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ARTICLE

Mitochondrial DNA haplogroup K is associated with a lower risk of Parkinson's disease in Italians

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It has been proposed that European mitochondrial DNA (mtDNA) haplogroups J and K, and their shared 10398G single-nucleotide polymorphism (SNP) in the *ND3* gene, are protective from Parkinson's disease (PD). We evaluated the distribution of the different mtDNA haplogroups in a large cohort of 620 Italian patients with adult-onset (>50, <65 years of age) idiopathic PD *vs* two groups of ethnic-matched controls. Neither the frequencies of haplogroup J nor that of 10398G were significantly different. However, the frequency of haplogroup K was significantly lower in PD. Stratification by sex and age indicated that the difference in the distribution of haplogroup K was more prominent in >50year old males. In spite of the common 10398G SNP, haplogroups J and K belong to widely diverging mitochondrial clades, a consideration that may explain the different results obtained for the two haplogroups in our cohorts. Our study suggests that haplogroup K might confer a lower risk for PD in Italians, corroborating the idea that the mitochondrial oxidative phosphorylation pathway is involved in the susceptibility to idiopathic PD.
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Introduction

Mitochondrial involvement in the pathogenesis of Parkinson's disease (PD) stems from the observation that parkinsonism can be induced by the neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which inhibits complex I of the mitochondrial respiratory chain through the metabolite MPP^+.[1] More recently, a PD-like phenotype has been induced in rodents using

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rotenone, another potent inhibitor of complex I.[2] Partially defective complex I activity has also been documented in tissues[3] and cybrids derived from PD patients.[4] The latter observation suggests that defects in mitochondrial DNA (mtDNA) may be responsible for biochemical dysfunction. However, although parkinsonian features have occasionally been reported in mtDNA-related encephalomyopathies,[5] neither maternal transmission nor specific mtDNA mutations have been associated with idiopathic PD.[6] Nevertheless, the analysis of mtDNA haplogroups in a large cohort of idiopathic PD patients from the white US population suggested that European mtDNA haplogroups J and K, and their common single-nucleotide polymorphism (SNP) 10398G in the *ND3* gene, exert a protective effect from the risk of PD compared to haplogroup H – the most common mtDNA haplogroup in Europe (~40%) – which carries the 10398A SNP.[7] On the contrary, Autere and colleagues[8] have found that the cluster made up of haplogroups J and T, defined by the mutation 4216C in the *ND1* gene, was more frequent among PD patients than the supercluster HVKU. In this study, to further evaluate the role of mtDNA backgrounds in PD, we have genotyped 620 mtDNAs from Italian idiopathic PD patients.

Materials and methods

Samples

All PD patients were Italians. Inclusion criteria included:

(1) disease onset after the age of 50 years;
(2) disease duration of at least 5 years;
(3) asymmetric onset;
(4) presence of at least two cardinal signs including:
  (a) tremor at rest,
  (b) nonspastic rigidity,
  (c) hypokinesia;
(5) good and sustained response to l-DOPA.

Exclusion criteria included:

(1) a positive family history up to second-degree relatives for PD or other movement disorders;
(2) the presence of additional neurological symptoms referable to vascular accidents or non-PD neurodegenerative syndromes;
(3) age of disease onset >65 years.

Controls included two groups, which are hereafter referred to as CT1 and CT2. CT1 was composed of 1486 random Italian subjects. For this control group, sex and age of individual subjects were not available thus it is not sex- and age-matched with the PD cohort. However, CT1 is by far the largest and most representative data set on the distribution of mtDNA haplogroups in Italians. CT2 was made up of 509 unrelated adult Italian subjects, of these 232 were over the age of 50 years, with no significant signs of cognitive or neurological impairment when enrolled in the study. The age-at-onset (AAO) mean (±SD) in PD individuals was 57.3±5.1 years. AAO was self-reported by the PD patients and defined as the age at which the affected individual first noticed one of the cardinal signs of PD. The age-at-examination (AAE) mean was 67.4±6.3 years in PD patients and 47.9±15.3 years in the CT2 controls. AAE was defined as the age at which the affected or unaffected participant was clinically examined and enrolled in the study. The mean age in the 232 CT2 individuals who were >50 years old was 62.1±7.8 years. Written consent was obtained from all participants in agreement with guidelines from the Ethical Committee of the Istituto Nazionale Neurologico C. Besta. For all subjects enrolled in the study, 10 mtDNA SNPs defining different haplogroups were surveyed by RFLP analysis (Table 1).

Statistics

Statistical analyses were performed using the SSPS statistical package, v. 12.0, and statistical significance was

Table 1 Diagnostic RFLP markers used for haplogroup classification

	Diagnostic RFLP markers									
Haplogroups	*DdeI 10394*	*AluI 7025*	*NlaIII 4216*	*HinfI 12308*[a]	*AluI 10032*	*MnlI 10871*	*HpaI 3592*	*AluI 10397*	*BstNI 13704*	*HaeII 9052*
I	+		−	−	+					
J	+		+						−	
K	+		−	+						−
L0–L1–L2	+		−	−	−		+			
L3	+		−	−	−	+	−	−		
M	+		−	−	−		−	+		
Others (10394 *Dde*I+)	+		−	−	−	−				
H	−	−								
U	−	+		+						
T	−	+	+	−						
Others (10394 *Dde*I−)	−	+	−	−		−				

A plus indicates the presence of the restriction site, a minus its absence.
[a]This RFLP site can be detected only by using a mismatched oligonucleotide.

established at $\alpha \leqslant 0.05$. Binary logistic regression analysis was used to assess the risk of mtDNA SNPs and haplogroups with PD. Taking into account that SNP genotype/haplogroup affiliation is a categorical independent variable with more than two categories, we performed two logistic regressions: one of the SNP genotype categories all at once, and one of the haplogroup categories all at once (to look for any effect). In the first logistic regression, the risk of PD associated with the mutations 4216C and 10398G was considered by using the SNP 7028C, which is diagnostic of haplogroup H (Table 1), as a reference level. In the second logistic regression analysis, we analyzed the haplogroup categories (H, J, K, T, U) in patients and controls using the common haplogroup H as a reference level. In both tests, we evaluated the likelihood of the entire model by the *P*-value associated with the likelihood ratio test. The odds ratio (OR) values for each SNP/haplogroup, with their associated 95% confidence intervals (CI), were also obtained. The analyses were performed by the use of either CT1 + CT2, CT1 alone or CT2 alone as controls. Moreover, to further evaluate the differential distribution of haplogroup K between PD and control subjects (maintaining haplogroup H as a reference group), the Fisher's exact test was performed on a 2×2 contingency table.

Results

The results of the RFLP survey of Table 1 were used to determine the frequencies of mtDNAs harboring 4216C and 10398G, as well as the frequencies of haplogroups H, I, J, K, T, U (w/t K) and L–M in PD patients and control groups (Table 2). Table 3 reports the ORs (with relative lower and upper CI values) obtained from the binary logistic regressions in numerous comparisons between patients and controls groups. As the power of the analyses depends on sample size, the most reliable among all comparisons is that employing the combined set (CT1 + CT2) of 1995 control subjects. As for the SNPs 4216C and 10398G, we noted that, employing the likelihood ratio test statistic, for each comparison, the general model was always superior to the interaction model in terms of overall fit (*P*-values >0.05), with only one exception, the comparison PD (males) *vs* CT2 (males), which suggests a potential protective role of 13098G in males (OR = 0.59; 95% CI: 0.39–0.91).

The role of 10398G and 4216C in PD was further evaluated by taking into account that 10398G characterizes haplogroups J and K (haplogroup I is too rare for reliable comparisons), while 4216C marks haplogroups J and T. Haplogroup U was used as a control. When age and sex were not taken into account, the logistic regressions showed a significant likelihood only in the comparison of PD *vs* CT1 + CT2 (*P*-value = 0.048), thus highlighting the importance of the sample size. Overall, the haplogroup comparisons of Table 3, which did not consider age and sex, support a potential protective role of 10398G in the context of haplogroup K, but never on a haplogroup J background. Indeed, significant differences for K were observed in PD *vs* CT1 + CT2 (OR = 0.54; 95% CI: 0.35–0.83), PD *vs* CT1 alone (OR = 0.53; 95% CI: 0.34–0.83) and PD *vs* CT2 alone (OR = 0.56; 95% CI: 0.32–0.97). Stratification by age and sex (in other words, comparisons involving only the CT2 cohort) appears to further support the protective effect of haplogroup K (PD *vs* CT2 >50 years; OR = 0.44; 95% CI: 0.24–0.84), particularly in males as shown by PD (males) *vs* CT2 (males) (OR = 0.42; 95% CI: 0.21–0.87), and PD (males) *vs* CT2 (males) >50 years (OR = 0.37; 95% CI: 0.16–0.89). However, in these comparisons, a significant likelihood is observed only in two comparisons – PD *vs* CT2 >50 years (*P*-value = 0.026), and PD (females) *vs* CT2 (females) >50 years (*P*-value = 0.011). Again, this could be due to the small sample size of CT2.

The potential effect of haplogroup K on PD was further evaluated by using the Fisher's exact test (Table 4). All comparisons involving haplogroup K, with the exception

Table 2 Haplogroup affiliation of mtDNAs from Italian PD patients and controls

		Frequencies (%)									
		SNPs		Haplogroups							
Samples	*No. of subjects*	*4216C*	*10398G*	*H*	*I*	*J*	*K*	*T*	*U*[a]	*L–M*	*Other*
CT1	1486	18.1	20.1	43.5	2.4	8.5	7.7	9.6	12.5	1.5	14.3
CT2[b]	509	19.7	19.5	38.7	—	7.5	6.5	12.2	12.4	—	22.7
CT2 (>50 years)	232	18.5	18.5	38.8	—	5.6	8.2	12.9	11.6	—	22.9
CT2 males	281	19.9	22.4	38.1	—	8.5	7.1	11.4	11.4	—	23.5
CT2 males (>50 years)	114	17.5	22.8	41.2	—	8.8	8.8	8.8	11.4	—	21.0
CT2 females	224	19.7	15.6	39.7	—	6.3	5.4	13.4	13.8	—	21.4
CT2 females (>50 years)	118	19.5	14.4	36.4	—	2.5	7.6	16.9	11.9	—	24.7
PD patients[c]	620	17.3	16.5	44.7	2.3	8.6	4.2	8.7	12.7	0.7	18.1
PD males	386	17.4	16.1	45.9	2.1	8.3	3.6	9.1	13.7	1.0	16.3
PD females	232	16.9	17.2	42.7	2.6	9.1	5.2	7.8	11.2	—	21.4

[a]'Superhaplogroup' U does not include mtDNAs belonging to haplogroup K.
[b]Sex was unknown for four of the CT2 subjects.
[c]Sex was unknown for two of the PD patients.

Table 3 ORs[a] of mtDNA SNPs and haplogroups

	SNPs		*Haplogroups*			
	4216C	*10398G*	*J*	*K*	*T*	*U*[b]
PD vs CT1+CT2						
OR	0.88	0.78	0.98	0.54	0.81	0.97
95% CI	(0.69–1.14)	(0.61–1.01)	(0.70–1.38)	(0.35–0.83)	(0.58–1.12)	(0.72–1.29)
P-value[c]	0.152			0.048		
PD vs CT1						
OR	0.93	0.80	0.98	0.53	0.89	0.99
95% CI	(0.71–1.21)	(0.61–1.04)	(0.69–1.39)	(0.34–0.83)	(0.63–1.25)	(0.73–1.34)
P-value	0.244			0.067		
PD vs CT2						
OR	0.76	0.73	0.99	0.56	0.62	0.89
95% CI	(0.55–1.06)	(0.53–1.02)	(0.63–1.56)	(0.32–0.97)	(0.41–0.93)	(0.61–1.30)
P-value	0.098			0.067		
PD[d] *vs CT2 >50 years*						
OR	0.81	0.77	1.32	0.44	0.58	0.95
95% CI	(0.53–1.24)	(0.53–1.24)	(0.69–2.54)	(0.24–0.84)	(0.35–0.97)	(0.58–1.56)
P-value	0.401			0.026		
PD (males) vs CT2 (males)						
OR	0.72	0.59	0.81	0.42	0.66	1.00
95% CI	(0.47–1.11)	(0.39–0.91)	(0.45–1.44)	(0.21–0.87)	(0.39–1.13)	(0.61–1.65)
P-value	0.042			0.116		
PD (males)[d] *vs CT2 (males) >50 years*						
OR	0.89	0.63	0.85	0.37	0.93	1.08
95% CI	(0.49–1.61)	(0.36–1.11)	(0.39–1.85)	(0.16–0.89)	(0.43–2.01)	(0.54–2.15)
P-value	0.285			0.285		
PD (females) vs CT2 (females)						
OR	0.80	1.03	1.35	0.90	0.54	0.75
95% CI	(0.47–1.34)	(0.60–1.76)	(0.65–2.81)	(0.38–2.10)	(0.28–1.03)	(0.42–1.37)
P-value	0.645			0.247		
PD (females)[d] *vs CT2 (females) >50 years*						
OR	0.74	1.02	3.04	0.58	0.39	0.81
95% CI	(0.39–1.38)	(0.52–2.00)	(0.86–10.47)	(0.23–1.48)	(0.19–0.81)	(0.38–1.69)
P-value	0.595			0.011		

[a]Two binary logistic regressions were performed using SNP status and haplogroup affiliation, respectively, as an independent variable.
[b]'Superhaplogroup' U does not include mtDNAs belonging to haplogroup K.
[c]*P*-values relative to the general model (all slopes = zero) obtained by the likelihood ratio test.
[d]All PDs were >50 years old.

of those in females, provided significant probability values thus supporting a role of haplogroup K in reducing the risk of PD.

As for the 4216C mutation, the absence in all comparisons of significant differences for haplogroup J does not support an involvement of this SNP in modulating PD, while some significant differences are observed for haplogroup T (Table 3), the other haplogroup harboring this mutation (Table 1). These significant differences always involve the CT2 cohort: PD *vs* CT2 (OR = 0.62; 95% CI: 0.41–0.93), PD *vs* CT2 >50 years (OR = 0.58; 95% CI: 0.35–0.97) and PD (females) *vs* CT2 (females) >50 years (OR = 0.39; 95% CI: 0.19–0.81). However, we performed an additional binary logistic regression in which the two control groups were compared (CT2 *vs* CT1) in terms of haplogroup distributions. A significant difference was observed only for haplogroup T (OR = 1.46; 95% CI: 1.04–2.04) indicating an excess of mtDNAs belonging to haplogroup T in CT2, thus suggesting that the differences observed between PD and CT2 are due to the smaller sample size of this control sample.

Discussion

The contribution of the 4216C and 10398G SNPs in modulating the risk of PD is controversial. Both higher[8] and lower[7] frequencies of the cluster JT in PD *vs* control cohorts have been reported in different European populations. In our study, the 4216C mutation was equally

Table 4 Probability values of Fisher's exact test

					CT2			
					Males		Females	
	CT1+CT2	*CT1*	*All*	*>50 years*	*All*	*>50 years*	*All*	*>50 years*
K[a]	0.005	0.005	0.050	0.019	0.025	0.038	0.831	0.316

[a]*P*-values were obtained comparing the frequencies of haplogroups K and H in PD patients and controls.

distributed in PD and non-PD subjects. We conclude that neither haplogroup J alone nor the supercluster JT, or the polymorphism 10398G, significantly influence the risk of PD in the general Italian population. A significant effect in modulating the risk of PD was obtained only for haplogroup K. This haplogroup was less frequent in PD patients than in controls, and even less so in >50 year old males. Our findings differ from those obtained by van der Walt and colleagues,[7] who observed a reduced risk for PD in association with 10398G, a polymorphism which among Europeans characterizes only haplogroups J, K and I. However, these haplogroups do not belong to the same clade. Haplogroup K is a clade within haplogroup U, J is a sister group of haplogroup T and haplogroup I is a subset of the cluster N1.[9] Therefore, the 10398G in J, K and I has not been transmitted by descent, rather it is the result of three mutational events that have occurred independently on different mtDNA backgrounds. In addition, the frequency of 10398G-positive mtDNAs, which is only 20–25% in Europeans, is much higher in other human populations. 10398G characterizes super-haplogroup M – the most common haplogroup in Asia – and virtually all sub-Saharan African mtDNAs (haplogroups L0–L3). Since there is no evidence of major ethnic biases in the prevalence of PD, the very high frequency of the 10398G in non-European populations makes it unlikely that this polymorphism can confer *per se* a significant protection from PD. Concordant with our own findings, the reduced risk for PD in the supercluster HVKU compared to the supercluster JTIWX found in Finns was exclusively contributed to by the KU cluster.[8] The accumulation of nonsynonymous changes in some mtDNA haplogroups can modulate either the efficiency in coupling respiration rate to ATP synthesis or the production of reactive oxygen species during respiration, or both. These effects could play a role in determining neuronal damage or, instead, neuronal protection. Interestingly, the KU cluster carries the lowest average number of amino-acid replacements in the *ND* genes of complex I, which is a key enzyme for energy production and a site of ROS production. It is possible that the relative paucity of nonsynonymous mutations in the ND subunits characterizing haplogroup K may contribute to the decreased risk for PD. Incidentally, haplogroup K is extremely common in the Ashkenazi Jews (more than 30%).[10] Therefore, if haplogroup K reduces the risk of PD, a verifiable consequence would be the observation of a lower frequency of PD in Ashkenazi Jews, relative to other European populations – a possibility that has not yet been investigated.

The modifying role of an mtDNA haplogroup on disease risk is most probably due to the synergistic action of a set of different polymorphisms rather than to the effect of a single polymorphism. Further analysis of haplogroup K within the KU cluster will allow us to recognize founder events and specific mtDNA subclades relevant for PD.

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