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Mitochondrial genomic variation in dementia with Lewy bodies: association with disease risk and neuropathological measures



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Abstract

Dementia with Lewy bodies (DLB) is clinically diagnosed when patients develop dementia less than a year after parkinsonism onset. Age is the primary risk factor for DLB and mitochondrial health influences ageing through effective oxidative phosphorylation (OXPHOS). Patterns of stable polymorphisms in the mitochondrial genome (mtDNA) alter OXPHOS efficiency and define individuals to specific mtDNA haplogroups. This study investigates if mtDNA haplogroup background affects clinical DLB risk and neuropathological disease severity. 360 clinical DLB cases, 446 neuropathologically confirmed Lewy body disease (LBD) cases with a high likelihood of having DLB (LBD-hDLB), and 910 neurologically normal controls had European mtDNA haplogroups defined using Agena Biosciences MassARRAY iPlex technology. 39 unique mtDNA variants were genotyped and mtDNA haplogroups were assigned to mitochondrial phylogeny. Striatal dopaminergic degeneration, neuronal loss, and Lewy body counts were also assessed in different brain regions in LBD-hDLB cases. Logistic regression models adjusted for age and sex were used to assess associations between mtDNA haplogroups and risk of DLB or LBD-hDLB versus controls in a case-control analysis. Additional appropriate regression models, adjusted for age at death and sex, assessed associations of haplogroups with each different neuropathological outcome measure. No mtDNA haplogroups were significantly associated with DLB or LBDhDLB risk after Bonferroni correction. Haplogroup H suggests a nominally significant reduced risk of DLB (OR=0.61, P=0.006) but no association of LBD-hDLB (OR=0.87, P=0.34). The haplogroup H observation in DLB was consistent after additionally adjusting for the number of APOE ɛ4 alleles (OR=0.59, P=0.004). Haplogroup H also showed a suggestive association with reduced ventrolateral substantia nigra neuronal loss (OR=0.44, P=0.033). Mitochondrial haplogroup H may be protective against DLB risk and neuronal loss in substantia nigra regions in LBD-hDLB cases but further validation is warranted.

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Keywords: Dementia with Lewy-bodies, Lewy body disease, Mitochondrial DNA, Mitochondrial haplogroups, Neuropathology

Introduction

Lewy body dementia are comprised of two distinct, but clinically related, disorders—Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) [22, 30]. The timing of dementia onset determines the exact clinical diagnosis, whereby dementia onset before or less than a year after parkinsonism is classified as DLB, and dementia onset more than one year after parkinsonism is classified as PDD [30]. DLB is one of the most common forms of dementia after Alzheimer's disease (AD), accounting for approximately 23% of all dementia cases [49]. Currently there is no treatment to prevent or cure DLB and disease course is progressive and eventually fatal.

Neuropathologically, DLB and PDD are very similar and fall under the pathological term of Lewy body disease (LBD). Lewy body disorders are characterized by the presence of Lewy bodies (LB) and Lewy neurites in the brain, causing neurodegeneration. LB are complex masses of aggregated phosphorylated alpha-synuclein (aSyn), p62, and ubiquitin proteins, as well as lipids and membranous organelles [29]. The location and distribution of LBs in the brain and the associated neuronal dysfunction determines clinical phenotypes observed. For example, LB accumulation in the brainstem and midbrain regions, and the associated neurodegeneration, typically induces Parkinson's disease (PD) symptoms of tremor, rigidity, and slowness of movement [38], whereas LB accumulation in the neocortical and limbic regions is associated with cognitive and neuropsychiatric symptoms, such as cognitive impairment, fluctuations, visual hallucinations, and behavioral changes—which are reflective of PDD or DLB [12, 30, 37]. Classical brainstem and nigral LB consist of dense, spherical cores with irradiating filaments, and a surrounding halo (when stained with hematoxylin/eosin), whereas LB in neocortical regions typically have pale, fibrillary structures without a halo or central core [29, 42]. Paler, fibrillary LB have been described as premature and are thought to develop into classical LB structures with disease progression [17]. In addition to LB, pathological aggregates of extracellular amyloid-beta (Abeta) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau proteins are often present in LBD making the disease spectrum very heterogenous [10]. Neuropathologists use defined criteria to assess aSyn and tau Braak stage, as well as betaamyloid Thal phase, to neuropathologically determine accurate LBD diagnosis and characterize disease severity, and use available medical records to determine the likelihood of clinical phenotypes [6].

Within the past decade, ongoing efforts have continued to work towards understanding genetic markers influencing LB disorders, particularly PD, whereby current casecontrol studies consist of tens of thousands of cases [18, 34, 35]. Recent smaller case-control studies of DLB have identified overlapping genetic markers between PD and AD [3, 18, 27], further demonstrating the overlapping pathologies of these diseases, but despite such efforts, the genetic etiology of DLB is yet to be defined. Thus, providing additional scope to characterize other genetic factors which may be driving dementia onset in DLB.

Age consistently remains the major risk factor for neurodegeneration and both healthy ageing and aSyn accumulation is influenced by mitochondrial health, whereby increased reactive oxygen species (ROS) production accelerates ageing and aSyn aggregation over time [20, 28]. ROS are a byproduct from oxidative phosphorylation (OXPHOS) which occurs on the inner mitochondrial membrane [26]. Mitochondria contain their own genomic information (mtDNA), independent to the nuclear genome, which codes for 13 essential subunits in OXPHOS complexes. Patterns of stable polymorphisms across the mtDNA molecule define individuals to specific mtDNA haplogroups, and each mtDNA haplogroup has a unique metabolic profile which influences ROS production over time [15, 16]. As a result of their distinct metabolic backgrounds, mtDNA haplogroups have been associated with age-related and multiple neurodegenerative diseases, including PD and AD [4, 21], but have not been examined in relation to dementia onset in large cohorts of patients. Therefore, the aims of this study were to evaluate the association between mtDNA haplogroups and risk of clinical DLB and pathologically confirmed LBD cases with a high likelihood of having clinical DLB (LBD-hDLB) in a case-control analysis. In analysis of the LBD-hDLB group, we also examined associations of mtDNA haplogroups with severity of neuropathological measures, such as LB counts and distribution, neuronal loss, and dopaminergic degeneration across several brain regions.

Material and methods

Study subjects and data collection

A total of 806 DLB subjects (N=360 clinically diagnosed DLB cases and N=446 autopsy-confirmed Lewy body disease cases that were assessed as having a high likelihood of DLB (LBD-hDLB) - of which N=48 were present in both series) and 910 controls were included in this study. Clinical DLB patients were diagnosed by neurologists at Mayo Clinic in Jacksonville, FL or Rochester, MN and were recruited as part of the Alzheimer's Disease Research Center and the Mayo Clinic Study of Aging. Pathologically confirmed LBD cases were obtained from the brain bank for neurodegenerative disorders at Mayo Clinic in Jacksonville, FL and were evaluated by a single neuropathologist (Dr. Dennis Dickson). LBD cases were all assessed as having a high likelihood of DLB according to the criteria of the fourth report of the DLB consortium [30]. Controls were recruited by Dr. Zbigniew Wszolek and his colleagues from Mayo Clinic in Jacksonville, FL and were absent of neurological disease. All subjects provided written consent prior to study commencement and were Caucasian, non-Hispanic, and unrelated. Age at DLB diagnosis in clinically diagnosed DLB cases, age at death in pathologically confirmed LBD-hDLB cases, and age at blood draw in controls, and sex was collected for all subjects (Table 1). Additionally, neuropathological measures for Lewy body counts and substantia nigra (SN) neuronal loss were available for 242 (54.3%) LBDhDLB cases (Table 1).

Neuropathological assessment in LBD-hDLB Assessment of neurofibrillary tangles, senile plaques, and Lewy bodies

Neuropathological methodologies used to assess neurofibrillary tangles (NFTs), senile plaques (SPs), and Lewy bodies (LBs) have been described previously [33]. Briefly, neuroanatomical sampling and thioflavin-S fluorescence microscopy was performed, where counts of NFTs and SPs were measured manually in six cortical regions, four sections of the hippocampus, and two regions of the amygdala [43]. Formalin-fixed, paraffin-embedded tissue samples from limbic and cortical regions were sectioned and mounted on glass slides. Assessment of LB pathology was performed using an aSyn antibody (NACP, 1:3000 rabbit polyclonal, Mayo Clinic antibody) with formic acid pretreatment for 30 minutes and was processed using the DAKO Autostainer (DAKO Auto Machine Corporation, Carpinteria, CA) with DAKO Envision+ HRP System. LB counts were measured in five cortical regions-middle frontal, superior temporal, inferior parietal, cingulate, and parahippocampal. The distribution of LB pathology was assessed using the staging scheme defined by Kosaka et al. to categorize samples as either brainstem, transitional, or diffuse [25]. Braak NFT stage [1] and Thal amyloid phase [44] were assigned according to the distributions of NFTs and SPs respectively. These neuropathologic measures are summarized in Table 1.

Quantification of striatal dopaminergic degeneration

Quantitative assessment of striatal dopaminergic degeneration by measurement of tyrosine hydroxylase immunoreactivity (TH-ir) has been described previously [24]. To summarize, the putamen was assessed at the level of the anterior commissure from a section made from the hemi-brain in a standardized dissection plane defined by three points in the fundibulum, uncus, and posterior margin of the anterior commissure in the third ventricle. Digital images of the putamen were parcelled into ventromedial and dorsolateral areas [19], and dopaminergic degeneration was quantitatively assessed.

The basal ganglia section was processed for immunohistochemistry with a commercially available antibody to TH (rabbit polyclonal, 1:600; Affinity Bioreagants, Golden, Colorado) with Proteinase K pretreatment for 5 minutes. The immunostained sections were captured by ScanScope XT (Aperio Technologies, Vista, California), and images were annotated with ImageScope (version 12.1). Regions of interest were manually edited to exclude artifacts, large blood vessels and their perivascular spaces, and large fiber bundles. The putamen was divided into ventromedial and dorsolateral regions. Quantification of TH-ir used an algorithm that detected positive pixels based on optical density. TH-ir was expressed as a percentage, calculated as the number of positive pixels divided by the sum of inverse pixels and background pixels. A lower TH-ir value represents a greater degree of putaminal dopaminergic degeneration. Table 1 summarizes dorsolateral and ventromedial putaminal TH-ir in DLB cases.

Assessment of substantia nigra pigmented neuronal loss

The midbrain was a transverse section at the level of the third nerve, similar to what has been recommended for diagnostic evaluation of PD [7]. A semi-quantitative assessment of SN cell groups was ascertained on hematoxylin and eosin-stained sections at 100x magnification. Our assessment was restricted to pigmented neurons of SN pars compacta and divided into medial and ventrolateral sections—similar to previous studies [14, 39]. We used a human atlas of SN cell groups to identify medial and ventrolateral regions of the SN [36]. The density of nonpigmented neurons was not taken into consideration for the assessment of the semi-quantitative scores, which were based on a 4-point scale (0=none, 1=mild, 2=moderate, and 3=severe) (Table 1).

Genetic analysis

Peripheral blood was collected from clinical DLB patients and control subjects, and frozen cerebellum brain tissue was provided from pathologically confirmed LBD-hDLB

Table 1 Patient characteristics

Variable	Controls (N = 910)	Clinical DLB (N = 360)	LBD with a high likelihood of DLB (N=446)
Age (years) ^a	79 (41, 102)	73 (50, 100)	78 (48, 103)
Sex			- (-))
Male	388 (42.6%)	270 (75.0%)	292 (65.5%)
Female	522 (57.4%)	90 (25.0%)	154 (34.5%)
Braak NFT stage			
0	N/A	N/A	13 (2.9%)
	N/A	N/A	23 (5.2%)
	N/A	N/A	138 (30.9%)
	N/A	N/A	143 (32 1%)
IV.	N/A	N/A	129 (28 9%)
V	N/A	N/A	0 (0 0%)
VI	N/A	N/A	0 (0.0%)
Thal amyloid phase			0 (0.073)
0	N/A	N/A	40 (10.7%)
1	N/A	N/A	37 (9.9%)
2	N/A	N/A	23 (6.1%)
3	N/A	N/A	129 (34.4%)
4	N/A	N/A	56 (14.9%)
5	N/A	N/A	90 (24.0%)
LBD subtype			
Transitional	N/A	N/A	89 (20.0%)
Diffuse	N/A	N/A	357 (80.0%)
Lewy body counts			
Middle frontal gyrus	N/A	N/A	5 (0, 35)
Superior temporal gyrus	N/A	N/A	10 (0, 50)
Inferior parietal gyrus	N/A	N/A	4 (0, 30)
Cingulate gyrus	N/A	N/A	12 (2, 32)
Parahippocampal gyrus	N/A	N/A	16 (1,45)
Putaminal TH-ir			
Dorsolateral	N/A	N/A	2.93 (0.26, 21.61)
Ventromedial	N/A	N/A	8.99 (0.26, 27.42)
Substantia nigra neuronal loss score			
Ventrolateral			
0.0 = none	N/A	N/A	0 (0.0%)
0.5 = none/mild	N/A	N/A	2 (1.0%)
1.0 = mild	N/A	N/A	10 (5.1%)
1.5 = mild/moderate	N/A	N/A	7 (3.6%)
2.0 = moderate	N/A	N/A	21 (10.8%)
2.5 = moderate/severe	N/A	N/A	23 (11.8%)
3.0 = severe	N/A	N/A	132 (67.7%)
Medial			
0.0 = none	N/A	N/A	2 (1.1%)
0.5 = none/mild	N/A	N/A	1 (0.6%)
1.0 = mild	N/A	N/A	25 (14.0%)
1.5 = mild/moderate	N/A	N/A	16 (8.9%)
2.0 = moderate	N/A	N/A	26 (14.5%)
2.5 = moderate/severe	N/A	N/A	25 (14.0%)
3.0 = severe	N/A	N/A	84 (46.9%)

Table 1 (continued)

Sample median (minimum, maximum) is given for continuous variables

For LBD cases with a high likelihood of DLB, information was unavailable for Thal amyloid phase (N=71), middle frontal gyrus Lewy body count (N=211), superior temporal gyrus Lewy body count (N=213), inferior parietal gyrus Lewy body count (N=211), cingulate gyrus Lewy body count (N=213), parahippocampal gyrus Lewy body count (N=232), dorsolateral putaminal TH-ir (N=250), ventromedial putaminal TH-ir (N=250), ventrolateral substantia nigra neuronal loss score (N=251), and medial substantia nigra neuronal loss score (N=267).

^a Age represents age at blood draw for controls, age at DLB onset for clinical DLB cases, and age at death for LBD with a high likelihood of DLB cases

cases. Genomic DNA was extracted from peripheral blood lymphocytes and cerebellum tissue using Autogen Flex Star and Autogen 245T (Holliston, MA) methods respectively. DNA was diluted to 15 ng/ μ l and 39 unique mitochondrial DNA variants were genotyped by a singleuser (RRV) using two custom-designed Agena Bioscience iPLEX arrays on Sequenom MassARRAY technology [11]. More detailed methods for genetic assessments have been previously published [46, 47]. Individual mitochondrial DNA haplogroups were defined to mitochondrial phylogeny for each subject [48] (Table 2). Phylogenetically related haplogroups were also grouped into family haplogroups (e.g. sub-haplogroups H, H1, H2, H3, and H4 are all part of the family H haplogroup) and four different super-haplogroups (e.g. family J and family T haplogroups are super-haplogroup JT) for secondary analysis assessments. Haplogroups that occurred in fewer than 10 subjects in a given association analysis were not analyzed in that specific analysis. All cases were examined for population stratification prior to conducting this study [3].

Statistical analysis

Associations of mitochondrial haplogroups with risk of clinical DLB and LBD-hDLB, each separately versus controls, were examined using logistic regression models that were adjusted for age and sex. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Additionally, clinical DLB and LBD-hDLB series were combined into one overall DLB series, and associations of haplogroups with risk of DLB in comparison to controls were assessed. The 48 cases that were present in both the clinical DLB series and the LBD-hDLB series, were only included once in the overall DLB series.

In the LBD-hDLB series, associations of haplogroups with each different neuropathological outcome measure were assessed using age at death and sex-adjusted regression models that are appropriate for the nature of the given outcome measure. Specifically, associations of haplogroups with dorsolateral and ventromedial putaminal TH-ir were examined using linear regression models, where due to their skewed distributions, lateral putaminal TH-ir was considered on the logarithm (base-10) scale and medial putaminal TH-ir was considered on the square root scale. Regression coefficients and 95% CIs were estimated and are interpreted as the additive increase on the mean outcome measure (on the logarithm or square root scale) for the given haplogroup. Associations of haplogroups with ventrolateral and medial SN neuronal loss scores were assessed using proportional odds logistic regression models. ORs and 95% CIs were estimated and are interpreted at the multiplicative increase on the odds or a more severe neuronal loss score for the given haplogroup. Neuronal loss scores <1(ventrolateral) and <1.5 (medial) were combined into one category in proportional odds logistic regression analysis due to their low frequencies. Associations between haplogroups and cortical LB counts were evaluated using negative binomial regression models. Multiplicative effects and 95% CIs were estimated and are interpreted as the multiplicative increase on the mean LB count for the given haplogroup. Finally, binary logistic regression models were used to assess associations between haplogroups and LBD subtype. ORs and 95% CIs for presence of diffuse LBD were estimated.

We utilized a Bonferroni correction for multiple testing separately for each outcome measure in the primary analysis that did not involve super-haplogroups (*P*-values <0.05 were considered statistically significant in secondary super-haplogroup analysis). As haplogroups that occurred in less than 10 subjects in a given association analysis were not analyzed in that specific analysis, and the degree of missing data differed between outcomes, the Bonferroni-corrected statistical significance level correspondingly varied between outcomes (see table footnotes for details). All statistical tests were two-sided. Statistical analyses were performed using R Statistical Software (version 3.6.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Associations of haplogroups with risk of clinical DLB and LBD-hDLB are detailed in Table 2. After adjusting for age and sex, no statistically significant associations were observed after Bonferroni correction (P < 0.0024 considered significant). However, a nominally significant (P < 0.05) association was reported between sub-haplogroup H and lower risk of clinical DLB (OR=0.61, P=0.006). This association was consistent when additionally adjusting for the number of apolipoprotein E4 (APOE4) alleles (OR=0.59, P = 0.004). No other associations approached statistical significance in any other series (all $P \ge 0.057$, Table 2). Interestingly though, despite mitochondrial

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Mitochondrial DNA Haplogroup	Haplogroup freque	ency, No. (%)			Clinical DLB vs. controls	LBD with a high likelihood of DLB vs. controls	Overall DLB vs. o	controls
	Controls (N=910)	Clinical DLB (N=360)	LBD high likelihood of DLB (N= 446)	Overall DLB (N=758)	OR (95% Cl) P-val	ue OR (95% Cl) <i>P</i> -valu	ie OR (95% Cl)	P-value
Na	2 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1		
N1 ^a	5 (0.5%)	4 (1.1%)	2 (0.4%)	6 (0.8%)	1	I	1.31 (0.38, 4.49)	0.66
_	31 (3.4%)	12 (3.3%)	13 (2.9%)	22 (2.9%)	0.77 (0.38, 1.56) 0.47	0.83 (0.41, 1.60) 0.60	0.77 (0.44, 1.38)	0.38
W	15 (1.6%)	9 (2.5%)	8 (1.8%)	15 (2.0%)	1.71 (0.69, 4.23) 0.24	1.11 (0.44, 2.64) 0.81	1.30 (0.61, 2.76)	0.50
×	8 (0.9%)	2 (0.6%)	7 (1.6%)	9 (1.2%)	0.60 (0.12, 3.10) 0.55	1.68 (0.57, 4.82) 0.33	1.36 (0.50, 3.69)	0.55
R or R0 ^a	6 (0.7%)	1 (0.3%)	1 (0.2%)	2 (0.3%)	1	1	I	1
HV or HV0a	22 (2.4%)	6 (1.7%)	13 (2.9%)	17 (2.2%)	0.86 (0.33, 2.26) 0.76	1.36 (0.65, 2.74) 0.40	1.02 (0.53, 2.00)	0.94
H, H1, H2, H3, and H4	423 (46.5%)	149 (41.4%)	183 (41.0%)	316 (41.7%)	0.85 (0.65, 1.10) 0.22	0.83 (0.66, 1.05) 0.13	0.85 (0.70, 1.04)	0.12
Т	199 (21.9%)	51 (14.2%)	85 (19.1%)	133 (17.5%)	0.61 (0.43, 0.87) 0.006	0.87 (0.65, 1.16) 0.34	0.78 (0.61, 1.01)	0.057
H	145 (15.9%)	67 (18.6%)	56 (12.6%)	116 (15.3%)	1.22 (0.87, 1.71) 0.26	0.78 (0.55, 1.09) 0.16	0.97 (0.74, 1.28)	0.85
H2	36 (4.0%)	6 (1.7%)	12 (2.7%)	17 (2.2%)	0.44 (0.18, 1.09) 0.076	0.67 (0.33, 1.28) 0.24	0.56 (0.30, 1.02)	0.057
H3	32 (3.5%)	16 (4.4%)	23 (5.2%)	36 (4.7%)	1.42 (0.73, 2.75) 0.30	1.59 (0.89, 2.78) 0.11	1.48 (0.89, 2.47)	0.13
H4	11 (1.2%)	9 (2.5%)	7 (1.6%)	14 (1.8%)	1.76 (0.70, 4.46) 0.23	1.14 (0.41, 2.98) 0.80	1.33 (0.59, 3.01)	0.50
>	18 (2.0%)	14 (3.9%)	12 (2.7%)	26 (3.4%)	1.99 (0.94, 4.22) 0.073	1.37 (0.63, 2.91) 0.41	1.82 (0.97, 3.42)	0.064
JТа	2 (0.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	Ι	I	I	1
J, J1, J1d, J2, J2a, and J2b	93 (10.2%)	38 (10.6%)	52 (11.7%)	87 (1 1.5%)	1.02 (0.67, 1.55) 0.94	1.13 (0.78, 1.63) 0.51	1.11 (0.81, 1.54)	0.51
Ĺ	0 (0.0%)	1 (0.3%)	0 (0.0%)	1 (0.1%)	1	1		I
11	72 (7.9%)	25 (6.9%)	41 (9.2%)	64 (8.4%)	0.82 (0.50, 1.35) 0.44	1.11 (0.73, 1.67) 0.61	1.02 (0.71, 1.46)	0.93
J1d	1 (0.1%)	1 (0.3%)	0 (0.0%)	1 (0.1%)	Ι	I	I	1
J2a	13 (1.4%)	7 (1.9%)	9 (2.0%)	15 (2.0%)	1.80 (0.67, 4.89) 0.25	1.74 (0.70, 4.16) 0.22	1.66 (0.76, 3.62)	0.20
J2b	7 (0.8%)	4 (1.1%)	2 (0.4%)	6 (0.8%)	1.20 (0.32, 4.48) 0.79	1	0.95 (0.30, 2.95)	0.92
T, T1, and T2	77 (8.5%)	32 (8.9%)	45 (10.1%)	71 (9.4%)	1.16 (0.73, 1.84) 0.53	1.25 (0.84, 1.86) 0.26	1.17 (0.83, 1.67)	0.38
Ta	0 (0.0%)	1 (0.3%)	0 (0.0%)	1 (0.1%)	1	I	I	1
T1	17 (1.9%)	5 (1.4%)	8 (1.8%)	13 (1.7%)	0.64 (0.22, 1.85) 0.41	0.85 (0.34, 1.96) 0.71	0.83 (0.39, 1.76)	0.62
Τ2	60 (6.6%)	26 (7.2%)	37 (8.3%)	57 (7.5%)	1.27 (0.76, 2.12) 0.35	1.38 (0.89, 2.14) 0.15	1.25 (0.84, 1.85)	0.26
U, U1, U3, U5, U6, and U8b'c	130 (14.3%)	53 (14.7%)	67 (15.0%)	110 (14.5%)	0.97 (0.67, 1.40) 0.89	1.01 (0.73, 1.40) 0.95	0.96 (0.72, 1.28)	0.79
N	44 (4.8%)	22 (6.1%)	21 (4.7%)	40 (5.3%)	1.12 (0.64, 1.96) 0.68	0.89 (0.51, 1.51) 0.66	0.97 (0.61, 1.52)	0.88
U1 ^a	1 (0.1%)	0 (0.0%)	2 (0.4%)	2 (0.3%)	Ι	I	I	
U3 ^a	8 (0.9%)	0 (0.0%)	1 (0.2%)	1 (0.1%)	Ι	I	I	I
U5	74 (8.1%)	30 (8.3%)	42 (9.4%)	65 (8.6%)	0.99 (0.62, 1.59) 0.97	1.13 (0.74, 1.68) 0.57	1.02 (0.71, 1.47)	06.0

Mitochondrial DNA Haplogroup	Haplogroup fr	equency, No. (%)			Clinical DLB vs. controls		LBD with a higl likelihood of D controls	LB vs.	Overall DLB vs.	controls
	Controls (N=9	010) Clinical DLB (N=360)	LBD high likelihood of DLB (N = 446)	Overall DLB (N=758)	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
N6ª	3 (0.3%)	1 (0.3%)	0 (0.0%)	1 (0.1%)	I	I	I	I	I	1
U8b'c ^a	0 (0:0%)	0 (0.0%)	1 (0.2%)	1 (0.1%)	I	I	I	I	I	I
×	78 (8.6%)	40 (11.1%)	43 (9.6%)	77 (10.2%)	1.23 (0.80, 1.88)	0.35	1.12 (0.74, 1.66)	0.59	1.15 (0.81, 1.62)	0.44
OR = odds ratio; CI = co	nfidence interval. ORs, 5	95% CIs, and p-values result from	logistic regression mod	dels that were adjusted for a	ige and sex. The 47	cases that v	vere in both the cli	nical DLB s	eries and the LBD v	vith a

Table 2 (continued)

high litetinoid of DLB series were included only once in the overaction series. After applying a Bonferroni correction for multiple testing, *P*-values < 0.0023 (clinical DLB vs. controls), < 0.0024 (LBD with a high likelihood of DLB vs. controls), < 0.0024 (LBD with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls), < 0.0024 (Clab with a high likelihood of DLB vs. controls)

 a Haplogroups that occurred in < 10 subjects in a given association analysis were not examined.

sub-haplogroup H not being strongly associated with LBD-hDLB (OR=0.87, P = 0.34), the protective association observed in the clinical DLB series was almost nominally significant when examining the combined DLB series (OR=0.78, P = 0.057) (Table 2).

In an exploratory analysis, we also evaluated associations of haplogroups with disease risks separately for males and females (Additional File 1: Tables S1 and S2). The aforementioned protective association between subhaplogroup H and clinical DLB was observed relatively consistently in males (OR=0.64, P = 0.042) and females (OR=0.56, P = 0.066). Also, haplogroup HV/HV0a was suggestively associated with an increased risk of LBD-hDLB in males (OR=3.33, P = 0.044) and haplogroup V suggested an association with increased risk of both clinical DLB (OR = 4.29, P = 0.009) and overall DLB (OR = 3.56, P = 0.006) in females.

Associations of individual mitochondrial haplogroups with putaminal TH-ir and SN neuronal loss (Table 3), cortical Lewy body counts (Table 4), and diffuse LBD subtype (Additional File1: Table S3) were also assessed. No statistically significant associations were observed after correcting for multiple testing. A nominally significant association was noted between sub-haplogroup H and a less severe ventrolateral SN neuronal loss score (OR = 0.44, P = 0.033, Table 3) and also between subhaplogroup T2 and a lower superior temporal LB count (multiplicative effect: 0.76, P = 0.044, Table 4). Both nominally significant associations remained consistent when additionally adjusting for the number of APOE $\varepsilon 4$ alleles (P = 0.033 and P = 0.036 respectively). No associations between super-haplogroups and either risk of DLB or neuropathological outcomes were observed (all P > 0.21).

Discussion

Efforts to understand the genetic etiology of DLB have identified shared genetic markers between PD and AD [3, 23, 27, 34]. Physiologically, mitochondrial dysfunction is consistently reported in synucleinopathies [32] and mitochondrial phenotypes are predisposed by variation in mtDNA [15, 16]. Interestingly, LB pathology is more prevalent in older individuals with mitochondrial disease compared to controls [9], further emphasizing the importance of mtDNA in disease pathology.

Acknowledging this, our examinations of mtDNA variation, in the form of mitochondrial haplogroups, with DLB risk and neuropathological measures in this study reported no statistically significant associations after applying Bonferroni correction. However, mtDNA subhaplogroup H reported a suggestive protective effect with clinical DLB risk (OR=0.61, P = 0.006) which was not observed in LBD-hDLB cases (OR=0.87, P = 0.34). Interestingly though, sub-haplogroup H also indicated an association with less severe ventrolateral SN neuronal loss (OR=0.44, P = 0.033) in LBD-hDLB cases.

Mitochondrial haplogroup H is the most common haplogroup in European populations, accounting for more than 40% of individuals [45]. Mitochondrial haplogroup H has more than 80 sub-haplogroups which are predominantly defined by variation in mtDNA coding regions [48], with sub-haplogroups H1 and H3 being the most common. Mitochondrial haplogroup H has previously been associated with increased risk of PD [21] and DLB [5]. Notably, the results reported in Hudson and colleagues' study was not an exact association of haplogroup H with PD risk, as they grouped haplogroup H and haplogroup V cases together. Although this was statistically more reliable, genetically the results do not clarify what mtDNA variants are driving PD risk because haplogroups H and V are phylogenetically related but are genetically different. Moreover, Chinnery et al. reported increased risk of haplogroup H with DLB in only 84 DLB cases and did not evaluate H sub-haplogroups. In this study, we assessed associations between mtDNA haplogroups and sub-haplogroups with DLB risk more comprehensively and in a much larger DLB cohort. It is possible that the elevated risk of DLB with mtDNA haplogroup H background reported by Chinnery et al. may be an artefact of common H sub-haplogroups, such as H1 and H3, inducing more detrimental risk outcomes with DLB-which is also observed in our data. Overall, these studies demonstrate the functional heterogeneity even within a given haplogroup and reinforce the need to stratify haplogroups into sub-haplogroups in genetic studies [13]. Replication will be important to validate our findings.

Disappointingly, we did not replicate the sub-haplogroup H association with reduced DLB risk in pathologically confirmed LBD-hDLB cases, nor in the combined cases group. This may be because one cohort was clinically defined whereas the other cohort was neuropathologically defined. Neuropathologically confirmed cases were included in this study if they were deemed as having a high likelihood of clinical dementia-which was determined from pathology propensity in cortical regions and available medical records. It is possible that the neuropathologically defined LBD-hDLB cohort may also contain clinical PDD cases. PDD has a different disease course to DLB and is diagnosed when dementia develops more than a year after parkinsonism onset and can be considered a much slower progression of dementia than DLB [30]. Interestingly, this data suggests that mitochondrial sub-haplogroup H may be protective against DLB but not PDD, which may suggest that mitochondrial background influences rate of dementia progression in PD.

Table 3	Associations	of	haplogroups	with	putaminal	TH-ir	(dorsolateral	and	ventromedial)	and	substantia	nigra	neuronal	loss
(ventrola	iteral and med	dial))											

Regression Cy1P-aleRegression configuenceP-aleP-aleP-aleP-aleP-aleP-aleN ¹ 0.00% </th <th>Mitochondrial DNA Haplogroup</th> <th>Haplogroup frequency, No. (%), N=242</th> <th>Association with d putaminal TH—ir</th> <th>orsolateral</th> <th>Association with ventromedial puta TH—ir</th> <th>minal</th> <th>Association wit ventrolateral su nigra neuronal loss s</th> <th>h Jbstantia core</th> <th>Association wit medial substan neuronal loss score</th> <th>h tia nigra</th>	Mitochondrial DNA Haplogroup	Haplogroup frequency, No. (%), N=242	Association with d putaminal TH—ir	orsolateral	Association with ventromedial puta TH—ir	minal	Association wit ventrolateral su nigra neuronal loss s	h Jbstantia core	Association wit medial substan neuronal loss score	h tia nigra
N ⁴ 0 (0,0%) - <th< th=""><th></th><th></th><th>Regression coefficient (95% CI)</th><th>P-value</th><th>Regression coefficient (95% CI)</th><th>P-value</th><th>OR (95% CI)</th><th>P-value</th><th>OR (95% CI)</th><th>P-value</th></th<>			Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
NIA0,00% <td>N^a</td> <td>0 (0.0%)</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td>	N ^a	0 (0.0%)	_	_	_	_	_	_	_	_
Pi8(3.3%) </td <td>N1^a</td> <td>0 (0.0%)</td> <td>_</td> <td>_</td> <td>-</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>-</td>	N1 ^a	0 (0.0%)	_	_	-	_	_	_	_	-
W ^A 2(03%)<	la	8 (3.3%)	_	_	_	_	_	_	_	_
X ^A 4(17%)6(1)	Wa	2 (0.8%)	_	_	_	_	_	_	_	_
RandR0 ⁶ 10.40%11111111111111111111111111111111111	Xa	4 (1.7%)	_	_	_	_	_	_	_	_
HY and HV0a ^a 8(3%) </td <td>R and R0ª</td> <td>1 (0.4%)</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td>	R and R0ª	1 (0.4%)	_	_	_	_	_	_	_	_
H,H,H2,H3 and H4-0.05 (-0.15,00)0.32-0.11 (-0.40,0.18)0.450.80 (0.43,1.46)0.461.12 (0.63,1.97)0.70H40 (16.5%)0.05 (-0.09,0.18)0.110.11 (-0.27,0.49)0.550.44 (0.21,0.3)0.330.54 (0.26,1.19)0.09H28 (3.3%)-0.07 (-0.23,0.09)0.42-0.13 (-0.58,0.23)0.571.33 (0.48,3.68)0.581.28 (0.52,3.13)0.51H3 ³ 12 (5.0%)-0.04 (-0.26,0.19)0.75-0.29 (-0.92,0.34)0.560.90 (0.25,3.31)0.88-0.01-0.14H4 ⁴ 41 (0.7%)-0.04 (-0.26,0.19)0.75-0.29 (-0.92,0.24)0.560.90 (0.25,3.31)0.88-0.01-0.14H4 ⁴ 61 (0.7%)-0.04 (-0.26,0.19)0.75-0.20 (-0.27,0.27)0.810.87 (0.36,0.20)0.750.20 (2.8,1.39)0.25J ¹ 0.06(5-0.07,0.02)0.420.55 (-0.37,0.47)0.810.81 (0.310,3.23)0.870.20 (2.8,1.39)0.25J ¹ 0.00%-0.07 (-0.09,0.22)0.390.60 (-0.38,0.50)0.780.81 (0.310,3.23)0.870.20 (2.9,1.19)0.20J ¹ 0.00%-0.07 (-0.90,0.23)0.36-0.07 (-0.52,0.30)0.780.93 (0.37,2.33)0.870.72 (0.31,16)0.40J ¹ 0.00%-0.07 (-0.90,0.23)0.36-0.07 (-0.52,0.30)0.741.42 (0.49,1.11)0.522.60 (0.87,1.50)0.88J ¹ 0.00%-0.07 (-0.52,0.38)0.741.20 (0.49,1.10)0.522.60 (0.87,1.50)	HV and HV0a ^a	8 (3.3%)	_	_	_	_	_	_	_	_
H40(165%)0.05(-0.09,0.18)0.110.110.110.440.440.330.540.540.540.540.540.540.540.550.540.550.	H, H1, H2, H3 and H4	91 (37.6%)	-0.05 (-0.15, 0.05)	0.32	-0.11 (-0.40, 0.18)	0.45	0.80 (0.43, 1.46)	0.46	1.12 (0.63, 1.97)	0.70
H127(11.2%)-0.07(-0.23,0%)0.4%-0.13(-0.58,0.2%)0.5%1.33(0.48,36%)0.5%1.28(0.52,3.1%)0.5%H2 ^a 8(3.3%)-0.04(-0.26,0.1%)0.7%-0.29(-0.92,0.34)0.360.90(0.25,3.3%)0.8%H4 ^a 4(1.7%)-0.0-0-0-0-0-0V ^a 8(3.3%)-0.0-0.0-0.0-0-0-0-0J ^a 0.00(0)-0.0-0.0-0.0-0.0-0.0-0.0-0.0	Н	40 (16.5%)	0.05 (-0.09, 0.18)	0.51	0.11 (-0.27, 0.49)	0.55	0.44 (0.21, 0.93)	0.033	0.54 (0.26, 1.10)	0.090
H2 ^a 8 (3.3%) - - - - - - - - - - H3 ^a 12 (5.0%) -0.04 (-0.26 (0.19) 0.75 -0.29 (-0.92, 0.34) 0.36 0.90 (0.25, 3.31) 0.88 - - H4 ^a 4 (1.7%) -	H1	27 (11.2%)	-0.07 (-0.23, 0.09)	0.42	-0.13 (-0.58, 0.32)	0.57	1.33 (0.48, 3.68)	0.58	1.28 (0.52, 3.13)	0.59
H3 1250% -004(-0.26,0.1) 0.75 -0.29(-0.92,0.34) 0.86 090(0.25,3.1) 0.88 - - H4 ³ 4(1.7%) -	H2ª	8 (3.3%)	_	_	_	_	_	_	_	_
Ha ⁴ 4 (17%) - - - - - - - V ⁴ 8 (3.3%) -	H3ª	12 (5.0%)	-0.04 (-0.26, 0.19)	0.75	-0.29 (-0.92, 0.34)	0.36	0.90 (0.25, 3.31)	0.88	_	_
Name Name <th< td=""><td>H4^a</td><td>4 (1.7%)</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td></th<>	H4 ^a	4 (1.7%)	_	_	_	_	_	_	_	_
Information Information <thinformation< th=""> <thinformation< th=""></thinformation<></thinformation<>	Va	8 (3.3%)	_	_	_	_	_	_	_	_
J.J. J.J. J.J. J.J. J.J. J.J. J.J. J.J	JTa	0 (0.0%)	_	_	_	_	_	_	_	_
ja 0 (0.0%) - - - - - - - - - - J1 23 (9.5%) 0.07 (-0.09, 0.22) 0.39 0.06 (-0.38, 0.50) 0.78 0.93 (0.37, 2.33) 0.87 0.72 (0.31, 1.69) 0.46 J1 ^a d 0.00% -	J, J1, J1d, J2a and J2b	26 (10.7%)	0.06 (-0.09, 0.21)	0.42	0.05 (-0.37, 0.47)	0.81	0.87 (0.36, 2.09)	0.75	0.62 (0.28, 1.39)	0.25
J1 23 (9.5%) 0.07 (-0.09, 0.22) 0.39 0.06 (-0.38, 0.50) 0.78 0.93 (0.37, 2.33) 0.87 0.72 (0.31, 1.69) 0.46 J1 ^a d 0 (0.0%) - <td>Ja</td> <td>0 (0.0%)</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td> <td>_</td>	Ja	0 (0.0%)	_	_	_	_	_	_	_	_
J1 ^a d 0 (0.0%) - <	11	23 (9 5%)	0.07 (-0.09 0.22)	039	0.06 (-0.38, 0.50)	0.78	0.93 (0.37, 2.33)	0.87	0.72 (0.31 1.69)	0.46
J2a ^a 2 (0.8%) - <	J1ªd	0 (0.0%)	_	_	-	_	-	_	-	_
J2b ^a 1 (0.4%) - - - - - - - - T,T and T2 30 (12.4%) -0.03 (-0.19,0.13) 0.70 -0.07 (-0.52,0.38) 0.74 1.42 (0.49, 4.11) 0.52 2.60 (0.87, 7.81) 0.088 T1 0 (0.0%) -	J2a ^a	2 (0.8%)	_	_	_	_	_	_	_	_
T,T1 and T2 30 (12.4%) -0.03 (-0.19, 0.13) 0.70 -0.07 (-0.52, 0.38) 0.74 1.42 (0.49, 4.11) 0.52 2.60 (0.87, 7.81) 0.088 T1 0 (0.0%) -	J2b ^a	1 (0.4%)	_	_	_	_	_	_	_	_
T1 0 (0.0%) -	T T1 and T2	30 (12 4%)	-0.03 (-0.19, 0.13)	0.70	-0.07 (-0.52, 0.38)	0 74	1 42 (0 49 4 11)	0.52	260(087 781)	0.088
T1 ^a 4 (1.7%) - <t< td=""><td>T1</td><td>0 (0.0%)</td><td>-</td><td>_</td><td>_</td><td>_</td><td>-</td><td>_</td><td>_</td><td>-</td></t<>	T1	0 (0.0%)	-	_	_	_	-	_	_	-
T2 26 (10.7%) 0.02 (-0.15, 0.19) 0.82 0.14 (-0.35, 0.63) 0.58 1.35 (0.42, 4.30) 0.62 2.52 (0.72, 8.80) 0.15 U, U1, U3, U5, U6 and U8bc 41 (16.9%) -0.05 (-0.18, 0.08) 0.47 -0.15 (-0.53, 0.23) 0.44 1.75 (0.72, 4.24) 0.21 1.21 (0.57, 2.55) 0.62 U1 12 (5.0%) - <t< td=""><td>T1^a</td><td>4 (1.7%)</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td></t<>	T1 ^a	4 (1.7%)	_	_	_	_	_	_	_	_
U, U1, U3, U5, U6 and U8b'c 41 (16.9%) -0.05 (-0.18, 0.08) 0.47 -0.15 (-0.53, 0.23) 0.44 1.75 (0.72, 4.24) 0.21 1.21 (0.57, 2.55) 0.62 U1 12 (5.0%) -<	Τ2	26 (10.7%)	0.02 (-0.15, 0.19)	0.82	0.14 (-0.35, 0.63)	0.58	1.35 (0.42, 4.30)	0.62	2.52 (0.72, 8.80)	0.15
U1 12 (5.0%) -	U, U1, U3, U5, U6 and U8b'c	41 (16.9%)	-0.05 (-0.18, 0.08)	0.47	-0.15 (-0.53, 0.23)	0.44	1.75 (0.72, 4.24)	0.21	1.21 (0.57, 2.55)	0.62
U1 ^a 2 (0.8%) - <t< td=""><td>U1</td><td>12 (5.0%)</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td></t<>	U1	12 (5.0%)	_	_	_	_	_	_	_	_
U3 ^a 1 (0.4%) - <t< td=""><td>U1^a</td><td>2 (0.8%)</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td></t<>	U1 ^a	2 (0.8%)	_	_	_	_	_	_	_	_
U5 25 (10.3%) -0.04 (-0.20, 0.12) 0.66 -0.17 (-0.62, 0.28) 0.45 1.59 (0.56, 4.51) 0.39 1.41 (0.56, 3.53) 0.47 U6 ^a 0 (0.0%) -	U3 ^a	1 (0.4%)	_	_	_	_	_	_	_	_
U6 ^a 0 (0.0%) - <t< td=""><td>U5</td><td>25 (10 3%)</td><td>-0.04 (-0.20, 0.12)</td><td>0.66</td><td>-017(-062028)</td><td>0.45</td><td>1 59 (0 56 4 51)</td><td>0.39</td><td>1 41 (0 56 3 53)</td><td>0.47</td></t<>	U5	25 (10 3%)	-0.04 (-0.20, 0.12)	0.66	-017(-062028)	0.45	1 59 (0 56 4 51)	0.39	1 41 (0 56 3 53)	0.47
U8b'c ^a 1 (0.4%) -	U6ª	0 (0 0%)	-	_	_	_	-	_	_	_
K 23 (9.5%) 0.00 (-0.18 0.17) 0.98 0.04 (-0.45 0.54) 0.87 0.60 (0.23 1.59) 0.31 1.29 (0.49 3.39) 0.61	U8b'c ^a	1 (0.4%)	_	_	_	_	_	_	_	_
	K	23 (9 5%)	0.00 (-0.18, 0.17)	0.98	0.04 (-0.45, 0.54)	0.87	0.60 (0.23 1.59)	0.31	1 29 (0 49 3 39)	0.61

For associations with putaminal TH-ir, regression coefficients, 95% Cls, and p-values result from linear regression models that were adjusted for age at death and sex, where due to their skewed distributions, lateral putaminal TH-ir was considered on the logarithm (base-10) scale and medial putaminal TH-ir was considered on the square root scale. Regression coefficients are interpreted as the additive increase on the mean outcome measure (on the logarithm or square root scale) for the given haplogroup. For associations with ventrolateral and medial substantia nigra neuronal loss scores, ORs, 95% Cls, and p-values result from proportional odds logistic regression models; ORs are interpreted at the multiplicative increase on the odds or a more severe neuronal loss score for the given haplogroup. After applying a Bonferroni correction for multiple testing separately for each outcome measure, *P*-values < 0.0045 (associations with lateral putaminal TH-ir, medial putaminal TH-ir, and ventrolateral substantia nigra neuronal loss score) and < 0.0050 (association with medial substantia nigra neuronal loss score) were considered statistically significant.

^a Haplogroups that occurred in < 10 subjects in a given association analysis not examined in that analysis. TH-ir=tyrosine hydroxylase immunoreactivity; CI = confidence interval; OR = odds ratio.

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of mitochondrial DNA
Associations of mitochondrial DNA

Mitochondrial DNA Haplogroup	Haplogroup frequency, No.(%),	Association witl frontal LB count	n middle	Association witl temporal LB cou	superior Int	Association with parietal LB coun	inferior t	Association with cingulate LB cou	_ t	Association with parahippocamp count	al LB
	N=242	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% CI)	<i>P</i> -value	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% CI)	<i>P</i> -value
Na	0 (0.0%)	I	I	I	I	I	1	I	1	I	1
N1 a	0 (0.0%)	I	I	I	I	I	I	I	I	I	I
la.	8 (3.3%)	I	I	I	I	I	I	I	I	I	I
Ma	2 (0.8%)	I	I	I	I	I	I	I	I	I	I
Xa	4 (1.7%)	I	I	I	I	I	I	I	I	I	I
R and R0 ^a	1 (0.4%)	I	I	I	I	I	I	I	I	I	I
HV and HV0a ^a	8 (3.3%)	I	I	I	I	I	I	I	I	I	I
H, H1, H2, H3 and H4	91 (37.6%)	1.16 (0.95, 1.43)	0.15	1.05 (0.89, 1.25)	0.56	1.12 (0.91, 1.39)	0.28	1.06 (0.92, 1.22)	0.41	1.02 (0.88, 1.18)	0.83
Т	40 (16.5%)	1.01 (0.77, 1.34)	0.93	0.93 (0.74, 1.18)	0.57	0.89 (0.67, 1.18)	0.41	0.94 (0.78, 1.14)	0.54	0.96 (0.80, 1.17)	0.70
H1	27 (11.2%)	1.10 (0.80, 1.52)	0.57	0.94 (0.72, 1.24)	0.64	1.08 (0.78,1.50)	0.64	1.00 (0.80, 1.25)	0.98	0.90 (0.72, 1.13)	0.34
H2 ^a	8 (3.3%)	I	I	I	I	I	I	I	I	I	I
H3	12 (5.0%)	1.03 (0.66, 1.65)	0.91	1.18 (0.82, 1.75)	0.38	1.22 (0.78, 1.94)	0.40	1.10 (0.82, 1.51)	0.52	1.03 (0.76, 1.42)	0.84
Нд ^а	4 (1.7%)	I	I	I	I	I	I	I	I	I	I
Va	8 (3.3%)	I	I	I	I	I	I	1	I	I	I
JТ ^а	0 (0.0%)	I	I	I	I	I	I	I	I	I	I
J, J1, J1d, J2a and J2b	26 (10.7%)	0.92 (0.67, 1.30)	0.64	0.99 (0.76, 1.31)	0.94	1.13 (0.81, 1.58)	0.48	0.99 (0.79, 1.25)	0.92	1.02 (0.81, 1.31)	0.85
þ	0 (0.0%)	1	I	I	I	I	I	I	I	I	I
۱۲	23 (9.5%)	0.87 (0.61, 1.25)	0.44	0.96 (0.72, 1.29)	0.77	1.02 (0.71, 1.47)	0.92	0.97 (0.76, 1.24)	0.79	1.03 (0.81, 1.34)	0.80
J1d ^a	0 (0.0%)	I	I	I	I	I	I	I	I	I	I
J2a ^a	2 (0.8%)	I	I	I	I	I	I	I	I	I	I
J2b ^a	1 (0.4%)	I	I	I	I	I	I	I	Ι	I	I
T, T1 and T2	30 (12.4%)	1.05(0.78, 1.43)	0.75	0.81 (0.63, 1.06)	0.11	0.88 (0.64, 1.21)	0.42	0.95 (0.77, 1.16)	0.59	0.91 (0.74, 1.12)	0.37
Ta	0 (0.0%)	I	I	I	I	I	I	I	I	I	I
T1 ^a	4 (1.7%)	I	I	I	I	I	I	1	I	I	I
T2	26 (10.7%)	1.03 (0.75, 1.43)	0.86	0.76 (0.58, 1.00)	0.044	0.79 (0.57, 1.12)	0.18	0.93 (0.75, 1.16)	0.50	0.90 (0.72, 1.13)	0.34
U, U1, U3, U5, U6 and U8b'c	41 (16.9%)	0.84 (0.64, 1.10)	0.20	1.02 (0.82, 1.28)	0.85	0.91 (0.69, 1.20)	0.49	0.97 (0.81, 1.16)	0.70	0.97 (0.80, 1.18)	0.75
	12 (5.0%)	0.94 (0.60, 1.52)	0.79	1.16 (0.80, 1.71)	0.45	0.95 (0.59, 1.55)	0.82	1.11 (0.82, 1.52)	0.49	I	I
U1 ^a	2 (0.8%)	I	I	I	I	I	I	I	Ι	I	I
U3ª	1 (0.4%)	I	I	I	I	I	I	I	I	I	I
U5	25 (10.3%)	0.82 (0.59, 1.15)	0.23	0.95 (0.72, 1.25)	0.69	0.95 (0.68, 1.33)	0.74	0.91 (0.73, 1.14)	0.40	0.92 (0.72, 1.18)	0.50
UGa	0 (0.0%)	I	I	I	I	I	I	I	I	I	I
U8b/c ^a	1 (0.4%)	I	I	I	I	I	I	1	I	I	1

(continued)
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Tab

Mitochondrial DNA Haplogroup	Haplogroup frequency, No.(%),	Association with frontal LB count	middle	Association with temporal LB cou	h superior unt	Association with parietal LB cour	n inferior Nt	Association with cingulate LB cou	, ut	Association wit parahippocamp count	h al LB
	N = 242	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% CI)	P-value	Multiplicative effect (95% Cl)	<i>P</i> -value	Multiplicative effect (95% Cl)	P-value
	23 (9.5%)	1.15 (0.82, 1.64)	0.43	1.17 (0.88, 1.57)	0.30	1.10 (0.77, 1.59)	0.60	1.06 (0.84,1.35)	0.64	1.13 (0.87, 1.48)	0.36

Multiplicative effects, 95% CIs, and p-values result from negative binomial regression models that were adjusted for age at death and sex. Multiplicative effects are interpreted as the multiplicative increase on the mean LB count for the given haplogroup. After applying a Bonferroni correction for multiple testing separately for each outcome measure, *P*-values < 0.0042 (associations with middle frontal, superior temporal, inferior parietal, and cingulate LB counts) and < 0.0045 (associations with middle frontal, superior temporal, inferior parietal, and cingulate LB counts) and < 0.0045 (associations with parahippocampal LB count) were considered statistically significant.

^a Haplogroups that occurred in < 10 subjects in a given association analysis not examined in that analysis. LB = Lewy body. Cl = confidence interval

Interestingly, we did observe a suggestive association between mtDNA sub-haplogroup H background and less severe ventrolateral SN neuronal loss in LBDhDLB cases. Albeit not statistically significant, this data is important because SN degeneration is a classical hallmark of PD and may behave as an important mediator in LB spread in LBD and may be a defining mediator between DLB and PDD. This concept also supports the rationale that sub-haplogroup H may be protective against DLB risk, as reported in this study. Functionally this could be explained by SN cells being more sensitive to physiological pressures than other neuronal types. More specifically, dopaminergic SN cells are highly metabolically active, long and thin, and have little to no myelination [2], and they heavily rely on healthy mitochondria for efficient OXPHOS to ensure sufficient ATP is produced to maintain their metabolic capacity. Mitochondria carrying mtDNA haplogroup H are reported to have the most efficient OXPHOS coupling capacity in all European haplogroups and produce more ATP and ROS than other groups [50]. This may be advantageous in protecting SN cells from accumulating LB with age. On the contrary though, SN cells with a haplogroup H background may be more susceptible to physiological pressures as functional studies in cybrid cell lines have demonstrated these cells have an increased susceptibility to oxidative stress compared to non-haplogroup H cells [31]. This suggests that mitochondrial background may provide cell or regional-specific biological benefits, but under additional physiological pressures may enhance disease progression.

As LBD pathology is very heterogenous and presents with pathological aggregates of tau, beta-amyloid, and TDP-43 proteins, it is important to also consider the role nuclear genetic risk factors play in driving disease risk relative to mtDNA background. More specifically, APOE4 is consistently an increased genetic risk factor for clinical DLB [18, 40] and AD [23, 41], and APOE4 influences LB pathology independently to AD pathology [8]. The mtDNA haplogroup associations reported in this study were all adjusted for APOE4 allele status which did not change any observations after adjustments. Reassuringly, mtDNA haplogroup associations in DLB and AD have been reported independent of APOE4 status in prior studies [5], suggesting both mitochondrial and nuclear genomic background influence disease phenotypes. Future studies should consider evaluating major nuclear genetic risk factors relative to mitochondrial genetic background to avoid any possible bias.

Several limitations of our study are important to note. The main limitation being that even though the sample size of DLB and LBD-hDLB cases are relatively large given the prevalence of DLB, sample numbers are small for a genetic association study and therefore the possibility of a type II error is important to consider. This is especially true when considering adjustment for multiple testing and for rare haplogroups. In addition, although all cases in this study were examined for population stratification prior to conducting this work [3]; population stratification in the control cohort may influence false positive findings (noting all subjects carried European mtDNA haplogroups). Global access to well described cohorts of DLB cases is required and validation of this work in larger cohorts of DLB and LBD-hDLB cases will be important to further investigate the role mitochondrial haplogroup H has in DLB risk and neuropathological development.

Conclusions

We have conducted a comprehensive study of the role of mitochondrial genomic variation, in the form of mitochondrial haplogroups and sub-haplogroups, in clinical DLB and pathologically confirmed LBD-hDLB. Moreover, this is one of the first studies to explore the association of mtDNA background with neuropathological LB counts and neuronal loss measures in LBD-hDLB brains. Our data suggests that mitochondrial sub-haplogroup H may be protective against clinical DLB risk, independent of *APOE4* background, and this may be indirectly influenced by the suggestive association that sub-haplogroup H is protective against neuronal loss in substantia nigra tissue. Additional assessments and replication studies are warranted to further validate and expand on this data.

Abbreviations

AD: Alzheimer's disease; APOE4: Apolipoprotein E4 allele; aSyn: Alpha-synuclein; ATP: Adenosine triphosphate; CIs: Confidence intervals; DLB: Dementia with Lewy bodies; DNA: Deoxyribonucleic acid; LB: Lewy bodies; LBD: Lewy body disease; LBD-hDLB: Lewy body disease with a high likelihood of having clinical DLB; mtDNA: Mitochondrial DNA; NFTs: Neurofibrillary tangles; ORs: Odds ratios; OXPHOS: Oxidative phosphorylation; PDD: Parkinson's disease dementia; ROS: Reactive oxygen species; SN: Substantia nigra; SP: Senile plaques; TH-ir: Tyrosine hydroxylase immunoreactivity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40478-022-01399-4.

Additional file 1. Supplementary Tables.

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Author contributions

Rebecca R. Valentino designed mitochondrial DNA genotyping assays, performed all genotyping and quality control assessments, and drafted the manuscript. Chloe Ramnarine assisted with genotyping samples. Michael G. Heckman and Patrick W. Johnson performed all statistical analysis and Michael G. Heckman provided manuscript improvements. Alexandra I. Soto-Beasley provided training for genotyping design and methods. Ronald L. Walton prepared all genomic DNA extracts for all samples from donated human tissues. Shunsuke Koga and Dennis W. Dickson provided brain tissue samples for all cases and Dennis W. Dickson performed neuropathological assessments. of LBD cases. Koji Kasanuki conducted all TH-ir stainings and evaluations in LBD cases. Ryan J. Uitti, Julie A. Fields, P, Hugo Botha, Vijay K. Ramanan, Kejal Kantarci, Val J. Lowe, Clifford R. Jack, Nilufer Ertekin-Taner, Rodolfo Savica, Jonathan Graff-Radford, Ronald C. Petersen, Joseph E. Parisi, R. Ross Reichard, Neill R. Graff-Radford, Tanis J. Ferman, Bradley F. Boeve, Zbigniew K. Wszolek, recruited clinical patients, characterized clinical, radiological, and pathological phenotypes and organized blood collections. Zbigniew K. Wszolek also obtained funding for the collection of control subjects, recruited all control subjects, and maintained IRB protocols. Owen A. Ross led the study and oversaw all method developments and analysis. He takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets that were used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This research was approved by the Mayo Clinic Institutional Review Board and all human participants involved in this work provided written consent prior to study commencement.

Consent for publication

Not applicable.

Competing interests

Melissa E. Murray personally receives consulting fees from VID Radiopharmaceuticals and has received reimbursements for Mild Cognitive Impairment and NIH study section. MEM is also Co-chair of Digital Pathology working group, Chair/Immediate Past Chair of Atypical Alzheimer's disease Professional Interest Area group and is on the organizing committee of the Southeastern Neurodegeneration Conference. Julie A. Fields is on the Oversight and Monitoring Board for the SWAN-Aging study that is funded by the NIA but receives no compensation. Hugo Botha received free AAN registration for the Geschwind Award, Vijay K. Ramanan consults for Bayer Schering Pharma, Piramal Life Sciences, Life Molecular Imaging, Eisai Inc., AVID Radiopharmaceuticals, and Merck Research, and receives research support from GE Healthcare, Siemens Molecular Imaging, and AVID Radiopharmaceuticals. Kejal Kantarci consults Biogen Inc, and receives research support from Avid Radiopharmaceuticals and Eli Lilly, and funding from NIH and Alzheimer's Drug Discovery Foundation. Val J. Lowe receives research support from AVID Radiopharmaceuticals and Siemens Healthcare and is a consultant for AVID Radiopharmaceuticals, Eisai, Inc., Bayer Schering Pharma, and Merck Research. Clifford R. Jack is on the Roche advisory board but receives no payments. CRJ is also on the writing committee for the NIA AA research framework. Jonathan Graff-Radford has received payment from American Academy of Neurology for lecturing. Nilufer Ertekin-Taner received payments as a conference speaker for 11th ISABS and the Department of Electrical and Computer Engineering and Computer Science, University of Urbana-Champaigne, and additionally for being a visiting professor at the Department of Neurology, Indiana University School of Medicine, Bloomington, Indiana, as well as at National Center for Geriatrics and Gerontology Nagoya, Japan. NET is a Framingham Heat Study Executive Board member and NIH/NIA TREAT-AD external advisory Board member. Jonathan Graff-Radford received a payment from American Academy of Neurology for presenting a lecture. Ronald C. Petersen receives royalties from Oxford University Press in UpToDate and consulting fees from Roche, Merck, Biogen, Genentech, and Eisai. RCP is also on the Genentech DSMB advisory board. R. Ross Reichard is the President of American Association of Neuropathologists. Neill R. Graff-Radford receives research support from multicenter studies with Biogen, Abbvie, Lilly, and Novartis, and receives personal royalties for a chapter on NPH in UpToDate. Bradley F. Boeve has served as an investigator for clinical trials sponsored by Biogen, Alector, and EIP Pharma, and serves on the Scientific Advisory Board of the Tau Consortium. Zbigniew K. Wszolek serves as PI or Co-PI on Biohaven Pharmaceuticals, Inc. (BHV4157-206 and BHV3241-301) and Neuraly, Inc. (NLY01-PD-1), and serves as Co-PI of the Mayo Clinic APDA Center for Advanced Research and as an external advisory board member for the Vigil Neuroscience, Inc. All other authors declare that they have no competing interests to report.

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