perhaps they are not prioritized by bioinformatics pipelines. It is also important to sequence the whole family or family trio (patient and both unaffected parents) when we employ WES or WGS. Trio sequencing helps to prioritize *de novo* variants based on knowledge of segregation within the family. At present, WES is used mostly but it is expected to change to WGS soon as this approach has many advantages, as it will allow investigation of both genomes, allow the genetic diagnosis of other rate diseases, and allow new disease gene discovery if all the data are shared globally. Hence, it can be said that WES will form a central role in diagnosing but will require some functional validation.[12]

Diagnostic WGS typically yields ~40–60-fold coverage across the nuclear genome to ensure that the nuclear genetic variant reads are detected with confidence.[13] More copies of mtDNA are present in white blood cells as compare to nuclear DNA, and therefore, the coverage of mtDNA is much higher approx. ~2000-fold in the blood genomic DNA sample by utilizing a shotgun sequencing technique.[14] This raises the possibility of detecting low variant mtDNA heteroplasmy in a sample submitted for WGS, enabling variant calling across both the mitochondrial genome and the nuclear genome using a single blood DNA test.[15]

Validation of WES/WGS findings is important for concluding some diagnosis. For proving pathogenicity, the gold standard is to experiment by incorporating a wild copy of the gene into the fibroblasts. If the biochemical phenotype is restored, then it confirms the disease. It is also important when we study the pathogenic role of a single-nucleotide variant in a single affected individual. There are also some interesting methods such as taking human DNA having identified SNV and then testing in complementation of yeast mutants, as mitochondrial proteins are conserved between the human and yeast, the gold standard for proving pathogenicity.[16]

NGS is much more effective than the Sanger method as it produces 100 times more data in a short period. The other advantages of NGS are high accuracy and reduced cost. These tests provide the clinicians to perform prenatal genetic testing and deliver genetic counseling. There is a high demand for bioinformatics experts and geneticists in coordination with clinicians to reach a final diagnosis. Thus, the interdisciplinary approach toward the NGS provides a significant breakthrough in the identification of the genetic makeup of inherited disorders, especially mitochondrial diseases and the researchers involved in NGS technology-based interventions must think of a database across the globe that eventually accelerates gene discovery and diagnostics.

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