Neurobiology of Aging xxx (2013) 1–10

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

The protective effect of LRRK2 p.R1398H on risk of Parkinson's disease is independent of *MAPT* and *SNCA* variants

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0197-4580/$ see front matter
http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.013

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ARTICLE INFO

Article history:
Received 9 April 2013
Received in revised form 10 July 2013
Accepted 15 July 2013

Keywords:
Parkinson's disease
LRRK2
SNCA
MAPT
Interaction
Genetics

ABSTRACT

The best validated susceptibility variants for Parkinson's disease are located in the α-synuclein (*SNCA*) and microtubule-associated protein tau (*MAPT*) genes. Recently, a protective p.N551K-R1398H-K1423K haplotype in the leucine-rich repeat kinase 2 (*LRRK2*) gene was identified, with p.R1398H appearing to be the most likely functional variant. To date, the consistency of the protective effect of LRRK2 p.R1398H across *MAPT* and *SNCA* variant genotypes has not been assessed. To address this, we examined 4 *SNCA* variants (rs181489, rs356219, rs11931074, and rs2583988), the *MAPT* H1-haplotype–defining variant rs1052553, and LRRK2 p.R1398H (rs7133914) in Caucasian (n 10,322) and Asian (n 2289) series. There was no evidence of an interaction of LRRK2 p.R1398H with *MAPT* or *SNCA* variants (all $p \geq 0.10$); the protective effect of p.R1398H was observed at similar magnitude across *MAPT* and *SNCA* genotypes, and the risk effects of *MAPT* and *SNCA* variants were observed consistently for LRRK2 p.R1398H genotypes. Our results indicate that the association of LRRK2 p.R1398H with Parkinson's disease is independent of *SNCA* and *MAPT* variants, and vice versa, in Caucasian and Asian populations.

1. Introduction

With an estimated prevalence of between 1% and 2% in individuals more than 65 years of age, Parkinson's disease (PD) is one of the most common age-related neurodegenerative disorders (de Lau and Breteler, 2006; Postuma and Montplaisir, 2009). Long thought of as a sporadic disease, PD now has a well-established genetic component that includes both disease-causing mutations as well as risk-modifying susceptibility variants (Gasser et al., 2011). Of the PD susceptibility variants that have been identified thus far, the best validated have involved those located in the α-synuclein (*SNCA*) gene, which also contains several pathogenic mutations that are linked to familial PD, and in the microtubule-associated protein tau (*MAPT*) gene (Gasser et al., 2011). More specifically, associations with PD have been identified in both Caucasian and Asian populations at the 3′ and 5′ ends of the *SNCA* gene (Mizuta et al., 2006; Mueller et al., 2005; Pankratz et al., 2009; Ross et al., 2007; Satake et al., 2009; Simón-Sánchez et al., 2009; Winkler et al., 2007), whereas the H1 haplotype in *MAPT* is associated with PD in Caucasians but not in Asians, owing to the almost complete absence of the H2 haplotype in the latter group (Evans et al., 2004; Healy et al., 2004; Skipper et al., 2004; Tobin et al., 2008; Wider et al., 2010).

Variation in the leucine-rich repeat kinase 2 (*LRRK2*) gene, which like *SNCA* harbors disease-causing mutations of its own has also been associated with susceptibility to PD in both Caucasian and Asian populations. The majority of proposed *LRRK2* PD risk variants have been relatively rare (minor allele frequencies [MAFs] between 1% and 5%) and have included p.G2385R and p.R1628P in Asian populations as well as the more recently identified p.A419V (in Asians), and p.M1646T (in Caucasians) (Di Fonzo et al., 2006; Farrer et al., 2007; Ross et al., 2008, 2011; Tan et al., 2010). The most common *LRRK2* PD risk factor to date, identified by several groups including our own, has involved a 3-variant (p.N551K-R1398H-K1423K) protective haplotype in both populations (Ross et al., 2011; Tan et al., 2010). It has been shown that the p.1398H variant has reduced kinase activity in comparison to the wild-type p.R1398 (Tan et al. 2010). Given these data, the p.R1398H (rs7133914) substitution, which occurs with a MAF of approximately 7% in Caucasians and 10% in Asians (Heckman et al., in press; Tan et al., 2010), is the most likely functional variant on the haplotype. The protective effect of p.R1398H appears to be strongest in Asians, in whom consistent odds ratios of 0.75 and 0.73 have been observed in studies by Tan et al. (2010) and Ross et al. (2011), with a similar odds ratio of 0.79 observed in a smaller study by Chen et al. (2011). In Caucasians, the odds ratio for p.R1398H observed in the aforementioned study by Ross et al. in a series of 6995 patients and 5595 control subjects was 0.89. This is very similar to the findings of a large meta-analysis of genome-wide association studies, in which, albeit not nominally significant, LRRK2 p.R1398H (MAF ~ 6.7%) had a protective odds ratio of 0.92 and 95% confidence limits ranging from 0.83 to 1.02 in regard to susceptibility to PD (Nalls et al., 2011; personal communication).

To best determine risk of PD for a given individual and to elucidate potential future therapeutic implications, it is important not only to identify individual genetic risk factors but also to understand how these risk factors interact with one another. However, sample sizes needed to reasonably evaluate evidence of such gene–gene interactions are usually fairly large and can be difficult to achieve. This is because the risk factor of interest in an interaction study (presence of the genotype of interest for both variants) occurs much less frequently than the genotype for the individual variants, which can result in a lack of precision in estimated interaction effects. Collaboration between members of the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium and the resulting large number of patients with PD and controls offers the opportunity to effectively examine how recognized susceptibility variants for PD may or may not interact with one another. Such a study was previously undertaken by the GEO-PD Consortium, in which *SNCA* and *MAPT* variants were

examined in relation to risk of PD and found to have independent effects (Elbaz et al., 2011). The identification of PD susceptibility variants in *LRRK2* raises the question of whether the effects of these variants may be modified by those in *SNCA* or *MAPT*, or vice versa. The aim of this study was to evaluate the interaction of the common *LRRK2* susceptibility variant p.R1398H with *SNCA* and *MAPT* variants in relation to risk of PD using Caucasian and Asian patient–control subject series obtained through the GEO-PD Consortium.

2. Methods

2.1. Subjects

As of 2013, the GEO-PD Consortium includes 57 sites from 29 countries and 6 continents that have agreed to share DNA and data for 38,686 patients with PD and 34,871 control subjects (http://www.geopd.org/). A total of 20 sites participating in the GEO-PD Consortium provided data to be used in the current study as part of a project initiated in 2009. The majority of the Caucasian subjects used in this study were also included in the previously mentioned GEO-PD *SNCA-MAPT* interaction study (Elbaz et al., 2011), and the subjects included in this study are a subset of those included in the previously referred to investigation of *LRRK2* exonic variants in relation to PD (Ross et al., 2011). To be consistent with the association analysis in the latter study involving *LRRK2* exonic variants, carriers of *LRRK2* pathogenic variants (n = 64) were excluded. Subjects were not genotyped for known pathogenic *SNCA* mutations and therefore this was not part of our exclusion criteria. In total, 7342 patients with PD and 5269 control subjects from 13 different countries on 4 continents were studied. These subjects were divided into a Caucasian series (5991 patients with PD, 4331 controls, 16 sites, 10 countries) and an Asian series (1351 patients with PD, 938 controls, 4 sites, 3 countries). Table 1 provides demographic information for the Caucasian and Asians series, whereas site-specific information is displayed in Supplementary Table 1.

Patients were diagnosed with PD using standard criteria (Bower et al., 1999; Gelb et al., 1999; Hughes et al., 1992). Controls were individuals free of PD or a related movement disorder at the time of examination. All subjects were unrelated within and between diagnosis groups. The Mayo Clinic Institutional Review Board approved the study; each individual site received local institutional review board approval, and all subjects provided informed consent.

2.2. Genetic analysis

Four *SNCA* variants (3′ end of gene: rs181489, rs356219, rs11931074; 5′ end of gene: rs2583988) as well as the *MAPT* H1-haplotype defining variant rs1052553 were genotyped because of consistently replicated associations with PD (Healy et al., 2004; Mueller et al., 2005; Mizuta et al., 2006; Pankratz et al., 2009; Ross et al., 2007; Satake et al., 2009; Skipper et al., 2004; Simón-Sánchez et al., 2009; Tobin et al., 2008; Wider et al., 2010; Winkler et al., 2007). These 5 variants were chosen for the aforementioned GEO-PD *SNCA-MAPT* interaction study (Elbaz et al., 2011). The REP1 polymorphism located in the *SNCA* promoter has also been associated with PD (Krüger et al., 1999; Maraganore et al., 2006); however, because the 263-bp allele (which has shown the strongest association with PD) is relatively rare, we did not evaluate REP1 in the current study. The *LRRK2* variant rs7133914 (p.R1398H) was also selected for inclusion because of the aforementioned findings demonstrating that is the most likely functional variant on a 3-variant haplotype (all 3 variants in strong linkage disequilibrium with $r^2 > 0.84$ in controls) that affects risk of PD in a protective manner (Ross et al., 2011; Tan et al., 2010).

Table 1
Subject characteristics for the Caucasian and Asian series

Variable	Patients with PD	Controls
Caucasian series	n = 5991	n = 4331
Age, y	69 ± 11 (18–106)	65 ± 15 (21–107)
Gender		
Male	3453 (58%)	2045 (47%)
Female	2538 (42%)	2286 (53%)
Age at onset, y	59 ± 12 (18–96)	NA
Asian series	n = 1351	n = 938
Age, y	61 ± 12 (20–91)	60 ± 11 (23–89)
Gender		
Male	672 (50%)	322 (34%)
Female	679 (50%)	616 (66%)
Age at onset, y	54 ± 12 (20–89)	NA

Sample mean ± SD (minimum–maximum) is given for age of subjects and age at onset. Information was unavailable regarding age in the Caucasian series (147 patients with PD, 21 controls) and Asian series (371 patients with PD, 298 controls). Information was unavailable regarding age at onset in the Caucasian series (723 patients) and Asian series (8 patients).
Key: NA, not applicable; PD, Parkinson's disease; SD, standard deviation.

DNA was sourced from blood and was stored in a freezer at −80 °C. All samples were de-identified with an anonymous code from each site and only a minimal clinical dataset. All *LRRK2* and *SNCA* genotyping was done using MassArray iPLEX chemistry and analyzed using Typer 4.0 (Sequenom, San Diego, CA). *MAPT* rs1052553 was genotyped using an ABI Taqman genotyping assay on an ABI 7900HT Fast Real-Time PCR system and analyzed using SDS 2.2.2 software (Applied Biosystems, Foster City, CA). All genotyping was performed at the Mayo Clinic Florida neurogenetics laboratory (Jacksonville, FL). Primer sequences are provided in Supplementary Table 2 for all variants except for *MAPT* rs1052553. Positive control DNA was run for each variant. Call rates in each series were > 95%. There was no evidence of departure from Hardy–Weinberg equilibrium in controls for any of the sites (all $p > 0.05$ after Bonferroni correction).

2.3. Statistical analysis

All analysis was performed separately for the Caucasian and Asian series. Associations of individual *SNCA* variants, *MAPT* rs1052553, and LRRK2 p.R1398H with PD, and pairwise interactions of LRRK2 p.R1398H with *SNCA* and *MAPT* variants in relation to PD, were evaluated using odds ratios (ORs) and 95% confidence intervals (CIs) from fixed-effects logistic regression models adjusted for site. Interactions were evaluated on a multiplicative scale only because it has been shown that when at least one of the interacting factors is protective, biological interactions are expected to result in departure from multiplicative effects (Weinberg, 1986).

We considered LRRK2 p.R1398H under a dominant model (presence vs. absence of the minor allele) in all analyses owing to the very small number of homozygotes of the minor allele, whereas *SNCA* variants were evaluated under an additive model (effect of each additional minor allele), dominant model, recessive model (presence of 2 copies vs. 0 or 1 copy of the minor allele) and genotype model (general comparison across genotypes). *MAPT* rs1052553 was also evaluated under additive, dominant, recessive, and genotype models, but with effects corresponding to the major allele to be consistent with previous reports in which ORs correspond to the H1 risk allele. In Caucasians, 3-gene interactions were also examined. Sensitivity of results to model adjustment for age and gender and to the use of random-effects models (DerSimonian and Laird, 1986) were also assessed when evaluating interactions.

Between-site heterogeneity in interaction ORs was examined using χ^2 tests based on the Q statistic, and also by estimating the I^2 statistic, which measures the proportion of variation in interaction ORs between sites due to heterogeneity beyond chance (Higgins and Thompson, 2002).

A relatively large number of statistical tests of gene–gene interaction were performed in our analyses (24 in the Caucasian series and 8 in the Asian series). To adjust for multiple testing and to control the family-wise error rate at 5%, we used a Bonferroni correction separately for each series, after which p values <0.0021 (Caucasian series) and <0.00625 (Asian series) were considered as statistically significant. All statistical analyses were performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

A summary of allele and genotype frequencies for *SNCA* variants, *MAPT* rs1052553, and LRRK2 p.R1398H in our Caucasian and Asian patient-control series is provided in Supplementary Table 3, along with country-specific frequencies. The *SNCA* variants rs181489 and rs2583988 as well as *MAPT* rs1052553 were observed extremely rarely in Asian patients and controls and, as such, were not assessed in association analysis. *SNCA* variants were in relatively weak linkage disequilibrium in controls ($r^2 < 0.32$) with the exception of rs181489 and rs356219 in the Caucasian series ($r^2 = 0.58$), rs181489 and rs2583988 in the Caucasian series ($r^2 = 0.53$), and rs356219 and rs11931074 in the Asian series ($r^2 = 0.97$).

To best interpret the results of gene–gene interaction analysis, it is helpful to first understand the effects of individual variants on risk of PD, and therefore single-variant associations with PD for the *SNCA*, *MAPT*, and *LRRK2* variants, which have largely been reported before in the aforementioned GEO-PD studies (Elbaz et al., 2011; Ross et al., 2011), are displayed in Supplementary Table 4. As has been previously shown, all variants were significantly associated with PD.

Evaluations of pairwise interactions of LRRK2 p.R1398H with *SNCA* variants and *MAPT* rs1052553 in relation to PD for the Caucasian series are shown in Table 2. To simplify our presentation of interaction results, we have focused on additive and genotype models for *SNCA* and *MAPT* variants in Table 2, because all of these variants had the strongest association with PD under an additive model except *SNCA* rs11931074 (which was also strongly associated with PD under an additive model), and because genotype models allow for the most general test of interaction. Gene–gene interactions under dominant and

Table 2
Interactions of LRRK2 p.R1398H with *SNCA* and *MAPT* variants in regard to susceptibility to Parkinson's disease (PD) in the Caucasian series under additive and genotype models

Variant/genotype	LRRK2 p.R1398H	Sample genotype count and frequency	Test of association		Test of interaction
			OR (95% CI)	p value	
SNCA rs181489					
CC	GG	3908 (39.9%)	1.00 (reference)	NA	Additive model
CC	GA or AA	599 (6.1%)	0.82 (0.69 0.98)	0.030	OR − 1.06
CT	GG	3636 (37.1%)	1.14 (1.04 1.25)	0.0070	95% CI − 0.88 1.28
CT	GA or AA	542 (5.5%)	1.08 (0.90 1.30)	0.42	p − 0.52
TT	GG	967 (9.9%)	1.65 (1.42 1.92)	1.4E-10	Genotype model[a]
TT	GA or AA	136 (1.4%)	1.40 (0.98 2.00)	0.066	p − 0.14
SNCA rs356219					
AA	GG	3087 (30.9%)	1.00 (reference)	NA	Additive model
AA	GA or AA	440 (4.4%)	0.82 (0.67 1.01)	0.060	OR − 0.98
AG	GG	4142 (41.5%)	1.15 (1.04 1.26)	0.0060	95% CI − 0.82 1.17
AG	GA or AA	628 (6.3%)	1.11 (0.93 1.32)	0.27	p − 0.81
GG	GG	1476 (14.8%)	1.51 (1.33 1.73)	7.2E-10	Genotype model[a]
GG	GA or AA	219 (2.2%)	1.10 (0.83 1.46)	0.51	p − 0.32
SNCA rs11931074					
GG	GG	7443 (74.6%)	1.00 (reference)	NA	Additive model
GG	GA or AA	1061 (10.5%)	0.85 (0.74 0.97)	0.017	OR − 1.06
GT	GG	1300 (12.9%)	1.34 (1.18 1.51)	6.8E-6	95% CI − 0.79 1.43
GT	GA or AA	232 (2.3%)	1.32 (1.00 1.74)	0.052	p − 0.69
TT	GG	59 (0.6%)	1.46 (0.84 2.62)	0.19	Genotype model[a]
TT	GA or AA	12 (0.1%)	0.67 (0.20 2.24)	0.51	p − 0.61
SNCA rs2583988					
CC	GG	4495 (44.6%)	1.00 (reference)	NA	Additive model
CC	GA or AA	677 (6.7%)	0.82 (0.69 0.97)	0.019	OR − 1.07
CT	GG	3480 (34.6%)	1.20 (1.09 1.31)	0.0001	95% CI − 0.89 1.29
CT	GA or AA	500 (5.0%)	1.13 (0.93 1.37)	0.23	p − 0.47
TT	GG	800 (7.9%)	1.42 (1.21 1.67)	1.9E-5	Genotype model[a]
TT	GA or AA	117 (1.2%)	1.22 (0.84 1.80)	0.30	p − 0.56
MAPT rs1052553[b]					
GG	GG	364 (3.6%)	1.00 (reference)	NA	Additive model
GG	GA or AA	58 (0.6%)	0.54 (0.30 0.97)	0.041	OR − 1.05
GA	GG	2617 (25.7%)	1.10 (0.88 1.38)	0.41	95% CI − 0.85 1.30
GA	GA or AA	398 (3.9%)	1.05 (0.78 1.41)	0.75	p − 0.65
AA	GG	5881 (58.0%)	1.36 (1.10 1.70)	0.0055	Genotype model[a]
AA	GA or AA	858 (8.4%)	1.19 (0.92 1.53)	0.19	p − 0.29

ORs and P values result from fixed-effects logistic regression models. For tests of association, the 2 given variants were combined into 1 variable, and the model was adjusted for site. For tests of interaction, models included each of the 2 variants, their interaction, and site. Additive models and genotype models refer to the characterization of *SNCA* and *MAPT* variants; only dominant models were considered for LRRK2 p.R1398H because of the small number of rare homozygotes for this variant. Interaction ORs under an additive model are interpreted as the multiplicative increase in the effect of the minor allele for LRRK2 p.R1398H on PD corresponding to each additional risk allele for *SNCA* and *MAPT* variants, or alternatively as the multiplicative increase in the effect of each additional risk allele for *SNCA* and *MAPT* variants on PD corresponding to presence of the minor allele for LRRK2 p.R1398H.

Key: CI, confidence interval; OR, odds ratio.

[a] Tests of interaction under a genotype model do not produce a single interaction OR, and therefore only a P value is given.

[b] The A allele for *MAPT* rs1052553 corresponds to the H1 haplotype.

recessive models for *SNCA* and *MAPT* variants are shown in Supplementary Tables 5 and 6. In site-adjusted analyses, no interactions of LRRK2 p.R1398H with *SNCA* and *MAPT* variants approached significance after multiple testing adjustment under any statistical model (all interaction $p > 0.10$); the protective effect of p.R1398H on risk of PD observed in similar magnitude for different genotypes of *SNCA* and *MAPT* variants, whereas the risk effects of *SNCA* and *MAPT* variants were seen similarly for subjects with and without a copy of the minor allele for p.R1398H. All interaction ORs were close to 1.0 in magnitude indicating lack of any interaction with LRRK2 p.R1398H, the only exceptions involving rare genotypes for *MAPT* rs1052553 under a dominant model (Supplementary Table 5) and *SNCA* rs11931074 under a recessive model (Supplementary Table 6), which are best interpreted with caution owing to the non-significant interactions and very low genotype frequencies. The lack of interaction of LRRK2 p.R1398H with *MAPT* and *SNCA* variants was also observed when adjusting for age and gender (Supplementary Table 7) in those subjects with that information available (98%) and also when using a random effects model (Supplementary Table 8). Results of country-specific interaction analysis are shown in Supplementary Table 9. Between-site heterogeneity regarding interactions with LRRK2 p.R1398H was low for *SNCA* rs356219, rs11931074, and rs2583988 ($I^2 = 0\%$, $p > 0.45$) and moderate for *SNCA* rs181489 and *MAPT* rs1052553 ($I^2 = 25\%$–36%, $p > 0.075$) (Supplementary Table 8).

More detailed analysis combining genotypes across all 3 genes for *SNCA* variants, *MAPT* rs1052553, and LRRK2 p.R1398H in the Caucasian series is displayed in Supplementary Table 10 and Fig. 1, where rare homozygotes were collapsed with heterozygotes for each variant to avoid extremely rare 3-variant genotype combinations. There was no evidence of any interaction in these 3-gene analyses (all, $p > 0.63$).

Interactions of LRRK2 p.R1398H with *SNCA* variants rs356219 and rs11931074 in the Asian series are examined in Table 3 in analyses adjusted for site. Individual effects of LRRK2 p.R1398H and *SNCA* variants on risk of PD were observed consistently across variants in the other gene, with no statistically significant evidence of gene–gene interaction (all interaction, $p > 0.14$). All interaction ORs were between 1.17 and 1.39, indicating a slight but nonsignificant reduction of the protective effect of LRRK2 p.R1398H on risk of PD when the risk allele for *SNCA* variants was present, and a similar small and nonsignificant enhancement of the *SNCA* risk effects, given the protective genotype for p.R1398H (Fig. 2). Results were similar when adjusting for age and gender (Supplementary Table 7) in the

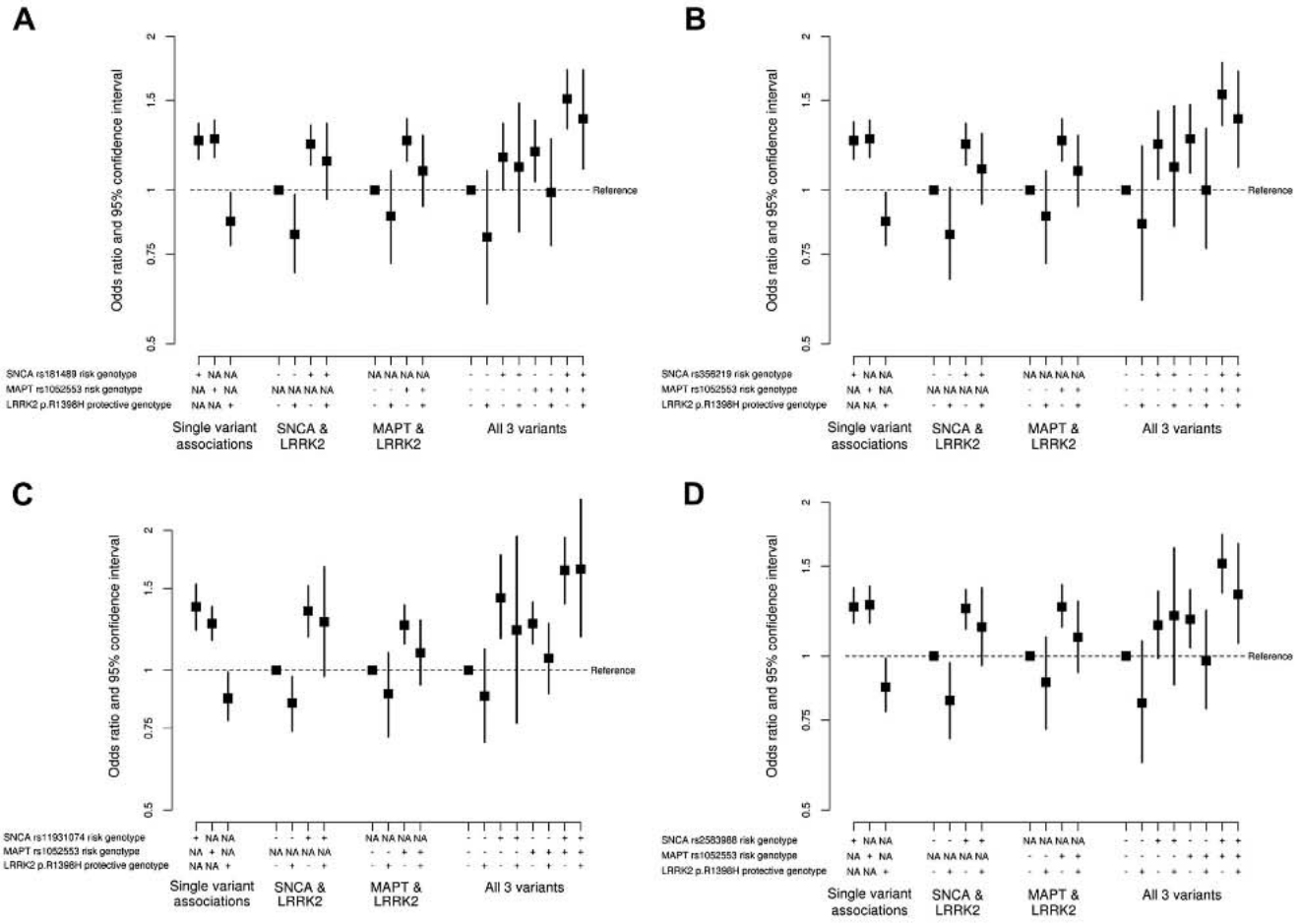

Fig. 1. (A) Individual and combined effects of *SNCA* rs181489, *MAPT* rs1052553, and LRRK2 p.R1398H on risk of Parkinson's disease (PD) in the Caucasian series. For *SNCA* rs181489, the risk genotype was CT or TT (i.e., presence of the minor allele). (B) Individual and combined effects of *SNCA* rs356129, *MAPT* rs1052553, and LRRK2 p.R1398H on risk of PD in the Caucasian series. For *SNCA* rs356129, the risk genotype was AG or GG (i.e., presence of the minor allele). (C) Individual and combined effects of *SNCA* rs11931074, *MAPT* rs1052553, and LRRK2 p.R1398H on risk of PD in the Caucasian series. For *SNCA* rs11931074, the risk genotype was GT or TT (i.e., presence of the minor allele). (D) Individual and combined effects of *SNCA* rs2583988, *MAPT* rs1052553, and LRRK2 p.R1398H on risk of PD in the Caucasian series. For *SNCA* rs2583988, the risk genotype was CT or TT (i.e., presence of the minor allele). (A–D) For *MAPT* rs1052553, the risk genotype was AA (i.e., presence of 2 copies of the major allele); for LRRK2 p.R1398H, the protective genotype was GA or AA (i.e., presence of the minor allele). NA indicates that a given SNP was not involved in the particular portion of the analysis.

Table 3
Interactions of LRRK2 p.R1398H with *SNCA* variants in regard to susceptibility to Parkinson's disease (PD) in the Asian series

Variant/genotype	LRRK2 p.R1398H	Sample genotype count and frequency	Test of association		Test of interaction
			OR (95% CI)	*p* value	
Additive/genotype models[a]					
SNCA rs356219					
AA	GG	282 (12.9%)	1.00 (reference)	N/A	Additive model
AA	GA or AA	83 (3.8%)	0.64 (0.39–1.06)	0.087	OR = 1.17
AG	GG	808 (37.0%)	1.59 (1.21–2.09)	0.0009	95% CI = 0.87–1.59
AG	GA or AA	232 (10.6%)	1.19 (0.84–1.69)	0.33	*p* = 0.30
GG	GG	623 (28.5%)	2.09 (1.56–2.79)	6E-7	Genotype model[d]
GG	GA or AA	156 (7.1%)	1.84 (1.23–2.77)	0.0031	*p* = 0.59
SNCA rs11931074					
GG	GG	302 (13.3%)	1.00 (reference)	N/A	Additive model
GG	GA or AA	89 (3.9%)	0.61 (0.37–0.98)	0.044	OR = 1.25
GT	GG	843 (37.2%)	1.55 (1.19–2.02)	0.0012	95% CI = 0.93–1.69
GT	GA or AA	243 (10.7%)	1.06 (0.75–1.49)	0.75	*p* = 0.14
TT	GG	630 (27.8%)	1.90 (1.43–2.51)	7.8E-6	Genotype model[d]
TT	GA or AA	158 (7.0%)	1.75 (1.18–2.61)	0.0059	*p* = 0.31
Dominant model[b]					
SNCA rs356219					
AA	GG	282 (12.9%)	1.00 (reference)	N/A	OR = 1.23
AA	GA or AA	83 (3.8%)	0.64 (0.39–1.06)	0.087	95% CI = 0.71–2.14
AG or GG	GG	1431 (65.5%)	1.78 (1.38–2.31)	1.10E-5	*p* = 0.47
AG or GG	GA or AA	388 (17.8%)	1.41 (1.03–1.92)	0.030	
SNCA rs11931074					
GG	GG	302 (13.3%)	1.00 (reference)	N/A	OR = 1.25
GG	GA or AA	89 (3.9%)	0.61 (0.37–0.98)	0.043	95% CI = 0.74–2.15
GT or TT	GG	1473 (65.0%)	1.69 (1.31–2.17)	4.3E-5	*p* = 0.41
GT or TT	GA or AA	401 (17.7%)	1.28 (0.95–1.73)	0.11	
Recessive model[c]					
SNCA rs356219					
AA or AG	GG	1090 (49.9%)	1.00 (reference)	N/A	OR = 1.22
AA or AG	GA or AA	315 (14.4%)	0.72 (0.56–0.93)	0.011	95% CI = 0.78–1.92
GG	GG	623 (28.5%)	1.48 (1.21–1.83)	0.0002	*p* = 0.38
GG	GA or AA	156 (7.1%)	1.31 (0.93–1.87)	0.13	
SNCA rs11931074					
GG or GT	GG	1145 (50.6%)	1.00 (reference)	N/A	OR = 1.39
GG or GT	GA or AA	332 (14.7%)	0.66 (0.52–0.85)	0.0011	95% CI = 0.90–2.17
TT	GG	630 (27.8%)	1.38 (1.12–1.69)	0.0020	*p* = 0.14
TT	GA or AA	158 (7.0%)	1.27 (0.90–1.80)	0.18	

ORs and *p* values result from fixed-effects logistic regression models. For tests of association, the 2 given variants were combined into 1 variable, and the model was adjusted for site. For tests of interaction, models included each of the 2 variants, their interaction, and site. Additive models, genotype models, dominant models, and recessive models refer to the characterization of *SNCA* variants; only dominant models were considered for LRRK2 p.R1398H because of the small number of rare homozygotes for this variant. Key: CI, confidence interval; OR, odds ratio.

[a] Interaction ORs under an additive model are interpreted as the multiplicative increase in the effect of the minor allele for LRRK2 p.R1398H on PD corresponding to each additional risk allele for *SNCA* variants, or alternatively as the as the multiplicative increase in the effect of each additional risk allele for *SNCA* variants on PD corresponding to presence of the minor allele for LRRK2 p.R1398H.

[b] Interaction ORs under a dominant model are interpreted as the multiplicative increase in the effect of the minor allele for LRRK2 p.R1398H on PD corresponding to presence of the risk allele for *SNCA* variants, or alternatively as the as the multiplicative increase in the effect of presence of the risk allele for *SNCA* variants on PD corresponding to presence of the minor allele for LRRK2 p.R1398H.

[c] Interaction ORs under a recessive model are interpreted as the multiplicative increase in the effect of the minor allele for LRRK2 p.R1398H on PD corresponding to presence of 2 risk alleles for *SNCA* variants, or alternatively as the as the multiplicative increase in the effect of presence of 2 risk alleles for *SNCA* variants on PD corresponding to presence of the minor allele for LRRK2 p.R1398H.

[d] Tests of interaction under a genotype model do not produce a single interaction OR, and therefore only a *p* value is given.

subgroup of Asian individuals for whom that information was available (71%) and also under a random effects model (Supplementary Table 8). Interactions between LRRK2 p.R1398H and *SNCA* variants under additive and recessive models are shown in Supplementary Table 11 separately for each Asian country; between-site heterogeneity in interactions with *LRRK2* p.R1398H was moderate for both *SNCA* rs356219 and rs11931074 in the Asian series (I^2 = 46%–55%, $p > 0.084$, Supplementary Table 8).

4. Discussion

Recently, a 3-variant (p.N551K-R1398H-K1423K) haplotype in the *LRRK2* gene was shown to affect susceptibility to PD in a protective manner in both Caucasian and Asian populations (Ross et al., 2011; Tan et al., 2010). The p.R1398H substitution appears to be the most likely functional variant, as it is located in the conserved Roc domain, and there is supporting evidence of reduced kinase activity (Tan et al., 2010). Although a number of previous investigations have examined interactions between the well-validated PD susceptibility variants located in the *SNCA* and *MAPT* genes (Biernacka et al., 2011; Elbaz et al., 2011; Goris et al., 2007; Mamah et al., 2005; McCulloch et al., 2008; Simón-Sánchez et al., 2009; Trotta et al., 2012; Wider et al., 2011), no study reported to date has examined interactions of LRRK2 p.R1398H with *SNCA* and *MAPT* variants. The results of our large case-control study involving both Caucasian and Asian individuals indicate that the protective effect of LRRK2 p.R1398H is observed consistently for different *SNCA* and *MAPT* genotypes, whereas, similarly, the *SNCA* and *MAPT* risk effects are observed for individuals with and without the protective p.R1398H allele.

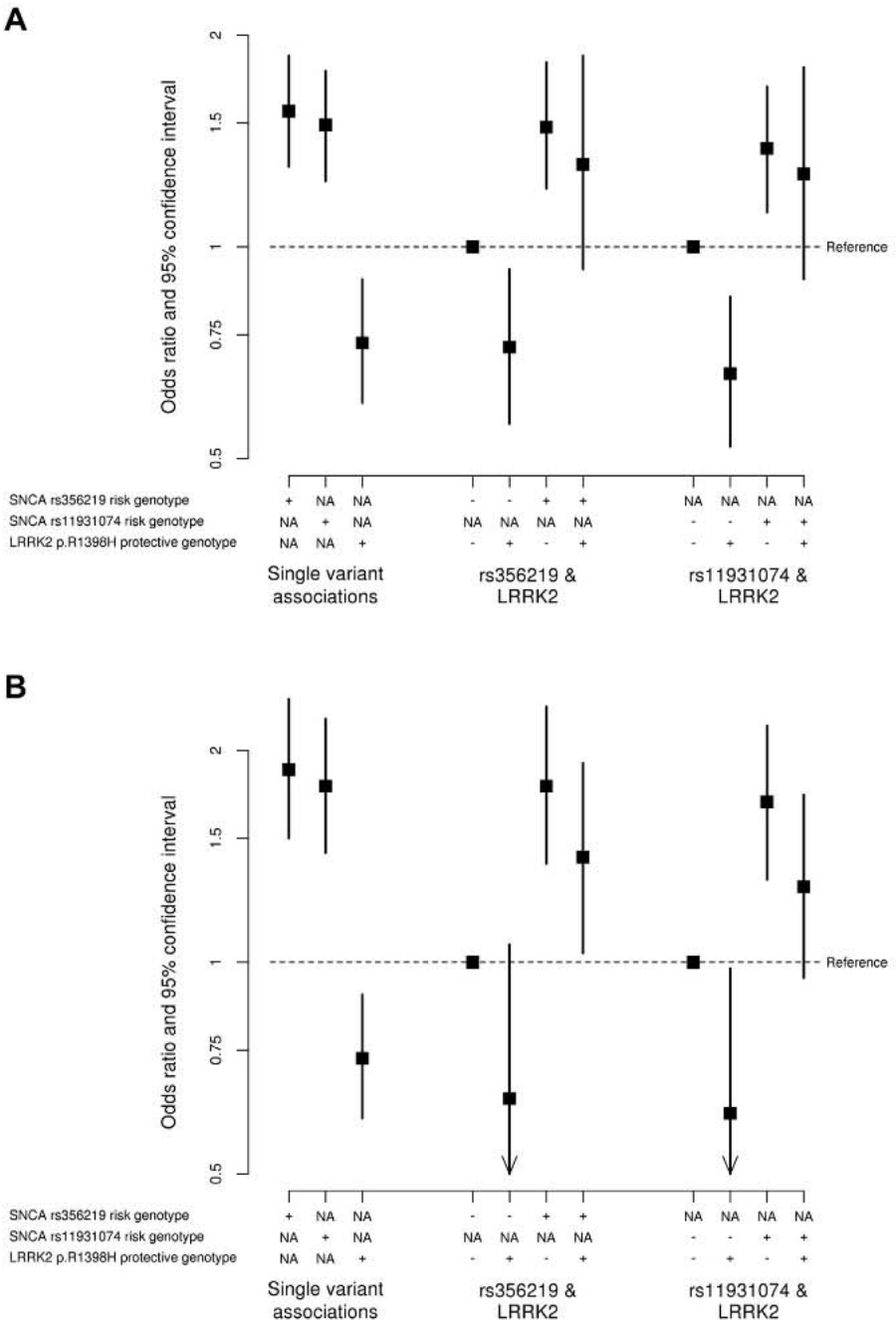

Fig. 2. (A) Individual and combined effects of *SNCA* rs356219, *SNCA* rs11931074, and LRRK2 p.R1398H on risk of Parkinson's disease (PD) in the Asian series. *SNCA* rs356219 and rs11931074 were considered under a recessive model (i.e., presence vs. absence of 2 copies of the minor allele). For *SNCA* rs356219, the risk genotype was GG. For *SNCA* rs11931074, the risk genotype was TT. (B) Individual and combined effects of *SNCA* rs356219, *SNCA* rs11931074, and LRRK2 p.R1398H on risk of PD in the Asian series. *SNCA* rs356219 and rs11931074 were considered under a dominant model (i.e., presence vs. absence of the minor allele). For *SNCA* rs356219, the risk genotype was AG or GG. For *SNCA* rs11931074, the risk genotype was GT or TT. (A and B) For LRRK2 p.R1398H, the protective genotype was GA or AA (i.e., presence of the minor allele). NA indicates that a given SNP was not involved in the particular portion of the analysis.

Despite the relatively large number of interactions and statistical models considered, the independent effects on PD risk for LRRK2 p.R1398H, *MAPT* rs1052553, and *SNCA* variants were observed with a very high level of consistency in our study. This was most apparent in the large Caucasian series, for which all interaction ORs were between 0.80 and 1.13, with the exception of the 2 aforementioned instances involving rare genotypes for *MAPT* rs1052553 and *SNCA* rs11931074. In addition, between-site heterogeneity in interaction effects was low to moderate in Caucasians. Although the protective effect of LRRK2 p.R1398H on risk of PD was observed consistently across *SNCA* variant genotypes in Asians, perhaps the least convincing evidence of lack of gene–gene interaction was observed in this series. Albeit not approaching significance even before adjustment for multiple testing, the magnitude of this observed protective effect was slightly smaller when the risk genotype for *SNCA* variants was present, whereas, conversely, the observed risk effects of *SNCA* variants were marginally stronger in individuals with the protective p.R1398H genotypes. In addition,

heterogeneity in interaction effects between sites was highest in the Asian series. However, it is important to highlight that it would be very unusual to observe a complete lack of gene–gene interaction (i.e., interaction OR = 1) in all scenarios simply because of natural sampling variability, particularly given the number of possible interactions that were examined. Nonetheless, given the smaller size of our Asian series in comparison to the Caucasian series, it will be important to validate our findings in larger series of Asian individuals.

Recent studies have supported our earlier work indicating that the effects of *SNCA* and *MAPT* variants on PD risk are independent of one another (Biernacka et al., 2011; Trotta et al., 2012; Wider et al., 2011). Although our current study is the first to date to examine the potential interaction of the protective LRRK2 p.R1398H substitution with *MAPT* and *SNCA* variants in regard to risk of PD, previous studies have evaluated interactions with, or combined effects of, *LRRK2* variants and those in *SNCA* and *MAPT*. In their analysis of 1098 patients with PD and 1098 matched controls from the United States (a subset of which were also used in the current study), Biernacka et al. (2011) found no statistically significant evidence of gene–gene interaction when considering 8 intronic *LRRK2* variants, 10 *SNCA* variants (8 intronic, 1 3′ downstream and 1 5′ Rep1), and 8 *MAPT* variants (6 intronic, 1 3′ UTR, and 1 H1/H2). Wang et al. (2012) concluded that other genes, including *MAPT* and *SNCA*, modified *LRRK2*-related risk for PD in a Chinese cohort of 2013 sporadic PD patients and 1971 controls. This was based on findings that, in comparison to individuals harboring only the LRRK2 p.G2385R or p.R1628P risk variants, the risk of PD is increased in individuals with these and other PD risk variants. However, it is unclear whether this represents independent or interactive effects, and the sample sizes of the combined risk-variant groups examined were quite small. The results of these studies are consistent with those of our own, with the effect of *LRRK2* variants on PD susceptibility appearing to be independent of *SNCA* and *MAPT* risk factors for PD.

The strengths of our study, including the large sample size and inclusion of subjects from a variety of different populations, are important to highlight; however, several limitations should also be acknowledged. A key question is whether the lack of interaction of LRRK2 p.R1398H with *SNCA* and *MAPT* variants is a consequence of sample size or the frequencies of the examined variants. To assess the possibility of a false-negative association, it is most helpful to examine 95% confidence limits for observed interaction odds ratio estimates (Goodman and Berlin, 1994). These confidence limits were generally relatively tight in the larger Caucasian series, indicating a lack of a biologically significant interaction in this population, but were wider in the Asian series, further highlighting the need for validation of our findings in that series. In addition, as is generally the case for large-scale collaborative studies attempting to address a focused research question that involves a small number of genetic variants, without available genome-wide population control markers, population stratification could potentially have had an impact on our results. However, this potential limitation is lessened by the fact that our logistic regression models were adjusted by site, which makes any possible population stratification a site-specific issue. Other limitations of our study include the different diagnostic criteria across the different sites and the lack of a standardized inclusion/exclusion criteria for patients with PD and controls.

In conclusion, our study provides evidence that the effect of LRRK2 p.R1398H on risk of PD is independent of the *MAPT* H1-haplotype defining variant rs1052553 and *SNCA* variants, and vice versa. This lack of gene–gene interaction was apparent in both our large Caucasian patient-control series and our smaller Asian series. Evaluation of interactions involving individuals of other ethnic backgrounds, other rarer *LRRK2* susceptibility variants, and PD susceptibility variants at other loci (Lill et al., 2012) is needed in order to move toward a fuller understanding of the genetic architecture of PD susceptibility.

Disclosure statement

J.O.A., M.J.F., and Z.K.W. report holding a patent on *LRRK2* genetic variability. M.J.F. has received royalties for licensing of genetically modified *LRRK2* mouse models. D.M.M. declares a patent pending entitled "Methods to Treat PD." C.K. and R.K. declare receiving payment in their role as consultants for Centogene and Takeda Pharmaceutical, respectively. All other authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by a grant from The Michael J. Fox Foundation for Parkinson's Research (O.A.R., M.J.F.). Original funding for the GEO-PD was supported by a grant from The Michael J. Fox Foundation for Parkinson's Research Edmond J. Safra Global Genetics Consortia program (D.M.M.). The Mayo Clinic Jacksonville is a Morris K. Udall Center of Excellence in Parkinson's Disease Research (grant no. P50 NS072187) and was supported by a the gift from the family of Carl Edward Bolch, Jr, and Susan Bass Bolch (R.J.U., Z.K.W., O.A.R.). O.A.R. acknowledges funding support from the National Institutes of Health (grant no. R01 NS078086). This research was undertaken, in part, thanks to funding from the Canada Excellence Research Chairs program (M.J.F., C.V.G.). Leading Edge Endowment Funds, provided by the Province of British Columbia, LifeLabs, and Genome BC, support the Dr Donald Rix BC Leadership Chair (M.J.F.). D.M.M. acknowledges the National Institutes of Health for funding support (grant no. R01ES10751). Studies at individual sites were supported by a number of different funding agencies world-wide including; Italian Ministry of Health (Ricerca Corrente, Ricerca Finalizzata); the Swedish Parkinson Academy; the Swedish Parkinson Foundation; the Federal Ministry for Education and Research (BMBF, NGFNplus; 01GS08134) (R.K.); the NGFNplus (Neuron-Parkinson-subproject 7) (S.G.); CHRU de Lille, University of Lille 2, INSERM; French Ministry PHRCs (1994/, 2002/1918, 2005/1914); Association France Parkinson (2005); Fondation de France 2004-013306; Fondation de la Recherche Médicale (2006); PPF (synucléothèque 2005–2009); the 2 Centres de Ressources Biologiques (IPL-Lille, CHRU-Lille) and its scientific committee (A.D., M.C.C.H., Philippe Amouyel, Florence Pasquier, Régis Bordet); funding from France-Parkinson Association and the program "Investissement d'avenir" ANR-10-IAIHU-06; the Swedish Research Council; the Swedish Society for Medical Research; the Swedish Society of Medicine; funds from the Karolinska Institutet and the Parkinson Foundation in Sweden (K.W.); the National Institutes of Health and National Institute of Neurological Disorders and Stroke (grant nos. 1RC2NS070276, NS057567, and P50NS072187); Mayo Clinic Florida Research Committee CR programs (M.J.F., Z.K.W.); the Geriatric Medical Foundation of Queensland (G.D.M.); a career development award from the Volkswagen Foundation and from the Hermann and Lilly Schilling Foundation (C.K.); the Research Committee of University of Thessaly (code 2845); and Laboratory of Neurogenetics, Biomedicine Department, CERETETH, Larissa, Greece (code 01-04-207) (G.H., E.D.).

A number of individuals must be acknowledged for their contributions to make this work possible; Ferdinanda Annesi, PhD; Patrizia Tarantino, PhD (Institute of Neurological Sciences, National Research Council); Monica Gagliardi, PhD, (Institute of Neurological Sciences, National Research Council, Cosenza Italy), Chiara Riva,

PhD (Department of Neuroscience and Biomedical Technologies, University of Milano-Bicocca, Monza, Italy); Roberto Piolti, MD (Department of Neurology, Ospedale San Gerardo, Monza, Italy); Alessandro Ferraris MD, PhD (IRCCS Casa Sollievo della Sofferenza Hospital, Mendel Laboratory, San Giovanni Rotondo, Italy); Aurélie Duflot, (UMR837 Inserm-Univ Lille 2, CHRU de Lille), Jean-Philippe Legendre, Nawal Waucquier (Neurologie et Pathologie du Mouvement, Clinique de Neurologie du CHU de Lille). Anna Rita Bentivoglio, MD, PhD, Tamara Ialongo, MD, PhD, Arianna Guidubaldi, MD, Carla Piano, MD (Institute of Neurology, Catholic University, Rome, Italy); Phil Hyu Lee, MD, PhD (Department of Neurology, Yonsei University College of Medicine, Seoul, Korea); Jan Reimer (Department of Neurology, Skåne University Hospital, Sweden); Hiroyo Yoshino, PhD, Manabu Funayama, PhD, Yuanzhe Li, MD, PhD (Juntendo University School of Medicine, Tokyo, Japan). From the Queensland Parkinson's Project: R.S. Boyle and A. Sellbach (Princess Alexandra Hospital, Brisbane), J. D. O'Sullivan (Royal Brisbane and Women's Hospital, Brisbane), G.T. Sutherland, G.A. Siebert and N.N.W. Dissanayaka (Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, QLD).

Finally, we acknowledge all of the patients and control subjects who kindly donated DNA to make collaborative studies like these possible.

A full list of GEO-PD consortium member sites is provided in the Supplementary Text.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.013.

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