Review

BRJ
Biomedical Research Journal

Role of Mitochondrial Genetics in Complex Diseases

Namrata Londhe and Jaya Vyas*

Genetics Department, Lab Medicine, Kokilaben Dhirubhai Ambani Hospital, Four Bunglows, Andheri (W), Mumbai – 400053, India

The challenge in managing patients with mitochondrial diseases is in its complex nature as exemplified in the inheritance pattern and clinical presentation of mitochondrial diseases. Besides involvement of the nuclear genes, mitochondrial genes independently or together add to the complexity. Transmission of mitochondrial genome mutations by maternal inheritance and presence of heteroplasmy, high mitochondrial mutation rates and absence of introns poses problems in diagnosis and protocols for identification, treatment, prevention and management of the patients. With the use of advanced techniques including next generation sequencing to simultaneously screen multiple genes for alterations, identification of targets for therapy and better patient management is envisaged. The current review attempts to understand the role of mitochondrial genome in complex diseases and the utility of technological advances in diagnosis of mitochondrial diseases.

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

Key words: Mitochondrial diseases, mitochondrial genome complexities, inheritance, heteroplasmy, diagnosis, Next Generation Sequencing.
***Corresponding Author:** Jaya Vyas, Genetics Department, Lab medicine, Kokilaben Dhiru bhai Ambani Hospital, Four Bunglows, Andheri (W), Mumbai–400053, India.
Email: jaya.vyas@relianceada.com; Tel: +91-22-30977012

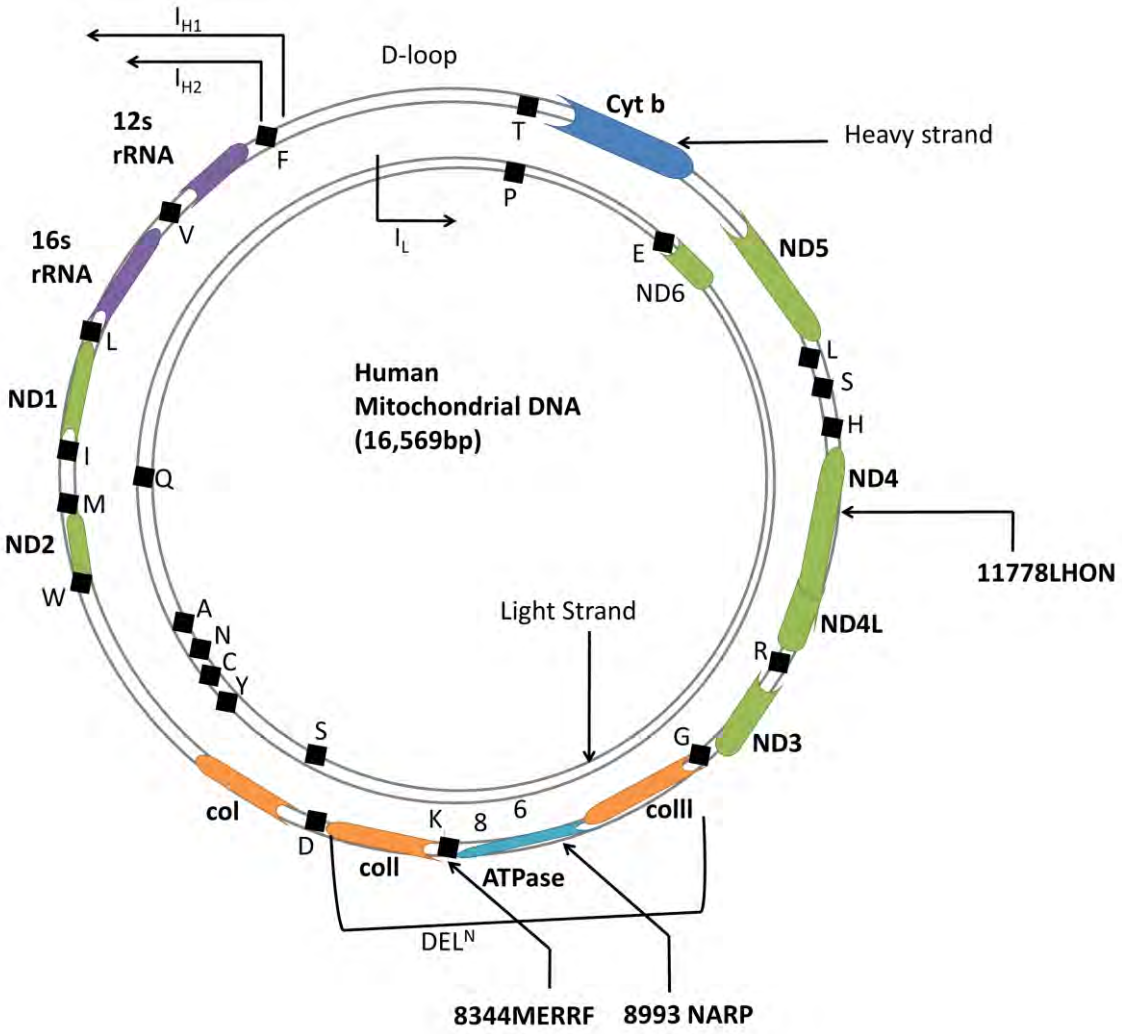

Figure 1: Human Mitochondrial Genome, consisting of 16,569bp genome. It is transcribed from both heavy and light strands. All promoters and other elements involved in replication initiation are located in displacement (D) loop. (Adapted from: Kyriakouli *et al.*, 2008).

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

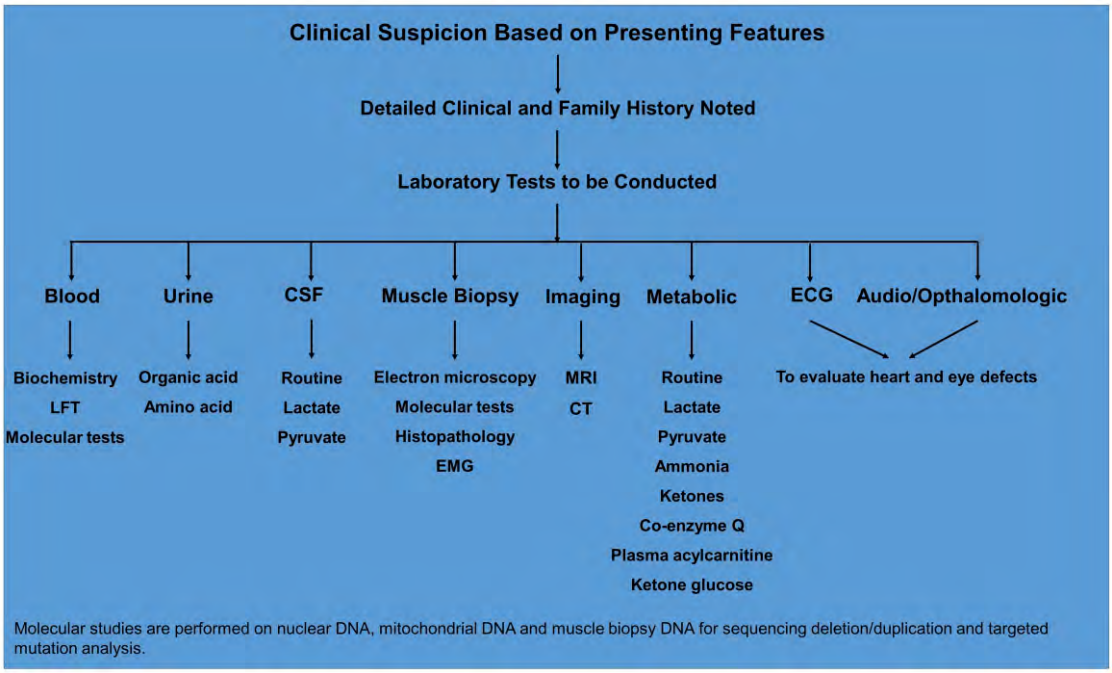

Figure 2: Diagnostic Workup for a Patient suspected for Mitochondrial Diseases.
MRI – Magnetic resonance imaging, CT – Computerized tomography, LFT – Liver function test, EMG – Electromyography
Disclaimer: This flowchart is not exhaustive and complete. This is just for the purpose of guidance and overview. Additional tests may have to be added or bypassed depending on the clinical presentation and the type of mitochondrial disease suspected in the patient..

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

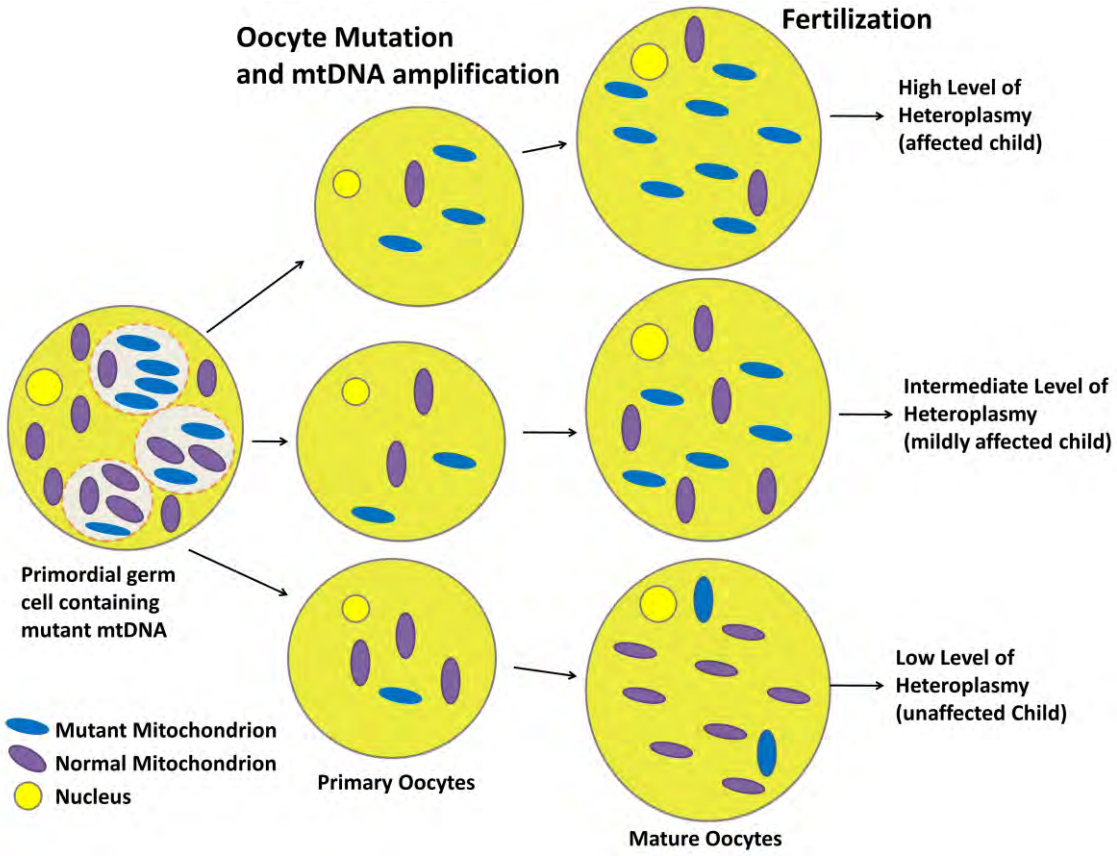

Figure 3: The Mitochondrial genome transmission: Mitochondrial genome is transmitted maternally and following possibilities exist. (As primary oocytes are formed, only a selected number of mitochondrial DNA (mtDNA) molecules are transferred into each oocyte. Due to rapid replication of this mtDNA population during oocyte maturation leading to restriction-amplification event which in turn leads to a random shift of mtDNA mutational load between generations. This is responsible for the variable levels of mutated mtDNA observed in affected offspring from mothers with pathogenic mtDNA mutations. (Adapted from: Taylor et al., 2005).

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 heteroplasmies 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.,*

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). Specific techniques which target mtDNA give coverage of 10,000 and above throughout the mitochondrial genome (Ameur *et al*., 2011; Guo *et al*., 2012; He *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.

REFERENCES

1000 Genomes Project Consortium, Abecasis GR, Altshuler D, Auton A, Brooks LD, *et al*. A map of human genome variation from population-scale sequencing. *Nature* 2010; 467:1061–1073.

Ameur A, Stewart JB, Freyer C, Hagstrom E, Ingman M, Larsson NG, *et al*. Ultra-deep sequencing of mouse DNA: mutational patterns and their origins. *PLoS Genet* 2011; 7:e1002028.

Anderson CD, Biffi A, Rahman R., Ross OA, Jagiella JM, Kissela B, *et al*. Common mitochondrial sequence variants in ischemic stroke. *Ann Neurol* 2011; 69:471–480.

Arning L, Haghikia A, Taherzadeh-Fard E, Saft C, Andrich J, Pula B *et al*. Mitochondrial haplogroup H correlates with ATP levels and age at onset in Huntington disease. *J Mol Med* 2010;88:431–436.

Bai RK, Chang J, Yeh KT, Lou MA, Lu JF, Tan DJ, *et al*. Mitochondrial DNA content varies with pathological characteristics of breast cancer. *J Oncol* 2011;2011:496189.

Bai RK, Leal SM, Covarrubias D, Liu A, Wong LJ. Mitochondrial genetic background modifies breast cancer risk. *Cancer Res* 2007;67:4687– 4694

Battersby, BJ, Loredo-Osti JC, Shoubridge EA. Nuclear genetic control of mitochondrial DNA segregation. *Nature Genet* 2003;33: 183–186.

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, *et al*. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;287:848–851.

Bendall KE, Macaulay VA, Baker JR, Sykes BC. Heteroplasmic point mutations in the human mtDNA control region. *Am J Hum Genet* 1996;59:1276–1287.

Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, *et al*. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nature Genet* 2006;38:515–517.

Bhat HK, Epelboym I. Quantitative analysis of total mitochondrial DNA: competitive polymerase chain reaction versus real-time polymerase chain reaction. *J Biochem Mol Toxicol* 2004;18:180–186.

Brown DT, Samuels DC, Michael EM, Turnbull DM, Chinnery PF. Random genetic drift determines the level of mutant mtDNA in human primary oocytes. *Am J Hum Genet* 2001;68:533–536.

Calloway CD, Reynolds RL, Herrin GL, Jr., Anderson WW. The frequency of heteroplasmy in the HVII region of mtDNA differs across tissue types and increases with age. *Am J Hum Genet* 2000;66:1384–1397.

Calvo SE, Compton AG, Hershman SG, Lim SC, Lieber DS, Tucker EJ, *et al*. Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. *Sci Transl Med* 2012;4:118ra110

Cancer Genome Atlas Network 2012. http://cancergenome.nih.gov.

Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. *Cancer Res* 2005;65:8028–8033.

Castle JC, Biery M, Bouzek H, Xie T, Chen R, Misura K *et al*. DNA copy number, including telomeres and mitochondria, assayed using next generation sequencing. *BMC Genomics* 2010;11:244.

Carroll CJ, Brilhante V, Suomalainen A. Next-generation sequencing for mitochondrial disorders. *Brit J Pharmacol* 2014;171:1837–1853

Chinnery PF, Turnbull DM. Clinical features, investigation, and management of patients with defects of mitochondrial DNA. *J Neurol Neurosug Psychiatry* 1997;63:559–563.

Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nat Genet1992*;2:324–329.

Cortopassi GA, Shibata D, Soong NW, Arnheim N. A pattern of accumulation of a somatic deletion of mitochondrial DNA in aging human tissues. *Proc Natl Acad Sci USA* 1992;89:7370–7374.

da Costa CK, Kiyomoto BH, Schmidt B, Oliveira AS, Gabbai AA, Tengan CH Age-related mitochondrial DNA point mutations in patients with mitochondrial myopathy. *J Neurol Sci* 2007;263:139–144.

Dasgupta S, Hoque MO, Upadhyay S, Sidransky D. Mitochondrial cytochrome B gene mutation promotes tumor growth in bladder cancer. *Cancer Res* 2008;68:700–706.

de Camargo MA, Paneto GG, de Mello AC, Martins JA, Barcellos W, Cicarelli RM. No relationship found between point heteroplasmy in mitochondrial DNA control region and age range, sex and haplogroup in human hairs. *Mol Bio. Rep* 2010;38:1219–1223.

Del Bo R, Bordoni A, Martinelli Boneschi F, Crimi M, Sciacco M, Bresolin N, *et al*. Evidence and age-related distribution of mtDNA D-loop point mutations in skeletal muscle from healthy subjects and mitochondrial patients. *J Neurol Sci* 2002; 202:85–91.

Ericson NG, Kulawiec M, Vermulst M, Sheahan K, O'Sullivan J, Salk JJ, *et al*. Decreased mitochondrial DNA mutagenesis in human colorectal cancer. *PLoS Genet* 2012;8: e1002689.

Fliss MS, Usadel H, Caballero OL, Wu L, Buta MR, *et al*. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science* 2000;287:2017–2019.

Galmiche L, Serre V, Beinat M, Assouline Z, Lebre AS, Chretien D, *et al*. Exome sequencing identifies MRPL3 mutation in mitochondrial cardiomyopathy. *Hum Mutat* 2011;32:1225– 1231.

Goto H, Dickins B, Afgan E, Paul IM, Taylor J, Makova KD, *et al*. Dynamics of mitochondrial heteroplasmy in three families investigated via a repeatable re-sequencing study. *Genome Biol* 2011;12:R59.

Greaves LC, Beadle NE, Taylor GA, Commane D, Mathers JC, Khrapko K, Turnbull DM. Quantification of mitochondrial DNA mutation load. *Aging Cell* 2009;8,566–572.

Guo Y CQ, Samuels DC, Ye F, Long J, Li CI, Winther JF, *et al*. The use of next generation sequencing technology to study the effect of radiation therapy on mitochondrial DNA mutation. *Mutat Res* 2012;744(2):154–160.

Guo Y, Li J, Li CI, Shyr Y, Samuels DC.

MitoSeek: extracting mitochondria information and performing high-throughput mitochondria sequencing analysis. *Bioinformatics* 2013;29: 1210–1211.

He Y, Wu J, Dressman DC, Iacobuzio-Donahue C, Markowitz SD, Velculescu VE, *et al*. Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature* 2010; 464(7288):610–614.

Herrmann PC, Gillespie JW, Charboneau L, Bichsel VE, Paweletz CP, Calvert VS, *et al.,* Mitochondrial proteome: altered cytochrome c oxidase subunit levels in prostate cancer. *Proteomics* 2003;3:1801–1810.

Hudson G, Gomez-Duran A, Wilson IJ, Chinnery PF. Recent Mitochondrial DNA Mutations Increase the Risk of Developing Common Late-Onset Human Diseases. *PLOS Genetics* 2014;10(5):e1004369

Ingram CJ, Weale ME, Plaster CA, Morrison KE, Goodall EF, Pall HS, *et al.,* Analysis of European case-control studies suggests that common inherited variation in mitochondrial DNA is not involved in susceptibility to amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2012;13(4):341–346.

Iwata N, Zhang J, Atzmon G, Leanza S, Cho J, Chomyn A, *et al*. Aging-related occurrence in Ashkenazi Jews of leukocyte heteroplasmic mtDNA mutation adjacent to replication origin frequently remodeled in Italian centenarians. *Mitochondrion* 2007;7: 267–272.

Khan NA, Govindaraj P, Meena AK, Thangaraj K. Mitochondrial disorders: Challenges in diagnosis and treatment. *Indian J Med Res* 2015;141(1):13–26.

Khusnutdinova E, Gilyazova I, Ruiz-Pesini E, Derbeneva O, Khusainova R, Khidiyatova I, *et al*. A mitochondrial etiology of neuro-degenerative diseases: evidence from Parkinson's disease. *Ann N Y Acad Sci* 2008; 1147:1–20.

Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet* 2006; 38:518–520.

Kyriakouli DS, Boesch P, Taylor RW, Lightowlers RN. Progress and prospects: gene therapy for mitochondrial DNA disease. *Gene Ther* 2008;15:1017–1023.

Larman TC, Depalma SR, Hadjipanayis AG, Protopopov A, Zhang J, Gabriel SB, *et al*. Spectrum of somatic mitochondrial mutations in five cancers. *Proc Natl Acad Sci USA* 2012; 109:14087–14091.

Lee HC, Lu CY, Fahn HJ, Wei YH. Aging- and smoking-associated alteration in the relative content of mitochondrial DNA in human lung. *FEBS Lett* 1998;441:292–296.

Li M, Schonberg A., Schaefer M, Schroeder R, Nasidze I, Stoneking M. Detecting hetero-plasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. *Am J Hum Genet* 2010;87:237–249.

Lièvre A, Chapusot C, Bouvier AM, Zinzindohoué F, Piard F, *et al*. Clinical value of mito-chondrial mutations in colorectal cancer. *J Clin Oncol* 2005;23:3517–3525.

Maassen JA, Biberoglu S, 't Hart LM, Bakker E, de Knijff P. A case of a de novo A3243G

mutation in mitochondrial DNA in a patient with diabetes and deafness. *Arch Physiol Biochem* 2002;110:186–188.

Mancuso M, Filosto M, Orsucci D, Siciliano G. Mitochondrial DNA sequence variation and neurodegeneration. *Hum Genom* 2008;3:71–78.

Matsuyama W, Nakagawa M, Wakimoto J, Hirotsu Y, Kawabata M, *et al*. Mitochondrial DNA mutation correlates with stage progression and prognosis in non-small cell lung cancer. *Hum Mutat* 2003;21:441–443.

McFarland R, Taylor RW, Turnbull RW. Mitochondrial disease—its impact, etiology, and pathology. *Curr. Top. Dev. Biol* 2007;77: 113–155.

Narendra DP, Youle RJ. Targeting mitochondrial dysfunction: role for PINK1 and Parkin in mitochondrial quality control. *Antioxid Redox Signal* 2011;14:1929–1938.

Naviaux RK. Developing a systematic approach to the diagnosis and classification of mitochondrial disease. *Mitochondrion* 2004; 4:351–361.

Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, *et al*. Exome sequencing identifies the cause of a Mendelian disorder. *Nat Genet* 2010;42: 30–35.

Pallotti F, Chen X, Bonilla E, Schon EA. Evidence that specific mtDNA point mutations may not accumulate in skeletal muscle during normal human aging. *Am J Hum Genet* 1996;59:591– 602.

Petrosillo G, Di Venosa N, Ruggiero FM, Pistolese M, D'Agostino D, Tiravanti E. *et. al*. Mitochondrial dysfunction associated with cardiac ischemia/ reperfusion can be attenuated by oxygen tension control. Role of oxygenfree radicals and cardiolipin. *Biochim Biophys Acta* 2005;1710:78–86.

Pfeffer G, Horvath R, Klopstock T, Mootha VK, Suomalainen A, Koene S *et al*. New treatments for mitochondrial disease-no time to drop our standards. *Nat Rev Neurol* 2013; 9(8):474–481.

Picardi E, Pesole G. Mitochondrial genomes gleaned from human whole-exome sequencing. *Nat Methods* 2012;9:523–524.

Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD *et al*. Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nat Genet* 1998;20:291–293.

Rahman S, Poulton J, Marchington D, Suomalainen A. Decrease of 3243AG mtDNA mutation from blood in MELAS syndrome: A longitudinal study. *Am J Hum Genet* 2001;68:238–240.

Rajasimha HK, Chinnery PF, Samuels DC. Selection against pathogenic mtDNA mutations in a stem cell population leads to the loss of the 3243AG mutation in blood. *Am J Hum Genet* 2008:82:333–343.

Renis M, Cantatore P, LoguercioPolosa P, Fracasso F, Gadaleta MN. Content of mitochondrial DNA and of three mitochondrial RNAs in developing and adult rat cerebellum. *J Neurochem* 1989;52:750–754.

Rose G, Longo T, Maletta R, Passarino G, Bruni AC, De Benedictis G. No evidence of association between frontotemporal dementia and major European mtDNA

haplogroups. *Eur J Neurol* 2008;15(9):1006–1008.

Rose G, Passarino G, Scornaienchi V, Romeo G, Dato S, Bellizzi D, *et al*. The mitochondrial DNA control region shows genetically correlated levels of heteroplasmy in leukocytes of centenarians and their offspring. *BMC Genomics* 2007;8:293.

Samuels DC, Han L, Li J, Quanghu S, Clark TA, Shyr Y *et al*. Finding the lost treasures in exome sequencing data. *Trends Genet* 2013; 29:593–599.

SanGiovanni JP, Arking DE, Iyengar SK, Elashoff M, Clemons TE, Reed GF, *et al*. Mitochondrial DNA variants of respiratory complex I that uniquely characterize haplogroup T2 are associated with increased risk of age-related macular degeneration. *PLoS ONE* 2009;4: e5508.

Santoro A, Balbi V, Balducci E, Pirazzini C, Rosini F, Tavano F, *et al*. Evidence for sub-haplogroup h5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. *PLoS ONE* 2012;5:e12037.

Schaefer AM, Walker M, Turnbull DM, Taylor RW. Endocrine disorders in mitochondrial disease. *Mol Cell Endocrinol* 2013;379(1–2): 2–11.

Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, *et al*. Detection of ultra-rare mutations by next-generation sequencing. *Proc Natl Acad Sci USA* 2012;109:14508–14513.

Schon EA, DiMauro S and Hirano M. Human mitochondrial DNA: roles of inherited and somatic Mutations. *Nat Rev Genet* 2012; 13(12):878–890.

Schon EA, Przedborski S. Mitochondria: the next (neurode) generation. *Neuron* 2011;70(6): 1033–1053.

Sequeira A, Martin MV, Rollins B, Moon EA, Bunney WE, Macciardi F *et al*. Mitochondrial mutations and polymorphisms in psychiatric disorders. *Front Genet* 2012;3: 103.

Shen J, Platek M, Mahasneh A, Ambrosone CB, Zhao H Mitochondrial copy number and risk of breast cancer: a pilot study. *Mitochondrion* 2010;10:62–68.

Sondheimer N, Glatz CE, Tirone JE, Deardorff MA, Krieger AM, Hakonarson H. Neutral mitochondrial heteroplasmy and the influence of aging. *Human Mol Genet* 2011;20:1653– 1659.

Sun W, Zhou S, Chang SS, McFate T, Verma A, Califano JA, *et al*. Mitochondrial mutations contribute to HIF1alpha accumulation via increased reactive oxygen species and up-regulated pyruvate dehydrogenease kinase 2 in head and neck squamous cell carcinoma. *Clin Cancer Res* 2009;15:476–484.

Tang S, Huang T. Characterization of mitochondrial DNA heteroplasmy using a parallel sequencing system. *Biotechniques* 2010;48:287–296.

Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 2005;6:389–402.

Tranah GJ. Mitochondrial-nuclear epistasis: implications for human aging and longevity. *Ageing Res Rev* 2012;10:238–252.

Tseng LM, Yin PH, Chi CW, Hsu CY, Wu CW, Lee LM, *et al*. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. *Genes Chromosomes*

Cancer 2006;45:629– 638.

Tuppen H, Blakely EL, Turnbull D, Taylor RW. Mitochondrial DNA mutations and human disease. *Biochimica et Biophysica Acta* 2010; 1797:113–128.

Vanharanta S, Buchta M, McWhinney SR. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Human Genet* 2004;74:153–159

Verma M, Naviaux RK, Tanaka M, Kumar D, Franceschi C, Singh KK. Meeting report: mitochondrial DNA and cancer epidemiology. *Cancer Res* 2007;67:437–439

Verschoor ML, Ungard R, Harbottle A, Jakupciak JP, Parr RL, G. Singh. Mitochondria and cancer: past, present, and future. *BioMed Res Int* 2013;612369

Vives-Bauza C, Tocilescu M, Devries RL, Alessi DM, Jackson-Lewis V, Przedborski S, *et al*. Control of mitochondrial integrity in Parkinson's disease. *Prog Brain Res* 2010; 183:99–113.

Wallace DC, Chalkia D, Mitochondrial DNA Genetics and the Heteroplasmy Conundrum in Evolution and Disease. *Cold Spring Harb Perspect Biol* 2013;5:a021220

Wallace DC. Mitochondria as chi. *Genetics* 2008; 179(2):727–735.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TA, Lezza TM *et al*. Mitochondrial DNA mutation associated with Leber's hereditaryoptic neuropathy. *Science* 1988; 242:1427–1430.

Wang J, Lin F, Guo L, Xiong X, Fan X. Cardiovascular disease, mitochondria, and traditional Chinese medicine. *Evid Based Complement Alternat Med* 2015;143145.

Wang Y, Michikawa Y, Mallidis C, Bai Y, Woodhouse L, Yarasheski KE, *et al*. Muscle–specific mutations accumulate with aging in critical human mtDNA control sites for replication. *Proc Natl Acad Sci USA* 2001;98:4022–4027.

Wardell TM, Ferguson E, Chinnery PF. Changes in the human mitochondrial genome after treatment of malignant disease. *Mutat Res* 2003;525:19–27.

Wong LJ, Scaglia F, Graham BH, Craigen WJ. Current molecular diagnostic algorithm for mitochondrial disorders. *Mol Genet Metab* 2010;100:111–117.

www.mitomap.org

www.mtdb.igp.uu.se

Ye F, Samuels DC, Guo Y. Detection of Mitochondrial Mutations in Cancer by Next Generation Sequencing. *Next Generat Sequenc Applic* 2014;1:102.

Yu M, Zhou Y, Shi Y, Ning L, Yang Y, Wei X, *et al*. Reduced mitochondrial DNA copy number is correlated with tumor progression and prognosis in Chinese breast cancer patients. *IUBMB Life* 2007;59:450–457.