ELSEVIER

Neurobiology of Aging 31 (2010) 1355–1363

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Association between mitochondrial DNA variations and Alzheimer's disease in the ADNI cohort

Anita Lakatos[a,*], Olga Derbeneva[b], Danny Younes[c], David Keator[a], Trygve Bakken[d], Maria Lvova[b], Marty Brandon[b], Guia Guffanti[a], Dora Reglodi[e], Andrew Saykin[f], Michael Weiner[g], Fabio Macciardi[a], Nicholas Schork[d], Douglas C. Wallace[b], Steven G. Potkin[a], and the Alzheimer's Disease Neuroimaging Initiative

[a] *Department of Psychiatry and Human Behavior, University of California, Irvine, CA, USA*
[b] *Center for Molecular and Mitochondrial Medicine and Genetics, University of California, Irvine, CA, USA*
[c] *University of California Medical School, San Francisco, San Francisco, CA, USA*
[d] *The Scripps Translational Science Institute, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA*
[e] *Department of Anatomy, University of Pécs, Pécs, Hungary*
[f] *Center for Neuroimaging, Department of Radiology, Indiana University School of Medicine, Indianapolis, IN, USA*
[g] *Department of Radiology, Psychiatry and Neurology, University of California San Francisco, San Francisco, CA, USA*

Received 16 February 2010; received in revised form 24 April 2010; accepted 27 April 2010

Abstract

Despite the central role of amyloid deposition in the development of Alzheimer's disease (AD), the pathogenesis of AD still remains elusive at the molecular level. Increasing evidence suggests that compromised mitochondrial function contributes to the aging process and thus may increase the risk of AD. Dysfunctional mitochondria contribute to reactive oxygen species (ROS) which can lead to extensive macromolecule oxidative damage and the progression of amyloid pathology. Oxidative stress and amyloid toxicity leave neurons chemically vulnerable. Because the brain relies on aerobic metabolism, it is apparent that mitochondria are critical for the cerebral function. Mitochondrial DNA sequence changes could shift cell dynamics and facilitate neuronal vulnerability. Therefore we postulated that mitochondrial DNA sequence polymorphisms may increase the risk of AD. We evaluated the role of mitochondrial haplogroups derived from 138 mitochondrial polymorphisms in 358 Caucasian Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects. Our results indicate that the mitochondrial haplogroup UK may confer genetic susceptibility to AD independently of the apolipoprotein E4 (APOE4) allele.
Published by Elsevier Inc.

Keywords: ADNI; Alzheimer's disease; Mitochondrial polymorphism; Mitochondrial haplogroups

1. Introduction

Despite the remarkable effort and resources devoted to Alzheimer's disease (AD) this last decade, the pathophysiology of AD has not been well characterized. The current animal models of AD pathogenesis and the human genome-wide association studies (GWAS) have not resulted in a single common origin for the irregular but common incidences of AD, thus implying extensive heterogeneity in the underlying pathophysiology. One clear underlying pathophysiologic feature of AD has been identified and this centered on "the amyloid and tau pathology" that is believed to lead to neuronal loss, decreased synaptic density, brain atrophy, and a subsequent progressive cognitive decline associated with AD (Yankner et al., 2008). Nevertheless, recent research into AD provides compelling evidence for a

* Corresponding author at: Department of Psychiatry and Human Behavior, University of California, 5251 California Ave, Suite 240, Irvine, CA 92617, USA. Tel.: +1 949 824 4423; fax: +1 949 824 3324.
E-mail address: alakatos@uci.edu (A. Lakatos).

0197-4580/$ – see front matter Published by Elsevier Inc.
doi:10.1016/j.neurobiolaging.2010.04.031

variety of additional pathological events including oxidative stress and apoptosis (Mamelak, 2007).

It is has been postulated that the accumulation of reactive oxygen species (ROS) over a period of time is negatively correlated with mitochondrial function and significantly contributes to the aging process (Wallace and Fan, 2009). As the primary source of ROS, mitochondria play pivotal roles in maintaining cellular energy balance and lie at the nexus of the signaling pathways controlling apoptosis. As such, it is conceivable that mitochondria may mediate the development and clinical outcome of AD. In fact, numerous research findings suggest a link between altered cerebral metabolic rate for glucose (CMR_{glc}) in subjects with mitochondrial mutations (Lindroos et al., 2009). PET imaging studies have demonstrated a detectable decline in cerebral metabolism before any brain atrophy or abnormality found by neuropsychiatric testing in subjects who later developed AD (Reiman et al., 2004). Postmortem studies on AD brains have revealed a reduction in the mitochondrial respiratory chain proteins in the posterior cingulate (Liang et al., 2008). The projection neurons of the cerebral cortex which tend to die first in AD, show increased vulnerability to decreased mitochondrial efficiency (Gotz et al., 2009).

The Down syndrome (DS) brain, with accelerated amyloid deposition and high propensity for AD, implies a possible synergy between amyloid deposition and ROS production. The structural changes that caused DS harbor amyloid precursor protein (APP), a precursor molecule of amyloid β as well as gene SOD1 encoding an enzyme named superoxide dismutase 1. SOD1 marks the first important step in scavenging and neutralizing ROS in cells (Engidawork and Lubec, 2001). The amyloid β, a product of APP processing, has been shown to be transported into the mitochondria through the translocase of outer mitochondrial membrane (TOMM) complex (Devi et al., 2006) and inhibit the oxidative phosphorylation system (OXPHOS) in line with apolipoprotein E (APOE) (Crouch et al., 2005). In addition, DNA sequence variations within both the APOE and TOMM40 genes have been found to be significantly associated with AD in genome-wide association studies (Potkin et al., 2009; Roses et al., 2009; Shen et al., 2010). Mitochondria efficiency may decrease in response to amyloid toxicity and interfering proteins like APOE and dynamin-related protein 1 (Drp1) (Cho et al., 2009) that initiate a "vicious cycle" in ROS production facilitating apoptosis and leading to cell death (Reddy, 2009).

Therefore, it is plausible to link AD to the functional status of mitochondria that critically relies on the mitochondrially-expressed oxidative phosphorylation system proteins (Fosslien, 2001). Because the mitochondria genome differs from the nuclear genome in the rate of accumulation of mutations, it has been proposed that some common mitochondrial DNA (mtDNA) polymorphisms probably alter protein functions and compromise mitochondria efficiency (Tuppen et al., 2009). There is growing evidence that certain mtDNA clusters and polymorphisms as well as the somatically acquired mutations could predispose to psychiatric disorders (Coskun et al., 2004; Jou et al., 2009; McMahon et al., 2000; Zecavati and Spence, 2009).

To further elucidate the relation between mitochondrial DNA sequence polymorphisms and risk of AD, we calculated the association of mitochondrial haplogroups derived from 138 mitochondrial polymorphisms in 358 Alzheimer's Disease Neuroimaging Initiative (ADNI) subjects. To the best of our knowledge this is the first report considering mitochondrial single nucleotide polymorphisms (SNPs) in the context of a longitudinal clinical study of AD.

2. Methods

2.1. Ethics

The ADNI data were previously collected across 50 research sites. Study subjects gave written informed consent at the time of enrollment for imaging and genetic sample collection and completed questionnaires approved by each participating sites' Institutional Review Board (IRB).

2.2. The Alzheimer's Disease Neuroimaging Initiative (ADNI)

ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies, and nonprofit organizations as a $60 million, 5-year public-private partnership. The primary goal of ADNI is to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment (MCI) and AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians in the development of new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The principal investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California, San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the USA and Canada. ADNI participants include approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. Participants are evaluated at baseline, 6, 12, 18 (for MCI only), 24, and 36 months (although AD participants do not have a 36-month evaluation). For additional information see www.adni-info.org.

Table 1
Annotations of a subset of 138 mtDNA polymorphic sites interrogated on the Illumina Human610-Quad Infinium HD platform (Illumina, Inc., San Diego, CA)

AF347015.1 mtDNA SNP	Corresponding rCRS SNP	Change	Type	Function	Haplogroup
MitoT9699C	9698	T > C	Synonymous	Cytochtome C oxidase III (COIII)	U8
MitoA11252G	11251	A > G	Synonymous	NADH dehydrogenase 4 (ND4)	JT
MitoG12373A	12372	G > A	Synonymous	NADH dehydrogenase 5 (ND5)	UK

The table illustrates a random subset of the 138 annotated mitochondrial DNA (mtDNA) polymorphisms. The first column represents single nucleotide polymorphisms (SNPs) derived from the AF347015.1 mtDNA sequence. The second column represents the corresponding rCRS mtDNA sequence position. The last 4 columns describe the corresponding rCRS SNP characteristics.

2.3. Identification of 138 mitochondrial SNPs

The genotyping procedure for the mtDNA and APOE variations was executed as described in Potkin et al. (2009). Genotyping was performed on the Illumina Human610-Quad Infinium HD platform (Illumina, Inc., San Diego, CA; www.illumina.com). The Illumina Human610-Quad BeadChip has of 550,000 polymorphic sites (SNP), plus an additional 60,000 genetic markers including 138 mitochondrial DNA sequence polymorphic sites. The 138 mitochondrial SNPs are based on the AF347015.1 mtDNA reference sequences, one of 53 African sequences deposited in GenBank by Ingman et al. in 2001 (Ingman and Gyllensten, 2001). As the first step, the 138 mitochondrial AF347015.1 DNA SNPs were mapped to revised Cambridge Reference Sequence (rCRS) and manually annotated. The 138 SNPs consist of 21 noncoding, 91 protein coding, 4 ribosomal RNA (rRNA), 11 transfer RNA (tRNA), and 1 termination sites. A subset of the 138 SNPs is shown in Table 1.

2.4. Haplotyping and haplogroup assignment

Because variation in the mtDNA genome arose as result of the sequential accumulation of mutations over time (1 mutation/10,000 years), specific combinations of these ancient mutations can cluster as haplotypes, and subsequently define haplogroups. The hierarchical relationship among these haplogroups based on mutations can be represented by a phylogenetic tree via available programs (www.phylotree.org). The nucleotide changes of the 138 mitochondrial SNPs for each subject were compared with rCRS and used to identify motifs that characterize mitochondrial haplotype (van Oven and Kayser, 2009). Assignment of each individual mtDNA "sequence" to specific haplogroups was performed according to criteria as published (Torroni et al., 1996) (e.g., haplogroup J is define by mutations at locations 11251, 16126, and no mutation at 16294). Based on each subject's available genotyping, all 816 ADNI subjects were haplotyped and assigned to a haplogroup.

2.5. Population and stratification

Population heterogeneity has been cited as 1 of many difficulties in studying complex diseases (Schork et al., 2001). Although race and ethnicity were available as surrogates to evaluate genetic similarity among individuals, race does not always correctly reflect population of origin, particularly in heterogeneous admixed groups — as expected for the ADNI dataset. In order to avoid the confounding effect due to population stratification, we assessed evidence for genetic background heterogeneity and by leveraging publicly available, well defined populations. Briefly, Phase 2 HapMap dataset ($n = 270$ subjects) were merged with the entire genome-wide scan of 816 ADNI subjects (pngu.mgh.harvard.edu/~purcell/plink/res.shtml) (Enoch et al., 2006). For all individuals in the merged dataset, a pair-wise identity-by-state (IBS) distance matrix was created by a linkage agglomerative algorithm implemented in PLINK (Purcell et al., 2007). Subsequently, multidimensional distance scaling (MDS) analysis was carried out on the genome-wide identity-by-state pair-wise-distance matrix of the merged dataset to display the structure of the distance between individuals as a geometric picture (JMP Genomics 4.00; SAS Institute, Inc., Cary, NC, 1989–2007). For the purpose of this study, the ADNI individuals that clustered with CEU founders (Utah resident with ancestry from northern and western Europe), and belonged to the diagnostic AD ($n = 170$) or control ($n = 188$) groups were selected for further statistical analyses. Analysis comparing nuclear DNA genetic background differences among individuals in the different mitochondrial haplogroups did not reveal any obvious associations among the European sample of individuals chosen for study.

2.6. Statistical analyses

Student t tests were used to evaluate the differences in means between AD and control (CTRL) groups for continuous variables. Statistical significance was assessed on the basis of a 2-sided test with $\alpha = 0.05$. Frequency differences for categorical outcomes in AD and CTRL groups were assessed via χ^2 test statistic or Fisher's exact test (Table 2). Mitochondrial haplogroups and allelic frequencies were compared between AD and CTRL using χ^2 test or Fisher's test. Effect size for the association was measured as an odds ratio (OR) with a 95% confidence interval (CI). Cochran-Mantel-Haenszel (CMH) tests were performed to adjust for apolipoprotein E4 (APOE4) allele genotype (i.e., E4 "dose"). The homogeneity of odds ratios across strata was tested by the Breslow-Day test. Logistic regression was

Table 2
Summary of demographic and clinical data of the 358 participating subjects

	CTRL	AD	Statistical significance
n	188	170	
Mean age (years)	75.93 ± 5.01	75.79 ± 7.57	$p \geq 0.83$
Gender (male/female)	98/90	101/91	$p \geq 0.706$
Smoker/nonsmoker	69/101	222/135	$p \geq 0.26$
Handedness (right/left)	171/17	158/12	$p \geq 0.5$
Mean MMSE	29.11 ± 0.93	23.40 ± 2.07	$p \leq 0.00001$
Mean ADAS-cog	9.37 ± 4.09	28.65 ± 8.7	$p \leq 0.00001$
Mean years of education	16.18 ± 2.81	14.9 ± 3.0	$p \leq 5.4E\text{-}5$
APOE (ε2/ε3/ε4)	27/297/52	8/189/143	$p \leq 4.77E\text{-}17$

Mean ± SD and the frequency of each category are represented with test statistics.
Key: AD, Alzheimer's disease; ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive; APOE, apolipoprotein E; CTRL, control; MMSE, Mini Mental State Examination.

performed to assess the contribution of mtDNA haplogroup, APOE4 allele, and their potential interaction effects to AD risk. Adaptive permutation tests were employed for accommodating multiple testing.

2.6.1. Statistical packages

For the analyses we used the computer programs, JMP Genomics version 4.00 (SAS Institute, Inc., Cary, NC, 1989–2007). PLINK (Purcell et al., 2007); STATA10 (Stata Statistical Software, release 10, 2007, StataCorp, College Station, TX).

3. Results

3.1. Demographic

All subjects were part of the Alzheimer's Disease Neuroimaging Initiative (ADNI), a longitudinal multisite observational study. All the participants in this study were confined to group case (AD) or control (CTRL) defined at baseline diagnosis. Following multidimensional distance scaling analysis (Section 2.7), a total of 170 AD subjects and 188 healthy controls were included in this analysis. The 2 groups were compared at different variables and summarized in Table 2.

AD and cognitively normal participant (CTRL) groups do not differ in age, gender, smoking, and handedness. However, AD subjects had a lower education level ($p \leq 5.4E\text{-}5$) and had a disproportionally higher APOE4 allele frequency ($p \leq 4.77E\text{-}17$) than the CTRL group. The 2 groups also significantly differed in Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog) ($p \leq 0.000\ 01$) and Mini Mental State Examination (MMSE) scores ($p \leq 0.000\ 01$) reflecting the enrollment criteria for ADNI.

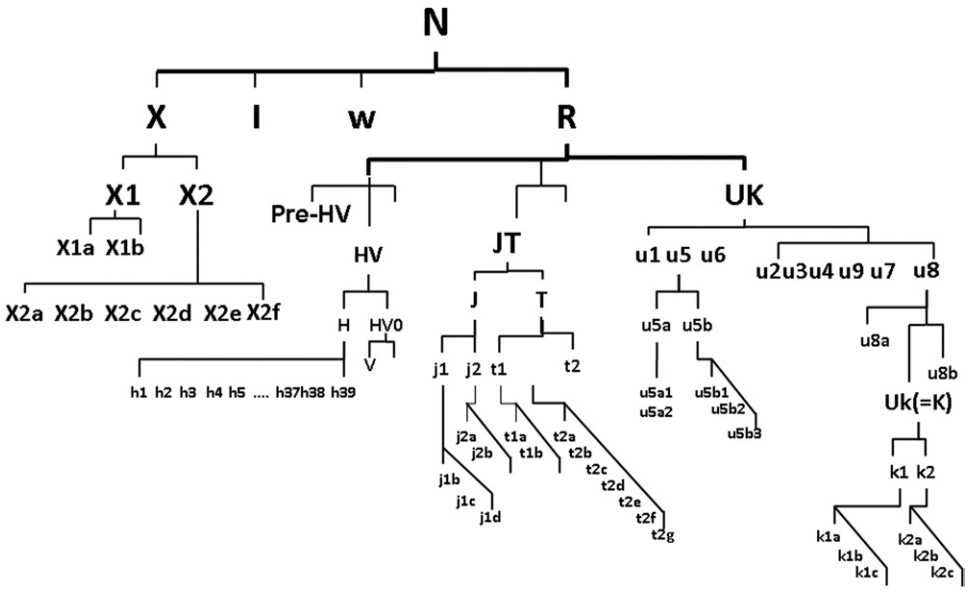

Fig. 1. Schematic phylogeny of the basal European mitochondrial DNA (mtDNA) haplogroups. Simplified mtDNA phylogeny tree demonstrate the Caucasian lineages exclusively originated from haplogroup N approximately 50,000–70,000 years before present (YBP). The alphabetical symbols illustrate the descendant lineages of macrograph N and indicate the phylogenic relationship among haplogroups as a basis of the haplogroup designation to clusters. The global mtDNA variations are available at www.phylotree.org.

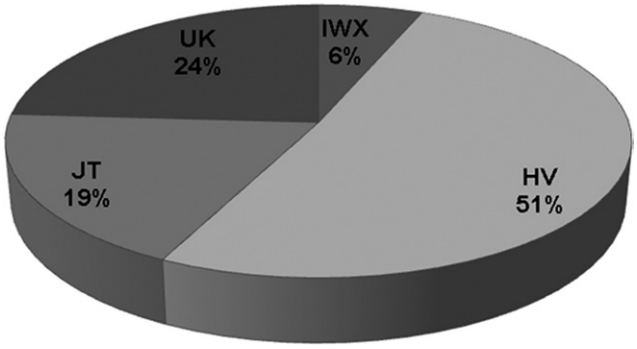

Fig. 2. Overall distribution of mitochondrial DNA (mtDNA) haplogroups of 358 subjects in the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. Labels designate the name of the haplogroup clusters and the relative frequencies of the haplogroup expressed in percentages.

3.2. Association analysis

3.2.1. Case-control differences in mitochondrial haplogroups

All subjects with Caucasian origin belonged to a major haplogroup N which was rooted approximately 70,000 years before present (YBP). Haplotype N consisted of 358 subjects who were distributed among the 9 designated haplogroups: H, I, J, K, T, U, V, W, and X. These 9 haplogroups were partitioned into 4 encompassing haplogroup clusters: (1) HV; (2) JT; (3) UK; and (4) IWX, based on their relation to ancestor lineage N (Finnila et al., 2001; Richards et al., 1996; Richards et al., 1998) as detailed in Fig. 1.

The frequency of the haplogroups for each of the 4 clusters is demonstrated in Fig. 2. The frequency distribution of the clusters is consistent with the reported worldwide mitochondrial haplogroup distribution reported at the MITOMAP database (www.mitomap.org). From the 358 ADNI participants, 86 subjects (24%) belong to haplogroup UK, 67 subjects (19%) belong to haplogroup JT, 183 (51%) to haplogroup HV, and 22 subjects (6%) to haplogroup IWX.

The χ^2 test statistics revealed significant association between disease status and mitochondrial haplogroups ($\chi^2 = 7.99$, $df = 3$, $p \leq 0.046$). (The observed and computed expected frequencies for each mitochondrial haplogroup are shown in Table 3.) The strongest association among the 4 haplogroups involved in the UK haplogroup with a contrast between CTRL ($\chi^2 = 2.75$) versus AD ($\chi^2 = 3.05$) and associated odds ratio of (1.92, 95% CI, 1.13–3.26; $p < 0.013$). A formal test for homogeneity confirmed that UK haplogroup appeared to have a stronger association with AD than the other haplogroups ($\chi^2 = 7.97$, $df = 3$ $p < 0.045$). A score test for trend of odds supported this ($\chi^2 = 4.76$, $p < 0.03$). The magnitude of association remained significant ($\chi^2 = 7.63$, $df = 3$, $p \leq 0.0057$, 95% CI 1.12–195) after adjusting for APOE4 allele dose.

3.2.2. Case-control differences in mitochondrial single nucleotide polymorphism

An allelic association analysis was conducted for each of the polymorphic mtDNA sites. In the analysis, all the 138 SNPs were calculated for association regardless of haplogroup specificity or minor allele frequency. Adaptive permutation analysis for each polymorphism was performed to determine an empirical p-value. All the mitochondrial SNPs (mtSNPs) with an empirical p-value < 0.05 were considered statistically significant. The SNP DNA position, minor allele frequency in AD and CTRL, asymptotic p-value, odds ratio, confidence interval, functional consequence, and haplogroup specificity are reported in Table 4.

Analysis of individual SNPs revealed that risk of AD was increased in subjects who carried minor allele MitoA11467G (OR = 2.22; 95% CI, 1.30–3.78; $p \leq 0.003$). The MitoA11467G is a synonymous polymorphism located in gene NADH dehydroenase 4 (ND4). The encoded protein of this gene is a subunit of a large enzyme complex known as complex I. Complex I is responsible for the first step in the oxidative phosphorylation process by transferring electrons from NADH to ubiquinone. The second most significant SNP was MitoA12308G (OR = 2.03; 95% CI, 1.23–3.34; $p \leq 0.006$) located in a tRNA which transfers the amino acid leucine for protein synthesis. This polymorphism was found highly significant in an interaction with 10398G (empirical p value = 0.0028) suggesting some women are at increased risk to develop breast cancer (Kinoshita et al., 1996). MitoG12372A (OR = 1.996; 95% CI, 1.21–3.27, $p \leq 0.006$) located in gene NADH dehydrogenase 5 (ND5) that encodes a subunit for complex I. MitoC9698T (OR = 2.265; 95% CI, 1.16–4.41; $p \leq 0.003$) is synonymous variant encoding cytochrome C oxidase III (COIII) enzyme found in complex IV. Defects in mitochondrial COIII gene implicated in Leber hereditary optic neuropathy (LHON) (Brown et al., 1992; Eichhorn-Mulligan and Cestari, 2008) and age-dependent accumulation of mutations in mitochondrial DNA (mtDNA) in cytochrome c oxidase has been implicated in the onset of sporadic AD (Davis et al., 1997; Lin and Beal, 2006). MitoC16270T (OR = 2.527; 95% CI, 1.16–4.41; $p \leq 0.048$) is located in the hypervariable segment assumed to represent a mutational hotspot (see summary in Table 4).

Besides age as a major risk for AD, APOE4 is the most consistently replicated genetic risk factor for late onset AD.

Table 3
The percentage of expected and observed subjects in each haplogroup

Diagnostic category	Haplogroup frequency	HV	JT	IWX	UK
CTRL	Observed (%)	28.50%	3.35%	11.10%	9.50%
	Expected (%)	26.80%	3.22%	9.80%	12.61%
AD	Observed (%)	22.63%	2.80%	7.60%	14.53%
	Expected (%)	24.27%	2.91%	8.90%	11.40%

Key: AD, Alzheimer's disease; CTRL, control.

Table 4
The results of the allelic association study by significant SNPs

SNP	Position in mtDNA (bp)	Allele frequency		*p*-value	OR	CI		Function	Haplogroup
		AD	CTRL			L95	U95		
MitoA11467G	11467	0.28	0.14	0.003	2.22	1.3	3.78	NADH dehydrogenase (ND4)	U and UK
MitoA12308G	12308	0.3	0.17	0.006	2.03	1.23	3.34	tRNA for leucine (CUN)	U and UK
MitoG12372A	12372	0.3	0.18	0.006	1.99	1.21	3.27	NADH dehydrogenase (ND5)	U and UK
MitoC9698T	9698	0.17	0.08	0.021	2.26	1.16	4.41	Cytochrome C oxidase (COIII)	U8 (UK)
MitoC16270T	16270	0.09	0.03	0.048	2.52	1	6.36	Noncoding region	Not specific

Key: AD, Alzheimer's disease; bp, basepair; CI, confidence interval; L95, lower 95%; U95, upper 95%; CTRL, control; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide; OR, odds ratio; SNP, single nucleotide polymorphism; tRNA, transfer RNA.

Because there is a significant APOE allele frequency difference in our cohort, a subsequent analysis was performed to assess the potential for an APOE4 cofounding or interactive effect. Logistic regression revealed no evidence for an interaction effect. However, APOE revealed strong association with AD at level of OR = 4.11–5.35 independently of mitochondrial SNPs.

The location and potential effect on gene function of the significant mutations in relation to mtDNA are depicted in Fig. 3.

Thus, based on χ^2 tests and logistic regression analysis, the APOE4 allele, and the mitochondrial haplogroup UK, as well as specific mitochondrial polymorphisms, are significantly associated with AD in the ADNI cohort. Given the functional consequences of the significant mitochondrial polymorphisms, the possession of these polymorphisms may predispose to AD.

4. Discussion

Recent research studies have found links between mitochondrial dysfunction and common diseases of aging, such as Parkinson's disease (PD) and Alzheimer's disease (AD) (Wang et al., 2007, 2009). A growing body of evidence suggests a reasonable association between amyloid-β toxicity, mitochondrial dysfunction, oxidative stress, and neu-

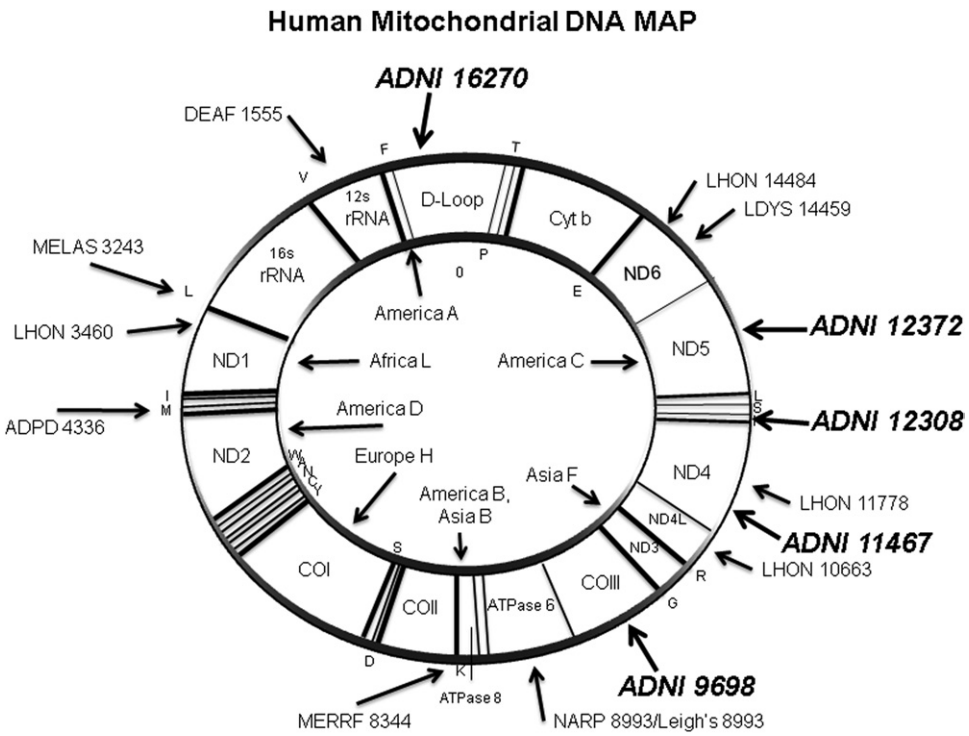

Fig. 3. Semantic illustration of the human mitochondrial genome. The 5 significant mitochondrial polymorphisms in *italics* (Alzheimer's Disease Neuroimaging Initiative [ADNI] prefix followed by single nucleotide polymorphism [SNP] number) are mapped to mitochondrial DNA (mtDNA). The most well known pathogenic mutations are also outlined. ADPD, Alzheimer's and Parkinson's disease; DEAF, deafness; LDYS, LHON + dystonia. LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy and ragged red fiber disease; NARP, neurogenic muscle weakness, ataxia, retinitis pigmentosum: Modified from Wallace (2005).

ronal damage in AD pathophysiology (Mancuso et al., 2006). The impact of the dysfunctional mitochondria on the integrity of neuronal cells is not fully understood. Mitochondria are exclusively positioned to play a pivotal role in neuronal cell survival or death by controlling energy metabolism and apoptotic pathways. Mitochondrial malfunction can alter the delicate bioenergetics balance making neuronal cells vulnerable to challenge (Wallace et al., 2010). Mitochondrial haplogroups and polymorphisms have also received substantial consideration in psychiatric and neurodegenerative diseases (Tuppen et al., 2009). Mitochondrial associations were found between AD and haplogroup J with increased susceptibility, while haplogroup T was found to have protective effect (Chagnon et al., 1999). Increased risk of AD in males with haplogroup U (van der Walt et al., 2004) as well as the mitochondrial tRNA(Glu) 4336 SNP have been reported (Brown et al., 1996; Shoffner et al., 1993). In addition, we report 5 mitochondrial SNPs, 3 of which define haplogroup UK and 2 of them specific to certain UK haplotypes in association exhibit associations with AD in the ADNI cohort.

One potential explanation for the associations is that mtDNA haplogroups correlate with genetic backgrounds that are distinctive between geographically separated populations. However, our population stratification analysis did not find significant differences between the major haplogroups and nuclear DNA-based genetic background and hence argues against a population substructure confounding effect. Another potential explanation is that the underlying mechanism for the predisposition for Alzheimer disease is related to energy deficiency. Observations on longevity, neurodegenerative disease susceptibility (van der Walt et al., 2004; Wallace et al., 1998), sperm viability (Montiel-Sosa et al., 2006), and climate adaptation propose association between functional mtDNA variations and adenosine triphosphate (ATP) production efficiency and correlated ROS and heat generation in different haplogroups (Arning et al., 2010). The mtDNA haplogroup most prone to energy deficiency in Europe are haplogroups U and UK (Hendrickson et al., 2008). It is conceivable that additional genetic risk factors further compromise the mitochondrial ATP production causing it to fall below the threshold level needed for optimal neuronal functioning. It is important to note that mitochondria genomes express 37 genes and other approximately 1500–2000 nuclear genes may play critical role in optimal mitochondria function (Wallace, 2008). This interaction between the 2 genomes may influence brain function (Roubertoux et al., 2003) and may contribute to the complexity of AD pathophysiology.

Despite few contradictory research findings in haplogroup association to AD (Chinnery et al., 2000; Coppede et al., 2007; Elson et al., 2006), the design of ADNI provides the opportunity to follow up our findings with neuroimaging and psychometric examination in addition to a nuclear DNA-based genome-wide association studies interrogation. This comprehensive approach has the potential to provide novel insight into the underlying pathomechanisms of Alzheimer's disease and possibly open up a new prospect for novel pharmacological targets and therapeutic strategies (Wallace, 2005).

Disclosure statement

The authors declare no competing interest.

This work was supported by National Institutes of Health grants AG16573, NS211328, AG24373, DK73691, AG16573, and NS41850; a Doris Duke Clinical Interfaces Award, and CIRM Comprehensive Grant RC1-00353-1 awarded to DCW. Dr. Schork is funded in part by The National Institute on Aging Longevity Consortium [grant number U19 AG023122-01] and the Scripps Translational Sciences Institute Clinical Translational Science Award [NIH/NCRR grant number UL1 RR025774]. This analysis was supported by grants to The Transdisciplinary Imaging Genetics Center (TIGC-P20 RR020837-01), the Alzheimer's Disease Neuroimaging Initiative (ADNI U01 AG024904-01, and supplement 3U01AG024904-03S5), the National Institute of Aging, the National Institute of Biomedical Imaging and Bioengineering (NIH), the Functional Imaging Biomedical Informatics Research Network (FBIRN U24-RR021992, National Center for Research Resources), commercial support from Vanda Pharmaceuticals, and private support from an anonymous foundation and anonymous donations. Additional contributions made through the Foundation for the NIH from Merck & Co. Inc., Pfizer, Inc., and Gene Network Sciences, Inc., partially supported the genotyping results reported here. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI; Principal Investigator: Michael Weiner; NIH grant and supplement). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering (NIBIB), and through generous contributions from the following: Pfizer Inc., Wyeth Research, Bristol-Myers Squibb, Eli Lilly and Company, GlaxoSmithKline, Merck & Co. Inc., AstraZeneca AB, Novartis Pharmaceuticals Corporation, Alzheimer's Association, Eisai Global Clinical Development, Elan Corporation plc, Forest Laboratories, and the Institute for the Study of Aging, with participation from the US Food and Drug Administration. Industry partnerships are coordinated through the Foundation for the National Institutes of Health. The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory of Neuro Imaging at the University of California, Los Angeles. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The ADNI data were previously collected across 50 research sites. Study subjects gave written informed consent at the time of enrollment for imaging and genetic sample collection and completed questionnaires approved by each participating sites' Institutional Review Board (IRB).

Acknowledgements

The authors express their appreciation to Jordan Hiller (JMP Genomics), Liv McMillan, Divya Rajpoot, Jacklyn Walter, Dosu Doherty, and Jerod Rasmussen for their technical support. The genotyping was performed by Jennifer Webster and Drs. David Craig and Matt Huentelman of TGen. We want to acknowledge the support and numerous contributions by the ADNI investigators and the ADNI Industry Advisory Board (Pfizer Inc., Wyeth Research, Bristol-Myers Squibb, Eli Lilly and Company, GlaxoSmith-Kline, Merck & Co. Inc., AstraZeneca AB, Novartis Pharmaceuticals Corporation, Alzheimer's Association, Eisai Global Clinical Development, Elan Corporation plc, Forest Laboratories), and the Institute for the Study of Aging, with participation from the US Food and Drug Administration and the Foundation for the National Institutes of Health. We gratefully acknowledge the participation of the ADNI subjects and their family members.

Genotyping was performed at The Translational Genomics Institute, Phoenix, AZ, by Jennifer Webster and Drs. David Craig and Matt Huentelman. Sample processing, storage, and distribution were provided by the NIA-sponsored National Cell Repository for Alzheimer's Disease by Dr. Tatiana Foroud and Kelley Faber. Sample verification and quality control bioinformatics were provided by Drs. Li Shen, SungEun Kim and Kwangsik Nho of the Indiana University Center for Neuroimaging, Nathan Pankratz of the IU Department of Medical and Molecular Genetics, and Bryan DeChairo of Pfizer, Inc.

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators is available at www.loni.ucla.edu/ADNI/Data/ADNI_Authorship_List.pdf.

The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), and private pharmaceutical companies and nonprofit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principle Investigator of this initiative is Michael W. Weiner MD, VA Medical Center and University of California, San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the USA and Canada.

References

Arning, L., Haghikia, A., Taherzadeh-Fard, E., Saft, C., Andrich, J., Pula, B., Hoxtermann, S., Wieczorek, S., Akkad, D.A., Perrech, M., Gold, R., Epplen, J.T., Chan, A., 2010. Mitochondrial haplogroup H correlates with ATP levels and age at onset in Huntington disease. J Mol Med. 88, 431–436.

Brown, M.D., Shoffner, J.M., Kim, Y.L., Jun, A.S., Graham, B.H., Cabell, M.F., Gurley, D.S., Wallace, D.C., 1996. Mitochondrial DNA sequence analysis of four Alzheimer's and Parkinson's disease patients. Am J Med Genet. 61, 283–289.

Brown, M.D., Voljavec, A.S., Lott, M.T., MacDonald, I., Wallace, D.C., 1992. Leber's hereditary optic neuropathy: a model for mitochondrial neurodegenerative diseases. FASEB J. 6, 2791–2799.

Chagnon, P., Gee, M., Filion, M., Robitaille, Y., Belouchi, M., Gauvreau, D., 1999. Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. Am J Med Genet. 85, 20–30.

Chinnery, P.F., Taylor, G.A., Howell, N., Andrews, R.M., Morris, C.M., Taylor, R.W., McKeith, I.G., Perry, R.H., Edwardson, J.A., Turnbull, D.M., 2000. Mitochondrial DNA haplogroups and susceptibility to AD and dementia with Lewy bodies. Neurology. 55, 302–304.

Cho, D.H., Nakamura, T., Fang, J., Cieplak, P., Godzik, A., Gu, Z., Lipton, S.A., 2009. S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. Science. 324, 102–105.

Coppede, F., Mancuso, M., Lo Gerfo, A., Manca, M.L., Petrozzi, L., Migliore, L., Siciliano, G., Murri, L., 2007. A Ser326Cys polymorphism in the DNA repair gene hOGG1 is not associated with sporadic Alzheimer's disease. Neurosci Lett. 414, 282–285.

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 U S A. 101, 10726–10731.

Crouch, P.J., Blake, R., Duce, J.A., Ciccotosto, G.D., Li, Q.X., Barnham, K.J., Curtain, C.C., Cherny, R.A., Cappai, R., Dyrks, T., Masters, C.L., Trounce, I.A., 2005. Copper-dependent inhibition of human cytochrome c oxidase by a dimeric conformer of amyloid-beta1–42. J Neurosci. 25, 672–679.

Davis, R.E., Miller, S., Herrnstadt, C., Ghosh, S.S., Fahy, E., Shinobu, L.A., Galasko, D., Thal, L.J., Beal, M.F., Howell, N., Parker, W.D., Jr, 1997. Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc Natl Acad Sci U S A. 94, 4526–4531.

Devi, L., Prabhu, B.M., Galati, D.F., Avadhani, N.G., Anandatheerthavarada, H.K., 2006. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. J Neurosci. 26, 9057–9068.

Eichhorn-Mulligan, K., Cestari, D.M., 2008. The genetics of leber hereditary optic neuropathy − prototype of an inherited optic neuropathy with mitochondrial dysfunction. Semin Ophthalmol. 23, 27–37.

Elson, J.L., Herrnstadt, C., Preston, G., Thal, L., Morris, C.M., Edwardson, J.A., Beal, M.F., Turnbull, D.M., Howell, N., 2006. Does the mito-

chondrial genome play a role in the etiology of Alzheimer's disease? Hum Genet. 119, 241–254.
Engidawork, E., Lubec, G., 2001. Protein expression in Down syndrome brain. Amino Acids. 21, 331–361.
Enoch, M.A., Shen, P.H., Xu, K., Hodgkinson, C., Goldman, D., 2006. Using ancestry-informative markers to define populations and detect population stratification. J Psychopharmacol. 20, 19–26.
Finnila, S., Lehtonen, M.S., Majamaa, K., 2001. Phylogenetic network for European mtDNA. Am J Hum Genet. 68, 1475–1484.
Fosslien, E., 2001. Mitochondrial medicine − molecular pathology of defective oxidative phosphorylation. Ann Clin Lab Sci. 31, 25–67.
Gotz, J., Schonrock, N., Vissel, B., Ittner, L.M., 2009. Alzheimer's disease selective vulnerability and modeling in transgenic mice. J Alzheimers Dis. 18, 243–251.
Hendrickson, S.L., Hutcheson, H.B., Ruiz-Pesini, E., Poole, J.C., Lautenberger, J., Sezgin, E., Kingsley, L., Goedert, J.J., Vlahov, D., Donfield, S., Wallace, D.C., O'Brien, S.J., 2008. Mitochondrial DNA haplogroups influence AIDS progression. AIDS. 22, 2429–2439.
Ingman, M., Gyllensten, U., 2001. Analysis of the complete human mtDNA genome: methodology and inferences for human evolution. J Hered. 92, 454–461.
Jou, S.H., Chiu, N.Y., Liu, C.S., 2009. Mitochondrial dysfunction and psychiatric disorders. Chang Gung Med J. 32, 370–379.
Kinoshita, H., Imayama, H., Sou, H., Shibata, J., Ogami, N., Tamae, T., Nakayama, T., 1996. A case of obstructive icterus caused by incarceration of a pancreatic stone in the common channel of the pancreatobiliary ducts. Kurume Med J. 43, 79–85.
Liang, W.S., Reiman, E.M., Valla, J., Dunckley, T., Beach, T.G., Grover, A., Niedzielko, T.L., Schneider, L.E., Mastroeni, D., Caselli, R., Kukull, W., Morris, J.C., Hulette, C.M., Schmechel, D., Rogers, J., Stephan, D.A., 2008. Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. Proc Natl Acad Sci U S A. 105, 4441–4446.
Lin, M.T., Beal, M.F., 2006. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature. 443, 787–795.
Lindroos, M.M., Borra, R.J., Parkkola, R., Virtanen, S.M., Lepomaki, V., Bucci, M., Virta, J.R., Rinne, J.O., Nuutila, P., Majamaa, K., 2009. Cerebral oxygen and glucose metabolism in patients with mitochondrial m.3243A > G mutation. Brain. 132, 3274–3284.
Mamelak, M., 2007. Alzheimer's disease, oxidative stress and gammahydroxybutyrate. Neurobiol Aging. 28, 1340–1360.
Mancuso, M., Coppede, F., Migliore, L., Siciliano, G., Murri, L., 2006. Mitochondrial dysfunction, oxidative stress and neurodegeneration. J Alzheimers Dis. 10, 59–73.
McMahon, F.J., Chen, Y.S., Patel, S., Kokoszka, J., Brown, M.D., Torroni, A., dePaulo, J.R., Wallace, D.C., 2000. Mitochondrial DNA sequence diversity in bipolar affective disorder. Am J Psychiatry. 157, 1058–1064.
Montiel-Sosa, F., Ruiz-Pesini, E., Enriquez, J.A., Marcuello, A., Diez-Sanchez, C., Montoya, J., Wallace, D.C., Lopez-Perez, M.J., 2006. Differences of sperm motility in mitochondrial DNA haplogroup U sublineages. Gene. 368, 21–27.
Potkin, S.G., Guffanti, G., Lakatos, A., Turner, J.A., Kruggel, F., Fallon, J.H., Saykin, A.J., Orro, A., Lupoli, S., Salvi, E., Weiner, M., Macciardi, F., 2009. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS One. 4, e6501.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81, 559–575.
Reddy, P.H., 2009. Amyloid beta, mitochondrial structural and functional dynamics in Alzheimer's disease. Exp Neurol. 218, 286–292.
Reiman, E.M., Chen, K., Alexander, G.E., Caselli, R.J., Bandy, D., Osborne, D., Saunders, A.M., Hardy, J., 2004. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. Proc Natl Acad Sci U S A. 101, 284–289.
Richards, M., Corte-Real, H., Forster, P., Macaulay, V., Wilkinson-Herbots, H., Demaine, A., Papiha, S., Hedges, R., Bandelt, H.J., Sykes, B., 1996. Paleolithic and neolithic lineages in the European mitochondrial gene pool. Am J Hum Genet. 59, 185–203.
Richards, M.B., Macaulay, V.A., Bandelt, H.J., Sykes, B.C., 1998. Phylogeography of mitochondrial DNA in western Europe. Ann Hum Genet. 62, 241–260.
Roses, A.D., Lutz, M.W., Amrine-Madsen, H., Saunders, A.M., Crenshaw, D.G., Sundseth, S.S., Huentelman, M.J., Welsh-Bohmer, K.A., Reiman, E.M., 2009. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. Pharmacogenomics J., 1-10.
Roubertoux, P.L., Sluyter, F., Carlier, M., Marcet, B., Maarouf-Veray, F., Cherif, C., Marican, C., Arrechi, P., Godin, F., Jamon, M., Verrier, B., Cohen-Salmon, C., 2003. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. Nat Genet. 35, 65–69.
Schork, N.J., Fallin, D., Thiel, B., Xu, X., Broeckel, U., Jacob, H.J., Cohen, D., 2001. The future of genetic case-control studies. Adv Genet. 42, 191–212.
Shen, L., Kim, S., Risacher, S.L., Nho, K., Swaminathan, S., West, J.D., Foroud, T., Pankratz, N., Moore, J.H., Sloan, C.D., Huentelman, M.J., Craig, D.W., Dechairo, B.M., Potkin, S.G., Jack, C.R., Jr, Weiner, M.W., Saykin, A.J., 2010. Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: A study of the ADNI cohort. Neuroimage.
Shoffner, J.M., Brown, M.D., Torroni, A., Lott, M.T., Cabell, M.F., Mirra, S.S., Beal, M.F., Yang, C.C., Gearing, M., Salvo, R., 1993. Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. Genomics. 17, 171–184.
Torroni, A., Huoponen, K., Francalacci, P., Petrozzi, M., Morelli, L., Scozzari, R., Obinu, D., Savontaus, M.L., Wallace, D.C., 1996. Classification of European mtDNAs from an analysis of three European populations. Genetics. 144, 1835–1850.
Tuppen, H.A., Blakely, E.L., Turnbull, D.M., Taylor, R.W., 2009. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta. 1797, 113–128.
van der Walt, J.M., Dementieva, Y.A., Martin, E.R., Scott, W.K., Nicodemus, K.K., Kroner, C.C., Welsh-Bohmer, K.A., Saunders, A.M., Roses, A.D., Small, G.W., Schmechel, D.E., Murali Doraiswamy, P., Gilbert, J.R., Haines, J.L., Vance, J.M., Pericak-Vance, M.A., 2004. Analysis of European mitochondrial haplogroups with Alzheimer disease risk. Neurosci Lett. 365, 28–32.
van Oven, M., Kayser, M., 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 30, E386–E394.
Wallace, D.C., 2005. The mitochondrial genome in human adaptive radiation and disease: on the road to therapeutics and performance enhancement. Gene. 354, 169–180.
Wallace, D.C., 2008. Mitochondria as chi. Genetics. 179, 727–735.
Wallace, D.C., Brown, M.D., Melov, S., Graham, B., Lott, M., 1998. Mitochondrial biology, degenerative diseases and aging. Biofactors. 7, 187–190.
Wallace, D.C., Fan, W., 2009. Energetics, epigenetics, mitochondrial genetics. Mitochondrion. 10, 12–31.
Wallace, D.C., Fan, W., Procaccio, V., 2010. Mitochondrial energetics and therapeutics. Annu Rev Pathologe. 5, 297–348.
Wang, X., Su, B., Lee, H.G., Li, X., Perry, G., Smith, M.A., Zhu, X., 2009. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. J Neurosci. 29, 9090–9103.
Wang, X., Su, B., Perry, G., Smith, M.A., Zhu, X., 2007. Insights into amyloid-beta-induced mitochondrial dysfunction in Alzheimer disease. Free Radic Biol Med. 43, 1569–1573.
Yankner, B.A., Lu, T., Loerch, P., 2008. The aging brain. Annu Rev Pathologe. 3, 41–66.
Zecavati, N., Spence, S.J., 2009. Neurometabolic disorders and dysfunction in autism spectrum disorders. Curr Neurol Neurosci Rep. 9, 129–136.