INTRODUCTION

The study of genetic disease is often centered on the human nuclear genome consisting of ~3.1 billion nucleotides and 25,000–30,000 genes located on the 23 pairs of chromosomes, whereas the mitochondrial genome has received less attention. Each cell contains numerous mitochondria and each mitochondrion contains several copies of mitochondrial DNA (mtDNA). Thus, a cell contains several thousand copies of mitochondrial genome. The mitochondrial genome is a short, circular genome with 16,569 nucleotides and 37 genes (Fig. 1) encoding 13 proteins, 22 tRNAs, and 2 rRNAs, and gene density of 1 per 450 bp. Introns are absent in mtDNA and coding DNA constitutes 93% of mtDNA in contrast to ~3% in nuclear DNA.

Mitochondria play a critical role in energy metabolism and Reactive Oxygen Species (ROS) production, and mutations in mtDNA are risk factors for various complex disorders. Thus, mutations of...
mtDNA are associated with several clinical manifestations affecting different systems with multi-organ involvement.

**Mutations in Mitochondrial Genome**

The mtDNA mutations may be deletions, point mutations of rRNA and tRNA genes, and point mutations of coding genes (Tuppen *et al.*, 2010). Wallace *et al.* (1988) described the first human pathogenic mtDNA mutation, a homoplasmic point mutation in *MTND4* (m.11778A > G), a common pathogenic mtDNA mutation. Since then, over 250 pathogenic mtDNA mutations including point mutations and rearrangements have been characterized in a wide variety of diseases with phenotypic heterogeneity.
and variable age of onset (McFarland et al., 2007). The prevalence of mtDNA disease is difficult to accurately assess due to clinical heterogeneity and a large number of causative mutations (Tuppen et al., 2010).

At the population level, mtDNA is highly polymorphic with specific mtDNA variants affecting mitochondrial function. The mtDNA genes play a central role in oxidative phosphorylation, and identical genetic variants often lead to different disorders. Hudson et al. (2014) examined the role of common mtDNA variants in several complex diseases. The authors examined 50,000 individuals in 11 different diseases in tandem and showed that mtDNA variants can increase or decrease the risk of disease by replicating and expanding the variants, and confirmed association of the same mtDNA variant in several distinct disease phenotypes. These shared genetic associations implicated an underlying functional effect such as changing cellular energy, which manifested as distinct phenotypes. The authors confirmed the important role of mtDNA variation in complex traits and supported Genome Wide Association Study (GWAS)-based approach for analyses of mitochondrial genetics (Hudson et al., 2014).

The mutation rate in the mitochondrial genome is 10–17 fold higher than that in nuclear DNA (Tuppen et al., 2010), and the mitochondrial pathologies with multisystem disorders are a result of failing to produce an adequate amount of ATP or energy. The clinical implications of mitochondrial dysfunction are severe in tissues particularly skeletal muscles, central nervous system and heart muscles that have high energy demand; however, other organs may also be affected (Chinnery et al., 1997; Khan et al., 2015; Naviaux et al., 2004).

Diagnosis and Management of Patients with Mitochondrial Disease
Diagnosis of mitochondrial disorders is complex and integration of medical history, laboratory tests, imaging and muscle biopsy are necessary. Diagnosis of mitochondrial respiratory chain disorder requires an amalgamation of clinical, biochemical, enzymatic, histopathological and molecular data (Fig. 2). However, a better understanding of the various aspects of mitochondrial diseases is necessary for patient management. The current review focuses
on molecular aspects of mitochondrial diseases and its implications.

**Molecular Implications**

Identification and diagnosis of specific inherited mitochondrial diseases is facilitated by mtDNA analysis (Wong et al., 2010). Testing of skeletal muscle mtDNA is preferred due to relative abundance and retention of mtDNA mutations, however urine and blood may also be tested depending on the mutation type. Diagnosis of a mitochondrial disease can be confirmed by identifying a common known pathogenic mtDNA mutation in symptomatic patients. However, depending on the percentage load of pathogenic mtDNA mutation due to the degree of heteroplasmy, a patient with known pathogenic mtDNA mutation may be asymptomatic. Hence, it is important that on identification of the index case with a mtDNA mutation, pretest genetic counseling be offered to family members regarding the implications, limitations, and potential benefits of mtDNA testing. The clinical presentation of mitochondrial disorders...
can indicate a particular disease phenotype with well recognized symptoms, suggesting specific mtDNA defect (Chinnery et al., 1997; Naviaux et al., 2004). However, large numbers of patients presented with clinical symptoms suggestive of particular mitochondrial disorders, do not precisely fit in a specific disease category. A number of factors, including the threshold effect, mitotic segregation, clonal expansion and genetic ambiguity affect the onset of clinical symptoms, phenotypic variability and variable penetrance of mitochondrial diseases (Tuppen et al., 2010).

Inheritance pattern of mitochondrial genes
Mitochondrial genetics differs considerably from Mendelian genetics with respect to uniparental inheritance, cellular polyploidy and deviation from the standard genetic code. These features directly bear upon the functional consequences of pathogenic mtDNA mutations (Tuppen et al., 2010). The heteroplasmy and polyploid nature of mitochondrial genome is one of the basic mechanism of mitochondrial genome expression with manifestations into severity of the clinical phenotypes. The mutations may affect limited copies (heteroplasmic mutation) or all copies (homoplasmic mutation) of the mitochondrial genome, with a threshold level of mutation for the clinical phenotype of the disease and biochemical defects (Taylor et al., 2005).

mtDNA is transmitted through maternal lineage and hence are clonal and do not show Mendelian inheritance. The transmission of heteroplasmic mtDNA point mutations is even more complex (Battersby et al., 2003). In addition to the effect of nuclear genetic and environmental factors on expression of the mitochondrial disease phenotype, the amount of mutated mtDNA transmitted to the offspring is variable (Khan et al., 2015; Taylor et al., 2005). As several clinical features depend on the relative proportions of mutated versus wild-type mtDNA, the outcome for each pregnancy is difficult to predict (Fig. 3).

Role of mtDNA in complex disorders
The genetics of mtDNA provides new perspectives on the etiology of common complex mitochondrial diseases. The maternally inherited mtDNA that codes for the essential energy genes, is present
in thousands of copies per cell, and has a very high mutation rate. Besides, the mtDNA is prone to additional new mutations. The mechanism of predominance of these “heteroplasmic” mtDNA mutations in the female germline and somatic tissues is not well understood. The maternal inheritance and heteroplasmy make the diagnosis and prevention of mtDNA disease more difficult.

Due to the unique quantitative genetics of the maternally inherited mtDNA, the subtle bioenergetic alterations lead to major clinical consequences and mitochondrial defects. As a result, mitochondrial dysfunction has been overlooked in case of “complex” diseases (Wallace et al., 2013).

There is evidence for and against the linkage of mtDNA susceptibility
polymorphisms and/or haplogroups in diseases, including age-related macular degeneration, Alzheimer's disease, amyotrophic lateral sclerosis, frontotemporal dementia, Huntington's disease, ischaemic stroke, psychiatric disorders, Parkinson's disease (Anderson et al., 2011; Arning et al., 2010; Ingram et al., 2012; Khusnutdinova et al., 2008; Mancuso et al., 2008; Rose et al., 2008; SanGiovanni et al., 2009; Santoro et al., 2012; Sequeira et al., 2012; Tranah, 2012). To decipher the relationship of mtDNA mutations to complex traits, advanced technology and various 'omics' approaches of proteomics, lipidomics and metabolomics may enable better understanding and define genotype–phenotype relationship (Schon, 2012).

**Aging and Mitochondrial Genome**

Analysis of mutations in the D-loop, a major control site for mtDNA expression region, indicates correlation of heteroplasmy and somatic aging (Sondheimer et al., 2011). The dynamics of somatic heteroplasmy over the course of lifespan, has been comprehensively analysed by Sondheimer and colleagues (2011) across a range of ages. The authors noted increase or decrease in heteroplasmy was observed at various sites, with several sites demonstrating an increase in homoplasmy associated with aging. The frequency of heteroplasmy has been estimated in two studies of the hypervariable regions of mtDNA, indicating 10–25% of individuals as heteroplasmic within specific regions and across the mitochondrial genome (Calloway et al., 2000; de Camargo et al., 2010; Li et al., 2010). Changes in heteroplasmy between generations have been reported (Bendall et al., 1996). Disease associated mutations, not clinically detectable in one generation, may increase in the offspring and cause disease (Brown et al., 2001; Maassen et al., 2002). Besides shifts in heteroplasmy between generations, mitochondrial allelic frequencies vary in multiple tissues in an individual (Bendall et al., 1996; He et al., 2010). The heteroplasmy may also alter over time in individuals.

In mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome, A3243G mutation in blood sample may decline with age due to mutation bearing cells being selected out (Rahman et al., 2001; Rajasimha et al., 2008). On the other hand associations between increase in
human mitochondrial heteroplasmy and aging have been detected including point mutations, large deletions or polymorphic variants (Corral-Debrinski et al., 1992; Cortopassi et al., 1992; da Costa et al., 2007; Del Bo et al 2002; Wang et al., 2001). Several heteroplasmys may be beneficial and are inherited by long-lived individuals and their offspring (Iwata et al 2007; Rose et al., 2007). On the other hand several studies reported absence of link between mutational load and age (de Camargo et al., 2010; Greaves et al., 2009; Pallotti et al., 1996). Analysis of mitochondrial heteroplasmy is necessary for understanding the phenomenon underlying increase or decrease of mitochondrial genome alterations. The degradation of mtDNA with aging process may be indicative of the role of mitochondrial genome as a biological clock. The disease pathology associated with mitochondrial genome may be amenable to specific understanding of the biological basis of these phenomena will be crucial to the development of therapies dependent on alteration of the heteroplasmy towards normalcy (Sondheimer et al., 2011).

**Endocrinal Pathology and Mitochondrial Genome**

Endocrine dysfunctions are not common in mitochondrial diseases although mtDNA mutation m.3243A>G has been reported in diabetes mellitus (Schaefer et al., 2013). Thus, presence of endocrine involvement may indicate investigations for mitochondrial disorder. Study of at risk endocrine organs for mtDNA genotype and phenotype correlation may provide key to patient diagnosis and management.

**Neurological Pathology and Mitochondrial Genome**

mtDNA mutations have been associated with late-onset neurodegenerative diseases including Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and hereditary spastic paraplegias, although the precise role of the mitochondrial mutations in neurological diseases is not clear (Schon et al., 2011). A secondary role has been noted in Parkinson's disease. Familial Parkinson's disease has been associated with PINK1 (PTEN-induced putative kinase 1), a mitochondrially targeted kinase (Vives-Bauza et al., 2010), and cytosolic ubiquitin
ligase translocation to dysfunctional mitochondria (Narendra et al., 2011). The substantia nigra affected in Parkinson's disease, the main site of dopaminergic neuron degeneration in these patients, harbors deletion and in mtDNAs as in Kearns-Sayre syndrome (KSS) and aging (Bender et al., 2006; Kraytsberg et al., 2006). These deletions in mtDNAs may cause bioenergetic deficits in the disease.

**Cardiac Pathology and Mitochondrial Genome**

Mitochondria play an important role in the cardiovascular system as the functioning of heart is dependent on oxidative energy generated in mitochondria. Thus, mutations of mtDNA can affect coronary artery disease leading to hypertension, atherosclerosis and cardiomyopathy (Wang et al., 2015). Human mtDNA mutations cause a large spectrum of clinically critical cardiovascular events. Studies using MITOCHIP, a mitochondrial cDNA expression array to analyze about 1000 genes involved in energy production, ROS biology, and apoptosis (Wallace, 2008). Wang et al. (2015) have reported that traditional Chinese medicine used for thousands of years to treat cardiovascular disease, targeted mitochondrial function aimed at treatment.

**Cancer and Mitochondrial Genome**

The influence of mitochondrial mutations on cancers is known, although the mechanism of action is not well understood. Polyak and colleagues demonstrated homoplasmic mitochondrial mutations in tumor DNA and not in matched control tissues (Baysal et al., 2000; Polyak et al., 1998; Vanharanta et al., 2004). Association of mitochondrial mutations with cancers of breast (Canter et al., 2005), prostate (Herrmann et al., 2003; Petrosillo et al., 2005), head and neck (Sun et al., 2009), colon (Ericson et al., 2012) and bladder (Dasgupta et al., 2008; Fliss et al., 2000) have been reported. Alterations in the mtDNA genome including copy number variations may be the overall result of gene (hereditary) and environmental interactions leading to oxidative stress (Lee et al., 1998; Renis et al., 1989; Verma et al., 2007). Increasing evidence indicates association of mutated mtDNA with bad prognosis and poor survival (Lièvre et al., 2005; Matsuyama et al., 2008).
2003), cancer recurrence (Fliss et al., 2000) and genotoxic damage (Wardell et al., 2003). Role of mtDNA mutations in metastasis has been demonstrated in Non-Small-Cell Lung Cancer (NSCLC) at different stages of tumor formation, and indicated a significantly decreased survival in advanced NSCLC patients harboring mtDNA mutations (Matsuyama et al., 2003). Studies investigating the role of mtDNA in breast cancer patients demonstrated contradictory results indicating increased mtDNA copy number in DNA from peripheral blood (Shen et al., 2010), and decreased mtDNA copy number in tissues (Bai et al., 2011; Tseng et al., 2006; Yu et al., 2007). The mutations were linked to increased cancer risk through a deficient Electron Transport Chain (ETC) function and altered ROS levels (Bai et al., 2007).

Next Generation Sequencing (NGS) for Mitochondrial Disorders

The advanced sequencing technology of next generation sequencing (NGS) with the power of high coverage and ability to study entire mitochondrial genome in a single experiment has the potential to change diagnosis of mitochondrial disorders. Highly variable mtDNA copy numbers are classically associated with several mitochondrial diseases. Traditionally, a PCR based method is used for assessing limited numbers of mtDNA copy number (Bhat et al., 2004). NGS is a useful method for the DNA sequencing and RNA sequencing will enable expression profiling as well (Castle et al., 2010). It has been shown that the amount of mitochondrial sequences captured in exome sequences is proportional to abundance of mitochondrial genome in original DNA extracted (Bai et al., 2007; Picardi et al., 2012; Schmitt et al., 2012). This is useful while studying tumors samples for clinical association and correlating with tumor type and metastasis (Ye et al., 2014).

Tumor cells often produce energy by the process of glycolysis and lactic acid fermentation, whereas normal cells primarily produce energy through pyruvate oxidation in mitochondria, known as “Warburg Effect”. Study of changes in mitochondrial DNA leading to altered energy metabolism using NGS will confirm the Warburg effect and indicate clinical applications (Verschoor et al., 2013). Whole exome sequencing, essentially a targeted sequencing
approach is a possible technique for studying the genetic aspects of mitochondrial diseases (Galmiche et al., 2011). NGS is used in studying of RNA sequencing and expression profiles, thus establishing link between mitochondrial gene expression and mutations (Ye et al., 2014). mtDNA sequencing data may also be obtained from exome, whole genome and RNA sequencing (Samuel et al., 2013). A small portion of reads from exome sequencing data align to mitochondrial genome although mtDNA is not the target during exome sequencing as reported (Larman et al., 2012). The average coverage of mitochondrial genome from exome sequencing is more than 100X, often better than mtDNA targeted sequencing (Picardi et al., 2012), due to the presence of high copy number of mtDNA per cell. The advantage of NGS technique and high coverage of mtDNA is a robust mode of study of mitochondrial DNA heteroplasmy (1000 Genomes project consortium; Goto et al., 2011; Guo et al., 2012; Ng et al., 2010, Tang et al., 2010) and detect heteroplasmy as less as 0.1% (Guo et al., 2013). Several groups have reported mitochondrial mutation analysis using exome sequencing data as in The Cancer Genome Atlas (TCGA) project with huge exome sequencing data from different cancers. The TCGA (Cancer Genome Atlas Network 2012) defined 325 mitochondrial DNA somatic mutations in breast cancer derived from off-target reads of the 776 exome sequencing data.

Challenges Associated with NGS and Mitochondrial Disorder Studies
In most studies, usefulness of NGS as method for detection of molecular diagnosis of mitochondrial diseases has been reported in a cohort of 50–100 patients (Carroll et al., 2014). Calvo et al. (2012) reported that targeted MitoExome data failed to diagnose 50% patients due to underdetection of indel, uncovered targets, intronic or regulatory regions, non-inclusion of pathogenic heterozygous variants which may actually be de novo dominating or incomplete penetrance. The findings also suggest that the large number of undiagnosed cases may be due to the wide spectrum of genetic disorders in
patients affected with mitochondrial disorders. NGS may be considered a diagnostic tool with exome sequencing as first line diagnostics in children with mtDNA coding sequence, obviating the need for invasive muscle biopsy. In specialized clinical laboratories it can serve as a routine diagnostic test (Carroll et al., 2014). The problem arises when NGS data indicates misannotated variants leading to complication in interpretation of results with variants of unknown significance and oligogenic variants, necessitating parental and additional family members to be analyzed. The identified pathogenic variants should be informed to the patient, and variants of unknown significance may be reanalyzed after a time period when information about pathogenic variants is accumulated. Alternatively, new NGS methodology with better coverage and capture can be repeated, and the clinicians informed accordingly (Carroll et al., 2014). Counseling patients and information on unrelated incidental finding is also an area of concern with NGS data with respect to ethical considerations. Costs associated with Sequencing show a downward trend, resulting in increased use in clinical applications (Carroll et al., 2014). Detection and reporting of molecular changes in rare genetic disorder necessitates national and international communication and collaborations. Although databases for mitochondrial variants like Mitomap (www.mitomap.org) and mtDB (www.mtdb.igp.uu.se/) exist independently, a common resource of genomic data should be created where all the molecular changes and findings in cases suspected for mitochondrial disorders should be reported, advancing diagnosis and providing opportunities for identification of therapeutic targets for mitochondrial disorders (Carroll et al., 2014).

An important drawback in the mitochondrial disease studies is the small sample sizes and the cases are non-randomized and non-blinded, leading to biased results (Pfeffer et al., 2013). With the awareness of limitations and lacunae in mtDNA studies, and constant advances in sequencing methodology diagnosis and multiple treatment options including targeted therapy may result in better patient management.

CONFLICT OF INTEREST
The authors claim no conflict of interest.
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