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A Mitochondrial Perspective on Human Origins and Disease**

Western medicine is based on an anatomical perspective of disease and on a Mendelian perspective of genetics. Yet these concepts have failed to provide a compelling understanding of the complexities of the common diseases. Clearly, something has been missing from our understanding for both disease and heredity [1-5].

At the beginning of the 1970's, I reasoned that defects in energy metabolism could also cause clinical problems, that the mitochondria were the source on most cellular energy, and that the newly identified mitochondrial DNA, mtDNA, could mutate and give rise to clinical manifestations. I then set out to define the genetics of the mtDNA. My colleagues and I were the first to demonstrate that the human and mammalian mtDNAs code for heritable traits by inventing the transmitochondrial cybrid system in cultured cells [6,7]. We then used this somatic cell genetic system to explore the implications of intracellular mixtures of mutant and normal mtDNAs (heteroplasmy) and to demonstrate the quantitative threshold expression parameters of mtDNA mutant alleles [8-13]. We helped to define the energetic function of the mtDNA genes [14-17], cloned and characterized critical nuclear DNA (nDNA) coded mitochondrial genes including the adenine nucleotide translocator (ANT) and the ATP synthase subunit [18-25], demonstrated the extraordinarily high sequence evolution rate and diversity of the mtDNA [18], and showed the close cooperation

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between the mtDNA and nuclear DNA (nDNA) in shaping cellular phenotypes [26-30]. With these basic science insights, I moved into clinical genetics. We were the first to demonstrate in 1980 the maternal inheritance of the human mtDNA [31, 32], report the surprisingly high level of sequence variation in the mtDNA [33], and show that mtDNA variation correlated with the continental origins of indigenous peoples [33].

Because of the strict maternal inheritance of the mtDNA, I reasoned that it could only evolve by the sequential accumulation of mutations on radiating maternal lineages. Because of its high mutation rate, I argued there should be sufficient variation within human populations to differentiate them and to link them through a mtDNA mutational tree. We then set out to survey the sequence variation of indigenous peoples from around the world [34-41]. This revealed that indigenous populations have their own distinct mtDNA sequences all of which could be linked together in a global sequential mtDNA mutational tree [42-44]. Moreover, important geographic transitions were associated with the appearance of functionally important mtDNA variants that founded the next regional group of descendant mtDNA haplotypes, known as haplogroups [44-46]. By calculating the mtDNA sequence evolution rate [47], we showed that women left Africa 65,000 years ago, with only two African mtDNAs successfully colonizing the rest of the world [44, 46]. Similarly, only three mtDNAs left Siberia 20,000 years ago to become the first Native Americans [41]. From these and related observations, we reasoned that the founding mtDNA haplogroup mutations must have changed individual energetic physiology thus permitting these individuals to adapt to new environments [2, 3, 48]. Therefore, mtDNA variants are the adaptive engine for human radiation. However, a mtDNA variant that is adaptive in one environment may be maladaptive in another [49]. This has proven to be the case by ours and others demonstration that different mtDNA haplogroups correlate with predisposition to a wide spectrum of metabolic and degenerative diseases, cancer, and aging [48, 50-52].

By defining the genetics of the mtDNA in the 1970s and 1980s, we could begin the search for mtDNA diseases. With the demonstration of the maternal inheritance of the mtDNA, Mendelian geneticists erroneously assumed that all maternal descendants should be affected. Since that was not seen, it was argued that mtDNA diseases did not exist. However, I knew from my 1970s research on heteroplasmy and threshold expression that variable expressivity of mtDNA disease mutations would be the norm. By using a more integrated understanding of mtDNA genetics, I was able to rapidly identify multiple pedigrees of potential mtDNA origin starting in the late 1970s [53, 54]. As molecular sequencing tools became available, I chose to concentrate on the mtDNAs of Leber Hereditary Optic Neuropathy (LHON) and Myoclonic Epilepsy and Ragged Red Fiber (MERRF) disease. This led to three seminal papers, the publication in *Science* in 1988 that LHON was caused by a mtDNA missense mutation (*ND4* nucleotide (nt) 11778 G>A arginine 340 to histidine (R340H)) [55] and the combined reports in *Cell* in 1988 and 1990 that MERRF is

caused by a mtDNA $tRNA^{Lys}$ nt 8344 A>G mutation [56, 57]. In addition to neurological diseases we also demonstrated the importance of mtDNA variation in cancer [52, 58, 59].

Analysis of MERRF pedigrees revealed to me that both the mtDNA 8344G heteroplasmy level plus the age of the patient determined the severity of the phenotype. This led to an extensive survey of the accumulation of mtDNA mutations in somatic human and mouse tissues. This showed that the accumulation of mtDNA mutations in somatic tissues correlated with aging [60] and that an array of age-related, «spontaneous», diseases, including cardiac [61] and neurodegenerative diseases [62-66] harbored high levels of somatic mtDNA mutations. Hence, while the predisposition to a disease was the result of the inheritance of haplogroup variants or new deleterious mutations, the delayed onset and progressive course was explained by the age-related accumulation of somatic mtDNA mutations that augment the inherited mitochondrial defects [3].

Since our studies in the late 1980s and early 1990s, hundreds of mtDNA mutations have been associated with a broad spectrum of clinical phenotypes from diabetes and obesity [67, 68], to blindness and deafness [69], to myopathy and cardiomyopathy [70], to epilepsy and Alzheimer disease [71]. In the 1980s, I started an information service to catalogue known mtDNA variation. This evolved into our curated, on-line, website, MITOMAP.org. MITOMAP catalogues currently contain 32,059 whole mtDNA sequences encompassing >12,000 variants, plus 658 putative mtDNA disease mutations, 333 occurring in polypeptide genes and 323 occurring in tRNAs & rRNAs genes. MITOMAP is the global authoritative source of mtDNA information and was accessed in the 2016-20167 year 109,547 times [72].

While the avalanche of mtDNA mutations associated with disease was being reported in the 1990s, the general belief in the medical genetics community remained that the mitochondrial dysfunction observed in common diseases was secondary to as yet unidentified nuclear gene defects. To counter this Mendelian bias, I set out in 1990 to introduce mitochondrial gene mutations into the mouse. We initially genetically inactivated the mouse nDNA heart-muscle-brain ANT isoform gene (*Ant1*) and showed that this resulted in myopathy and cardiomyopathy [73-75]. We also reported a 13 generation human pedigree with a null *ANT1* mutation with myopathy and cardiomyopathy, the severity of the cardiomyopathy being determined by the background mtDNA haplogroup [76]. Additional studies on the ANT1-deficient mouse revealed that partial energetic defects in the brain impair cortical interneuron migration resulting in autism endophenotypes [77]. Systemic and tissue specific knockouts of the mouse *Ant2* gene altered the mitochondrial permeability transition pore (mtPTP) [78] and impaired cardiac development [79]. We also inactivated the various nDNA-coded mitochondrial antioxidant genes and demonstrated their effects on myopathy, cardiomyopathy, neurodegeneration, and aging [80-84]. Our group also collaborated in creating a mitochondrially targeted catalase (mCAT) gene and showing that mCAT mice have reduced mtDNA mutations and extended lifespan [85] thus proving that somatic mtDNA mutations are the aging clock.

I also wanted to prove conclusively that mtDNA mutations could cause disease. Therefore, we set out to develop a procedure for introducing mtDNA mutations into the mouse female germline. First, we developed methods for generating clinically relevant mtDNA mutations in cultured mouse cells [86]. Next, we identified and characterized a mouse female embryonic stem cell line (mfESC), enucleated the mtDNA mutant mouse cell lines and fused the mitochondria-containing cytoplasts to the mfESCs creating mfESC transmitochondrial cybrids. The mtDNA mutant mfESCs were injected into mouse blastocysts, the female chimeras bred, and female mice harboring the mutant mtDNAs identified [87]. These mice immediately showed that single mtDNA base substitutions were sufficient by themselves to impart a wide range of common disease phenotypes from diabetes, to cardiomyopathy, to neurodegeneration [88-90]. Since the mtDNA only codes for energy genes, these experiments proved beyond doubt that bioenergetic defects can cause the full range of metabolic and degenerative diseases.

The creation of mice heteroplasmic for severe mutations led to an extraordinary discovery. Severe mtDNA mutations are rapidly and directionally eliminated from the female germline, often within four maternal generations [89]. Hence, the female germline has a mechanism for detecting and eliminating the most severe mtDNA mutations prior to development. This means that the mtDNA can have a very high germline mutation rate, without imparting crippling genetic loads. The high mtDNA mutation rate can thus rapidly generate extensive sequence and physiological variation, providing the evolutionary diversity necessary to permit individuals and thus populations to adapt to environmental challenges. This mtDNA adaptive system now fills a critical gap in neo-Darwinian evolutionary theory. Previous theory postulated that the accumulation of nDNA genetic variation permits adaptation to new environments leading to speciation. The problem is that there is no mechanism for a species to survive in a marginal environment long enough for sufficient nDNA anatomical gene mutations to accumulate to permit the switch a more prevalent energy source in the new environment. By permitting species to rapidly adjust their physiology to survive in marginal environments for prolonged periods, the mtDNA fills this gap [48, 92].

To prove that mtDNA variation is sufficiently physiologically robust to permit meaningful phenotypic changes, we created mice in which we mixed two normal but different mtDNAs, thus obviating uniparental inheritance. These mice appeared normal, but proved to have marked behavioral and learning defects [92]. Hence, mtDNA haplogroups are physiologically relevant and maternal inheritance is required to avoid their mixing.

While mitochondrial genetics and mitochondrial medicine are what our laboratory is best known for, we have also made exciting observations on the biophysics of the mitochondrion. In the 1960s, Peter Mitchell proposed the chemi-osmotic theory for mitochondrial oxidative phosphorylation (OXPHOS) [93]. In his model, the electron transport chain arrayed within the mitochondrial inner membrane burns calo-

ries with oxygen and traps the energy by pumping protons out across the mitochondrial inner membrane to create a capacitor. This capacitance is utilized by the ATP synthase to generate ATP. The problem with Mitchell's conceptualization is that the protons on the outside of the mitochondrial inner membrane are in direct contact with the huge buffering capacity of the cytosol through the porin-containing mitochondrial outer membrane. Combining ultrastructural and cell biological studies with collaborations with electrical engineers, we used microfluidic and microelectronic systems to readdress this model. We discovered that the mitochondrial inner membrane is involuted into closed chambers we call "cristae lumens." The electron transport chain pumps protons into these chambers where the charge is isolated and concentrated [94]. We found that the cristae within and between mitochondria are aligned indicating that the highly charged cristae and mitochondria interact with each other, probably electromagnetically [27]. This is particularly important for the high energy tissues such as the heart, muscle, and brain. This new concept of mitochondrial bioenergetics is permitting me to redraw our understanding of mitochondrial metabolism and regulation of apoptosis and thus to redefine the physics of life and death.

Because I have unwaveringly argued that bioenergetic defects can cause disease and that non-Mendelian genetics can play an important role in human genetics, I have regularly been at odds with the entrenched anatomical and Mendelian paradigms of Western medicine. By contrast, my ideas have offered powerful new insights and empirical procedures that resonate with the more holistic Eastern medical concept of Qi, or vital force [95] It is my hope that the genetics, physiology, physics, and medicine of the mitochondrion can provide a bridge between Eastern and Western medicine, thus providing a new synthesis that could transform our understanding of human biology and our approaches to human health and disease.

REFERENCES

[1] Wallace D.C., (2011). Bioenergetic origins of complexity and disease. *Cold Spring Harb. Symp. Quant. Biol.* 76(Metabolism and Disease):1-16.

[2] Wallace D.C., (2013). Bioenergetics in human evolution and disease: Implications for the origins of biological complexity and the missing genetic variation of common diseases. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 368(1622):20120267.

[3] Wallace D.C., (2013). Mitochondrial bioenergetic etiology of disease. *J. Clin. Invest.* 123(4):1405-1412.

[4] Wallace D.C., (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39:359-407.

[5] Wallace D.C., (2007). Why do we have a maternally inherited mitochondrial DNA? Insights from Evolutionary Medicine. *Annu. Rev. Biochem.* 76:781-821.

[6] Bunn C.L., Wallace D.C., & Eisenstadt J.M., (1974). Cytoplasmic inheritance of chloramphenicol resistance in mouse tissue culture cells. *Proc. Natl. Acad. Sci. USA* 71(5):1681-1685.

[7] Wallace D.C., Bunn C.L., & Eisenstadt J.M., (1975). Cytoplasmic transfer of chloramphenicol resistance in human tissue culture cells. *J. Cell Biol.* 67(1):174-188.

[8] Bunn C.L., Wallace D.C., & Eisenstadt J.M., (1977). Mitotic segregation of cytoplasmic determinants for chloramphenicol resistance in mammalian cells. I: Fusions with mouse cell lines. *Somatic Cell Genet.* 3(1):71-92.

[9] Wallace D.C., Bunn C.L., & Eisenstadt J.M., (1977). Mitotic segregation of cytoplasmic inherited genes for chloramphenicol resistance in mammalian cells. II: Fusions with human cell lines. *Somatic Cell Genet.* 3(1):93-119.

[10] Wallace D.C., (1981). Assignment of the chloramphenicol resistance gene to mitochondrial deoxyribonucelic acid and analysis of its expression in cultured human cells. *Mol. Cell. Biol.* 1(8):697-710.

[11] Blanc H., Wright C.T., Bibb M.J., Wallace D.C., & Clayton D.A., (1981). Mitochondrial DNA of chloramphenicol-resistant mouse cells contains a single nucleotide change in the region encoding the 3' end of the large ribosomal RNA. *Proc. Natl. Acad. Sci. USA* 78(6):3789-3793.

[12] Blanc H., Adams C.W., & Wallace D.C., (1981). Different nucleotide changes in the large rRNA gene of the mitochondrial DNA confer chloramphenicol resistance on two human cell lines. *Nucleic Acids Res.* 9(21):5785-5795.

[13] Wallace D.C., Pollack Y., Bunn C.L., & Eisenstadt J.M., (1976). Cytoplasmic inheritance in mammalian tissue culture cells. *In Vitro* 12(11):758-776.

[14] Oliver N.A., Greenberg B.D., & Wallace D.C., (1983). Assignment of a polymorphic polypeptide to the human mitochondrial DNA unidentified reading frame 3 gene by a new peptide mapping strategy. *J. Biol. Chem.* 258(9):5834-5839.

[15] Oliver N.A. & Wallace D.C., (1982). Assignment of two mitochondrially synthesized polypeptides to human mitochondrial DNA and their use in the study of intracellular mitochondrial interaction. *Mol. Cell. Biol.* 2(1):30-41.

[16] Oliver N.A., McCarthy J., & Wallace D.C., (1984). Comparison of mitochondrially synthesized polypeptides of human, mouse, and monkey cell lines by a two-dimensional protease gel system. *Somat. Cell Mol. Genet.* 10(6):639-643.

[17] Yang J.H., Ye J.H., & Wallace D.C., (1984). Computer selection of oligonucleotide probes from amino acid sequences for use in gene library screening. *Nucleic Acids Res.* 12(1 Pt 2):837-843.

[18] Neckelmann N., Li K., Wade R.P., Shuster R., & Wallace D.C., (1987). cDNA sequence of a human skeletal muscle ADP/ATP translocator: lack of a leader peptide, divergence from a fibroblast translocator cDNA, and coevolution with mitochondrial DNA genes. *Proc. Natl. Acad. Sci. USA* 84(21):7580-7584.

[19] Neckelmann N., *et al.*, (1989). The human ATP synthase beta subunit gene: sequence analysis, chromosome assignment, and differential expression. *Genomics* 5(4):829-843.

[20] Li K., *et al.*, (1989). A human muscle adenine nucleotide translocator gene has four exons, is located on chromosome 4, and is differentially expressed. *J. Biol. Chem.* 264(24):13998-14004.

[21] Li K., Hodge J.A., & Wallace D.C., (1990). OXBOX, a positive transcriptional element of the heart-skeletal muscle ADP/ATP translocator gene. *J. Biol. Chem.* 265(33):20585-20588.

[22] Haraguchi Y., *et al.*, (1993). Genetic mapping of human heart-skeletal muscle adenine nucleotide translocator and its relationship to the facioscapulohumeral muscular dystrophy locus. *Genomics* 16(2):479-485.

[23] Haraguchi Y., Chung A.B., Neill S., & Wallace D.C., (1994). OXBOX and REBOX, overlapping promoter elements of the mitochondrial F_0F_1-ATP synthase beta subunit gene. OXBOX/REBOX in the ATPsyn beta promoter. *J. Biol. Chem.* 269(12):9330-9334.

[24] Chung A.B., Stepien G., Haraguchi Y., Li K., & Wallace D.C., (1992). Transcriptional control of nuclear genes for the mitochondrial muscle ADP/ATP translocator and the ATP synthase beta subunit. Multiple factors interact with the OXBOX/REBOX promoter sequences. *J. Biol. Chem.* 267(29):21154-21161.

[25] Wallace D.C., *et al.*, (1987). Sequence analysis of cDNAs for the human and bovine ATP synthase b-subunit: mitochondrial DNA genes sustain seventeen times more mutations. *Curr. Genet.* 12(2):81-90.
[26] Giles R.E., Stroynowski I., & Wallace D.C., (1980). Characterization of mitochondrial DNA in chloramphenicol-resistant interspecific hybrids and a cybrid. *Somatic Cell Genet.* 6(4):543-554.
[27] Picard M., *et al.*, (2015). Trans-mitochondrial coordination of cristae at regulated membrane junctions. *Nat. Commun.* 6:6259.
[28] Wallace D.C. & Fan W., (2010). Energetics, epigenetics, mitochondrial genetics. *Mitochondrion* 10(1):12-31.
[29] Wallace D.C., Fan W., & Procaccio V., (2010). Mitochondrial energetics and therapeutics. *Annu. Rev. Path.* 5:297-348.
[30] Wallace D.C., (2009). Mitochondria, bioenergetics, and the epigenome in eukaryotic and human evolution. *Cold Spring Harb. Symp. Quant. Biol.* 74:383-393.
[31] Giles R.E., Blanc H., Cann H.M., & Wallace D.C., (1980). Maternal inheritance of human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 77(11):6715-6719.
[32] Case J.T. & Wallace D.C., (1981). Maternal inheritance of mitochondrial DNA polymorphisms in cultured human fibroblasts. *Somatic Cell Genet.* 7(1):103-108.
[33] Denaro M., *et al.*, (1981). Ethnic variation in Hpa 1 endonuclease cleavage patterns of human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 78(9):5768-5772.
[34] Chen Y.S., Torroni A., Excoffier L., Santachiara-Benerecetti A.S., & Wallace D.C., (1995). Analysis of mtDNA variation in African populations reveals the most ancient of all human continent-specific haplogroups. *Am. J. Hum. Genet.* 57(1):133-149.
[35] Schurr T.G., Sukernik R.I., Starikovskaya Y.B., & Wallace D.C., (1999). Mitochondrial DNA variation in Koryaks and Itel'men: population replacement in the Okhotsk Sea-Bering Sea region during the Neolithic. *Am. J. Phys. Anthropol.* 108(1):1-39.
[36] Ballinger S.W., *et al.*, (1992). Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient mongoloid migrations [published erratum appears in Genetics 1992 Apr;130(4):957]. *Genetics* 130(1):139-152.
[37] Torroni A., *et al.*, (1996). Classification of European mtDNAs from an analysis of three European populations. *Genetics* 144(4):1835-1850.
[38] Torroni A., *et al.*, (1994). MtDNA and the origin of Caucasians. Identification of ancient Caucasian-specific haplogroups, one of which is prone to a recurrent somatic duplication in the D-loop region. *Am. J. Hum. Genet.* 55(4):760-776.
[39] Derbeneva O.A., Starikovskaya E.B., Wallace D.C., & Sukernik R.I., (2002). Traces of early Eurasians in the Mansi of northwest Siberia revealed by mitochondrial DNA analysis. *Am. J. Hum. Genet.* 70(4):1009-1014.
[40] Derbeneva O.A., *et al.*, (2002). Analysis of mitochondrial DNA diversity in the Aleuts of the Commander Islands and its implications for the genetic history of Beringia. *Am. J. Hum. Genet.* 71(2):415-421.
[41] Schurr T.G., *et al.*, (1990). Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. *Am. J. Hum. Genet.* 46(3):613-623.
[42] Johnson M.J., Wallace D.C., Ferris S.D., Rattazzi M.C., & Cavalli-Sforza L.L., (1983). Radiation of human mitochondria DNA types analyzed by restriction endonuclease cleavage patterns. *J. Mol. Evol.* 19(3-4):255-271.
[43] Merriwether D.A., *et al.*, (1991). The structure of human mitochondrial DNA variation. *J. Mol. Evol.* 33(6):543-555.
[44] Wallace D.C., Brown M.D., & Lott M.T., (1999). Mitochondrial DNA variation in human evolution and disease. *Gene* 238(1):211-230.

[45] Ruiz-Pesini E., Mishmar D., Brandon M., Procaccio V., & Wallace D.C., (2004). Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* 303(5655):223-226.
[46] Mishmar D., *et al.*, (2003). Natural selection shaped regional mtDNA variation in humans. *Proc. Natl. Acad. Sci. USA* 100(1):171-176.
[47] Torroni A., Neel J.V., Barrantes R., Schurr T.G., & Wallace D.C., (1994). A mitochondrial DNA «clock» for the Amerinds and its implication for timing their entry into North America. *Proc. Natl. Acad. Sci. USA* 91(3):1158-1162.
[48] Wallace D.C., (2015). Mitochondrial DNA variation in human radiation and disease. *Cell* 163(1):33-38.
[49] Ji F., *et al.*, (2012). Mitochondrial DNA variant associated with Leber hereditary optic neuropathy and high-altitude Tibetans. *Proc. Natl. Acad. Sci. USA* 109(19):7391-7396.
[50] Hendrickson S.L., *et al.*, (2008). Mitochondrial DNA haplogroups influence AIDS progression. *AIDS* 22(18):2429-2439.
[51] Lakatos A., *et al.*, (2010). Association between mitochondrial DNA variations and Alzheimer's disease in the ADNI cohort. *Neurobiol. Aging* 31(8):1355-1363.
[52] Wallace D.C., (2012). Mitochondria and cancer. *Nature Reviews Cancer* 12(10):685-698.
[53] Novotny E.J., *et al.*, (1986), Leber's disease and dystonia: a mitochondrial disease. *Neurology* 36(8):1053-1060.
[54] Wallace D.C., (1986). Mitochondrial genes and disease. *Hosp. Pract. (Off. Ed.)* 21(10):77-87, 90-92.
[55] Wallace D.C., *et al.*, (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science* 242(4884):1427-1430.
[56] Wallace D.C., *et al.*, (1988). Familial mitochondrial encephalomyopathy (MERRF): Genetic, pathophysiological, and biochemical characterization of a mitochondrial DNA disease. *Cell* 55(4):601-610.
[57] Shoffner J.M., *et al.*, (1990). Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. *Cell* 61(6):931-937.
[58] Petros J.A., *et al.*, (2005). mtDNA mutations increase tumorigenicity in prostate cancer. *Proc. Natl. Acad. Sci. USA* 102(3):719-724.
[59] Arnold R.S., *et al.*, (2013). An inherited heteroplasmic mutation in mitochondrial gene COI in a patient with prostate cancer alters reactive oxygen, reactive nitrogen and proliferation. *Biomed Res Int* 2013:239257.
[60] Corral-Debrinski M., *et al.*, (1992). Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nat. Genet.* 2(4):324-329.
[61] Corral-Debrinski M., *et al.*, (1991). Hypoxemia is associated with mitochondrial DNA damage and gene induction. Implications for cardiac disease. *JAMA* 266(13):1812-1816.
[62] Corral-Debrinski M., *et al.*, (1994). Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. *Genomics* 23(2):471-476.
[63] Coskun P.E., Beal M.F., & Wallace D.C., (2004). Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. *Proc. Natl. Acad. Sci. USA* 101(29):10726-10731.
[64] Coskun P.E., *et al.*, (2010). Systemic mitochondrial dysfunction and the etiology of Alzheimer's disease and down syndrome dementia. *J. Alzheimers Dis.* 20 Suppl 2:S293-S310.
[65] Coskun P., *et al.*, (2012). A mitochondrial etiology of Alzheimer and Parkinson disease. *Biochim. Biophys. Acta.* 1820(5):553-564.
[66] Coskun P., *et al.*, (2016). Metabolic and growth rate alterations in lymphoblastic cell lines discriminate between Down Syndrome and Alzheimer's Disease. *J. Alzheimers Dis.* ePub ahead of print, http://dx.doi.org/10.3233/JAD-160278
[67] Ballinger S.W., *et al.*, (1992). Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nat. Genet.* 1(1):11-15.

[68] Ballinger S.W., Shoffner J.M., Gebhart S., Koontz D.A., & Wallace D.C., (1994). Mitochondrial diabetes revisited. *Nat. Genet.* 7(4):458-459.
[69] Ortiz R.G., *et al.*, (1993). Variable retinal and neurologic manifestations in patients harboring the mitochondrial DNA 8993 mutation. *Arch. Ophthalmol.* 111(11):1525-1530.
[70] Zaragoza M.V., Brandon M.C., Diegoli M., Arbustini E., & Wallace D.C., (2011). Mitochondrial cardiomyopathies: how to identify candidate pathogenic mutations by mitochondrial DNA sequencing, MITOMASTER and phylogeny. *Eur. J. Hum. Genet.* 19(2):200-207.
[71] Shoffner J.M., *et al.*, (1993). Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. *Genomics* 17(1):171-184.
[72] MITOMAP, (2017). A Human Mitochondrial Genome Database. http://www.mitomap.org
[73] Graham B.H., *et al.*, (1997). A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/skeletal muscle isoform of the adenine nucleotide translocator. *Nat. Genet.* 16(3):226-234.
[74] Narula N., *et al.*, (2011). Adenine nucleotide translocase 1 deficiency results in dilated cardiomyopathy with defects in myocardial mechanics, histopathological alterations, and activation of apoptosis. *JACC Cardiovasc. Imaging* 4(1):1-10.
[75] Esposito L.A., Melov S., Panov A., Cottrell B.A., & Wallace D.C., (1999). Mitochondrial disease in mouse results in increased oxidative stress. *Proc. Natl. Acad. Sci. USA* 96(9):4820-4825.
[76] Strauss K.A., *et al.*, (2013). Severity of cardiomyopathy associated with adenine nucleotide translocator-1 deficiency correlates with mtDNA haplogroup. *Proc. Natl. Acad. Sci. USA* 110(9):3253-3458.
[77] Lin-Hendel E.G., McManus M.J., Wallace D.C., Anderson S.A., & Golden J.A., (2016). Differential mitochondrial requirements for radially and non-radially migrating cortical neurons: implications for mitochondrial disorders. *Cell Rep.* 15(2):229-237.
[78] Kokoszka J.E., *et al.*, (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427(6973):461-465.
[79] Kokoszka J.E., *et al.*, (2016). Deficiency in the mouse mitochondrial adenine nucleotide translocator isoform 2 gene is associated with cardiac noncompaction. *Biochim. Biophys. Acta.* 1857(8):1203-1212.
[80] Li Y., *et al.*, (1995). Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11(4):376-381.
[81] Esposito L.A., *et al.*, (2000). Mitochondrial oxidative stress in mice lacking the glutathione peroxidase-1 gene. *Free Radic. Biol. Med.* 28(5):754-766.
[82] Melov S., *et al.*, (1999). Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proc. Natl. Acad. Sci. USA* 96(3):846-851.
[83] Melov S., *et al.*, (2001). Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics. *Journal of Neuroscience* 21(21):8348-8353.
[84] Melov S., *et al.*, (1998). A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. *Nat. Genet.* 18(2):159-163.
[85] Schriner S.E., *et al.*, (2005). Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308(5730):1909-1911.
[86] Fan W., Lin C.S., Potluri P., Procaccio V., & Wallace D.C., (2012). MtDNA lineage analysis of mouse L cell lines reveals the accumulation of multiple mtDNA mutants and intermolecular recombination. *Genes Dev.* 26(4):384-394.
[87] MacGregor G.R., Fan W.W., Waymire K.G., & Wallace D.C., (2006). Generating animal models of human mitochondrial genetic disease using mouse ES cells. *Embryonic Stem Cells,* Practical Approach Series, eds Notarianni E. & Evans M.J. (Oxford University Press, New York, NY), pp. 72-104.
[88] Sligh J.E., *et al.*, (2000). Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice. *Proc. Natl. Acad. Sci. USA* 97(26):14461-14466.

[89] Fan W., *et al.*, (2008). A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science* 319(5865):958-962.
[90] Lin C.S., *et al.*, (2012). A mouse mtDNA mutant model of Leber's Hereditary Optic Neuropathy. *Proc. Natl. Acad. Sci. USA* 109(49):20065-20070.
[91] Wallace D.C., (2016). Genetics: mitochondrial DNA in evolution and disease. *Nature* 535(7613):498-500.
[92] Sharpley M.S., *et al.*, (2012). Heteroplasmy of mouse mtDNA Is genetically unstable and results in altered behavior and cognition. *Cell* 151(2):333-343.
[93] Mitchell P., (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191:144-148.
[94] Pham T.D., *et al.*, (2016). Cristae remodeling causes acidification detected by integrated graphene sensor during mitochondrial outer membrane permeabilization. *Sci. Rep.* 6:35907.
[95] Wallace DC (2008) Mitochondria as chi. *Genetics* 179(2):727-735.