Published in final edited form as:

Adv Exp Med Biol. 2012 ; 942: 39–51. doi:10.1007/978-94-007-2869-1_2.

Physiology and Pathophysiology of Mitochondrial DNA

Hongzhi Li,

Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China

Danhui Liu,

Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China

Jianxin Lu, and

Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China

Yidong Bai, Ph.D.

Zhejiang Provincial Key Laboratory of Medical Genetics, Wenzhou Medical College, Wenzhou, Zhejiang 325035, China. Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Yidong Bai: baiy@uthscsa.edu

Abstract

Mitochondria are the only organelles in animal cells which possess their own genomes. Mitochondrial DNA (mtDNA) alterations have been associated with various human conditions. Yet, their role in pathogenesis remains largely unclear. This review focuses on several major features of mtDNA: (1) mtDNA haplogroup, (2) mtDNA common deletion, (3) mtDNA mutations in the control region or D-loop, (4) mtDNA copy number alterations, (5) mtDNA mutations in translational machinery, (6) mtDNA mutations in protein coding genes (7) mtDNA heteroplasmy. We will also discuss their implications in various human diseases.

Keywords

Mitochondrial DNA mutation; Mitochondrial haplogroup; D-loop region; Common deletion; Heteroplasmy

2.1 Mitochondrial DNA

The mammalian mitochondrial genome is a double-stranded circular DNA of about 16,500 nucleotides. It encodes 13 peptides for the oxidative phosphorylation apparatus, 7 for subunits of complex I (ND1, 2, 3, 4L, 4, 5, 6), 1 for subunit of complex III (cytb), 3 for subunits of complex IV (COI, II, III) and 2 for subunits of complex V (ATP 6 & 8), as well as 22 tRNAs and 2 rRNAs (12S, 16S) which are essential for protein synthesis within

mitochondria (Fig. 2.1). Besides these coding regions, there is a main control region, or D-loop which contains the mtDNA replication origin (O_H) and promoters (P_H and P_L) for mtRNA transcription (Fig. 2.1).

mtDNA is predominantly maternal transmission. Most mammalian cells contain many copies of mitochondrial genomes. mtDNA within a cell could be a mixture of both wild-type and mutant species, a condition called “heteroplasmy”, while “homoplasmy” refers the situation when all mtDNAs are identical. The pathogenic mutations are usually heteroplasmic in nature. It is expected that, due to the multiplicity of mitochondrial genomes in each cell, a threshold of mutant mtDNA must be reached before cellular dysfunction caused by defective mitochondria becomes apparent. Thus mtDNA heteroplasmy is very important in regulating mitochondrial function.

2.2 mtDNA Alterations in Human Diseases

As discussed in previous chapters, mitochondria play important roles in regulating energy production, metabolism, signal transduction and apoptosis, it is not surprising that more and more mtDNA alterations and mitochondrial dysfunctions have been reported in various human diseases. Clinical manifestations that have been related to mtDNA mutations affect the brain, heart, skeletal muscle, kidney, endocrine system and other organs. Specific symptoms include forms of blindness, deafness, dementia, cardiovascular disease, muscle weakness, movement disorders, renal dysfunction, and endocrine disorders. Recently, mutations in both the non-coding and coding regions of the mtDNA have also been identified in almost all types of human cancer (Lu et al. 2009).

2.3 mtDNA Haplogroup

A human mtDNA haplogroup refers to a unique set of mtDNA polymorphisms, reflecting mutations accumulated by a discrete maternal lineage. The haplogroups are associated with region-specific mtDNA sequence variation as a result of genetic drift and/or adaptive selection for an environment-favored mitochondrial function. Difference in redox signaling as a consequence of haplogroup-associated oxidative phosphorylation capacity has been reported (Tanaka et al. 2004; Wallace 2005). Some typical haplogroups and their determination sites in Wenzhou population which we have studied were described as in Fig. 2.2.

The implications of mtDNA haplogroups in various conditions including aging, neurodegenerative diseases, metabolic diseases, infectious diseases and cancer have been explored. For example, haplogroups D4a and D5 were reported to be enriched in centenarians in Japanese population (Bilal et al. 2008), while D4 was found to be increased in female and N9 to be decreased in a Chinese population in Rugao area (Cai et al. 2009). mtSNP at 10398, which is a major diagnostic site of macro-haplogroups M and N, has been implicated not only in longevity, but also in Parkinson's disease and breast cancer, in various populations. The population-specific haplogroup implication was also reported on D4a as it was observed to increase susceptibility in a Chinese population to esophageal carcinoma (Li et al. 2007).

In addition, haplogroup T was associated with coronary artery disease and diabetic retinopathy in Middle European Caucasian populations (Kofler et al. 2009). H5 was reported as a risk factor for late onset Alzheimer's disease for females without APOE genotype (Santoro et al. 2010). The haplogroup cluster IWX was associated with front temporal lobar degeneration (Kruger et al. 2010). Haplogroup K was suggested as an independent determinant of risk of cerebral, but not coronary ischemic vascular events, which provided a new means for the identification of individuals with a high susceptibility of developing ischemic stroke (Chinnery et al. 2010). The LHON patients with the primary mtDNA mutation at 14484T>C had significantly higher frequency of haplogroups C, G, M10 and Y, but a lower frequency of haplogroup F (Yu et al. 2010). Our own study indicated that mitochondrial haplogroups could have a tissue-specific, population-specific and stage-specific role in modulating cancer development (Shen et al. 2010).

2.4 mtDNA Common Deletion

It has been shown that oxidative damaged DNA is especially prone to mispairing of repetitive elements and is correlated with DNA deletions. The uncharacterized DNA repairing machineries may mediate the formation of deleted species, by homologous recombination and non-homologous end-joining (Fukui and Moraes 2009; Vermulst et al. 2008). mtDNA deletions are most likely to occur during repair of damaged mtDNA (Krishnan et al. 2008). More than 100 mtDNA deletions have been reported to be associated with various diseases (<http://www.mitomap.org/>). In fact, large scale deletions were among the first mtDNA mutations identified to cause human diseases. In particular, large scale deletions in individual neurons from the substantia nigra of patients with Parkinson's disease were detected, with various sizes ranging from 1,763bp to 9,445bp within the major arc (Reeve et al. 2008).

Among these deletions, a 4,977-bp deletion (Fig. 2.1) occurring between two 13-bp direct repeats at positions 13,447–13,459 and 8,470–8,482 has attracted tremendous interests since it is the common cause of several sporadic diseases including Pearson's disease(PD), Kearns-Sayre syndrome (KSS), mitochondrial myopathies (MM) and progressive external ophthalmoplegia (PEO) (Sadikovic et al. 2010), and is therefore called the "common" deletion. This deletion also accumulates in many tissues during aging (Schroeder et al. 2008), and has been used as an mtDNA damage biomarker (Meissner et al. 2008). As shown in Fig. 2.1, the common deletion removes all or part of the genes encoding four complex I subunits, one complex IV subunit, two complex V subunits and five tRNA genes, which are indispensable for maintaining normal mitochondrial function. Consequently, the common deletion could lead to energy production catastrophes (Peng et al. 2006). We found that the mtDNA 4,977 bp deletion may play a role in the early stage of colorectal cancer, and it is also implicated in alteration of mtDNA content in cancer cells (Chen et al. 2011).

2.5 mtDNA Mutations in D-Loop

The mtDNA D-loop, is a DNA structure where the two strands of a double-stranded mtDNA molecule are separated and held apart by a third strand of DNA. The third strand has a sequence which is complementary to one of the main strands and pairs with it, thus

displacing the other main strand in the region. The D-loop locates in the main non-coding area, a segment also called the main control region (Fig. 2.1). D-loop is the most variable region in mtDNA. The mutation rate at two hypervariable regions (HV-I, HV-II) in D-loop was estimated 100- to 200-fold that of nuclear DNA (Sharawat et al. 2010). It was suggested that in the D-loop region, a poly-C stretch (poly-C tract) termed the D310 region is more susceptible to oxidative damage and electrophilic attack compared with other regions of mtDNA (Mambo et al. 2003).

mtDNA alterations in D-loop region have been reported as a frequent event in cervical cancer, breast cancer, gastric carcinoma, colorectal cancer, hepatocellular cancer, lung cancer and renal cell carcinoma in the forms of point mutations, insertions, deletions, and mitochondrial microsatellite instability (mtMSI) (Lu et al. 2009). Our own results indicate that mtDNA alterations in D-loop region could happen before tumorigenesis in thyroid, and they might also accumulate during tumorigenesis (Ding et al. 2010). Cancer patients with D-loop mutations (Lievre et al. 2005), or in particular with heteroplasmy of the mtDNA D-loop (CA) (n) polymorphism (Ye et al. 2008) were reported to have significantly poorer prognosis. One large investigation showed three variations in HV-I, namely m.16126T>C, m.16224T>C and m.16311T>C, could serve as a potential prognostic marker in paediatric acute myeloid leukemia (AML) (Sharawat et al. 2010).

D-loop mutations have also been associated with other diseases. For example, T16189C was reported in patients with coronary artery disease (CAD) in a Middle European population (Mueller et al. 2011). The same mutation was also found in European type 2 diabetes patients (Mueller et al. 2011).

2.6 mtDNA Mutations in Translational Machinery

Point mutations in mitochondrial protein synthesis genes (including 2 rRNA and 22 tRNA genes) can result in multisystem disorders with a wide range of symptoms, including deafness, diabetes, mitochondrial myopathy, movement disorders, cardiomyopathy, intestinal dysmotility, dementia, etc (Wallace 2005) (Fig. 2.3).

The tRNA (Lys) A8344G mutation is a classical one which was associated with myoclonic epilepsy and ragged red fiber (MERRF) disease (Fan et al. 2006). Perhaps the most common mtDNA protein synthesis mutation is the A3243G in the tRNA (Leu) gene. The A3243G mutation in the tRNA (Leu) was first associated with mitochondrial encephalomyopathy, lactic acid and stroke-like episodes, mitochondrial encephalomyopathy lactic acidosis and stroke-like syndrome (MELAS). This mutation is remarkable in the variability of its clinical manifestations. When the A3243G mutation is present at relatively low levels (10–30%) in the blood, the patient may manifest only type II diabetes with or without deafness, accounting for 0.5–1% of all type II diabetes worldwide. Interestingly, when the A3243G mutation is present at relatively high levels (>70% of the mtDNAs), it causes more severe symptoms including short stature, cardiomyopathy, chronic progressive external ophthalmoplegia (CPEO), mitochondrial encephalomyopathy, lactic acid and stroke-like episodes (Wallace 2005). Deleterious mutations in the mitochondrial tRNA (Phe) gene may solely manifest with epilepsy when segregating to homoplasmy (Zsurka et al. 2010). In

benign cytochrome c oxidase deficiency myopathy patients, a homoplasmic T14674C or T14674G tRNA (Glu) mutation within tRNA (Glu) gene was identified (Mimaki et al. 2010).

mtDNA mutations, those in the 12S rRNA gene and tRNA genes in particular have been associated with hearing loss (Xing et al. 2007). The A1555G mutation of 12S rRNA gene was a primary contributing factor underlying the development of deafness but not sufficient to produce a clinical phenotype. The T1095C mutation of 12S rRNA gene was suggested to play a role in the phenotypic expression of A1555G mutation, as it disrupted an evolutionarily conserved base pair at a stem-loop structure, resulting in impaired translation in mitochondrial protein synthesis and a significant reduction of cytochrome c oxidase activity (Dai et al. 2008). The alteration of the tertiary or quaternary structure of 12S rRNA by an A827G mutation may play a role in the pathogenesis of hearing loss and aminoglycoside hypersensitivity (Chaig et al. 2008).

2.7 mtDNA Mutations in Protein Coding Genes

All mtDNA-encoded 13 polypeptide are subunits of mitochondrial oxidative phosphorylation complexes, and mutations in these genes can result in an array of clinical manifestations (Wallace 2005) (Fig. 2.4).

The T8993G mutation in ATP6 gene is associated with neurogenic muscle weakness, ataxia, retinitis pigmentosa (NARP) when presents at lower percentages of mutant, and lethal childhood Leigh syndrome when present at higher percentages of mutant (Wallace 2005). Missense and nonsense mutations in the cytb gene have been linked to progressive muscle weakness (Wallace 2005). Rare nonsense or frameshift mutations in COI have been associated with encephalomyopathies (Wallace 2005). Missense mutations in mtDNA encoded complex I subunit genes have been linked to Leigh syndrome, generalized dystonia and deafness, and Leber hereditary optic neuropathy (LHON) (Wallace 2005). Three primary mutations at G3460A of ND1, G11778A of ND4, and T14484C of ND6 account for over 90% of LHON cases in European population (Yu-Wai-Man et al. 2011).

Among mitochondrial genes, ND5 encoded the largest peptide. Mutations in the ND5 gene are also a frequent cause of oxidative phosphorylation disease, especially for those with MELAS- and Leigh-like syndrome with a complex I deficiency (Blok et al. 2007). The sequence variant A13511T occurred in a patient with a Leigh-like syndrome. Mutation G13513A, associated with MELAS and MELAS/Leigh/LHON overlap syndrome, was found in two patients from two different families, one with a MELAS/Leigh phenotype and the other with a MELAS/CPEO phenotype. Mutation G13042A which was detected previously in a patient with a MELAS/MERRF phenotype and in a family with a prevalent ocular phenotype, was also found in a patient with a Leigh-like phenotype. The sequence variant G12622A which was reported once in a control database as a polymorphism, was reported to have a fatal effect in three brothers, all with infantile encephalopathy (Leigh syndrome).

2.8 mtDNA Heteroplasmy

The mtDNA has a relatively high mutation rate, presumably due to its chronic exposure to mitochondrial ROS. When a new mtDNA mutation arises in a cell, a mixed population of mtDNAs is generated, a state known as heteroplasmy. As mtDNA replicates and segregates, the mutant and normal molecules are randomly distributed into the daughter cells. As a consequence, the proportion of mutant and normal mtDNAs can drift toward homoplasmic mutant type or homoplasmic wild type. A threshold is reached when the mutant mtDNA accumulates to a certain level that the normal mitochondrial function can not be sustained.

The regulation of mtDNA heteroplasmy could happen at the replication level. A replicative advantage of the mutant mtDNA molecules was described for mtDNA carrying the mutation associated with the mitochondrial encephalomyopathy (Yoneda et al. 1992). As a result, mutant mtDNA enriched. The regulation could also be achieved by specific turnover of mitochondria. It was reported that long-term overexpression of cytosolic E3 ligase Parkin may signal the selective removal of defective mitochondria within cells. Parkin can eliminate mitochondria with deleterious COXI mutations in heteroplasmic cybrid cells, thereby enriching cells for wild-type mtDNA and restoring cytochrome c oxidase activity (Suen et al. 2010).

Although it had been considered to be homogeneous, one recent study found widespread heteroplasmy in the mtDNA of normal human cells. Moreover, the frequency of heteroplasmic variants varied considerably between different tissues in the same individual. Cancer cells have been reported to harbor both homoplasmic and heteroplasmic mtDNA mutations. These results provide insights into the nature and variability of mtDNA sequences. In particular, they demonstrate that individual humans are characterized by a complex mixture of mitochondrial genotypes rather than a single genotype (He et al. 2010).

mtDNA heteroplasmy has been related to severe inherited syndromes, such as myopathy, encephalopathy, MELAS, neuropathy, ataxia, NARP-MILS, and LHON (Suen et al. 2010).

We recently examined the contribution of heteroplasmic and homoplasmic ND5 mutations in tumorigenesis, the same mutation was previously identified in a human colorectal cancer cell line (Park et al. 2009). With increasing mutant ND5 mtDNA content, respiratory function, including oxygen consumption and ATP generation through oxidative phosphorylation, declined progressively, whereas lactate production and dependence on glucose increased. Both heteroplasmic and homoplasmic mtDNA mutation caused an increased production of mitochondrial ROS. However in cells with heteroplasmic ND5 mutation, the cytosolic ROS level was somewhat reduced, probably due to the upregulation of antioxidant enzymes. As a result, only cells with homoplasmic ND5 mutation exhibited enhanced apoptotic potency. Furthermore, anchorage dependence and tumor-forming capacity of cells carrying wild type and mutant mtDNA were tested by a growth assay in soft agar and subcutaneous implantation of the cells in nude mice. Surprisingly, the cell line carrying the heteroplasmic ND5 mtDNA mutation showed significantly enhanced tumor growth, whereas tumor formation was inhibited for cells with the homoplasmic form of the same mutation.

2.9 mtDNA Copy Number Alterations

Regulation of mitochondrial biogenesis is essential for proper cellular functioning. mtDNA depletion and the resulting mitochondrial malfunction have been implicated in cancer, neurodegeneration, diabetes, aging, and many other human diseases (Clay Montier et al. 2009). The mtDNA control region is believed to play an important role in mtDNA replication. The mutation T16189C at mtDNA D-loop cit was suggested to interfere with the replication process of mtDNA, which in turn decreased the mtDNA copy number and caused mitochondrial dysfunction (Liou et al. 2010). Large deletions in mtDNA control region are rarely found, as they are expected to interfere with the replication of mtDNA. However, a recent report showed a 50-bp deletion and a 154-bp deletion in the human mtDNA control region do not affect the mtDNA copy number, suggesting that the control of mtDNA replication may be more complex than we had thought (Bi et al. 2010).

Alterations in mtDNA content have been reported in increasing numbers of cancer types (Lee and Wei 2009). Low mtDNA content has been reported to be associated with increased risk of renal cancer carcinoma, and a decrease in mtDNA copy number was also found in gastric cancer, breast cancer and hepatocellular carcinoma. On the other hand, an increase in mtDNA copy number was reported in the majority of head and neck cancer, endometrial cancer, ovarian cancer and colorectal cancer (Lee and Wei 2009). Recent reports suggested that mtDNA copy number may be positively associated with subsequent risk of lung cancer (Hosgood et al. 2010) and non-Hodgkin lymphoma (Lan et al. 2008).

The age-related decrease in mtDNA copy number observed in human pancreatic islet preparations may explain the age-dependent decline in pancreatic beta cell insulin secretory capacity (Cree et al. 2008). The mtDNA copy number was reported decreased in Friedreich's ataxia (FRDA) patients. Adriamycin (ADR) is a commonly used chemotherapeutic agent that also produces significant tissue damage. mtDNA mutations and reductions in mtDNA copy number have been identified as contributors to ADR-induced injury (Papeta et al. 2010).

2.10 Pitfalls in mtDNA Studies

As described in previous sections, mtDNA alterations in human diseases have drawn more and more attentions and more and more mtDNA mutations have been associated expanding numbers of human diseases. To avoid flaws in the experimental procedures and interpretation of the data, some cautions are required. Following are some guidelines we have followed in our own research.

The DNA source for sequencing analysis is critical. In cancer study, it's very important to extract DNA from the very samples that used for pathological diagnosis. The percentages of cancer cells are different in different fractions of the cancer tissue. We suggest that one should extract DNA for sequencing analysis from the material on slides used for pathological diagnosis with microdissection technology (Eltoum et al. 2002).

Both positive and negative controls should be included in PCR, especially in cases where nested PCR strategy is utilized. Cautions have been taken according to Kraysberg's

suggestions to avoiding contamination in PCR (Kraytsberg and Khrapko 2005). It is important to include a mtDNA-less rho zero cell as a control to avoid amplification of mitochondrial pseudogenes in nuclear genome (Yao et al. 2008).

Artificially generated phantom mutations through the sequencing and editing process have been found in some reports (Bandelt et al. 2007). One should be very careful about phantom mutations when only one strand of mtDNA is analyzed.

Another important issue is that one should be very careful when they conclude that mtDNA mutations they found are novel and/or pathogenic. In fact, many reported 'novel' mtDNA mutations turned out to be having been reported previously, and some previously reported 'pathogenic' mutations are generally considered to be polymorphic variants now. MITOMAP is widely used for deciding if the mtDNA alteration is novel for it is a convenient source for the information required. But it may be misleading if the user was not aware of its limitations and did not perform multiple searches throughout the published record. We suggest other databases such as mtDB (<http://www.genpat.uu.se/mtDB>), mtDNA (<http://www.ianlogan.co.uk/mtdna.htm>), FBI mtDNA population database (<http://www.fbi.gov/hq/lab/fsc/backissu/april2002/miller1.htm>) or GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) should be included in data analysis. A straightforward Google or Yahoo search can help to define the "novelty" of mtDNA polymorphisms. The other useful advice is to identify potential pathogenic mutations with phylogenetic methods (Fang et al. 2009).

2.11 Experimental Approaches to Study mtDNA

Over the years, a set of unique experimental approaches to study of mitochondrial function has been established, and these tools are very useful in investigating physiology and pathology of mtDNA.

(1) Labeling mitochondrial proteins

Based on the difference in sensitivity to certain antibiotics, we can selectively label the mtDNA encoded proteins (Bai et al. 2004). In particular, mitochondrial proteins are labeled with [³⁵S] methionine in the presence of emetine, to inhibit cytoplasmic protein synthesis. The labeled products are then electrophoresed through SDS-polyacrylamide gradient gel. The electrophoretic patterns between wild-type and mutant cell lines could indicate the possible mutation site on mtDNA as shown in our studies in identification of mouse ND5 and ND6 mutation (Bai and Attardi 1998; Bai et al. 2000).

(2) Generation of cytoplasmic hybrid (cybrid)

Because mitochondria are under dual genetic control, nuclear and mitochondrial, mutations in either genome could potentially cause mitochondrial dysfunctions. To verify that a mutation in mtDNA is solely responsible for the respiration deficiency observed, an mtDNA-less (ρ^0) cell repopulation approach has been established (King and Attardi 1988). For example, mtDNAs carrying a mutation in the ND5 or ND6 gene were transferred to mouse ρ^0 cells by cytoplasm-cell fusion, thus placing those mutant genomes in a new nuclear

background. The co-segregation of mtDNA with the mitochondrial dysfunction usually indicates a pathogenic role of mutation residing on these mtDNA (Bai et al. 2004).

(3) Generation of trans-mitochondrial mice

Up to now, two procedures have been successfully established in introducing exogenous mtDNA mutations into the mouse female germline: (a) to fuse cytoplasts from mutant cells directly to mouse single-cell embryos and then to implant the embryos into the oviduct of pseudo-pregnant females, and (b) to fuse enucleated cell cytoplasts bearing mutant mtDNA to undifferentiated female mouse embryonic stem (ES) cells, and then to inject the stem cell cybrids into mouse blastocysts, and to implant the chimeric embryos into a foster mother (Wallace and Fan 2009). The former method was used to create mice harboring a heteroplasmic mtDNA deletion (Nakada et al. 2008), while the latter has permitted the creation of variety of mouse strains bearing heteroplasmic or homoplasmic mtDNA point mutations (Fan et al. 2008).

2.12 Perspectives

So far forward genetics has made important contributions in our understanding of the putative role of mtDNA mutations in various human diseases. It is well-established that accumulation of mtDNA mutations is associated with a wide variety of diseases. However, there is still no convincing evidence to explain whether accumulation of these pathogenic mutant mtDNAs in tissues is responsible for the expressions of various clinical phenotypes.

To complement, reverse genetic study is required to provide model systems for studying exactly how pathogenic mutant mtDNAs are transmitted and distributed in tissues and result in the pathogenesis of mitochondrial diseases with various clinical phenotypes. However, so far we are still facing serious technical challenges to perform such experiments. First there are no procedures available for introducing mutagenized mammalian whole mtDNA genome into mitochondria in living cells or even into isolated mitochondria (Nakada et al. 2008). The investigation of the effects of mtDNA mutations have lagged behind due to lack of effective technologies to modify the mammalian mtDNA.

We also need a better system to investigate the role of nuclear modifiers in manifestation of tissue-specific pathogenesis of various mtDNA mutations. The recently developed iPS systems might provide some hopes in this regard.

Finally animal models carrying the corresponding mtDNA mutations identified in human diseases would be great assets in understanding the molecular pathways mediating the clinical phenotypes, and the generation of such animals will open new ways to develop therapeutic approaches.

Acknowledgments

The work in the laboratory of the authors has been supported by NIH (R01 AG025223) and Chinese National Science Foundation (31070765/C050605, and 810004611/H1409).

References

- Bai Y, Attardi G. The mtDNA-encoded ND6 subunit of mitochondrial NADH dehydrogenase is essential for the assembly of the membrane arm and the respiratory function of the enzyme. *EMBO J*. 1998; 17:4848–4858. [PubMed: 9707444]
- Bai Y, Shakeley RM, Attardi G. Tight control of respiration by NADH dehydrogenase ND5 subunit gene expression in mouse mitochondria. *Mol Cell Biol*. 2000; 20:805–815. [PubMed: 10629037]
- Bai Y, Hu P, Park JS, Deng JH, Song X, Chomyn A, Yagi T, Attardi G. Genetic and functional analysis of mitochondrial DNA-encoded complex I genes. *Ann N Y Acad Sci*. 2004; 1011:272–283. [PubMed: 15126303]
- Bandelt HJ, Yao YG, Salas A, Kivisild T, Bravi CM. High penetrance of sequencing errors and interpretative shortcomings in mtDNA sequence analysis of LHON patients. *Biochem Biophys Res Commun*. 2007; 352:283–291. [PubMed: 17123466]
- Bi R, Zhang AM, Zhang W, Kong QP, Wu BL, Yang XH, Wang D, Zou Y, Zhang YP, Yao YG. The acquisition of an inheritable 50-bp deletion in the human mtDNA control region does not affect the mtDNA copy number in peripheral blood cells. *Hum Mutat*. 2010; 31:538–543. [PubMed: 20151402]
- Bilal E, Rabadan R, Alexe G, Fuku N, Ueno H, Nishigaki Y, Fujita Y, Ito M, Arai Y, Hirose N, Ruckenstein A, Bhanot G, Tanaka M. Mitochondrial DNA haplogroup D4a is a marker for extreme longevity in Japan. *PLoS One*. 2008; 3:e2421. [PubMed: 18545700]
- Blok MJ, Spruijt L, de Coo IF, Schoonderwoerd K, Hendrickx A, Smeets HJ. Mutations in the ND5 subunit of complex I of the mitochondrial DNA are a frequent cause of oxidative phosphorylation disease. *J Med Genet*. 2007; 44:e74. [PubMed: 17400793]
- Cai XY, Wang XF, Li SL, Qian J, Qian DG, Chen F, Yang YJ, Yuan ZY, Xu J, Bai Y, Yu SZ, Jin L. Association of mitochondrial DNA haplogroups with exceptional longevity in a Chinese population. *PLoS One*. 2009; 4:e6423. [PubMed: 19641616]
- Chaig MR, Zernotti ME, Soria NW, Romero OF, Romero MF, Gerez NM. A mutation in mitochondrial 12S rRNA, A827G, in Argentinean family with hearing loss after aminoglycoside treatment. *Biochem Biophys Res Commun*. 2008; 368:631–636. [PubMed: 18261986]
- Chen T, He J, Shen L, Fang H, Nie H, Jin T, Wei X, Xin Y, Jiang Y, Li H, Chen G, Lu J, Bai Y. The mitochondrial DNA 4,977-bp deletion and its implication in copy number alteration in colorectal cancer. *BMC Med Genet*. 2011; 12:8. [PubMed: 21232124]
- Chinnery PF, Elliott HR, Syed A, Rothwell PM. Mitochondrial DNA haplogroups and risk of transient ischaemic attack and ischaemic stroke: a genetic association study. *Lancet Neurol*. 2010; 9:498–503. [PubMed: 20362514]
- Clay Montier LL, Deng JJ, Bai Y. Number matters: control of mammalian mitochondrial DNA copy number. *J Genet Genomics*. 2009; 36:125–131. [PubMed: 19302968]
- Cree LM, Patel SK, Pyle A, Lynn S, Turnbull DM, Chinnery PF, Walker M. Age-related decline in mitochondrial DNA copy number in isolated human pancreatic islets. *Diabetologia*. 2008; 51:1440–1443. [PubMed: 18528676]
- Dai D, Lu Y, Chen Z, Wei Q, Cao X, Xing G. Co-segregation of the T1095C with the A1555G mutation of the mitochondrial 12S rRNA gene in a patient with non-syndromic hearing loss. *Biochem Biophys Res Commun*. 2008; 377:1152–1155. [PubMed: 18983818]
- Ding Z, Ji J, Chen G, Fang H, Yan S, Shen L, Wei J, Yang K, Lu J, Bai Y. Analysis of mitochondrial DNA mutations in D-loop region in thyroid lesions. *Biochim Biophys Acta*. 2010; 1800:271–274. [PubMed: 19463899]
- Eltoum IA, Siegal GP, Frost AR. Microdissection of histologic sections: past, present, and future. *Adv Anat Pathol*. 2002; 9:316–322. [PubMed: 12195221]
- Fan H, Civalier C, Booker JK, Gulley ML, Prior TW, Farber RA. Detection of common disease-causing mutations in mitochondrial DNA (mitochondrial encephalomyopathy, lactic acidosis with stroke-like episodes MTTL1 3243 A > G and myoclonic epilepsy associated with ragged-red fibers MTTK 8344A > G) by real-time polymerase chain reaction. *J Mol Diagn*. 2006; 8:277–281. [PubMed: 16645216]

- Fan W, Waymire KG, Narula N, Li P, Rocher C, Coskun PE, Vannan MA, Narula J, Macgregor GR, Wallace DC. A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science*. 2008; 319:958–962. [PubMed: 18276892]
- Fang H, Lu J, Wei J, Shen LJ, Ding Z, Li H, Bai Y. Mitochondrial DNA mutations in the D-loop region may not be frequent in cervical cancer: a discussion on pitfalls in mitochondrial DNA studies. *J Cancer Res Clin Oncol*. 2009; 135:649–651. [PubMed: 19142663]
- Fukui H, Moraes CT. Mechanisms of formation and accumulation of mitochondrial DNA deletions in aging neurons. *Hum Mol Genet*. 2009; 18:1028–1036. [PubMed: 19095717]
- He Y, Wu J, Dressman DC, Iacobuzio-Donahue C, Markowitz SD, Velculescu VE, Diaz LA JR, Kinzler KW, Vogelstein B, Papadopoulos N. Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature*. 2010; 464:610–614. [PubMed: 20200521]
- Hosgood HD 3rd, Liu CS, Rothman N, Weinstein SJ, Bonner MR, Shen M, Lim U, Virtamo J, Cheng WL, Albanes D, Lan Q. Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. *Carcinogenesis*. 2010; 31:847–849. [PubMed: 20176654]
- King MP, Attardi G. Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA. *Cell*. 1988; 52:811–819. [PubMed: 3349520]
- Kofler B, Mueller EE, Eder W, Stanger O, Maier R, Weger M, Haas A, Winker R, Schmut O, Paulweber B, Iglseder B, Renner W, Wiesbauer M, Aigner I, Santic D, Zimmermann FA, Mayr JA, Sperl W. Mitochondrial DNA haplogroup T is associated with coronary artery disease and diabetic retinopathy: a case control study. *BMC Med Genet*. 2009; 10:35. [PubMed: 19383124]
- Kraysberg Y, Khrapko K. Single-molecule PCR: an artifact-free PCR approach for the analysis of somatic mutations. *Expert Rev Mol Diagn*. 2005; 5:809–815. [PubMed: 16149882]
- Krishnan KJ, Reeve AK, Samuels DC, Chinnery PF, Blackwood JK, Taylor RW, Wanrooij S, Spelbrink JN, Lightowlers RN, Turnbull DM. What causes mitochondrial DNA deletions in human cells? *Nat Genet*. 2008; 40:275–279. [PubMed: 18305478]
- Kruger J, Hinttala R, Majamaa K, Remes AM. Mitochondrial DNA haplogroups in early-onset Alzheimer's disease and frontotemporal lobar degeneration. *Mol Neurodegen*. 2010; 5:8.
- Lan Q, Lim U, Liu CS, Weinstein SJ, Chanock S, Bonner MR, Virtamo J, Albanes D, Rothman N. A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Blood*. 2008; 112:4247–4249. [PubMed: 18711000]
- Lee HC, Wei YH. Mitochondrial DNA instability and metabolic shift in human cancers. *Int J Mol Sci*. 2009; 10:674–701. [PubMed: 19333428]
- Li XY, Su M, Huang HH, Li H, Tian DP, Gao YX. mtDNA evidence: genetic background associated with related populations at high risk for esophageal cancer between Chaoshan and Taihang Mountain areas in China. *Genomics*. 2007; 90:474–481. [PubMed: 17689918]
- Lievre A, Chapusot C, Bouvier AM, Zinzindohoue F, Piard F, Roignot P, Arnould L, Beaune P, Faivre J, Laurent-Puig P. Clinical value of mitochondrial mutations in colorectal cancer. *J Clin Oncol*. 2005; 23:3517–3525. [PubMed: 15908662]
- Liou CW, Lin TK, Chen JB, Tiao MM, Weng SW, Chen SD, Chuang YC, Chuang JH, Wang PW. Association between a common mitochondrial DNA D-loop polycytosine variant and alteration of mitochondrial copy number in human peripheral blood cells. *J Med Genet*. 2010; 47:723–728. [PubMed: 20837494]
- Lu J, Sharma LK, Bai Y. Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. *Cell Res*. 2009; 19:802–815. [PubMed: 19532122]
- Mambo E, Gao X, Cohen Y, Guo Z, Talalay P, Sidransky D. Electrophile and oxidant damage of mitochondrial DNA leading to rapid evolution of homoplasmic mutations. *Proc Natl Acad Sci USA*. 2003; 100:1838–1843. [PubMed: 12578990]
- Meissner C, Bruse P, Mohamed SA, Schulz A, Warnk H, Storm T, Oehmichen M. The 4977 bp deletion of mitochondrial DNA in human skeletal muscle, heart and different areas of the brain: a useful biomarker or more? *Exp Gerontol*. 2008; 43:645–652. [PubMed: 18439778]
- Mimaki M, Hatakeyama H, Komaki H, Yokoyama M, Arai H, Kirino Y, Suzuki T, Nishino I, Nonaka I, Goto Y. Reversible infantile respiratory chain deficiency: a clinical and molecular study. *Ann Neurol*. 2010; 68:845–854. [PubMed: 21194154]

- Mueller EE, Eder W, Ebner S, Schwaiger E, Santic D, Kreindl T, Stanger O, Paulweber B, Iglseder B, Oberkofler H, Maier R, Mayr JA, Krempler F, Weitgasser R, Patsch W, Sperl W, Kofler B. The Mitochondrial T16189C Polymorphism Is Associated with Coronary Artery Disease in Middle European Populations. *PLoS One*. 2011; 6:e16455. [PubMed: 21298061]
- Nakada K, Sato A, Hayashi J. Reverse genetic studies of mitochondrial DNA-based diseases using a mouse model. *Proc Jpn Acad Ser B Phys Biol Sci*. 2008; 84:155–165.
- Papeta N, Zheng Z, Schon EA, Brosel S, Altintas MM, Nasr SH, Reiser J, D'Agati VD, Gharavi AG. Prkdc participates in mitochondrial genome maintenance and prevents Adriamycin-induced nephropathy in mice. *J Clin Invest*. 2010; 120:4055–4064. [PubMed: 20978358]
- Park JS, Sharma LK, Li H, Xiang R, Holstein D, Wu J, Lechleiter J, Naylor SL, Deng JJ, Lu J, Bai Y. A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. *Hum Mol Genet*. 2009; 18:1578–1589. [PubMed: 19208652]
- Peng TI, Yu PR, Chen JY, Wang HL, Wu HY, Wei YH, Jou MJ. Visualizing common deletion of mitochondrial DNA-augmented mitochondrial reactive oxygen species generation and apoptosis upon oxidative stress. *Biochim Biophys Acta*. 2006; 1762:241–255. [PubMed: 16368227]
- Reeve AK, Krishnan KJ, Elson JL, Morris CM, Bender A, Lightowlers RN, Turnbull DM. Nature of mitochondrial DNA deletions in substantia nigra neurons. *Am J Hum Genet*. 2008; 82:228–235. [PubMed: 18179904]
- Sadikovic B, Wang J, El-Hattab A, Landsverk M, Douglas G, Brundage EK, Craigen WJ, Schmitt ES, Wong LJ. Sequence homology at the breakpoint and clinical phenotype of mitochondrial DNA deletion syndromes. *PLoS One*. 2010; 5:e15687. [PubMed: 21187929]
- Santoro A, Balbi V, Balducci E, Pirazzini C, Rosini F, Tavano F, Achilli A, Siviero P, Minicuci N, Bellavista E, Mishto M, Salvioli S, Marchegiani F, Cardelli M, Olivieri F, Nacmias B, Chiamanti AM, Benussi L, Ghidoni R, Rose G, Gabelli C, Binetti G, Sorbi S, Crepaldi G, Passarino G, Torroni A, Franceschi C. Evidence for sub-haplogroup h5 of mitochondrial DNA as a risk factor for late onset Alzheimer's disease. *PLoS One*. 2010; 5:e12037. [PubMed: 20700462]
- Schroeder P, Gremmel T, Berneburg M, Krutmann J. Partial depletion of mitochondrial DNA from human skin fibroblasts induces a gene expression profile reminiscent of photoaged skin. *J Invest Dermatol*. 2008; 128:2297–2303. [PubMed: 18337828]
- Sharawat SK, Bakhshi R, Vishnubhatla S, Bakhshi S. Mitochondrial D-loop variations in paediatric acute myeloid leukaemia: a potential prognostic marker. *Br J Haematol*. 2010; 149:391–398. [PubMed: 20230407]
- Shen L, Wei J, Chen T, He J, Qu J, He X, Jiang L, Qu Y, Fang H, Chen G, Lu J, Bai Y. Evaluating mitochondrial DNA in patients with breast cancer and benign breast disease. *J Cancer Res Clin Oncol*. 2010; 137:669–675. [PubMed: 20552226]
- Suen DF, Narendra DP, Tanaka A, Manfredi G, Youle RJ. Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic hybrid cells. *Proc Natl Acad Sci USA*. 2010; 107:11835–11840. [PubMed: 20547844]
- Tanaka M, Takeyasu T, Fuku N, Li-Jun G, Kurata M. Mitochondrial genome single nucleotide polymorphisms and their phenotypes in the Japanese. *Ann N Y Acad Sci*. 2004; 1011:7–20. [PubMed: 15126279]
- Vermulst M, Wanagat J, Kujoth GC, Bielas JH, Rabinovitch PS, Prolla TA, Loeb LA. DNA deletions and clonal mutations drive premature aging in mitochondrial mutator mice. *Nat Genet*. 2008; 40:392–394. [PubMed: 18311139]
- Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 2005; 39:359–407. [PubMed: 16285865]
- Wallace DC, Fan W. The pathophysiology of mitochondrial disease as modeled in the mouse. *Genes Dev*. 2009; 23:1714–1736. [PubMed: 19651984]
- Xing G, Chen Z, Cao X. Mitochondrial rRNA and tRNA and hearing function. *Cell Res*. 2007; 17:227–239. [PubMed: 17199108]
- Yao YG, Kong QP, Salas A, Bandelt HJ. Pseudomitochondrial genome haunts disease studies. *J Med Genet*. 2008; 45:769–772. [PubMed: 18611982]

- Ye C, Gao YT, Wen W, Breyer JP, Shu XO, Smith JR, Zheng W, Cai Q. Association of mitochondrial DNA displacement loop (CA)_n dinucleotide repeat polymorphism with breast cancer risk and survival among Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2008; 17:2117–2122. [PubMed: 18708405]
- Yoneda M, Chomyn A, Martinuzzi A, Hurko O, Attardi G. Marked replicative advantage of human mtDNA carrying a point mutation that causes the MELAS encephalomyopathy. *Proc Natl Acad Sci USA.* 1992; 89:11164–11168. [PubMed: 1454794]
- Yu D, Jia X, Zhang AM, Li S, Zou Y, Zhang Q, Yao YG. Mitochondrial DNA sequence variation and haplogroup distribution in Chinese patients with LHON and m.14484 T > C. *PLoS One.* 2010; 5:e13426. [PubMed: 20976138]
- Yu-Wai-man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies – disease mechanisms and therapeutic strategies. *Prog Retin Eye Res.* 2011; 30:81–114. [PubMed: 21112411]
- Zsurka G, Hampel KG, Nelson I, Jardel C, Mirandola SR, Sassen R, Kornblum C, Marcorelles P, Lavoue S, Lombes A, Kunz WS. Severe epilepsy as the major symptom of new mutations in the mitochondrial tRNA(Phe) gene. *Neurology.* 2010; 74:507–512. [PubMed: 20142618]

Fig. 2.1. Human mitochondrial genome. It encodes 13 peptides, 22 tRNA and 2 rRNA. The D-loop contains mtDNA replication origin and transcription promoters. The 4,977-bp common deletion is indicated

Fig. 2.2. Classification of mtDNA haplogroups in Wenzhou population. The defining sites utilized in this study are listed in the branches. “d” indicates a deletion; “9bpd” indicates a 9bp deletion in the intergenic mtDNA region between nucleotides 8,195–8,316

Fig. 2.3.

mtDNA tRNA/rRNA mutation map. *ADPD* Alzheimer's Disease and Parkinson's Disease, *CIPO* Chronic Intestinal Pseudoobstruction with myopathy and Ophthalmoplegia, *CPEO* Chronic Progressive External Ophthalmoplegia, *DEMCHO* Dementia and Chorea, *DM* Diabetes Mellitus, *DMDF* Diabetes Mellitus & Deafness, *EXIT* exercise intolerance, *FICP* Fatal Infantile Cardiomyopathy Plus, a MELAS-associated cardiomyopathy, *HCM* Hypertrophic Cardio Myopathy, *LS* Leigh Syndrome, *MELAS* Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes, *MERRF* Myoclonic Epilepsy and Ragged Red Muscle Fibers, *MHCM* Maternally Inherited Hypertrophic Cardiomyopathy, *MICM* Maternally Inherited Cardiomyopathy, *MM* Mitochondrial Myopathy, *SNHL* Sensorineural Hearing Loss

Fig. 2.4.

mtDNA coding region mutation map. *AD* Alzheimer's Disease, *ADPD* Alzheimer's Disease and Parkinson's Disease, *CPEO* Chronic Progressive External Ophthalmoplegia, *DM* Diabetes Mellitus, *EXIT* exercise intolerance, *FBSN* Familial Bilateral Striatal Necrosis, *HCM* Hypertrophic Cardio Myopathy, *LHON* Leber Hereditary Optic Neuropathy, *LS* Leigh Syndrome, *MELAS* Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes, *MM* Mitochondrial Myopathy, *NAION* Nonarteritic Anterior Ischemic Optic Neuropathy, *NARP* Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa, *SIDA* Sideroblastic Anemia, *SNHL* Sensorineural Hearing Loss