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Mitochondrial DNA sequence associations with dementia and amyloid-β in elderly African Americans

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Abstract

Mitochondrial dysfunction occurs early in the course of several neurodegenerative diseases, and is potentially related to increased oxidative damage and amyloid-β (Aβ) formation in Alzheimer's disease. The goals of this study were to assess mtDNA sequence associations with dementia risk, 10-year cognitive change, and markers of oxidative stress and Aβ among 1089 African-Americans in the population-based Health, Aging, and Body Composition Study. Participants were free of dementia at baseline, and incidence was determined in 187 (18%) cases over 10 to 12 follow-up years. Haplogroup L1 participants were at increased risk for developing dementia (odds ratio =

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1.88, 95% confidence interval = 1.23–2.88, $p = 0.004$), lower plasma Aβ42 levels ($p = 0.03$), and greater 10-year decline on the Digit Symbol Substitution Test ($p = 0.04$) when compared with common haplogroup L3. The p.V193I, *ND2* substitution was associated with significantly higher Aβ42 levels ($p = 0.0012$), and this association was present in haplogroup L3 ($p = 0.018$) but not L1 ($p = 0.90$) participants. All associations were independent of potential confounders, including APOEε4 status and nuclear genetic ancestry. Identification of mtDNA sequence variation associated with dementia risk and cognitive decline may contribute to the development of new treatment targets and diagnostic tests that identify responders to interventions targeting mitochondria.

Keywords

Dementia; Mitochondria; mtDNA; Amyloid-β; Oxidative stress

1. Introduction

Mitochondrial dysfunction is an especially important characteristic of most late-onset neurodegenerative diseases (Mattson, 2000) and is a prominent hallmark of Alzheimer's disease (AD) (Anglade et al., 1997, Blass et al., 2002, Castellani et al., 2002, Chinopoulos et al., 1999, Corral-Debrinski et al., 1994, Coskun et al., 2010, de la Monte et al., 2000, Dragicevic et al., 2010, Eckert et al., 2003, Gibson et al., 2000, Grazina et al., 2006, Hauptmann et al., 2009, Hinerfeld et al., 2004, Hirai et al., 2001, Hsiao et al., 1996, Leuner et al., 2012, Manczak et al., 2006, Mucke et al., 2000, Park et al., 1999, Pruijn et al., 1992, Shi et al., 2008 and Yao et al., 2009), the most common type of dementia. Considerable evidence suggests that the changes in mitochondrial function are causally linked to several early abnormalities that accompany AD and precede both neuronal loss and amyloid-β (Aβ) formation (de la Monte et al., 2000, Hauptmann et al., 2009, Leuner et al., 2012 and Yao et al., 2009), as well as the clinical manifestation of AD in humans (Gibson and Shi, 2010). These early alterations to mitochondria, which can induce multiple abnormalities, may present more desirable therapeutic targets than the reversal of the individual pathologies that occur later in the neurodegenerative process.

Several lines of evidence show that key enzymes responsible for mitochondrial energy metabolism are severely affected in AD (Blass et al., 2002, Eckert et al., 2003, Gibson et al., 1998, Grazina et al., 2006, Kish et al., 1999 and Swerdlow et al., 1997). For example, genes coding for respiratory chain subunits are differentially expressed in AD patients (Gibson et al., 1998 and Kish et al., 1999). In addition, it is known that cytoplasmic hybrids (cybrids) carrying mitochondrial DNA (mtDNA) from AD patients exhibit depressed Complex IV activity when compared with cybrids prepared with mtDNA from non-AD controls, suggesting that the deficit is in part encoded by mtDNA abnormalities (Swerdlow et al., 1997). The brain is particularly susceptible to defective mitochondrial function related to mtDNA mutations (Bishop et al., 2010 and Gibson and Shi, 2010). For example, mtDNA damage may be a major cause of abnormal reactive oxygen species (ROS) production in AD or may increase neuronal susceptibility to oxidative injury during aging.

Human mtDNA is a maternally inherited 16,569–base pair loop–containing genes critical to mitochondrial energy production (Wallace, 2010), and bioenergetic defects resulting from acquired and inherited mtDNA mutations may be critical for both age-related dementia and associated neuropathological changes observed in AD (Brown and Wallace, 1994, Corral-Debrinski et al., 1994 and Coskun et al., 2010; De Vivo, 1993, Graeber et al., 1998, Hutchin et al., 1997, Manczak et al., 2004, Tranah et al., 2012b and Wallace, 2001). Sequence variation within the 13 mtDNA-encoded oxidative phosphorylation (OXPHOS) genes may have an impact on superoxide production at OXPHOS Complexes I and III through respiratory chain impairment (Niemi et al., 2005), apoptosis (Li et al., 2003), and ATP generation efficiency (Tarnopolsky et al., 2004).

Individual mtDNA mutations have been identified in patients with AD (Brown et al., 1996, Edland et al., 1996, Edland et al., 2002, Egensperger et al., 1997, Grazina et al., 2005, Grazina et al., 2006, Hutchin and Cortopassi, 1995, Janetzky et al., 1996, Kosel et al., 1994, Lakatos et al., 2010, Lin et al., 1992, Petruzzella et al., 1992, Qiu et al., 2001, Shoffner et al., 1993, Tanno et al., 1998, Tranah et al., 2012b, Tysoe et al., 1996 and Wragg et al., 1995); yet, many of these studies were small, and most of the identified variants have not been replicated (Edland et al., 2002, Janetzky et al., 1996, Kosel et al., 1994, Petruzzella et al., 1992, Tanno et al., 1998, Tysoe et al., 1996 and Wragg et al., 1995). To date, the most comprehensive studies of mtDNA variation in AD (Lakatos et al., 2010), dementia (Tranah et al., 2012b), and cognitive decline (Tranah et al., 2012b) identified haplogroup and individual variant associations with disease that were independent of APOEε4 allele status. The majority of this previous work, however, has focused on white European and North American populations, and little is known about risk factors for understudied populations. Identifying risk factors for the African American population is of particular importance, as African Americans have substantially higher AD rates than white North Americans (Evans et al., 2003, Obisesan et al., 2012 and Tang et al., 2001). As the prevalence of AD reaches epidemic proportions in the United States and worldwide in the coming decades (Hebert et al., 2003), understanding the basis of cognitive impairment is critical to treating and preventing disease. In the present study, we extend our previous mtDNA work in the Health, Aging, and Body Composition (Health ABC) Study (Tranah et al., 2012b) by assessing dementia risk and cognitive decline in elderly African Americans, and by examining whether mtDNA variation is associated with circulating Aβ levels and markers of oxidative stress (plasma oxidized LDL and urinary 8-iso-prostaglandin $F_{2\alpha}$). We have shown that greater energy expenditure is associated with a reduced incidence of cognitive impairment in older adults from the Health ABC Study (Middleton et al., 2011) and have documented significant differences in metabolic rate among African and European mitochondrial haplogroups from this study (Tranah et al., 2011 and Tranah et al., 2012a). Considering the strong link between metabolic rate and cognitive impairment (Middleton et al., 2011), these previous studies provide the impetus for understanding the association between mitochondrial haplogroups and variants and cognition in late life. Uncovering specific mitochondrial variants that have an impact on dementia risk may lead to the development of new interventions or clinical strategies for improving mitochondrial function and delaying the onset of disease and cognitive decline, as well as genetic tests for identifying individuals who are more or less likely to respond to treatments that target the mitochondria.

2. Method

2.1. Study population

Participants were part of the Health ABC Study, a prospective cohort study of 3075 community-dwelling man and women of black and white ethnicities living in Memphis, TN, or Pittsburgh, PA, and aged 70 to 79 years at recruitment in 1996 to 1997 (Rooks et al., 2002). To identify potential participants, a random sample of white and all black Medicare-eligible elders, within designated zip code areas, were contacted. To be eligible, participants had to report no difficulty with activities of daily living, walking a quarter of a mile, or climbing 10 steps without resting. They also had to be free of life-threatening cancer diagnoses and have no plans to move out of the study area for at least 3 years. The sample was approximately balanced for sex (51% women), and 41% of participants were black. Participants self-designated race/ethnicity from a fixed set of options (Asian/Pacific Islander, black/African American, white/Caucasian, Latino/Hispanic, do not know, other). All eligible participants signed a written informed consent form, approved by the institutional review boards at the clinical sites. This study was approved by the institutional review boards of the clinical sites and the coordinating center (University of California–San Francisco).

2.2. Genotyping

Genomic DNA was extracted from buffy coat collected using a PUREGENE DNA Purification Kit during the baseline examination. Genotyping was performed by the Center for Inherited Disease Research (CIDR) using the Illumina Human1M-Duo BeadChip system. This platform includes 138 mtDNA SNPs including the majority of haplogroup-defining variants (Saxena et al., 2006). Samples were excluded from the dataset for the reasons of sample failure, genotypic sex mismatch, and first-degree relative of an included individual based on genotype data. Only SNPs with call rate ≥97% and Hardy–Weinberg equilibrium $p \geq 10^{-6}$ were analyzed. For African American Health ABC participants, autosomal genotypes were available on 1,007,948 high-quality autosomal SNPS. Genotyping of 138 mtDNA SNPs was successful for 1089 unrelated individuals of African genetic ancestry and yielded 94 polymorphic sites. The major African haplogroups were defined in 1029 African American participants using PhyloTree (van Oven and Kayser, 2009): L0 (n = 66, 6.4%), L1 (n = 188, 18.3%), L2 (n = 360, 35.0%), and L3 (n = 415, 40.3%). Sixty participants were identified as belonging to different rare African or Eurasian haplogroups. Nuclear genetic ancestry was determined using a set of 1332 ancestry informative markers that estimated the proportion of African and European ancestry in the Health ABC African Americans as previously described (Aldrich et al.).

2.3. Dementia incidence

All participants were free of dementia at baseline. Incident dementia was determined by the date of the first available record of a dementia diagnosis over 10 to 12 years of follow-up. Cases were identified through hospital records indicating a dementia-related hospital event, either as the primary or secondary diagnosis related to the hospitalization, or by record of prescribed dementia medication (i.e. galantamine, rivastigmine, memantine, donepezil, tacrine).

2.4. Cognitive function testing

The Modified Mini-Mental State Examination (3MS) was administered to participants at years 1, 3, 5, 7, 9 and 10. The 3MS is a brief, general cognitive battery with components for orientation, concentration, language, praxis, and immediate and delayed recall (episodic memory) (Teng and Chui, 1987). Possible scores range from 0 to 100, with higher scores indicating better cognitive function. The Digit Symbol Substitution Test (DSST) was administered to participants at years 1, 5, 7, 9 and 10. The DSST measures response speed, sustained attention, visual spatial skills, and set shifting, all of which reflect executive cognitive function (Beres and Baron, 1981 and Wechsler, 1981). The test is reported to distinguish mild dementia from healthy aging (Tierney et al., 1987). The DSST score is calculated as the total number of items correctly coded in 90 seconds, with a higher score indicating better cognitive function. Participant-specific slopes of 3MS and DSST scores were estimated by best linear unbiased predictions using mixed-effects models with random intercepts and slopes (Fiocco et al.) in STATA10 (StataCorp, College Station, TX).

2.5. Biomarkers

Plasma Aβ40 and Aβ42 levels were measured by the Mayo Clinic (Jacksonville, FL), using Innogenetics (Ghent, Belgium) INNO-BIA assays in samples obtained during the second Health ABC visit (Yaffe et al.). Plasma oxidized LDL (oxLDL) levels were measured in samples obtained during the first Health ABC visi (Cesari et al., 2005 and Njajou et al., 2009) by the Atherosclerosis and Metabolism Unit of the Katholieke Universiteit Leuven as previously described (Holvoet et al., 2006) using a monoclonal antibody (4E6)–based competition ELISA. Urinary 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) levels were measured in samples obtained at the second Health ABC visit (Cesari et al., 2012) by the Laboratory of Clinical Pharmacology of Eicosanoids and Pharmacodynamic located in the Center of Excellence on Aging at the “Gabriele D’Annnunzio” University Foundation (Chieti, Italy), using previously described radioimmunoassay methods (Ciabattoni et al., 1987 and Wang et al., 1995). Summary statistics for biomarkers are presented in Table 1.

2.6. Statistical analyses

We first analyzed dementia risk among the major African haplogroups and for each of 41 common variants (minor allele frequency [MAF] ≥5%). Unconditional logistic regression was used to obtain odds ratios (ORs) as estimates of relative risks (hereafter called risk) and 95% confidence intervals (CIs) for dementia involving haplogroups and common variants. Risk of dementia was examined for haplogroups L0, L1, and L2 as compared to the haplogroup L3 reference group. Haplogroup L3 was selected as the reference group because it is the most common African haplogroup in our study and this group gave rise to the major Eurasian haplogroups from which the vast majority of non-Africans are descended (Salas et al., 2002). The 94 individual mtDNA variants were examined for associations with dementia using logistic regression, testing risk associated with the rare allele as compared with the common allele. Aβ (40, 42, and 42/40), oxLDL, and 8-iso-$PGF_{2\alpha}$ were compared among the common African haplogroups and for individual mtDNA variants using the generalized linear model. All analyses were adjusted for age, sex, and clinic site using SAS version 9.2 (SAS Institute, Cary, NC). Haplogroup analyses were adjusted for APOEε4 allele carrier

status and estimated nuclear European ancestry in secondary models. To avoid false-positive results due to population stratification, analyses involving the 137 mtDNA variants were also adjusted for 6 eigenvectors of mitochondrial genetic ancestry derived from principal component analysis (PCA) (Biffi et al., 2010, Patterson et al., 2006, Price et al., 2006, Price et al., 2010 and Reich et al., 2008). In our previous mtDNA sequencing work the first 6 eigenvectors have accounted for 71% of the variance in the mtDNA sequence dataset (Tranah et al., 2011). Mitochondrial PCA has been shown to outperform haplogroup-stratified or adjusted association analyses with no loss in power for the detection of true associations (Biffi et al., 2010). Several in silico methods were used to examine mtDNA nucleotide conservation [PhastCons (Siepel et al., 2005) and PhyloP (Pollard et al., 2010)] for all variants and to predict the potential functional impact of non-synonymous substitutions on amino acid protein sequences [Sorting Tolerant From Intolerant (SIFT) (Kumar et al., 2009 and Ng and Henikoff, 2006), MutPred (Li et al., 2009), and PolyPhen2 (Adzhubei et al., 2010)].

3. Results

Among 1089 genotyped African American Health ABC participants, 187 (17%) developed dementia (Table 1). In general, dementia cases were more likely to be APOEε4 allele carriers, but there were no differences in age, sex, or levels of European nuclear genetic ancestry (Table 1). Haplogroup frequencies are consistent with mtDNA sequencing performed by us (Lam et al., 2012) and others (Saxena et al., 2006), and mean European nuclear genetic ancestry did not differ ($p = 0.38$) among the 4 major African haplogroups: L0 (19%), L1 (21%), L2 (20%), L3 (21%). Among the 94 polymorphic mtDNA sites detected after genotyping 138 SNPs, 53 occurred at a MAF of <5% and 41 at MAF of ≥5%.

Risk of developing dementia among the 4 African sub-haplogroups is reported in Table 2. Carriers of haplogroup L1 had an increased risk of developing dementia compared with the most common African haplogroup L3 in a model adjusted for age, sex, and clinic site (OR = 1.88, 95% CI = 1.23 – 2.88, $p = 0.004$) that was statistically significant after adjustment for multiple comparisons (3 haplogroups, critical $\alpha = 0.016$). Adjustment for either APOEε4 allele carrier status (OR = 1.78, 95% CI = 1.15–2.76, $p = 0.009$) or European ancestry (OR = 1.76, 95% CI = 1.14–2.73, $p = 0.010$) slightly attenuated the associations, but the results remained statistically significant at an adjusted threshold. Haplogroup L1 participants experienced a slightly greater 10-year decline in DSST ($\beta = -0.08$, ± standard error [SE] = 0.04) when compared with haplogroups L2 ($p = 0.02$) and L3 ($p = 0.04$) (Table 3). Adjustment for European ancestry and education level did not affect results (data not shown). There were no haplogroup differences in either baseline 3MS or 3MS slopes.

We examined haplogroup differences among the subsets of participants with plasma oxLDL, urinary 8-iso-$PGF_{2\alpha}$, and plasma Aβ40 and Aβ42. Among 433 African American participants with plasma Aβ42 levels measured, haplogroup L1 participants had nominally lower ($p = 0.03$) Aβ42 levels when compared with participants from haplogroup L3 (Table 4). No haplogroup differences were identified for oxLDL, 8-iso-$PGF_{2\alpha}$, and Aβ42/40 ratios.

Among the 41 common mtDNA variants genotyped, the m.5046G>A variant encoding a p.V193I, *ND2*substitution was associated with significantly higher (p = 0.001) Aβ42 levels after adjustment for multiple comparisons (critical α = 0.0012). Haplogroup L carriers of the m.5046A allele (n = 119) had an elevated mean (SE) Aβ42 of 43.0 (3.5) pg/mL when compared with a mean (SE) Aβ42 of 31.2 (0.59) pg/mL for carriers of the m.5046G allele (n = 968). The m.5046G>A variant was present only in haplogroups L1 and L3. We identified a statistically significant (p = 0.047) interaction between the m.5046A variant and haplogroup L1/L3 status in a model that included age, sex, m.5046G>A genotype, L1/L3 status, and an interaction term for m.5046G>A genotype and L1/L3 status. The m.5046A allele was significantly associated with elevated Aβ42 levels among haplogroup L3 (p = 0.018) but not L1 (p = 0.90) participants (Table 5). Specifically, haplogroup L3 carriers of the m.5046A allele had an elevated mean (SE) Aβ42 of 43.2 (4.5) pg/mL when compared with a mean (SE) Aβ42 of 33.4 (0.80) pg/mL for carriers of the m.5046G allele. There was no in silico evidence for nucleotide conservation (phastCons, phyloP) or predicted functional significance (SIFT, PolyPhen2, and MutPred) for the p.V193I, *ND2* substitution. None of the individual mtDNA variants were associated with dementia risk, 3MS, or DSST slopes after adjustment for multiple comparisons.

4. Discussion

Several mechanisms underlie the changes observed in the aging brain including mitochondrial function and oxidative stress, autophagy, and protein turnover (Bishop et al., 2010). Mitochondrial dysfunction, in particular, occurs early in the neurodegenerative process and precedes neuronal loss and early Aβ formation in AD (de la Monte et al., 2000, Hauptmann et al., 2009, Leuner et al., 2012 and Yao et al., 2009). Several key enzymes responsible for mitochondrial energy metabolism are severely affected in AD (Blass et al., 2002, Castellani et al., 2002, Eckert et al., 2003, Grazina et al., 2006 and Swerdlow et al., 1997), and the results presented herein suggest that Complex I genetic variation may be of particular importance to the neurodegenerative process. Complex I is a large, multi-subunit, membrane-bound protein that serves as the major entry point for most electrons into the electron transport chain. This complex is a major contributor to cellular ROS production (Hirst). Inhibition of Complex I leads to increased generation of ROS, decreased ATP levels, and induction of apoptosis (Langston and Ballard, 1983, Li et al., 2003 and Ramsay and Singer, 1986), all of which play a major role in neurodegeneration. In previous studies linking Complex I mtDNA sequence data to cognitive function and disease, non-synonymous *ND4* and *ND5* substitutions were associated with AD risk (Lakatos et al., 2010), and we reported *ND6* associations with decline in 3MS (Tranah et al., 2012b).

In the current study, Haplogroup L1 participants were at a significantly increased risk for developing dementia, experienced a significant 10-year decline in DSST, and had lower plasma Aβ42 levels [which has previously been associated with increased risk of developing AD (Graff-Radford et al., 2007, Lewczuk et al., 2010 and Pesaresi et al., 2006) and greater cognitive decline on the 3MS (Yaffe et al., 2011)]. The large number of variants that are closely associated with one another and that define haplogroup L1 (characterized by Complex I [*ND1*, *ND5*, *ND6*] and Complex IV [*COI*] variants) (van Oven and Kayser, 2009) complicate interpretation of haplogroup association data. In addition, 3 of these

haplogroup-defining variants encode amino acid substitutions with possible functional potential: p.Y485H, *ND5*, p.I166V, *ND6*, and p.Y496H, *COI*. We previously observed that European haplogroup T participants were at a significantly increased risk for developing dementia and that haplogroup J participants experienced a significant longitudinal decline in 3MS (Tranah et al., 2012b). Haplogroup T is defined by multiple variants in the 12S and 16S rRNAs, $tRNA_{Arg}$, $tRNA_{Thr}$, *ND2*, *ND5*, *CytB*, and *ATP6* (van Oven and Kayser, 2009) with only 1 of these variants having apparent functional potential: m.4917A>G, which encodes amino acid substitution p.N150D, *ND2* in Complex I. Haplogroup J is defined by variants in ND5 and the hypervariable region (van Oven and Kayser, 2009). Such haplogroup associations can mask the effects of individual nucleotide changes because most mitochondrial haplogroups can be defined by several control region and/or coding region variants. This implies that functional studies should account for all mtDNA variants within the mitochondrial genome, especially for haplogroups that can be defined by several variants.

The analysis of mtDNA is further complicated by recurrent mutation at the same mtDNA site across divergent haplogroups, which can sometimes hide diagnostic specificity of a particular variant. That the same mutations have been observed repeatedly on different mtDNA backgrounds (e.g., haplogroups) has been cited as evidence of convergent adaptive evolution of particular mtDNA mutations (Wallace, 2010). We observed that the m. 5046G>A variant encoding the p.V193I, *ND2* substitution was significantly associated with Aβ42 levels and that this association was specific to haplogroup L3 carriers of the variant but not haplogroup L1 carriers. Indeed, significant differences in the frequency of non-synonymous mutations among haplogroups (Moilanen et al., 2003) suggests that some mutations may be non-neutral within specific phylogenetic lineages but neutral within others.

This study has a number of strengths: a well-characterized population-based African-American cohort with longitudinal assessment of 3MS and DSST; a large sample size for assessing dementia risk and Aβ among African mtDNA haplogroups and variants; measurement of circulating Aβ levels, and markers of oxidative stress. Limited power to detect individual effects of rare variants is acknowledged. These preliminary results are based on a single cohort and further studies are needed to confirm these findings. In addition, plasma- and urine-derived biomarkers may not accurately reflect the state of brain levels.

In summary, dementia is a complex neurodegenerative disorder with a multifaceted genetic and clinical pathogenesis involving neurodegenerative, vascular, and metabolic causes. Mitochondrial dysfunction underlies the symptoms of many human neurological disorders, including AD (Mattson, 2000), which suggests the existence of a shared neurodegenerative mechanism operating across multiple pathologies. Identifying dementia-associated OXPHOS Complex I variants may lead to targeted interventions that affect superoxide production (Brand, 2010 and Li et al., 2003). For example, several natural compounds (Baur and Sinclair, 2006, Baur et al., 2006, Chowanadisai et al., 2010, Davis et al., 2009, Liu et al., 2007, Nogueira et al., 2011, Pratico, 2008, Rasbach and Schnellmann, 2008, Rodriguez et al., 2007, Stites et al., 2006, Tauskela, 2007 and Timmers et al., 2011), behavioral

interventions (Civitarese et al., 2007, Guarente, 2008, Johnston et al., 2008, Lopez-Lluch et al., 2006, Menshikova et al., 2006 and Nisoli et al., 2005), and pharmacologic agents (Bar-Am et al., 2009, Weinreb et al., 2008a, Weinreb et al., 2008b and Youdim and Buccafusco, 2005) target the mitochondria and have been shown to induce mitochondrial biogenesis and increase electron transport chain activity. Further studies confirming our findings may provide detailed clues about the mechanisms of disease related to specific mitochondrial variants, and ultimately may contribute to the development of genotype-specific therapeutic interventions for delaying the onset of disease and cognitive decline.

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Table 1

Baseline characteristics of dementia case individuals and controls among 1089 genotyped African American Health ABC participants

Characteristic	No dementia	Dementia
n (%)	902 (83)	187 (17)
Age at baseline, y, mean (SD)	73.3 (2.8)	74.1 (3.0)
APOEe4 carrier, n (%)	295 (33)	88 (48)[b]
Sex, n (%)		
Male	393 (44)	73 (39)
Female	509 (56)	114 (61)
Haplogroup, n[a] (%)		
L0	56 (84.85)	10 (15.15)
L1	139 (73.94)	49 (26.06)
L2	300 (83.33)	60 (16.67)
L3	351 (84.58)	64 (15.42)
European nuclear genetic ancestry, % (SE)	20 (12)	21 (12)
3MS	86.4 (9.4)	82.9 (12.1)
DSST	27.8 (14.5)	23.3 (14.2)
Aβ42 units	32.6 (9.7)	31.9 (9.8)
Aβ42/40	0.18 (0.06)	0.17 (0.04)
oxLDL units	1.41 (0.84)	1.27 (0.57)
8-iso-PGF2α units	753 (469)	793 (566)

Key: Aβ42, Plasma mean amyloid-beta-42 (pg/mL; n = 433); Aβ42/40, plasma mean amyloid-beta-42/40 ratio (n = 423); DSST, Digit Symbol Substitution Test (n = 1059); oxLDL, plasma oxidized low density lipoprotein (mg/dL; n = 1027); 3MS, Modified Mini-Mental State Examination (n = 1079); 8-iso-PGF2a, urinary 8-iso-prostaglandin, F2-alpha (pg/mg creatinine; n = 584).

[a]Numbers do not add up to total because of missing information for haplogroups.

[b]APOEe4 frequency significantly differs between dementia cases and controls, Fisher's exact test, $p = 0.0003$.

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for dementia associated with haplogroup L subgroups

Haplogroup	Cases (n, %)	Controls (n, %)	OR (95% CI)[a]	*p* Value	OR (95% CI)[b]	*p* Value
L3	64 (15.42)	351 (84.58)	Ref.		Ref.	
L2	60 (16.67)	300 (83.33)	1.07 (0.72–1.57)	0.97	1.05 (0.71–1.55)	0.93
L1	49 (26.06)	139 (73.94)	1.88 (1.23–2.88)	0.004	1.78 (1.15–2.76)	0.009
L0	10 (15.15)	56 (84.85)	1.00 (0.48–2.05)	0.74	1.04 (0.50–2.16)	0.83

[a] Adjusted for age, sex, and clinic site.

[b] Adjusted for age, sex, clinic site, and APOE*e4 status.

Table 3

Baseline and 10 year rate of change on the DSST and 3MS tests for haplogroup L subgroups

Haplogroup	Baseline DSST, mean (SE) (n = 981)	DSST slope, mean (SE) (n = 455)	Baseline 3MS, mean (SE) (n = 999)	3MS slope, mean (SE) (n = 421)
L3	27.1 (0.71)	0.012 (0.027)[a]	85.7 (0.50)	− 0.016 (0.052)
L2	27.2 (0.76)	0.030 (0.030)[b]	86.2 (0.54)	0.011 (0.057)
L1	27.1 (1.07)	− 0.084 (0.039)[ab]	85.3 (0.75)	− 0.002 (0.075)
L0	27.3 (1.84)	− 0.015 (0.060)	84.9 (1.28)	0.052 (0.12)

All values adjusted for age, sex, clinic site, and APOE*e4 status.

Pairwise comparisons.

[a] $p = 0.04$ for pairwise comparison.

[b] $p = 0.02$ for pairwise comparison.

Table 4

Comparison of amyloid-β levels and markers of oxidative damage among African American haplogroups

Haplogroup	Aβ42 (n = 433)	Aβ42/40 (n = 423)	oxLDL (n = 1,027)	8-iso-PGF2α (n = 584)
L3	33.70 (0.76)[a]	0.18 (0.005)	1.39 (0.04)	792 (32)
L2	32.14 (0.76)	0.18 (0.005)	1.38 (0.04)	742 (36)
L1	31.12 (1.09)[a]	0.17 (0.007)	1.45 (0.06)	757 (47)
L0	31.69 (1.77)	0.18 (0.011)	1.23 (0.10)	661 (71)

All values adjusted for age, sex, and clinic site.

Key (for pairwise comparisons): Aβ42/40, plasma mean amyloid-beta-42/40 ratio; oxLDL, plasma oxidized low density lipoprotein (mg/dL); 8-iso-PGF2α, urinary 8-iso-prostaglandin, F2-α (pg/mg creatinine).

[a] $p = 0.03$.

Table 5

Association among m.5046G>A, p.V193I, *ND2*, and Aβ42 levels among haplogroup L1 and L3 participants

	Haplogroup L1 Aβ42			Haplogroup L3 Aβ42		
	n (%)	**Mean (SE)**	***p* Value**	**n (%)**	**Mean (SE)**	***p* Value**
A	39 (49%)	31.0 (1.66)		5 (3%)	43.4 (4.50)	
G	40 (51%)	31.3 (1.64)	0.90	156 (97%)	33.4 (0.80)	0.018

All values adjusted for age and sex.