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Review

Next generation molecular diagnosis of mitochondrial disorders

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ABSTRACT

Mitochondrial disorders are by far the most genetically heterogeneous group of diseases, involving two genomes, the 16.6 kb mitochondrial genome and ~1500 genes encoded in the nuclear genome. For maternally inherited mitochondrial DNA disorders, a complete molecular diagnosis requires several different methods for the detection and quantification of mtDNA point mutations and large deletions. For mitochondrial disorders caused by autosomal recessive, dominant, and X-linked nuclear genes, the diagnosis has relied on clinical, biochemical, and molecular studies to point to a group of candidate genes followed by stepwise Sanger sequencing of the candidate genes one-by-one. The development of Next Generation Sequencing (NGS) has revolutionized the diagnostic approach. Using massively parallel sequencing (MPS) analysis of the entire mitochondrial genome, mtDNA point mutations and deletions can be detected and quantified in one single step. The NGS approach also allows simultaneous analyses of a group of genes or the whole exome, thus, the mutations in causative gene(s) can be identified in one-step. New approaches make genetic analyses much faster and more efficient. Huge amounts of sequencing data produced by the new technologies brought new challenges to bioinformatics, analytical pipelines, and interpretation of numerous novel variants. This article reviews the clinical utility of next generation sequencing for the molecular diagnoses of complex dual genome mitochondrial disorders.

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1. Introduction

Mitochondrial disorders are a group of the most clinically and genetically heterogeneous diseases known to date. Two genomes are involved, the tiny 16,569 bp circular double stranded mitochondrial DNA (mtDNA) and approximately 1500 nuclear genes related to mitochondrial structure and function (Calvo et al., 2006; Koopman et al., 2012; Scharfe et al., 2009). Most human cells contain hundreds to thousands of mitochondria, each of which contains multiple copies of mtDNA (Dimmock et al., 2010). Clinical phenotypes of the affected patient depend on the biochemical and molecular characteristics of the specific mutation and its tissue distribution (Freyer et al., 2012; Schon et al., 2012; Shanske and Wong, 2004; Smeitink et al., 2001; Wong and Boles, 2005).

Unlike nuclear genes, the mitochondrial genome contains 13 protein coding genes, two ribosomal RNAs and 22 tRNAs without the interruption of introns or noncoding regions, except for the 723 bp of displacement loop around the origin of replication. Pathogenic mutations, including point mutations and large deletions, occur throughout the entire mitochondrial genome (http://www.mitomap.org/MITOMAP). Thus, diagnosis of mtDNA-related disorders should include the detection and quantification of sequence changes at any position of the mitochondrial genome.

Several groups of nuclear genes are known to be associated with certain recognizable mitochondrial syndromes (DiMauro and Emmanuele, 2012; Koopman et al., 2012; Nunnari and Suomalainen, 2012; Poulton et al., 2009; Rotig, 2011; Spinazzola, 2011; Wong et al., 2010). For example, genes responsible for mtDNA biogenesis or maintenance of mtDNA integrity may cause mtDNA depletion syndromes, including the hepatocerebral and myopathic forms (Bourdon et al., 2007; Carrozzo et al., 2007; DiMauro, 2004; Elpeleg et al., 2005; Galbiati et al., 2006; Nishino et al., 1999; Ostergaard et al., 2007a, 2007b; Pitceathly et al., 2012; Sitarz et al., 2012; Spinazzola, 2011; Spinazzola and Zeviani, 2009; Spinazzola et al., 2009; Yu-Wai-Man and Chinnery, 2012). Genes involved in the respiratory chain complex or their assembly may cause isolated respiratory chain complex deficiency (Hoefs et al., 2012; Nouws et al., 2012; Saada et al., 2009; Smeitink et al., 2006; Smits et al., 2011), whereas genes encoding aminoacyl tRNA synthetases (Elo et al., 2012; Rotig, 2011; Tyynismaa, 2012), mitochondrial ribosomal protein subunits (Graham, 2012; Pearce et al., 2012), or mitochondrial protein translation factors may cause a generalized reduction in the synthesis of mitochondrial encoded proteins (Graham, 2012; Pearce et al., 2012). Therefore, a comprehensive molecular diagnosis should ultimately include the analysis of all nuclear genes whose defects may cause mitochondrial disorders (Baughman et al., 2009; Koopman et al., 2012; Pearce et al., 2012; Rotig, 2011; Scharfe et al., 2009). Table 1A lists groups of genes known to cause mitochondrial disorders. The list also includes related candidate genes whose protein products are components of mitochondrial structure and/or function.

The high throughput NGS technology is capable of sequencing a group of target genes in parallel and is an ideal approach for the diagnosis of complex dual genome mitochondrial disorders. The NGS approach has been used to discover many novel genes that cause mitochondrial related disorders on a research basis (Calvo et al., 2006, 2012; Casey et al., 2012; Galmiche et al., 2011; Gandre-Babbe and van der Bliek, 2008; Gerards et al., 2011; Ghezzi et al., 2012; Glazov et al., 2011; Gotz et al., 2011; Haack et al., 2010; Majczenko et al., 2012; Mayr et al., 2012; Pierce et al., 2011; Rotig, 2011; Sloan et al., 2011; Spiegel et al., 2012; Steenweg et al., 2012; Tucker et al., 2011; Watkins et al., 2011; Wortmann et al., 2012) (Table 1B). Some of these newly discovered genes have recently been included as target genes for the clinical diagnosis of complex mitochondrial disorders. This review describes how NGS has brought the next generation molecular diagnosis of mitochondrial disorders to clinical laboratories.

2. The Next Generation Sequencing (NGS) technology

2.1. Target gene enrichment

NGS utilizes high throughput parallel sequencing through target gene enrichment, library preparation, and sequencing. The aim of target gene enrichment is to selectively enrich coding regions of genes of interest, usually coding exons and 20 bp of the flanking introns. This is often achieved by PCR or hybridization with specific probes (Zhang et al., 2012a). In the case of mitochondrial disorders, the sequences of interest are the entire mitochondrial genome and any subset(s) or all of the 1500 candidate genes involved in mitochondrial structure and function. The choice of gene enrichment methods depends on the number and size of genes, or the total target size in megabases, to be analyzed. If the total size of target sequences to be enriched is below a few hundred kb, PCR or multiplex PCR methods using gene sequence specific primers can be used to amplify the coding regions of interest. However, if the size of target genes is too large, then it is not practical to synthesize thousands or hundreds of thousands of primers for PCR. In addition, PCR conditions are hard to optimize. In this case, capture by probes in solution will be easier. The capture approach is scalable from a small number of genes to hundreds, thousands, or even the whole exome (20,000–30,000) (Calvo et al., 2012; Galmiche et al., 2011; Glazov et al., 2011; Gotz et al., 2011; Haack et al., 2010; Jones et al., 2012; Sloan et al., 2011; Wang et al., 2012a).

Ideally, the mitochondrial genome and nuclear genes should be enriched and sequence analyzed together in one single step. Unfortunately, due to the presence of nuclear homologs of mitochondrial DNA (mtDNA) sequences (called NUMT), the entire mitochondrial genome has to be enriched separately in a single amplicon using long range PCR and then sequenced using a separate index to avoid the interference of NUMT in capture and sequencing steps (Cui et al., 2012).

Table 1A
Categories of nuclear genes targeted to mitochondria and estimated number.

Category	Number	Category	Number	Category	Number	Category	Number
Apoptosis	50	Metabolism[a]	400[a]	Proteases	25	aa-tRNA synthesis	23
Chaperone	25	Dynamics	12	Nucleases	5	Transporters[d]	75
Cytochromes	15	Replication	10	RNA mod	5	Protein folding/modification	15
Cytoskeletal	10	Transcription	30	Translation[b]	15	Protein import: TIMMs and TOMMs	30
DNA repair	12	Regulation	10	MRPLs[c]	55	RC complex and assembly	150
Fe–S cluster	5	Porins	4	MRPSs[c]	36	RNA modification/processing	15
Immune	3	ROS	15	Others	150	Unknown	150

[a] Metabolic pathways include amino acids, carbohydrates, creatine, iron, ketone, Kreb cycle, lipids, nucleotide, phospholipid, porphyrin, steroid, tetrahydrofolate, tRNA, CoQs, pyruvate, etc.
[b] Translation: initiation, elongation, termination proteins and factors.
[c] MRP: mitochondrial ribosomal proteins large and small subunits.
[d] Transporters: ATP Binding Cassette, iron, nucleotides, solutes, and uncouplers.

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