

Two-stage association study and meta-analysis of mitochondrial DNA variants in Parkinson disease

Gavin Hudson, PhD
Mike Nalls, PhD
Jonathan R. Evans,
MRCP, PhD
David P. Breen, MRCP
Sophie Winder-Rhodes,
BSc
Karen E. Morrison, MD
Huw R. Morris, MRCP,
PhD
Caroline H. Williams-
Gray, MCRP, PhD
Roger A. Barker, MCRP,
PhD
Andrew B. Singleton,
PhD
John Hardy, MD, PhD
Nicholas E. Wood, MD,
PhD
David J. Burn, MD
Patrick F. Chinnery, PhD,
FMedSci

Correspondence to
Dr. Chinnery:
patrick.chinnery@ncl.ac.uk

Supplemental data at
www.neurology.org

ABSTRACT

Objectives: Previous associations between mitochondrial DNA (mtDNA) and idiopathic Parkinson disease (PD) have been inconsistent and contradictory. Our aim was to resolve these inconsistencies and determine whether mtDNA has a significant role in the risk of developing PD.

Methods: Two-stage genetic association study of 138 common mtDNA variants in 3,074 PD cases and 5,659 ethnically matched controls followed by meta-analysis of 6,140 PD cases and 13,280 controls.

Results: In the association study, m.2158T>C and m.11251A>G were associated with a reduced risk of PD in both the discovery and replication cohorts. None of the common European mtDNA haplogroups were consistently associated with PD, but pooling of discovery and replication cohorts revealed a protective association with “super-haplogroup” JT. In the meta-analysis, there was a reduced risk of PD with haplogroups J, K, and T and super-haplogroup JT, and an increase in the risk of PD with super-haplogroup H.

Conclusions: In a 2-stage association study of mtDNA variants and PD, we confirm the reduced risk of PD with super-haplogroup JT and resolve this at the J1b level. Meta-analysis explains the previous inconsistent associations that likely arise through sampling effects. The reduced risk of PD with haplogroups J, K, and T is mirrored by an increased risk of PD in super-haplogroup HV, which increases survival after sepsis. Antagonistic pleiotropy between mtDNA haplogroups may thus be shaping the genetic landscape in humans, leading to an increased risk of PD in later life.

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GLOSSARY

58BC = 1958 Birth Cohort; **MAF** = minor allele frequency; **mtDNA** = mitochondrial DNA; **NBS** = National Blood Service; **OR** = odds ratio; **PD** = Parkinson disease; **QC** = quality control; **SNP** = single nucleotide polymorphism; **WTCCC** = Wellcome Trust Case Control Consortium.

Multiple lines of evidence implicate mitochondria in the pathogenesis of idiopathic Parkinson disease (PD). A defect of respiratory chain complex I has been documented in the substantia nigra of postmortem PD brains,¹ and the potent complex I inhibitor MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) causes parkinsonism and nigrostriatal dopaminergic degeneration in humans.² Exposing rats to the complex I inhibitor rotenone causes tremor and rigidity with associated loss of substantia nigra neurons and aggregation of α -synuclein, a major constituent of Lewy bodies, which characterize the neuropathology of PD.³ Furthermore, several monogenic forms of PD are due to mutations in genes coding for mitochondrial proteins or mitochondrial maintenance/autophagy (including *PARK2*,⁴ *PINK1*,⁵ and *DJF*), which compromise oxidative phosphorylation.

Mitochondrial DNA (mtDNA) codes for 13 respiratory chain proteins that are essential for the synthesis of the intracellular energy source adenosine triphosphate. Point mutations of mtDNA compromise oxidative phosphorylation and cause severe multisystem neurologic disorders with

From the Wellcome Centre for Mitochondrial Research (G.H., P.F.C.), Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK; Laboratory of Neurogenetics (M.N., A.B.S.), National Institute on Aging, National Institutes of Health, Bethesda, MD; Department of Clinical Neurosciences (J.R.E., D.P.B., S.W.-R., C.H.W.-G.), University of Cambridge, Cambridge; Division of Neurosciences (K.E.M., R.A.B.), University of Birmingham, Edgbaston, Birmingham; Medical Research Council Centre for Neuropsychiatric Genetics and Genomics (H.R.M.), Cardiff University School of Medicine, Cardiff; Department of Molecular Neuroscience (J.H., N.E.W.), Institute of Neurology, University College London, London; and Institute for Ageing and Health (D.J.B.), Newcastle University, Newcastle upon Tyne, UK.

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extrapyramidal features resembling PD, raising the possibility that more subtle polymorphic variants contribute to genetic susceptibility to PD.

mtDNA is strictly maternally inherited, and mutations acquired throughout human history have subdivided the population into discrete “haplogroups,”⁶ typically 5,000 to 10,000 years old and each harboring many additional single nucleotide polymorphisms (SNPs).⁶ Several studies have reported an association of mtDNA haplogroups and specific mtDNA SNPs with PD.^{7–16} These studies have consistently shown an association between mtDNA and PD, but the precise haplogroup or SNP associations have differed and have often been contradictory (table 1). These inconsistencies mean that the role of inherited mtDNA variants in PD has yet to be fully established.

Herein, we report a 2-stage association study of inherited mtDNA variants in PD, which is the largest study to date. Our results confirm earlier haplogroup findings and provide further resolution of the genetic association. In addition, a meta-analysis of the available published data

firmly establishes mtDNA as a major determinant of genetic risk in PD, even in the context of reported nuclear genome-wide associations.¹⁷

METHODS We studied 138 mitochondrial variants in 3,074 PD cases and 5,658 ethnically matched controls from the United Kingdom. All samples were Caucasian. We performed a 2-stage genetic association study with discovery and replication phases. In the discovery phase, 2,197 cases (PD1, previously described¹⁸) were compared with 2,930 controls from the Wellcome Trust Case Control Consortium–1958 Birth Cohort (WTCCC-58C). In the replication phase, 877 cases (PD2, previously described¹⁹) were compared with 2,729 controls from the UK National Blood Service (WTCCC-NBS). Discovery cases (PD1) were genotyped using the Illumina 610K quad array (Illumina, San Diego, CA) and replication cases (PD2) were genotyped using the Affymetrix 550K array (Affymetrix Inc., Santa Clara, CA). All controls were genotyped on the Illumina 1.2M Duo platform.

Stringent quality control (QC) filters were applied to remove poorly performing samples using tools implemented in PLINK v2.050²⁰ as previously described.²¹ Individuals were excluded from subsequent analysis if data were absent in >10% of SNPs²¹ (discovery phase, PD1 cases = 472 and WTCCC-58C = 36; replication phase, PD2 = 1 and WTCCC-NBS = 8). After sample QC, 1,725 PD cases and 2,894 controls remained in the discovery phase and 876 PD cases and 2,721 controls remained in the replication phase.

SNPs were excluded if >10% of genotypes were absent²¹ (discovery = 13 and replication = 6). We also excluded SNPs with a study-wide missing data rate of >5% (if minor allele frequency [MAF] was >5%) or >1% study-wide missing data rate

Table 1 Previously reported significant associations between mitochondrial DNA and Parkinson disease^a

Ref.	Year	Cases	Controls	Variant	Published haplogroup	Predicted haplogroup	p	OR (95% CI)	Sex	Present on array?
10	2008	146	122	m.13708G>A	J	X2b/R0a/HV1/J/T2	0.005	0.49 (0.07-0.53)	M	No
		332	414	m.497C>T	K1a	K1a	0.025	—		No
		332	414	m.498delC	K1c	L3c	0.011	—		No
12	2005	271	230	m.4336T>C	—	U6/H5a	0.011	4.45 (1.23-15.96)	F	Yes
				m.10398A>G	—	J/K1/R1112	0.009	0.53 (0.33-0.86)		Yes
11	2005	620	1,486	m.10398A>G and m.12308A>G	K	J/K1/R1112-U/U5	0.048	0.54 (0.35-0.83)		Yes/yes
13	2005	455	188	m.16069T>C	J	HV4/J	0.029	0.61 (0.39-0.94)		No
				m.12308A>G	U	U/U5	0.004	0.56 (0.37-0.83)		Yes
				—	JTUK		0.0001	0.50 (0.38-0.66)		—
16	2004	102	112	m.4336A>G	—	H5a/U6d	0.018	Increased risk ^b		Yes
				m.10398A>G	—	—	0.022	Increased risk ^b		Yes
14	2003	90	129	m.4216T>C	TJ	R2'JT/K1a/H1-10/X2b	0.014	—		No
15	2003	609	340	—	J		0.020	0.55 (0.34-0.91)		—
				—	K		0.020	0.52 (0.30-0.90)		—
				m.10398A>G	—	J/K1/R1112	0.0001	0.53 (0.39-0.73)		Yes
				m.13708G>A	—	J/T2/U4b/K2a	0.010	—		No

Abbreviations: CI = confidence interval; OR = odds ratio.

^aShown are the reference, year of publication, number of samples used in the study (both patients and controls), associated variant, corresponding published haplogroup, phylogenetically predicted mitochondrial haplogroup (www.phylotree.org), probability of association, ORs of association (95% CIs), and whether the effect was sex-specific (M or F). The table also indicates the availability of the variant in the current array-based study.

^bAbsolute values unavailable.

for SNP MAF of <5%²¹ (discovery = 3 and replication = 4); additionally, SNPs with MAF of <1% were removed (discovery = 43 and replication = 49). Finally, to verify the quality of genotypes, cluster plots of normalized intensity for each SNP were generated using R (<http://www.R-project.org>) and inspected. To exclude the potential for differential array-based genotyping artifacts, SNP frequencies and subsequent mtDNA haplogroup frequencies were compared between PD1 and PD2 using PLINK v2.050.²⁰ Variants showing significant ($p > 0.05$) population differences were excluded.

Subsequent analysis was restricted to a concordant dataset of 77 mtDNA variants passing QC in both the discovery and replication phases. Included in the final analysis were m.4667T>C, m.10398A>G, and m.12308A>G, all of which have been previously associated with PD (table 1). Differential missingness tests, which statistically compare the frequency of “missing” genotype data between cases and controls,²¹ revealed differences in 4 SNPs ($p = <10^{-4}$), reducing the final number of experimental SNPs to 74. Statistical significance was defined as $p < 0.05$ in both the discovery and replication phases. Missingness and χ^2 test were computed using PLINK v2.050.²⁰

mtDNA haplogroup determination. The hierarchical relationship among mtDNA variants is represented at www.phylotree.org. Each array variant (n = 74 post-QC) in each subject (n = 8,215 post-QC) was compared with the revised Cambridge Reference Sequence and used to identify haplogroup-specific motifs. Assignment of haplogroup was performed according to published criteria⁶; for example, haplogroup J is defined by variants m.295 and m.489 (table e-1 on the *Neurology*[®] Web site at www.neurology.org). If a no-call was detected in a major haplogroup-defining SNP, then clade-specific subtype variants were used to identify the mtDNA haplogroup. Based on available genotyping data, 99.51% of subjects were assigned to a major European haplogroup. As a final QC metric to remove the effects of phylogenetic heterogeneity, non-European samples (i.e., non-HVJTUKWXI and O haplogroups) were removed from subsequent analysis (discovery: cases = 6 and controls = 5; replication: cases = 25 and controls = 4). Subsequent statistical analysis was based on finalized discovery (PD1 = 1,719 vs

WTCCC-58C = 2,889) and replication cohorts (PD2 = 851 vs WTCCC-NBS = 2,717).

Meta-analysis. A meta-analysis of all available mtDNA haplogroup and PD studies^{7–15} was conducted using Comprehensive Meta-Analysis (version 2, www.meta-analysis.com).²² In total, 9 published studies (total cases = 3,030 and total controls = 4,862) were combined with our larger dataset (discovery: cases = 1,719 and controls = 2,889; replication: cases = 851 and controls = 2,717).

Standard protocol approvals, registrations, and patient consents. We received approval from the Northumberland Local Research Ethics Committee (REF 05/Q0902/51) to conduct a genetic association study of PD. Written informed consent was obtained from the patients in all cases.

RESULTS SNP association. In the discovery cohort, we identified 9 mtDNA variants associated with PD (table 2). Two of these associations were also seen in the replication cohort at the same order of magnitude. Both m.2158T>C (discovery: $p = 2.40 \times 10^{-2}$ and odds ratio [OR] = 0.51; replication: $p = 2.45 \times 10^{-2}$ and OR = 0.32) and m.11251A>G (discovery: $p = 2.92 \times 10^{-2}$ and OR = 0.84; replication: $p = 1.20 \times 10^{-3}$ and OR = 0.71) were associated with a reduced the risk of PD. We found no evidence of a consistent association between PD and m.4667T>C, m.12308A>G, or m.10398A>G, either in the individual cohorts or the combined cohort.

Haplogroup associations. A comparison of the major European mtDNA haplogroups did not identify a consistent association with PD in the discovery, replication, or combined cohorts (table e-2). However, given previous reports of a reduced risk of PD in

Table 2 mtDNA SNP association with idiopathic PD in discovery (PD1 vs WTCCC-58C) and replication (PD2 vs WTCCC-NBS) phases

Array ID ^a	rCRS	A1	Discovery (PD1 vs WTCCC-58C)				Replication (PD2 vs WTCCC-NBS)			
			FA	FC	p^b	OR (95% CI)	FA	FC	p^b	OR (95% CI)
MitoT2160C	m.2158T>C	C	0.008	0.016	2.40×10^{-2}	0.51 (0.28–0.92)	0.005	0.014	2.45×10^{-2}	0.32 (0.12–0.91)
MitoA9668G	m.9667A>G	G	0.017	0.008	7.68×10^{-3}	2.06 (1.20–3.56)	0.008	0.013	2.74×10^{-1}	0.64 (0.28–1.44)
MitoT10035C	m.10034T>C	C	0.026	0.037	4.68×10^{-2}	0.70 (0.49–1.00)	0.031	0.029	7.89×10^{-1}	1.06 (0.68–1.66)
MitoT10239C	m.10238T>C	C	0.028	0.039	4.02×10^{-2}	0.70 (0.50–0.99)	0.042	0.032	1.48×10^{-1}	1.34 (0.90–1.98)
MitoG10399A	m.10398G>A	G	0.199	0.236	3.84×10^{-3}	0.81 (0.70–0.93)	0.249	0.225	1.33×10^{-1}	1.15 (0.96–1.37)
MitoA11252G	m.11251A>G	G	0.186	0.213	2.92×10^{-2}	0.84 (0.73–0.98)	0.160	0.211	1.20×10^{-3}	0.71 (0.58–0.88)
MitoG12373A	m.12372G>A	A	0.237	0.210	3.47×10^{-2}	1.17 (1.01–1.35)	0.201	0.236	3.32×10^{-2}	0.82 (0.68–0.98)
MitoC16272T	m.16271C>T	T	0.103	0.082	1.83×10^{-2}	1.29 (1.04–1.59)	0.070	0.093	3.57×10^{-2}	0.73 (0.55–0.98)
MitoG16393A	m.16392G>A	A	0.025	0.038	2.00×10^{-2}	0.65 (0.45–0.94)	0.031	0.027	5.94×10^{-1}	1.13 (0.72–1.77)

Abbreviations: A1 = associated allele; CI = confidence interval; FA = allele frequency in cases; FC = allele frequency in controls; 58BC = 1958 Birth Cohort; mtDNA = mitochondrial DNA; NBS = National Blood Service; OR = odds ratio; PD = Parkinson disease; rCRS = revised Cambridge Reference Sequence (actual mtDNA bases position [numbered as per GenBank NC_012920]); SNP = single nucleotide polymorphism; WTCCC = Wellcome Trust Case Control Consortium.

^a Affymetrix and Illumina array identification.

^b Uncorrected Pearson probability.

subjects belonging to “super-haplogroup JT,” we combined the data from haplogroups J and T. The pooled analysis identified an association with the super-haplogroup JT ($p = 3.54 \times 10^{-2}$, OR = 0.87, 95% CI = 0.77–0.97).

Meta-analysis. A meta-analysis revealed a significant reduction in PD risk for haplogroups J ($p = 1.22 \times 10^{-2}$, OR = 0.88), T ($p = 2.45 \times 10^{-2}$, OR = 0.87), and K ($p = 3.63 \times 10^{-3}$, OR = 0.84) (figure 1). Combining haplogroups J and T increased the strength of the association but did not change the effect size ($p = 5.84 \times 10^{-4}$, OR = 0.85) (figure 2). When studied in isolation, a meta-analysis of haplogroups H and V failed to show an effect on PD risk (figure e-1). However, a significant increase in the risk of PD was identified when consolidated to the “super-haplogroup” HV ($p = 3.64 \times 10^{-3}$, OR = 1.112) (figure 2). No association was identified between haplogroups U, W, X, and I when analyzed in isolation, or when grouped into phylogenetically related super-haplogroups (figure e-1).

DISCUSSION Our 2-phased approach identified a consistent association between PD and both m.2158T>C and m.11251A>G. These 2 SNPs are phylogenetically linked, with m.11251A>G defining the mtDNA super-haplogroup JT and m.2158T>C residing on the J-specific subclade (J1b).²³ These findings are consistent with the association we observed with super-haplogroup JT, confirm some previous reports, and, more importantly, resolve this association at the subhaplogroup level (J1b). Variant m.11251A>G does not result in an amino acid substitution (*MTND4* L164L) and is therefore unlikely to directly contribute to disease pathogenesis. However, m.2158T>C is present in the highly heterogeneous mitochondrial 16s ribosomal RNA gene (*MTRNR2*). Although it is conceivable that m.2158T>C could alter intramitochondrial synthesis, there are other possibilities. *MTRNR2* shows 99% sequence homology to Humanin, a molecule shown to suppress neurotoxicity in Alzheimer disease, leading to the suggestion that Humanin is actually produced from *MTRNR2* within the mitochondrion.^{24,25} It is therefore possible that common mtDNA variation in *MTRNR2* has a direct neuroprotective effect.

Despite >99% power to detect the previously reported findings, we were unable to consistently replicate the previous associations to m.4336T>C, m.10398A>G, or m.12308A>G. It is therefore unlikely that these variants are relevant to the etiology of PD. Differences in sample size provide the likely explanation for the discrepancies, with our combined cohorts being >4-fold greater than the largest mtDNA study published to date in PD. We were

unable to test the direct association with 6 of the previously associated mtDNA variants because they were not present on the genotyping array; however, the phylogenetic nature of mtDNA allowed us to make direct haplogroup comparisons to these studies using correlated mtDNA variants (table e-1). Combining data from 10 studies (6,140 PD cases and 13,280 controls) confirmed the JT association and also provided sufficient statistical power to show a protective effect from haplogroups J, K, and T. Each one of these haplogroups had been associated with a reduced risk of PD previously but not in the same study. These disparate findings suggested that the associations were spurious false-positive results. However, the results of our meta-analysis indicate that the associations are likely to be robust, with the limited sample sizes in previous studies explaining the inconsistencies.

What are the functional consequences of our findings? mtDNA haplogroups have been shown to modulate the efficiency of adenosine triphosphate synthesis or the production of reactive oxygen species,²⁶ and these effects could directly modulate neuronal protection rather than damage. It is also likely that haplogroups J, T, and K are genetic tags of further mtDNA variation not directly genotyped in this study. For example, haplogroup K contains the lowest frequency of nonsynonymous complex I gene variants (compared with the other haplogroups), raising the possibility that natural selection against genetic variation of complex I reduces the risk of PD in haplogroup K individuals.

Conversely, by pooling all of the data, we were able to show that super-haplogroup HV is associated with an increased risk of PD. Whether the increased risk of PD in HV is directly attributable to functional variants within that haplogroup or simply reflects the “mirror image” of the reduced risk of PD in J, K, and T remains to be established. However, it is intriguing that haplogroup H has been associated with an increased propensity to survive severe infection.²⁷ This raises the possibility that natural selection has led to the emergence of genetic variants that predispose toward neurodegenerative disease through antagonistic pleiotropy. Similar to Huntington disease, whereby an increased CAG_(n) repeat length in the *huntingtin* gene causes a neurodegenerative disorder but is associated with a lower incidence of cancer, recent mutations in mtDNA may be tolerated in humans because they increase the chance of surviving early-life insults such as sepsis, but are pathogenic in later life through the increased generation of reactive oxygen species.

Finally, recent data suggest that α -synuclein is localized to mammalian neuronal mitochondria and that its expression is associated with a decrease in complex I activity.²⁸ It is also clear that functional

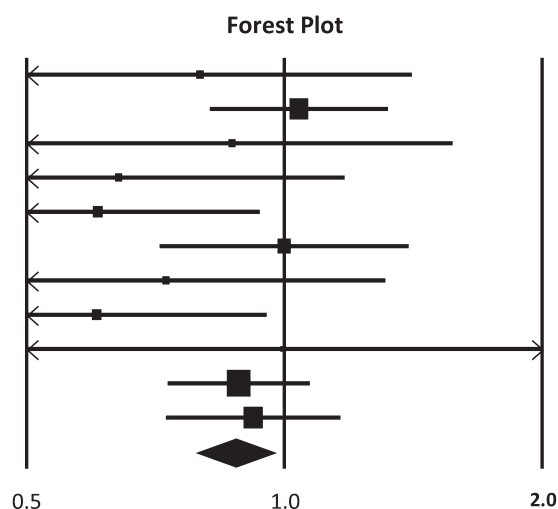
mtDNA is required to modulate α -synuclein toxicity,²⁹ but that overexpression of α -synuclein can result in mitochondrial dysfunction.³⁰ It is therefore likely that α -synuclein accumulation and mtDNA

variation interact synergistically to affect neuronal complex I activity. The resultant loss of activity could lead to an increase in reactive oxygen species and a reduction in cellular energy, culminating in the

Figure 1 Meta-analysis of previously reported PD and mtDNA haplogroup associations and the data from this study

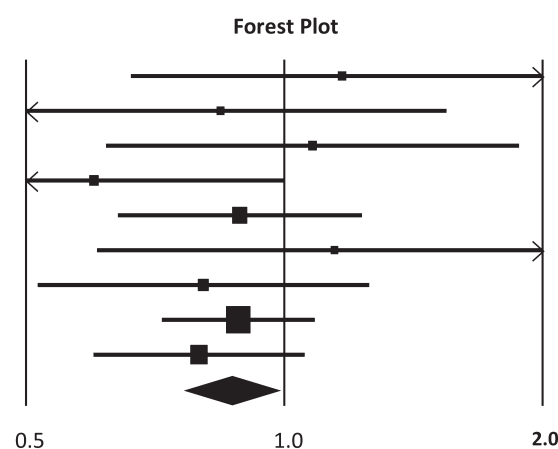
Haplogroup J

Study	OR	L95 CI	U95 CI	P Value
7	0.797	0.451	1.410	4.36E-01
8	1.040	0.818	1.322	7.49E-01
9	0.869	0.480	1.574	6.43E-01
10	0.641	0.349	1.177	1.51E-01
13	0.606	0.392	0.937	2.42E-02
11	1.000	0.715	1.398	9.98E-01
12	0.728	0.403	1.314	2.92E-01
15	0.604	0.382	0.955	3.09E-02
14	0.996	0.435	2.282	9.92E-01
Current study PD1	0.884	0.730	1.072	2.10E-01
Current study PD2	0.920	0.727	1.164	4.87E-01
Fixed	0.877	0.791	0.972	1.22E-02



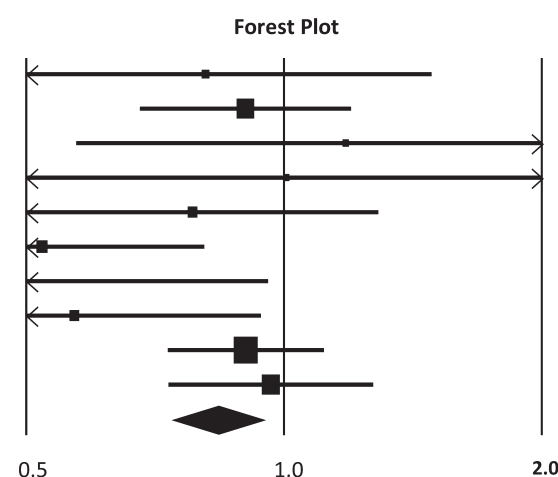
Haplogroup T

Study	OR	L95 CI	U95 CI	P Value
7	1.168	0.662	2.060	5.92E-01
9	0.843	0.459	1.547	5.81E-01
10	1.079	0.619	1.880	7.88E-01
13	0.600	0.360	0.999	4.96E-02
11	0.888	0.639	1.232	4.77E-01
12	1.144	0.605	2.166	6.79E-01
15	0.805	0.515	1.257	3.40E-01
Current study PD1	0.884	0.720	1.086	2.41E-01
Current study PD2	0.796	0.599	1.057	1.15E-01
Fixed	0.868	0.767	0.982	2.45E-02



Haplogroup K

Study	OR	L95 CI	U95 CI	P Value
7	0.810	0.440	1.489	4.97E-01
8	0.902	0.678	1.198	4.75E-01
9	1.180	0.571	2.438	6.54E-01
10	1.006	0.480	2.107	9.87E-01
13	0.782	0.474	1.290	3.36E-01
11	0.522	0.337	0.808	3.52E-03
12	0.461	0.222	0.959	3.83E-02
15	0.569	0.344	0.940	2.78E-02
Current study PD1	0.902	0.731	1.114	3.39E-01
Current study PD2	0.965	0.732	1.272	8.02E-01
Fixed	0.837	0.742	0.944	3.63E-03

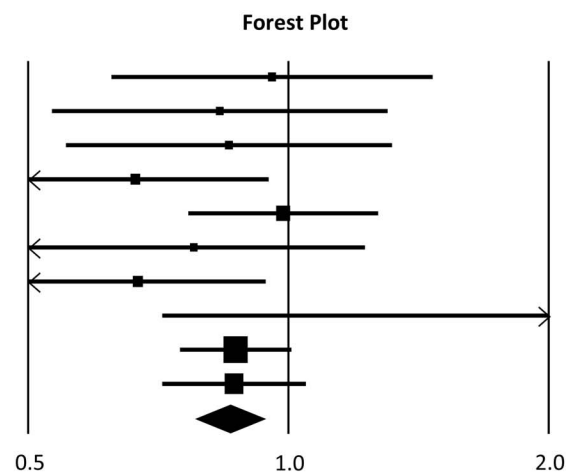


Meta-analysis of our data and published European mtDNA haplogroup vs PD associations in isolation. Discovery = Hudson et al., PD1; replication = Hudson et al., PD2. Boxes are proportional to study size, and fixed-model meta-analysis is denoted by a diamond symbol. CI = confidence interval; L95 = lower 95% CI; mtDNA = mitochondrial DNA; OR = odds ratio; PD = Parkinson disease; U95 = upper 95% CI.

Figure 2 Meta-analysis of previously reported PD and mtDNA “super-haplogroup” associations and the data from this study

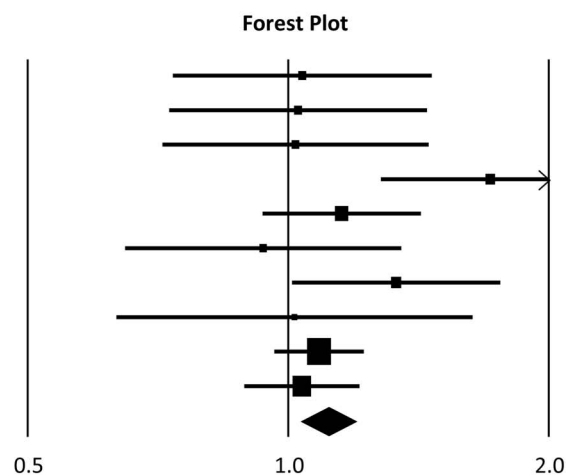
‘Super Haplogroup’ JT

Study	OR	L95 CI	U95 CI	P Value
7	0.957	0.624	1.470	8.42E-01
9	0.833	0.532	1.303	4.24E-01
10	0.854	0.552	1.319	4.76E-01
13	0.665	0.466	0.949	2.47E-02
11	0.986	0.765	1.271	9.14E-01
12	0.777	0.493	1.227	2.79E-01
15	0.670	0.476	0.942	2.13E-02
17	1.970	0.714	5.433	1.90E-01
Current study PD1	0.869	0.748	1.009	6.47E-02
Current study PD2	0.865	0.714	1.048	1.40E-01
Fixed	0.855	0.782	0.935	5.84E-04



‘Super Haplogroup’ HV

Study	OR	L95 CI	U95 CI	P Value
7	1.038	0.735	1.466	8.32E-01
9	1.027	0.728	1.448	8.81E-01
10	1.020	0.715	1.454	9.14E-01
13	1.712	1.279	2.292	3.03E-04
11	1.153	0.933	1.424	1.87E-01
12	0.936	0.648	1.352	7.23E-01
15	1.333	1.009	1.760	4.28E-02
17	1.017	0.633	1.634	9.45E-01
Current study PD1	1.085	0.963	1.224	1.80E-01
Current study PD2	1.037	0.889	1.210	6.43E-01
Fixed	1.112	1.035	1.194	3.64E-03



Meta-analysis of our data and published European mtDNA haplogroup vs PD associations when combined into super-haplogroups. Discovery = Hudson et al., PD1; replication = Hudson et al., PD2. Boxes are proportional to study size, and fixed-model meta-analysis is denoted by a diamond symbol. CI = confidence interval; L95 = lower 95% CI; mtDNA = mitochondrial DNA; OR = odds ratio; PD = Parkinson disease; U95 = upper 95% CI.

initiation of apoptotic pathways and eventual neuronal cell death.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: G. Hudson, D. Burn, and P.F. Chinnery. Contributed reagents/materials/analysis tools: G. Hudson, M. Nalls, J.R. Evans, D.P. Breen, S. Winder-Rhodes, K.E. Morrison, H.R. Morris, C.H. Williams-Gray, Wellcome Trust Case Control Consortium 2, R.A. Barker, A.B. Singleton, J. Hardy, N.E. Wood, D.J. Burn, and P.F. Chinnery. Wrote the manuscript: G. Hudson and P.F. Chinnery.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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