Mitochondrial haplogroups and cognitive progression in Parkinson's disease

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Mitochondrial haplogroups and cognitive progression in Parkinson's disease

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Abstract

Mitochondria are a culprit in the onset of Parkinson's disease, but their role during disease progression is unclear. Here we used Cox proportional hazards models to exam the effect of variation in the mitochondrial genome on longitudinal cognitive and motor progression over time in 4,064 patients with Parkinson's disease. Mitochondrial macro-haplogroup was associated with reduced risk of cognitive disease progression in the discovery and replication population. In the combined analysis, patients with the super macro-haplogroup J, T, U[#] had a 41% lower risk of cognitive progression with $P = 2.42 \times 10^{-6}$ compared to those with macro-haplogroup H. Exploratory analysis indicated that the common mitochondrial DNA variant, m.2706A>G, was associated with slower cognitive decline with a hazard ratio of 0.68 (95% CI 0.56-0.81) and $P = 2.46 \times 10^{-5}$. Mitochondrial haplogroups were not appreciably linked to motor progression. This initial genetic survival study of the mitochondrial genome suggests

that mitochondrial haplogroups may be associated with the pace of cognitive progression in Parkinson's disease over time.

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Running title: mtDNA variants link to cognitive decline in PD

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Abbreviations: GCI = global cognitive impairment; HY = Hoehn and Yahr stage; MDS-UPDRS = Movement Disorder Society-Unified Parkinson's disease Rating Scale; MMSE = Serial Mini Mental State Exam; PD = Parkinson's disease

Introduction

Disability and quality of life of patients with Parkinson's disease (PD) is affected by progressive cognitive impairment[1]. Increasing numbers of cognitively impaired patients with PD pose a medical and socio-economic challenge in many countries[2]. The pace of cognitive changes during the disease course, however, varies substantially from patient to patient[3] and the genetic architecture accounting for this heterogeneity in disease progression has not been well established.

Genome-wide association studies (GWAS) during the past decade have delineated the genetic architecture of disease susceptibility with 90 association signals in 78 common autosomal loci in PD patients of European ancestry[4]. Our recent genome-wide survival study identified associations with longitudinal progression from PD to Lewy body dementia in five loci, *RIMS2, GBA,* and *APOE*, *WWOX,* and *TMEM108*[5]. This extends and confirms longitudinal studies implicating *GBA* variants[6,7] and *APOE* ε4[8] in cognitive decline in PD. These genome-wide and targeted sequencing efforts have paved the way for unravelling the genetic architecture of disease progression in PD, but have not yet investigated the second critical source of human DNA – the mitochondrial genome (mtDNA).

MtDNA mutations contribute to a spectrum of human diseases[9] and in PD there is accumulating genetic and environmental evidence that mitochondrial dysfunction may play a key role in the pathogenesis of the disease[10,11]. There are high level of somatic mtDNA mutations in substantia nigra neurons in early PD[12] and dysregulation of mtDNA homeostasis

in sporadic PD[13]. Mutations in the nuclear-encoded *PINK1* and *PRKN* cause autosomal recessive PD and disrupt mitophagy[14]. Moreover, there is a pervasive defect in *PGC-1alpha*-regulated mitochondrial bioenergetics gene expression in nigral dopamine neurons and substantia nigra even in prodromal, subclinical Lewy body neuropathology[15].

The diversity of modern human mtDNA haplogroups (variants) has provided valuable information to trace the history of human evolution, and many studies in recent years have reported links between specific mtDNA haplogroups and susceptibility for PD[16], however, the impact of mtDNA haplogroups or variants on progression in PD has not been defined. To characterize whether genetic variation in the mitochondrial genome influences the progression of PD, we performed a longitudinal, multi-cohort analysis, and identified specific mitochondrial haplogroups linked to cognitive decline in PD. Further exploratory analysis indicated two single nucleotide polymorphisms (SNPs) in mtDNA specifically associated with cognitive progression.

Materials and Methods

Study participants

The cohorts included in this study were described in previous work form the IGPP Consortium[5-7]. In brief, 4,491 patients with PD (with available genotyping data and quality control) were longitudinally assessed with 33,406 study visits in 15 cohorts from North America and Europe between 1986 and 2017 (**Supplementary Methods**). Written informed

consent for DNA collection and phenotypic data collection for secondary research use for each cohort was obtained from the participants with approval from the local ethics committees. The Institutional Review Board of Mass General Brigham and the Institutional Review Board of the School of Medicine, Sun Yat-sun University approved the current analyses. Patients whose longitudinal follow-up evaluations were not consistent with a diagnosis of PD were excluded. Fifteen cohorts were *a priori* assigned to discovery or replication cohorts as we previously described[5] (**Supplementary Fig. 2**). This achieves an approximately two-thirds to one-third split among the two stages and a balanced distribution of the distinct types of cohorts (for example, purpose-designed biomarkers studies, phase 3 clinical trials, population-based cohorts) across stages.

Polymorphism identification and haplogroup classification

We analyzed 763 mitochondrial SNPs in 4,491 patients with PD and predicted their mitochondrial haplogroup using Haplogrep2.0[17] with default parameters using mitochondrial rCRS reference (**Supplmentary Fig. 1**). We next simplified the sub-haplogroups (455 sub-haplogroups) to the 34 haplogroups (**Supplementary Table 1**). After quality control (**Supplementary Methods**), 4,064 subjects with 30,515 study visits were used for haplogroup analysis (including H, HV*(excluding H, V), I, J, K, T, U# (excluding K) haplogroups). Out of 763 mitochondrial SNPs, 102 SNPs with allele frequency > 1% were used for single SNP Cox regression analysis.

Statistical analysis

The Cox proportional hazards (Cox PH) analysis was used to estimate the influence of different mitochondrial haplogroups on time (years from onset of PD) to reaching the endpoint of global cognitive impairment (GCI) as indicated by a MMSE ≤ 25 according to the recommendation the International Parkinson and Movement Disorder Society (MDS) Task Force[18] and adjusting for the covariates of age at onset, gender, years of education, and polygenic hazard score (PHS) as fixed effect, and for a cohort term as random effect. A second endpoint was time to motor disability with postural instability as indicated by Hoehn and Yahr stage 3 (HY3) adjusting for age at onset, gender, *GBA* carrier status and the cohort term similar to Ref.[6] (see **Supplementary Methods** for detail). For the single nucleotide variants, a similar Cox PH analysis was used (using the same co-variants as mentioned above) to investigate the effect of each SNP on time to cognitive impairment.

Generalized longitudinal mixed fixed and random effects analysis (LMM) of cognitive decline was performed with MMSE scores longitudinally assessed at varying times (enrollment visit and multiple longitudinal follow-up visits) in the combined data set (**Supplementary Methods**). All analyses were conducted in the R statistical environment version 4.0.2.

Data availability

The genotype and clinical data for PPMI included in this study are publicly available upon request to ppmi@loni.usc.edu through a PPMI Whole Genome Sequencing Data Agreement. Clinical data for PDBP included in this study are publicly available through

https://pdbp.ninds.nih.gov. Clinical longitudinal data and genotyping data for the other cohorts included are accessible through appropriate data sharing agreements that protect patient privacy with the institutions that conducted or are conducting study consents and clinical assessments under local institutional review board approvals.

Results

Mitochondrial haplogroup is associated with cognitive decline in patients with PD.

The genotyped data of 4,491 patients with PD across 15 cohorts from North America and Europe were used to estimate their mitochondrial haplogroups. 4,447 patients with 33,068 longitudinal study visits passed quality control (**Supplementary Fig. 1A**) and were classified into eight groups: seven macro-haplogroups (H, HV*, I, J, T, K, U$^{\#}$) and a group comprising various other haplogroups (**Supplementary Fig. 1B, Supplementary Table 1**). 41.13%, (1,829) patients belonged to macro-haplogroup H, which is a common mtDNA clade in Europe and it is found in approximately 43.10% of UK Biobank individuals[19]. There were no significant differences in demographic and clinical characteristics of the patients in the various macro-haplogroups (**Supplementary Table 2**). The proportion of the seven macro-haplogroups was consistent with a previous survey in various European countries (**Supplementary Table 3**) and did not differ between the 15 cohorts ($P \approx 1$, Fisher exact test, **Supplementary Fig. 2**). For 4,064 patients within seven macro-haplogroups, we assigned

2,811 patients and 12,605 longitudinal visits to the discovery population. 1,253 patients and 17,910 visits comprised the replicate population.

We then investigated the effect of seven macro-haplogroups on the risk of cognitive and motor impairment during the progression of Parkinson's disease in discovery and replicate populations. "Haplogroup" was an unordered categorical variable in our Cox PH model. An omnibus test for haplogroup variation with 6 degrees of freedom showed that the 7 haplogroups in general were differed from each other in their association with cognitive progression (the null hypothesis is that the haplogroups have the same effect) with an "omnibus" test P value < 0.001 in the discovery stage. We followed up this omnibus test with pertinent post hoc likelihood ratio tests which are the pairwise comparisons of each of the haplogroups against the "reference" haplogroup H. J, T, U$^{\#}$ haplogroups were associated with a reduced risk for GCI (MMSE ≤ 25) compared to the common haplogroup H with a hazard ratios (HR) of 0.65 (95% CI 0.44-0.97) and P = 0.033, HR of 0.53 (95% CI 0.34-0.83) and P = 0.0052 and HR of 0.68 (95% CI 0.49-0.96) with P = 0.028 in discovery stage, respectively (**Fig. 1A**). We further confirmed these associations in a replicate population, where the HRs were 0.45 (95% CI 0.22-0.94), 0.54 (95% CI 0.29-0.99), 0.51 (95% CI 0.28-0.92) with P values of 0.033, 0.047 and 0.025 for J, T, U$^{\#}$ compared with Haplogroup H, respectively (**Fig. 1B**). Consistently, in the combined analysis, HR were 0.58 (95% CI 0.41-0.82) with P = 0.0023, 0.53 (95% CI 0.37-0.77) with P = 0.0007, and 0.63 (95% CI 0.47-0.85) with P = 0.0023, respectively (**Fig. 1C**). For each haplogroup compared to haplogroup H, the

Cochran's Q-test and the I^2 index showed HRs across studies were homogeneous (**Supplementary Table 4**).

There was no difference in HR for GCI among sub-haplogroups of H (**Supplementary Fig. 3**). There was no difference in HR for motor progression to Hoehn & Yahr stage 3 (motor disability with postural instability in PD) for each of the seven macro-haplotypes in discovery, replication or combined populations (**Supplementary Fig. 4**). A polygenic hazard score (PHS) based on five nuclear genetic loci exhibited a substantial aggregate association with progression to PD dementia in our recent study[5]. Here, we calculated the PHS for each patient and found no association between PHS and mtDNA haplogroups (Kruskal-Wallis rank sum test $P = 0.59$, **Supplementary Fig. 5**). This suggests that mitochondrial and nuclear genome variants may play independent roles in the cognitive progression of PD.

Since subjects with macro-haplogroups J, T, $U^{\#}$ showed a protective effect compared to haplogroup H, we combined these subjects into a super-group (n = 1,298) and showed reduced risk for GCI with a HR = 0.59 (95% CI 0.48-0.74) and $P = 2.42 \times 10^{-6}$ (**Fig. 1D**) (macro-haplogroup H as reference) after adjusting for covariates. A liner mixed model analysis confirmed serial MMSE scores in patients with macro-haplogroups J, T, $U^{\#}$ declined more slowly over time compared to patients in the common macro-haplogroup H ($P = 0.018$).

Exploratory analysis of single nucleotide polymorphisms in mtDNA and cognitive decline in PD.

We next carried out an exploratory analysis to investigate the effect of single nucleotide polymorphisms in mtDNA on cognitive impairment during the progression of PD in combined population (**Methods**). We observed that two variants, m.2706A>G and m.14766C>T, were associated with cognitive decline (**Fig. 2A**). The common m.2706A>G variant (G allele carriers, 58.3% in our cohorts) is located in the 16S rRNA locus. Patients with the m.2706G allele had a reduced risk of developing GCI with a HR = 0.68 (95% CI 0.56-0.81) and $P = 2.46\times10^{-5}$ compared to patients with the A allele (**Fig. 2B**). The common variant m.14766C>T (C allele carrier, 47.5% in our cohorts) codes for an amino acid substitution of an isoleucine for threonine at amino acid site 7 in *CYTB*. Patients with PD and m.14766T had a reduced risk of developing GCI with a HR = 0.70 (95% CI 0.58-0.84) and $P = 1.15\times10^{-4}$ compared to patients carrying the C allele. For m.2706A>G and m.14766C>T, proportional HRs across studies were homogeneous with $P = 0.46$ ($I^2 = 0\%$) and $P = 0.44$ ($I^2 = 0.96\%$) respectively, by Cochran's Q-test for heterogeneity. Associations of these two variants remained significant after considering multiple-testing with both *P* values lower than the Bonferroni-corrected significance threshold (0.05/102 variants tested = 4.9×10^{-4}). 12 additional variants were associated with cognitive decline during the course of PD with a $P < 0.05$ (**Fig. 2A, Table 1**).

Both m.2706A and m.14766C are largely specific to the H or HV* haplogroup. The alternative alleles m.2706G and m.14766T occur in other haplogroups (**Fig. 2C**). These results are consistent with our haplogroup analysis as patients within haplogroup J, T, $U^{\#}$ have a lower risk for cognitive progression compared to those with haplogroup H. We found

high correlation ($r^2 = 0.78$) of these two common variants in our cohorts and 94.1% of patients carried the same risk/protective alleles (m.2706A/m.14766C or m.2706G/m.14766T). After correcting for the effect of m.2706A>G, conditional Cox PH analysis no longer showed an association of m.14766C>T with cognitive decline (HR = 0.92 (95% CI 0.62-1.38); $P = 0.7$). Thus, m.14766C>T was dependent with m.2706A>G in our cohorts.

Age at disease onset, years of education, sex, MMSE at enrollment, MDS-UPDRS III score at enrollment, depression at enrollment are clinical variables associated with cognitive decline in PD[7]. 2,629 patients were included in both our previous[7] and current study, and we used these 2,376 patients (253 left censored patients were removed) for further analyses (**Supplementary Methods**). m.2706A and m.14766C carriers showed significant hazard ratios of 1.48 (95% CI 1.18-1.86, $P = 8.21\times10^{-4}$) and 1.38 (95% CI 1.09-1.74, $P = 7.23\times10^{-3}$) for risk of progression to GCI, respectively, adjusting for all six clinical predictors (**Supplementary Fig. 6**).

Consistent with our previous genome-wide survival analysis for progression from PD to PD dementia, *GBA* carriers had a HR of 1.91 (95% 1.39-2.64) with $P = 7.76\times10^{-5}$ and *APOE* ε4 carriers had a HR of 1.29 (95% 1.03-1.62) with $P = 0.028$ for cognitive decline (without accounting for mitochondrial variants, **Supplementary Fig. 6**). *GBA* carriers who carried the mitochondrial m.2706A allele (linked to relatively more *rapid* progression compared to the m.2706G allele) had a HR of 2.92 (95% CI, 1.87-4.55, $P = 2.23\times10^{-6}$). *GBA*-positive non-m.2706A carriers had the second highest HR of 1.84 (95% CI 1.15-2.93, *P*

= 0.011), and *GBA*-negative m.2706A carriers had a HR of 1.46 (95% CI 1.14-1.88, *P* = 0.0028) compared to patients carrying neither *GBA* variants nor the m.2706A variant (**Fig. 3**). Thus, m.2706A>G and *GBA* variants may have additive effects. Moreover, patients homozygous for the *APOE* ε4 allele and carrying m.2706A had a substantially elevated risk for longitudinal cognitive decline with HR = 5.09 (95% CI 2.04-12.56 *P* = 0.0005) compared to patients carrying neither *APOE* ε4 allele nor the m.2706A variant (**Supplementary Fig. 7)**.

Discussion

This genetic survival study overall indicates that mitochondrial macro-haplogroups are associated with reduced risk of cognitive disease progression. Post-hoc analyses identified the haplogroups J, T, U# as the haplogroups associated with reduced risk compared to the macro-haplogroup H in Parkinson's patients, but further research is required to definitively identify the contribution and statistical significance of each individual haplogroup. Previous meta-analyses found that the haplogroups J, K, T are associated with reduced susceptibility for PD and the haplogroup H is linked to elevated susceptibility for PD[16].

About 41% of patients with PD in this study belong to the macro-haplogroup H, the most common genotype in Europeans. The European mtDNA haplogroup H is associated with a higher survival ratio after sepsis[20], but is linked to higher risk of developing PD in late life[16]. On the flip side our findings are consistent with a relatively more deleterious effect of haplogroup H on the progression of PD compared to haplogrups J, T, U#. This may represent an evolutionary trade-offs[21] where genetic variants that increase the chance of surviving early-life illness such as sepsis might contribute to pathogenic events later in life[21].

Alzheimer's disease-associated plaques and tangles are found in a substantial proportion of brains with of patients with PD dementia, in addition to Lewy bodies[22]. Haplogroups H and HV are a risk haplogroup for AD[23], while the JT haplogroup was protective in a prior study[24]; evidence for the other haplogroups (K, J, T, U) is limited and controversial (e.g. J[23,25]; **Supplementary Table 5)**. This is also consistent with our study,

where H carriers had a relatively more rapid cognitive progression compared to the protective haplogroups J, T, U[#].

Two common significant mtDNA variants showed effects on the risk of global cognitive impairment and are related to the haplogroups (**Fig. 2C**). The common m.2706A>G variant, located at 16S rRNA gene, is close to the ribosomal peptidyl transferase center, and might be relevant to many diseases, such as MELAS, Alzheimer's and PD[26]. This variant can induce substantial alterations in the mitochondrial 16S rRNA secondary structure[27]. The m.14766C variant might increase the risk for late-onset Alzheimer's disease[24] consistent with our findings. Interestingly, contrary to our data, m.2706G was associated with faster cognitive aging in a large longitudinal cohort of African Americans, but not Caucasian Americans[28].

Our study is limited in sample size and statistical power. *P* values for individual haplogroups were not adjusted for multiple testing. Another limitation of this study is that we evaluated the effects of mitochondrial genetic variants during the progression of PD with European ancestry only. The mtDNA variants (m.2706A>G or m.14766C>T) are rare in populations from East Asia or Africa (**Table 1**). Further studies in other populations are urgently needed due to difference of mtDNA haplogroups, considering that more than 60% of PD patients are expected to live in the Western Pacific Region by 2030[29], most of them belonging to haplogroups A, B, C, D, F, G. Moreover, replication of our exploratory findings in additional longitudinal patient populations of European ancestry is needed.

This study suggests that mitochondrial genotypes may not be innocent bystanders in the progression of Parkinson's, but, might play an role in modulating disease progression. Our study provides evidence for the role of mitochondrial haplogroups in the progression of PD towards Lewy body dementia, and this association appears independent of *GBA* and *APOE*.

Mitochondrial dysfunction[15] and alpha-synuclein accumulation are two pathologically[30] and biologically[31] linked culprits of PD. alpha-synclein triplication causes mitochondrial bioenergetics dysfunction[32]. Conversely, the mitochondrial toxin rotenone leads to alpha-synclein accumulation[33]. Taken together with our new findings, this body of evidence suggests that mitochondria might play a role not only in the onset, but also in the progression of Parkinson's disease.

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Competing interests

G.L., C.N., J.Z., W.L., J.L., J.J.L., W.X., L. C., Z.P., A.E., J.H.G., A.Y.H., S.K., P.T., S.S.A., J.S.P., and M.C.C. report no relevant financial or other conflicts of interest in relation to this study.

Outside this work, C.R.S. has served as consultant, scientific collaborator or on scientific advisory boards for Sanofi, Berg Health, Pfizer, Biogen, and has received grants from NIH, U.S. Department of Defense, American Parkinson Disease Association, and the Michael J Fox Foundation (MJFF).

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Supplementary material

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Figure legends

Figure 1 Mitochondrial haplogroups and risk for global cognitive impairment over time in patients with PD. The forest plot shows hazard ratios for global cognitive impairment in specific types of macro-haplogroups compared to macro-haplogroup H in patients with PD from the discovery **(A)**, replication **(B)** and combined **(C)** populations. The squares represent point estimates, with the sides of the square inversely proportional to the standard error of the estimates. The horizontal lines indicate 95% confidence intervals of the estimates. **(D)** Covariate-adjusted survival curves for patients with PD in macro-haplogroups J T, U$^{\#}$ (cyan line) and those in macro-haplogroups H (magenta line).

Figure 2 mtDNA variants associated with cognitive progression in patients with PD. (A) Association plot of SNPs in mtDNA associated with risk of developing global cognitive impairment (dot) in the combined population. The outside labels indicate mitochondrial genes; circular axis from outside to inside represents the value of -log10(*P*) from 0 to 5; SNPs with $P < 0.05$ are shown in magenta, while SNPs with $P \geq 0.05$ are shown in grey. **(B)** Covariate-adjusted survival curves for patients with PD carrying mtDNA m.2706G (cyan line) and those with m.2706A (magenta line). m.2706A was used as a reference allele to calculate HR from the Cox PH analysis and *P* values from two-sided Wald tests. **(C)** Overlap between carriers of the m.2706A>G and the m.14766C>T variant. Out of 2,611 m.2706G allele carriers and 2,347 m.14766T allele carriers, 2,342 individuals carried both alleles. Out of 1,830 m.2706A allele carriers and 2,080 m.14766C allele carriers, 1,819 individuals carried both alleles.

Figure 3 Effects of *GBA* variants and mtDNA genotype in predicting global cognitive impairment for patients with PD. Covariate-adjusted survival curves for patients with PD

stratified into four subgroups: *GBA*-negative and non-m.2706A carriers (n = 1,257), *GBA*-negative and m.2706A carriers (n = 891), *GBA*-positive and non-m.2706G carriers (n = 132), *GBA*-positive and m.2706A carriers (n = 96). HR and P values were calculated adjusting for clinical covariates and study cohort as a random term. The group of *GBA*-negative and non-m.2706A carriers is denoted as reference group (REF) in Cox PH analysis.

Tables

Table 1 mtDNA SNPs were associated with developing global cognitive impairment in the progression of Parkinson's

rCRS	Effect allele	Alternative allele	*P*	*P*[a]	HR (95%CI)	EAF[b]	EAF in European	EAF in East Asian	EAF in African
m.2706A>G	G	A	2.46×10^{-5}	0.003	0.68 (0.56-0.81)	0.5826	0.5746	0.9960	0.9970
m.14766C>T	T	C	1.15×10^{-4}	0.012	0.70 (0.58-0.84)	0.5249	0.5169	0.9960	0.9985
m.11251A>G	G	A	0.002	0.204	0.67 (0.52-0.86)	0.1942	0.1610	0.0000	0.0015
m.15452C>A	A	C	0.002	0.204	0.67 (0.52-0.87)	0.1951	0.1610	0.0000	0.0000
m.15607A>G	G	A	0.017	1	0.65 (0.46-0.93)	0.0984	0.0875	0.0000	0.0015
m.16162A>G	G	A	0.019	1	1.95 (1.12-3.40)	0.0235	0.0199	0.0417	0.0015
m.15928G>A	A	G	0.021	1	0.66 (0.47-0.94)	0.0998	0.0875	0.0080	0.0000
m.11812A>G	G	A	0.029	1	0.65 (0.44-0.96)	0.0787	0.0696	0.0000	0.0045
m.4917A>G	G	A	0.030	1	0.66 (0.46-0.96)	0.0971	0.0875	0.0000	0.0000
m.9477G>A	A	G	0.031	1	0.66 (0.46-0.96)	0.0926	0.1392	0.0000	0.0061
m.10589G>A	A	G	0.041	1	1.89 (1.03-3.47)	0.0110	0.0060	0.0020	0.0530
m.16482A>G	G	A	0.043	1	1.62 (1.02-2.59)	0.0200	0.0139	0.0020	0.0000
m.15218A>G	G	A	0.045	1	0.55 (0.31-0.99)	0.0453	0.0437	0.0119	0.0000
m.10463T>C	C	T	0.048	1	0.71 (0.51-1.00)	0.1030	0.0875	0.0040	0.0000

rCRS = revised Cambridge Reference Sequence; HR = hazard ratio; CI = confidence interval; *P* from the Cox proportional hazards statistic to estimate the influence of SNP on time (years from onset of PD) to reaching the endpoint of global cognitive impairment (GCI) as indicated by a MMSE ≤ 25 in exploratory analysis using combined population; age at onset of PD, sex, years of education, and polygenic hazard score (PHS including *GBA* mutation status, *APOE* ε4 allele haplotype and novel 3 SNPs loci rs182987047, rs138073281 and rs8050111) were included as covariates in the Cox analyses. A "cohort" term was included as a random effect; *P*[a]: Bonferroni correction based on the result of 102 mtDNA SNPs from combined analysis was performed using p.adjust function with "bonferroni" method in R. Effect allele frequency (EAF)[b] was calculated based on 4,491 patients with PD across 15 cohorts. EAF in 503 European, 503 East Asian or 661 African was calculated based on dataset of Phase 1 and 3 of the 1000 Genome Project mitochondrial variants calling by the MToolBox pipeline.

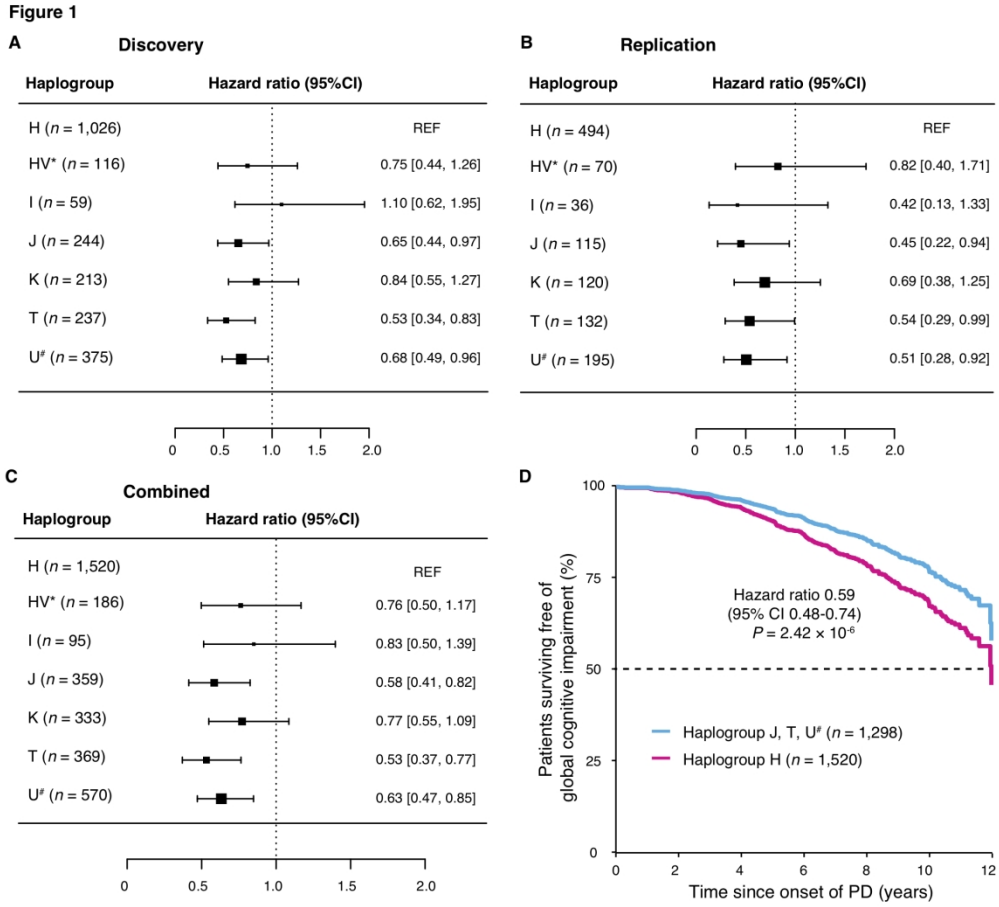

Figure 1 Mitochondrial haplogroups and risk for global cognitive impairment over time in patients with PD.

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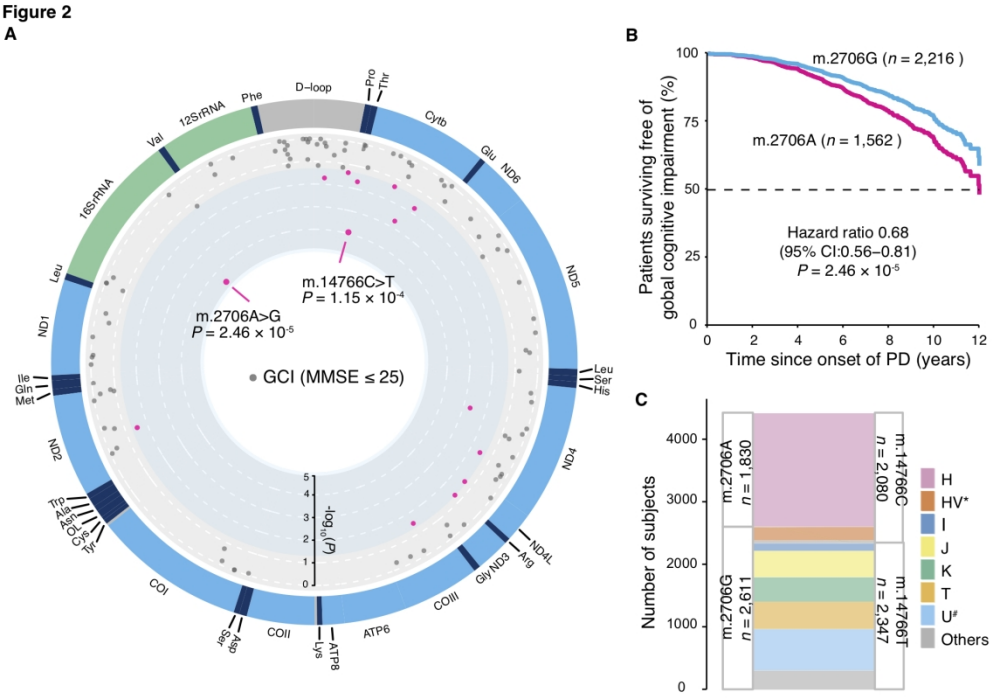

Figure 2 mtDNA variants associated with cognitive progression in patients with PD.

177x127mm (300 x 300 DPI)

Figure 3

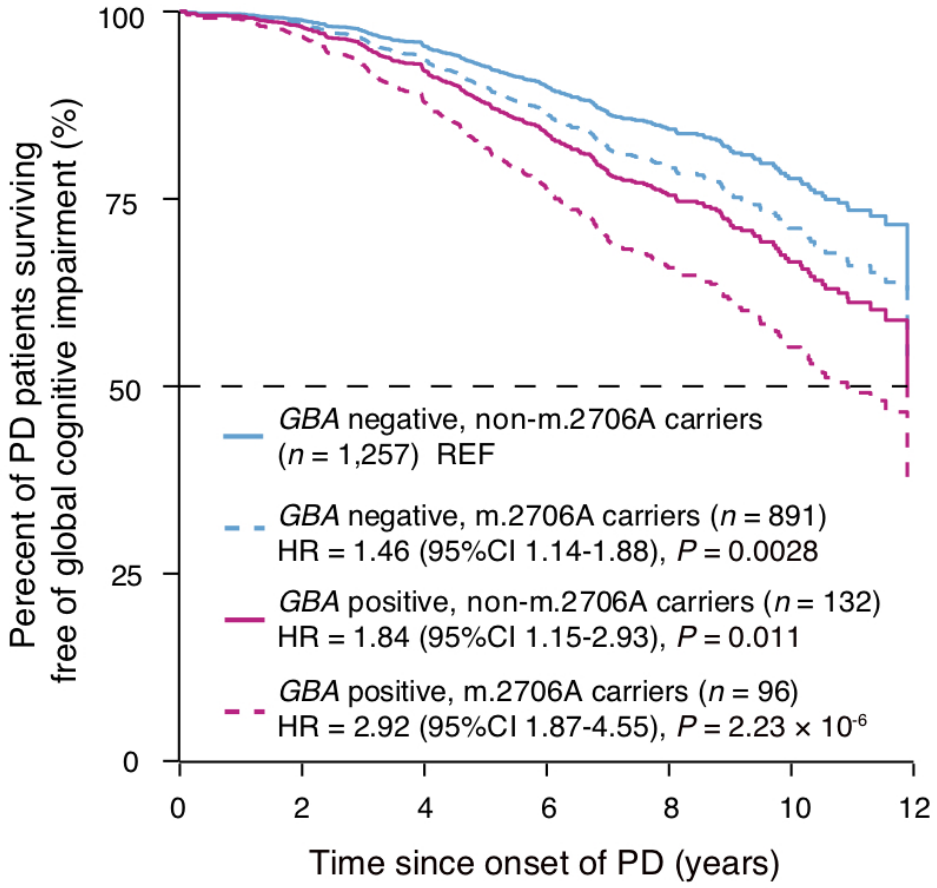

Figure 3 Effects of GBA variants and mtDNA genotype in predicting global cognitive impairment for patients with PD.

71x72mm (300 x 300 DPI)

Mitochondrial haplogroups and cognitive progression in Parkinson's disease

Supplementary material

Supplementary material 1

Study participants 7

Polymorphism identification and haplogroup classification 8

Statistical analysis 9

Comparison of models 10

Supplementary Table 1 Classification of mitochondrial haplogroups in patients with PD across the 15 cohorts 12

Supplementary Table 2 Clinical characteristics of patients with PD at enrollment with different macro-haplogroups across the 15 cohorts 13

Supplementary Table 3 The percentage (%) of different mitochondrial haplogroups in European population from literatures 14

Supplementary Table 4 Test for residual heterogeneity for each haplogroup compared to haplogroups of H in GCI combined analysis 15

Supplementary Table 5 The association of mitochondrial haplogroups in Alzheimer disease ... 16

Supplementary Figure 1 The classification of haplogroup in patients with PD across 15 cohorts. 17

Supplementary Figure 2 The stacking diagram for distribution of seven macro-haplogroups in patients with PD across 15 cohorts 18

Supplementary Figure 3 Patients with PD with major sub-haplogroups of H have similar risk of progression to global cognitive impairment 19

Supplementary Figure 4 Patients with PD in seven macro-haplogroups have similar risk of progression to HY3. 20

Supplementary Figure 5 Patients with PD in seven mitochondrial macro-haplogroups have similar polygenic hazard scores 21

Supplementary Figure 6 The exploratory analysis for global cognitive impairment models with different genetic factors. 22

Supplementary Figure 7 Exploratory analysis for global cognitive impairment models with APOE ε4 and m.2706A>G. 23

References 24

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ParkWest: Principal investigators: Guido Alves (Norwegian Centre for Movement Disorders, Stavanger University Hospital), Ole-Bjørn Tysnes (Haukeland University Hospital). Investigators and study coordinators: Karen Herlofson, Solgunn Ongre, Siri Bruun (Sørlandet Hospital Arendal); Ineke HogenEsch, Marianne Kjerandsen, Liv Kari Håland (Haugesund Hospital); Wenche Telstad, Aliaksei Labusau, Jane Kastet (Førde Hospital); Bernd Müller, Geir Olve Skeie, Charalampos Tzoulis (Haukeland University Hospital); Kenn Freddy Pedersen, Michaela Dreetz Gjerstad, Elin Bjelland Forsaa, Jodi Maple-Grødem, Johannes Lange, Veslemøy Hamre Frantzen, Anita Laugaland, Karen Simonsen, Ingvild Dalen (Stavanger University Hospital). The ParkWest study has received funding from the Western Norway Regional Health Authority (grant number 911218), and the Norwegian Parkinson's Disease Association, and the Research Council of Norway (grant number 287842).

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Supplementary Methods

Study participants

4,491 patients with PD (with available genotyping data and quality control) were longitudinally assessed with 3,3406 study visits in 15 cohorts from North America and Europe between 1986 and 2017: Harvard Biomarkers Study (HBS)[1], Neuroprotection Exploratory Trials in PD- Long term Study-1 (NET-PD LS1)[2], Drug Interaction with Genes in PD (DIGPD)[3] , PROfiling PARKinson's disease (PROPARK) study[4], PROPARK-Cross sectional cohort; Cambridgeshire Parkinson's Incidence from GP to Neurologist (CamPaIGN)[5-7]; Parkinsonism: Incidence, Cognition and Non-motor heterogeneity in Cambridgeshire (PICNICS)[8]; Parkinson's Disease Biomarkers Program (PDBP)[9]; Banner Health study(Arizona Study of Aging/Brain and Body Donation Program)[10]; ParkWest[11] and PIB[12]; Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP)[13]; Parkinson Research Examination of CEP-1347 Trial/A Longitudinal Follow-up of the PRECEPT Study Cohort (PreCEPT/PostCEPT)[14] and Tartu[15], Parkinson's Progression Markers Initiative (PPMI)[16] . For PPMI, approval was obtained to download and analyze the publicly accessible WGS and clinical data. 13 cohorts enrolled patients with a diagnosis of PD established according to modified UK PD Society Brain Bank diagnostic criteria as previously reported[1-5,8,9,11,12,14-18]. In DATATOP, the eligibility criteria required a clinical diagnosis of early, idiopathic PD (HY stages 1 or 2) with patients not on anti-parkinsonian medications[17]. Banner Health study: all subjects have come to autopsy and have had full neuropathological examinations with diagnosis[10]. Diagnostic certainty was increased by confirming the clinical diagnosis of PD during longitudinal follow-up visits[19] in all cohorts.

Serial Mini Mental State Exam (MMSE) scores[20] were longitudinally collected in 10 cohorts. Montreal Cognitive Assessment (MoCA)[21] scores were collected in the PDBP, PPMI study and converted to MMSE scores according to a published formula[22]. SCOPA-COG were collected in PROPARK, PROPARK-C and NET-PD LS1 cohort and converted to MMSE scores.

Polymorphism identification and haplogroup classification

The genotyping data of the 4,491 subjects with Parkinson's disease were reported in Ref.[23]. Briefly, the samples (excluded PPMI with WGS) were genotyped with Illumina Multi-Ethnic Genotyping Array (MEGA, Illumina), which includes 810 SNP markers in mtDNA after quality control as described in Ref.[23] 810 mtDNA variants were converted from "plink" format to "vcf" format according to the rCRS reference alleles. We removed 25 mismatched SNPs and InDel SNPs, 11 duplicated SNP probes on the array, and 11 variants with highly discordant MAF (> 5%) compared to Phase 1 and 3 of the 1000 Genomes Project[24] mitochondrial variants (n = 503 European) as called by the MToolBox pipeline[25]. The remained 763 SNPs were used to predict mitochondrial haplogroups using Haplogrep2.0[26] with default parameter using rCRS reference. Haplotype quality-control was performed according to the Haplogrep2 instruction and 44 subjects with quality score < 0.8 were excluded (**Supplementary Fig. 1**). We next simplified the sub-haplogroups (455 sub-haplogroups) to the 34 haplogroups (**Supplementary Table 1**) according to the mtDNA tree http://www.phylotree.org/tree/index.htm. 4,447 subjects were successfully assigned haplogroup, and 24 of these patients had no clinical records of note so were removed from the analysis. We further removed the haplogroups with less than 100 subjects (**Supplementary Table 1**, total 359 subjects), and the remaining 4,064 subjects with 30,515 study visits were used for haplogroup analysis (including H, HV* (excluding H, V), I, J, K, T, U#

(excluding K) haplogroups). It should be note that in our work, U[#] denotes all U haplogroups (U1, U2, U3, U4, U5, U6, U7…) but not haplogroup K as in Ref[27]. Out of 763 SNPs, 102 SNPs with allele frequency > 1% were used for single SNP Cox regression analysis. Notably, common mtDNA haplogroup or mtDNA variants are often ancient and are usually homoplasmic[28]. We did not analyze heteroplasmic mtDNA mutations in this study.

Statistical analysis

The Cox proportional hazards statistic was used to estimate the influence of different mitochondrial haplogroups on time (years from onset of PD) to reaching the endpoint of motor disability with postural instability (Hoehn and Yahr stage HY 3) or global cognitive impairment (GCI) as indicated by a MMSE ≤ 25 according to the recommendation the International Parkinson and Movement Disorder Society (MDS) Task Force[29] as in Ref.[30]. For HY analysis, age at onset of PD, sex and *GBA* carrier status were included as covariates. In the GCI analysis, age at onset of PD, sex, years of education, and polygenic hazard score (PHS including *GBA* carrier status, *APOE* ε4 allele haplotype and three novel progression variants rs182987047, rs138073281 and rs8050111 from Ref.[23]) were included as covariates in the Cox analyses. A "cohort" term was included as a random effect (a random effects Cox model is often termed a "frailty" model). 29,115 (95.4 %) of the visits from 4,088 patients with PD occurred within 12 years of longitudinal follow-up from disease onset with a median follow-up time of 6.7 years (inter-quartile range, 4.2 years), thus we focused our survival analyses on the 12-year time frame from disease onset. Patients were left-censored and those with missing or non-quality clinical data were excluded. Cox proportional hazards analyses were performed using the coxph function in the Survival package (v2.38-1)[31] and the "breslow" method was used for handling observations that have tied

survival times in the analysis and *P* values less than or equal to 0.05 were considered as indicative of haplogroup significance.

For single polymorphism variants analysis, we used a similar Cox proportional hazards regression model (same co-variants as mentioned above) to investigate each SNP effect on motor and cognitive impairment. Bonferroni correction was performed using p.adjust function with "bonferroni" in R.

Generalized longitudinal mixed fixed and random effects analysis (LMM)[32] of cognitive decline was performed with serial Mini Mental State Exam (MMSE) scores longitudinally assessed at varying times (enrollment visit and multiple longitudinal follow-up visits) in the combined data set. Two cohorts (PROPARK-C and Tartu) were excluded from the LMM because no longitudinal MMSE scores were available. The MMSE score was the dependent variable and the primary predictors were mitochondrial haplogroup status, time in the study (years), and their interaction. An intercept term and linear rate of change across time per subject were the random terms (permitted to be correlated). Subject level fixed covariates were age at baseline, sex, years of education, duration of PD illness at baseline, as well as PHS score. A study term was included as a random effect. This analysis was performed using the lme4 package (v1.1-23). All analyses were conducted in the R statistical environment version 4.0.2.

Comparison of models

The original multivariable Cox model from a previous study[33] included age at Parkinson's disease onset, years of education, sex, MMSE at enrolment, MDS-UPDRS III score at enrolment, depression at enrolment and *GBA* carrier status, and a cohort term was included as a random effect (using a frailty Cox model). 2,629 patients in the original nine longitudinal cohorts with available

mitochondrial variants, and 2,376 patients (253 left censored patients were removed) with 22,617 visits within 12 years of longitudinal follow-up from disease onset were used for comparison of different Cox regression genetic models (*GBA* carrier, *APOE* ε4, m.2706A>G, m.14766C>T), adjusting by age at Parkinson's disease onset, years of education, sex, MMSE at enrolment, MDS-UPDRS III score at enrolment, depression at enrolment, and a cohort term was included as a random effect.

Combination analysis of two genetic risk (*GBA* carrier and m.2706A>G variant/*APOE* ε4 and m.2706A>G variant) was performed using 2,376 patients from the Cox regression model, adjusting by the same six clinical predictors as mentioned bove, and a cohort term was included as a random effect.

Supplementary Table 1 Classification of mitochondrial haplogroups in patients with PD across the 15 cohorts

Haplogroups	Number	Sub-haplogroups of H	Number
H	1,829		
U#	666	H1	599
T	440	H2	599
J	427	H3	155
K	393	H5	146
HV*	218	H6	94
I	115	H4	75
W	93	H13	47
X	75	H46	20
N1	65	H15	11
V	42	H26	11
R0[&]	17	H7	10
L2	11	H41	8
M1	9	H14	6
D	7	H79	6
A	5	H85	6
C	5	H28	5
L1	4	H44	5
R1	4	H24	4
L3e	3	H100	3
B	2	H22	3
L3b	2	H81	3
M9	2	H56	2
N2	2	H94	2
N3	2	H17	1
G	1	H30	1
L0	1	H33	1
M7	1	H34	1
M30	1	H42	1
M33	1	H49	1
M49	1	H50	1
N9	1	H73	1
Y	1	H77	1
Z	1		

Haplogroups according to the mtDNA tree http://www.phylotree.org/tree/index.htm. 4,447 subjects were successfully assigned haplogroup. HV*: not including H, HV; U[#]: including all U haplogroups (U1, U2, U3, U4, U5, U6, U7…) but not haplogroup K as in Ref[27]; R0[&]: not including HV, H, V.

Supplementary Table 2 Clinical characteristics of patients with PD at enrollment with different macro-haplogroups across the 15 cohorts

***n* = 4,423**	**Marco-haplogroup**								***P*[&]**
	H	**HV***	**I**	**J**	**K**	**T**	**U[#]**	**Others**	
Number of men (*n*, %)	1,819 (63.2)	217 (65.4)	115 (57.4)	427 (61.8)	391 (65.7)	435 (62.1)	660 (63.8)	359 (62.1)	0.76
Age at onset, mean (SD), years	60.6 (10.6)	61.4 (10.1)	61.0 (10.8)	61.6 (10.9)	61.1 (9.6)	60.9 (10.5)	60.7 (11.0)	61.3 (10.1)	0.45
Age at enrollment, mean (SD), years	64.2 (10.2)	64.8 (9.8)	64.5 (10.5)	65.1 (10.4)	64.2 (9.3)	64.4 (10.1)	64.3 (10.5)	64.8 (10.3)	0.62
Years of education, mean (SD), years	14.2 (3.8)	14.6 (3.7)	14.1 (4.1)	14.4 (3.9)	14.6 (3.8)	14.1 (3.9)	14.2 (3.6)	14.7 (3.6)	0.08
Study years, mean (range), years	3.8 (0-19.9)	3.7 (0-9.3)	3.9 (0-8.3)	3.6 (0-13.1)	3.8 (0-14.5)	3.6 (0-13.5)	3.6 (0-12.6)	3.5 (0-12.3)	0.26
Hoehn and Yahr, mean (SD)	1.9 (0.8)	1.9 (0.7)	1.8 (0.6)	1.9 (0.8)	1.9 (0.7)	2.0 (0.7)	2.0 (0.7)	1.9 (0.7)	0.28
MDS-UPDRS III, mean (SD)	28.4 (14.2)	27.2 (13.5)	27.4 (13.7)	27.9 (14.5)	26.9 (13.0)	28.4 (13.7)	28.4 (14.0)	27.9 (14.9)	0.72
MMSE, mean(SD)	28.2 (2.2)	28.2 (1.9)	28.5 (1.5)	28.1 (2.3)	28.3 (2.0)	28.2 (2.1)	28.2 (2.2)	28.3 (2.4)	0.47
LED, mean(SD)	436.6 (439.9)	402.3 (470.0)	428.6 (458.2)	415.2 (428.0)	373.6 (398.2)	399.9 (416.8)	433.2 (446.0)	418.7 (447.7)	0.34

24 subjects have no available clinic data, the table showed clinical characteristics of 4,423 patients with PD.

[&] Fisher exact test was used for the number of men in each group. Group comparisons were performed using Kruskal-Wallis test for age at onset, age at enrollment, years of education, study years, HY, MDS-UPDRS III, MMSE, LED.

HV*: The sub-haplogroups of haplogroup HV, not including haplogroup H, V.

U[#]: The sub-haplogroups of haplogroup U, not including haplogroup K.

Supplementary Table 3 The percentage (%) of different mitochondrial haplogroups in European population from literatures

Haplogroup	Latvia[34] n=299	Spain[35] n=312	Portugal[36] n=241	France[37] n=210	Norway[34] n=397	Czech[38] n=300	Germany[34] n=333	Iceland[27] n=467	Italy[39] n=124
H	44.5	42.3	40.7	41.9	45.1	40.7	47.7	47.6	41.1
HV*	2.3	NA	NA	NA	0.3	2.7	0.6	NA	1.6
I	4.3	1.6	0.8	2.9	2.3	2.0	1.8	4.7	NA
J	6.4	6.7	6.6	5.2	12.6	8.3	8.4	14.1	4.8
K	2.3	4.8	5.4	11.4	5.0	4.0	7.5	7.7	1.6
T	9.4	8.3	10.8	11.9	9.8	8.0	9.0	10.1	8.1
U[#]	23.1	16.0	17.4	17.6	16.9	21.3	13.5	11.8	30.6

HV*: The sub-haplogroups of haplogroup HV, not including haplogroup H, V;
H: sum of available sub-haplogroups of H;
J: sum of available sub-haplogroups of J;
K: sum of available sub-haplogroups of K;
T: sum of available sub-haplogroups of T;
U[#]: sum of available sub-haplogroups of haplogroup U, but not including haplogroup K.

Supplementary Table 4 Test for residual heterogeneity for each haplogroup compared to haplogroups of H in GCI combined analysis

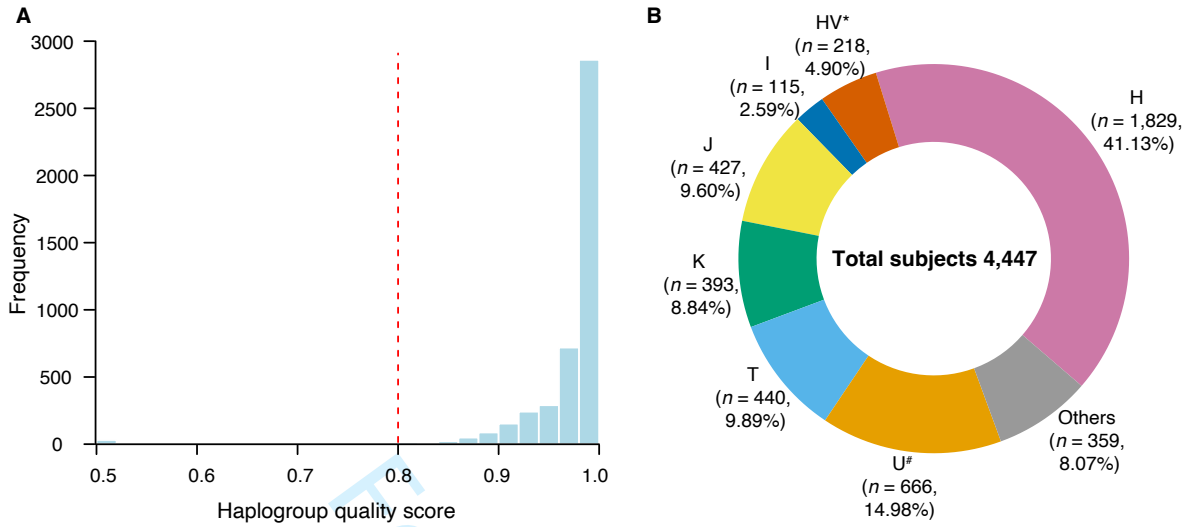
Haplogroups (H as reference)	Heterogeneity Q	*P* value[&]	I^2
HV*	5.32	0.87	0%
I	12.59	0.32	12.61%
J	4.88	0.96	0%
K	7.54	0.82	0%
T	5.05	0.96	0%
U[#]	15.90	0.20	24.54%

HV*: The sub-haplogroups of haplogroup HV, not including haplogroup H, V.
U[#]: The sub-haplogroups of haplogroup U, not including haplogroup K.
[&]The Cochran's Q-test was used to test for residual heterogeneity across studies via R metafor package (version 2.4-0). I^2 index (100%×(Q-df)/Q) was used to quantify the degree of heterogeneity.

Supplementary Table 5 The association of mitochondrial haplogroups in Alzheimer disease

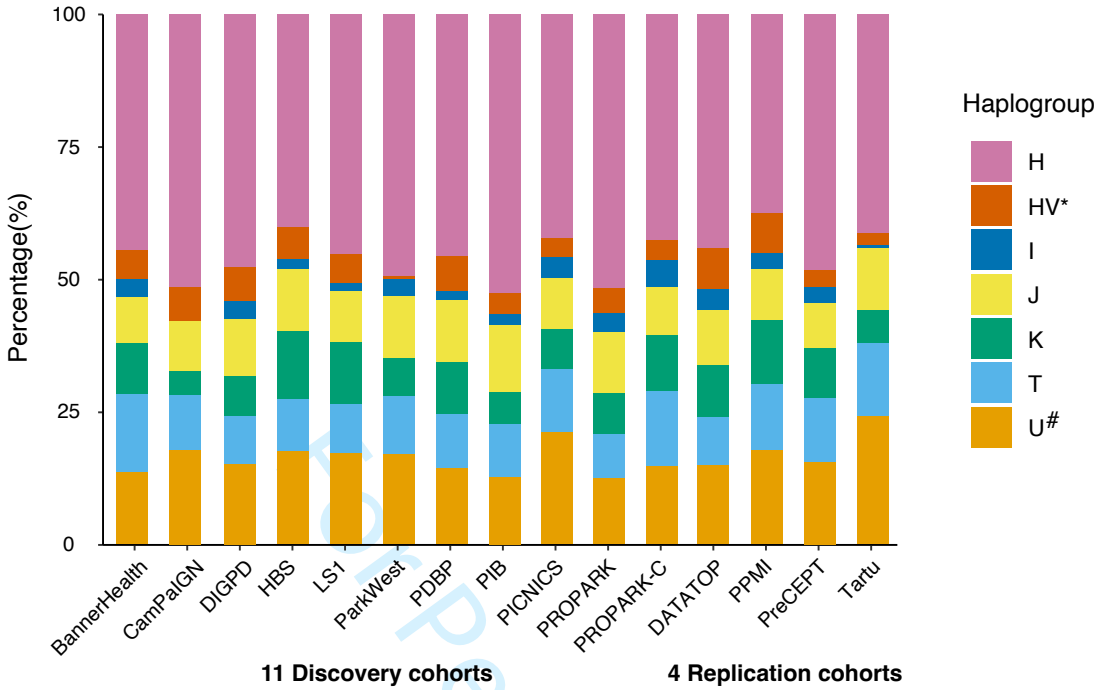
Haplogroup	Years	Effect	Ethnicity	Dataset size (case/control)	Dataset type
H	2009[40]	Risk	Poland	222/252	Whole mitochondrial genomics
	2011[41]	Risk	Caucasian	422/318	Control_region position (16624-576) + 9 coding SNPs
HV	2009[40]	Risk	Poland	222/252	Whole mitochondrial genomics
	2011[41]	Risk	Caucasian	422/318	Control_region position (16624-576) + 9 coding SNPs
K	2001[42]	Protective	Italian	213/389	10 restricted sites
	2011[41]	Protective	Caucasian	422/318	Control_region position (16624-576) + 9 coding SNPs
	2020[43]	Protective	American	309/507	Whole mitochondrial genomics
K1A1B	2013[44]	Risk	Caucasian	154/175	138SNPS
J	2009[40]	Protective in males	Poland	222/252	Whole mitochondrial genomics
	2020[43]	Risk	American	309/507	Whole mitochondrial genomics
T	2011[41]	Protective in females	Caucasian	422/318	Control_region position (16624-576) + 9 coding SNPs
JT	2011[41]	Protective in females	Caucasian	422/318	Control_region position (16624-576) + 9 coding SNPs
U	2001[42]	Protective	Italian	213/389	10 restricted sites
	2004[45]	Risk in males, protective in females	Caucasian	989/328	10 SNPs

Supplementary Figure 1 The classification of haplogroup in patients with PD across 15 cohorts.



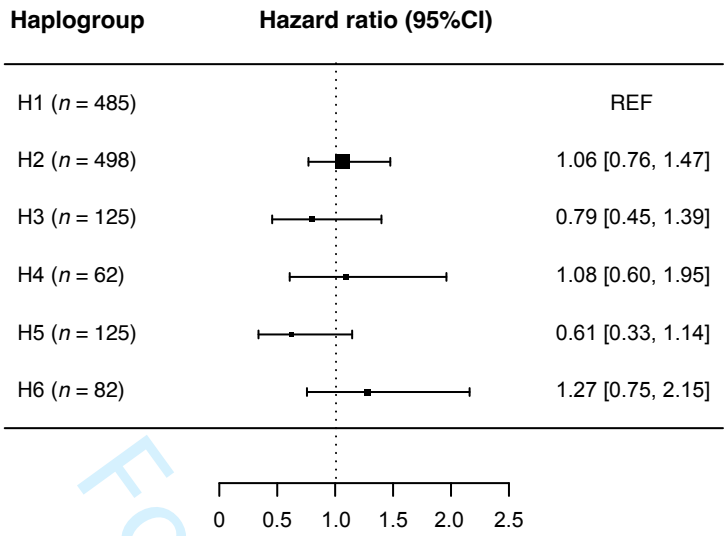
A The haplogroup quality score of 4,491 patients with PD was evaluated from HaploGrep2.0[26] based on Kulczynski measure: (HaplogroupWeight + SampleWeight) × 0.5. The HaploGrep2.0 applied this formula to all haplogroups in Phylotree and returned the overall best hit and the score represented its haplogroup quality. The quality of 0.8 as cutoff was recommended and 4,447 subjects were successfully assigned mitochondrial haplogroup. **B** The donut plot presents the proportion of patients with PD within diverse mitochondrial macro-haplogroups

Supplementary Figure 2 The stacking diagram for distribution of seven macro-haplogroups in patients with PD across 15 cohorts



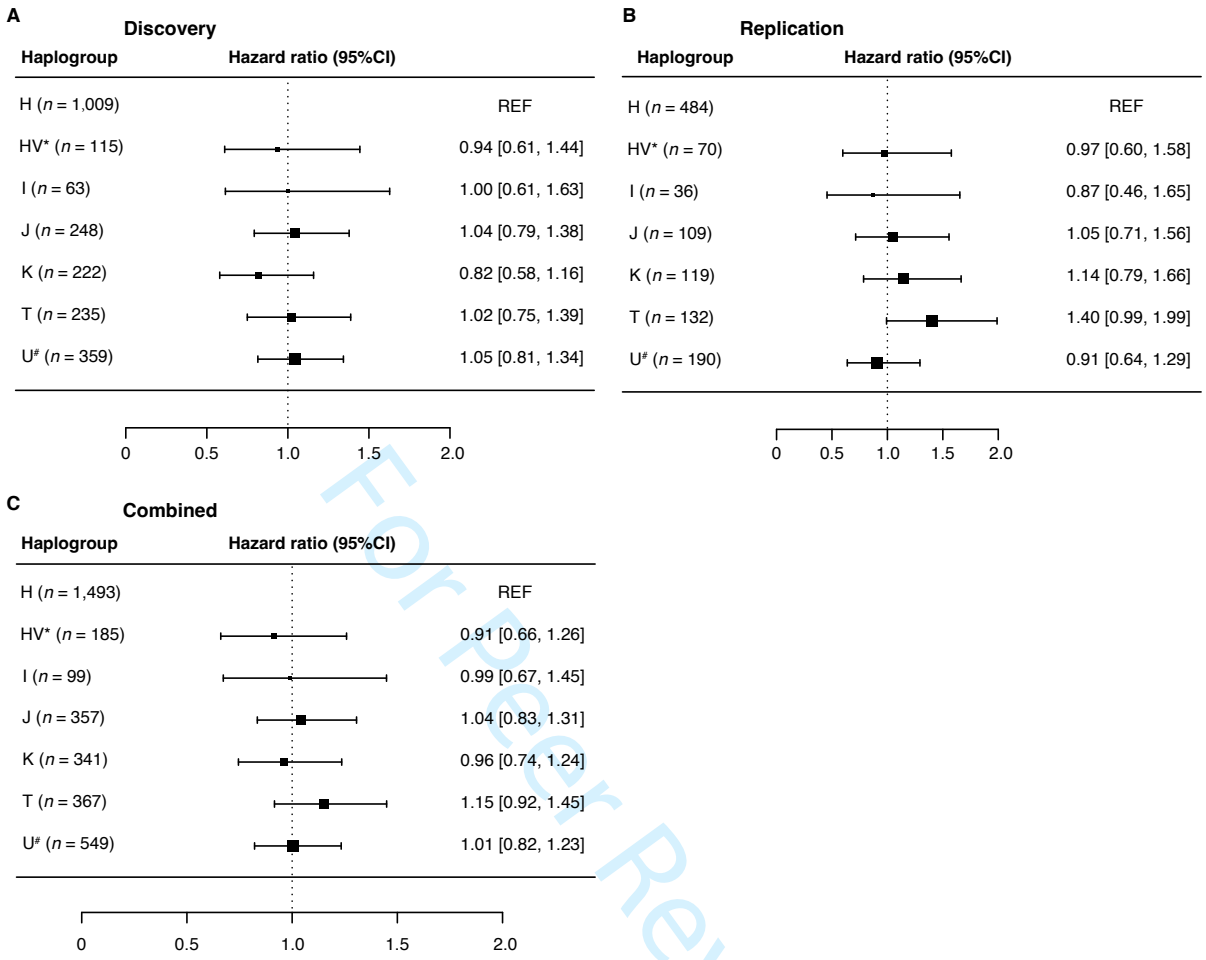
Each vertical bar corresponds to one cohort and consists of 7 sub-bars representing the proportions of the 7 macro-haplogroups H, HV*, I, J, K, T and U# in relevant cohort. There was no any difference in the proportion of seven macro-haplogroups in 15 cohorts ($P \approx 1$, Fisher exact test).

Supplementary Figure 3 Patients with PD with major sub-haplogroups of H have similar risk of progression to global cognitive impairment



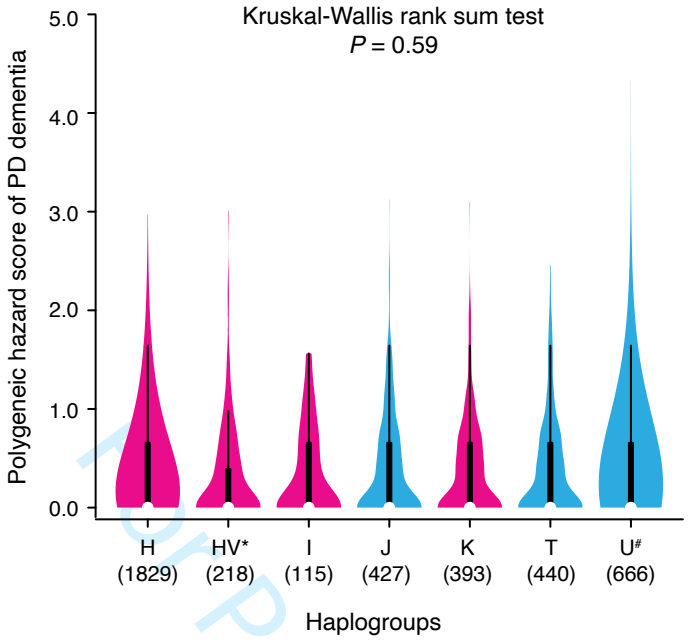
Cox regression analysis did not show any different hazard ratio (HR) to develop global cognitive impairment (MMSE ≤ 25) in combined population, according to the recommendation of the International Parkinson and Movement Disorder Society (MDS) Taks Force[29], among patients with PD in six major sub-haplogroups of H.

Supplementary Figure 4 Patients with PD in seven macro-haplogroups have similar risk of progression to HY3.



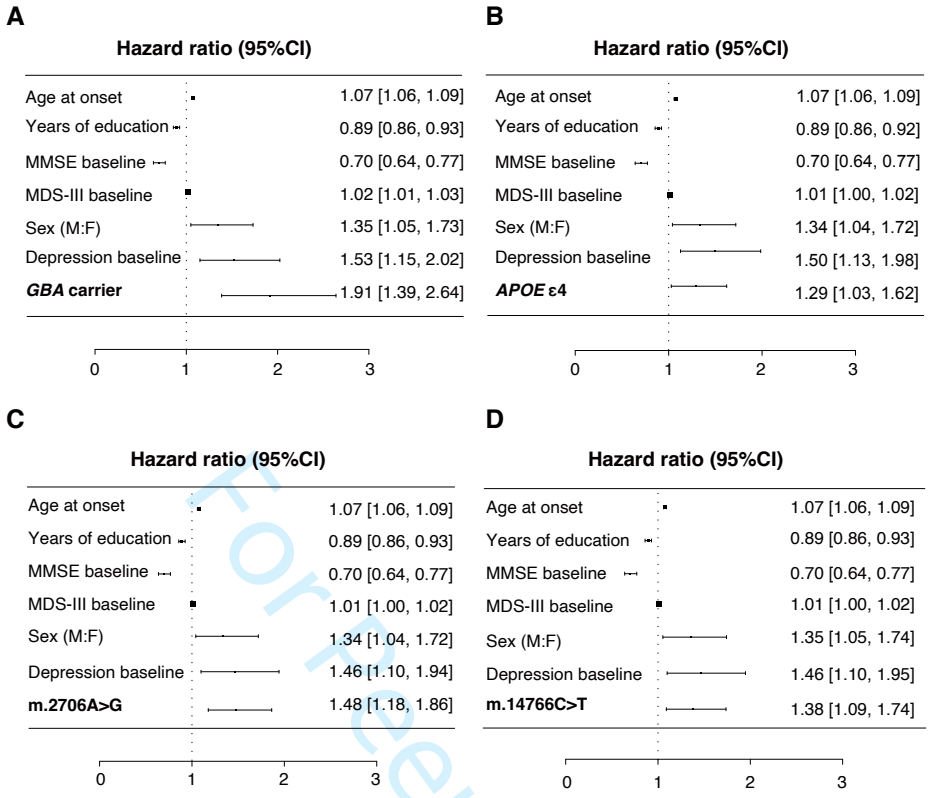
Cox regression analysis did not show any difference in hazard ratio (HR) for development of motor disability with postural instability (Hoehn & Yahr stage 3) during the progression of disease in seven macro-haplogroups from (**A**) discovery, (**B**) replication and (**C**) combined population.

Supplementary Figure 5 Patients with PD in seven mitochondrial macro-haplogroups have similar polygenic hazard scores



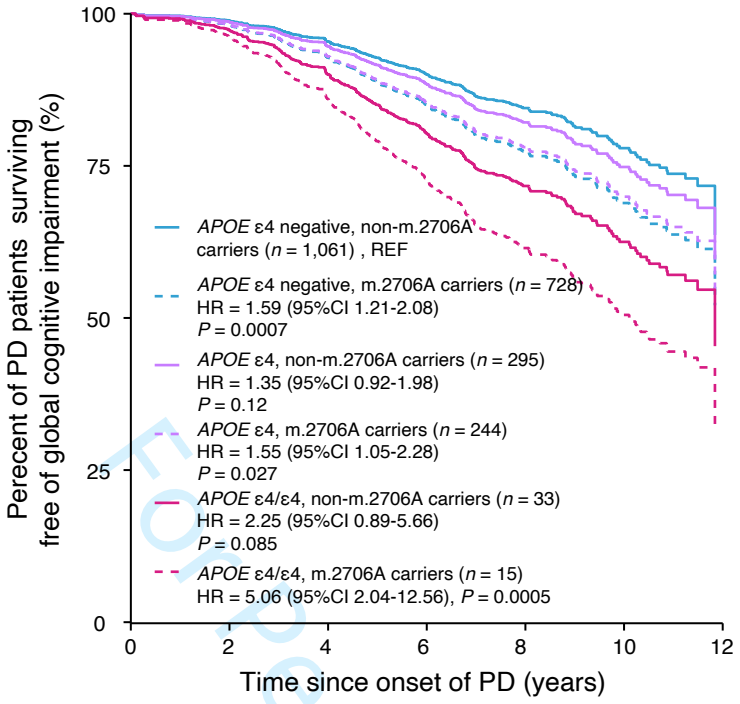
Violin-plot showed no significant difference between Polygenic hazard score to develop PD dementia among seven macro-haplogroups in combined population. Violin plot is a mixed of a box plot and a kernel density plot: the white dot represents the median, and black bar represents the interquartile range of score, the thin black line represents the rest of distribution and each side of the line is a kernel density estimation.

Supplementary Figure 6 The exploratory analysis for global cognitive impairment models with different genetic factors.



The forest plots show hazard ratios (Methods) for global cognitive impairment (GCI) in different genetic models (**A**) *GBA* carrier, (**B**) *APOE* ε4, (**C**) m.2706A>G and (**D**) m.14766C>T with the same six clinical risk factors. The squares represent point estimates, with the height of the square inversely proportional to the standard error of the estimates. The horizontal lines indicate 95% confidence intervals of the estimates.

Supplementary Figure 7 Exploratory analysis for global cognitive impairment models with *APOE* ε4 and m.2706A>G.



Covariate-adjusted survival curves for patients with PD stratified into six subgroups: *APOE* ε4 negative and non-m.2706A carriers (n = 1,061), *APOE* ε4 negative and m.2706A carriers (n = 728), *APOE* ε4 heterozygotes and non-m.2706A carriers (n = 295), *APOE* ε4 heterozygotes and m.2706A carriers (n = 244), *APOE* ε4/ε4 and non-m.2706A carriers (n = 33), *APOE* ε4/ε4 and m.2706A carriers (n = 15).

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Randomized Controlled Trial
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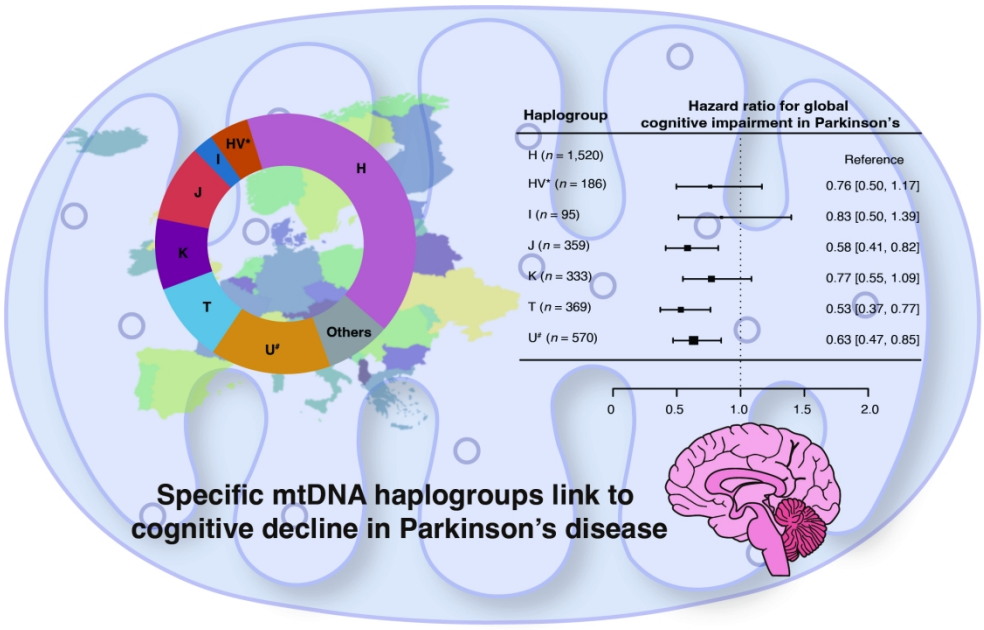

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STREGA statement: Reporting guidelines checklist for genetic association studies

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
TITLE AND ABSTRACT			
	1a	Indicate the study's design with a commonly used term in the title or the abstract	Page 1-2
	1b	Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1-2
INTRODUCTION			
Background and objectives	2	Explain the scientific background and rationale for the investigation being reported	Page 5
	3	State specific objectives, including any pre-specified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both	Page 6
METHODS			
Study design	4	Present key elements of study design early in the paper	Page 6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 6-7
Participants	6a	Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant	Page 7, Supplementary page 7
Variables	7	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin)	Page 7
Data sources/measurements	8*	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory/centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches	Page 7, Supplementary page 8
Bias	9	For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of	NA

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
		the potential bias, and explain what approach was used to deal with this	
Study size	10	Explain how the study size was arrived at	Page 6-7, Supplementary page 7
Quantitative variables	11	If applicable, describe how effects of treatment were dealt with	NA
Statistical methods	12a	Describe all statistical methods, including those used to control for confounding. State software version used and options (or settings) chosen	Page 8, Supplementary page 9-10
	12b	Describe any methods used to examine subgroups and interactions	NA
	12c	Explain how missing data were addressed	Supplementary page 9
	12d	Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	Supplementary page 9
	12e	Describe any sensitivity analyses	NA
	12f	State whether Hardy–Weinberg equilibrium was considered and, if so, how	NA
	12g	Describe any methods used for inferring genotypes or haplotypes	Page 7, Supplementary page 8
	12h	Describe any methods used to assess or address population stratification	NA
	12i	Describe any methods used to address multiple comparisons or to control risk of false-positive findings	Supplementary page 10
	12j	Describe any methods used to address and correct for relatedness among subjects	NA
RESULTS			

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
Participants	13a	Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful	Page 9, Supplementary page 8
	13b	Give reasons for non-participation at each stage	Supplementary page 8
	13c	Consider use of a flow diagram	NA
Descriptive Data	14a	Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders. Consider giving information by genotype	Page 9-10
	14b	Indicate number of participants with missing data for each variable of interest	Supplementary page 8
	14c	Cohort study—Summarise follow-up time (eg, average and total amount)	Supplementary page 9
Outcome Data	15*	Cohort study— Report outcomes (phenotypes) for each genotype category over time Case-control study— Report numbers in each genotype category Cross-sectional study— Report outcomes (phenotypes) for each genotype category	Page 10-11
Main Results	16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 10-11
	16b	Report category boundaries when continuous variables were categorized	Page 11
	16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
	16d	Report results of any adjustments for multiple comparisons	Page 12
Other Analyses	17a	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	Page 13-14
	17b	If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken	NA
	17c	If detailed results are available elsewhere, state how they can be accessed	NA
DISCUSSION			

SECTION	ITEM NUMBER	CHECKLIST ITEM	REPORTED ON PAGE NUMBER:
Key Results	18	Summarise key results with reference to study objectives	Page 15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 16-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 16-17
Generalisability	21	Discuss the generalisability (external validity) of the study results Other information	Page 17
FUNDING			
	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Reference: von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. This guideline was published simultaneously in 8 journals. Ann Intern Med. 2007; 147(8):573-577. PMID: 17938396, PLoS Med. 2007;4(10):e296. PMID: 17941714, BMJ. 2007;335(7624):806-808. PMID: 17947786, Prev Med. 2007;45(4):247-251. PMID: 17950122, Epidemiology. 2007;18(6):800-804. PMID: 18049194, Lancet. 2007;370(9596):1453-1457. PMID: 18064739 J Clin Epidemiol. 2008;61(4):344-349. PMID: 18313558, Bull World Health Organ. 2007;85(11):867-872. PMID: 18038077