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SNCA and *mTOR* Pathway Single Nucleotide Polymorphisms Interact to Modulate the Age at Onset of Parkinson's Disease

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Abstract

Background—Single nucleotide polymorphisms (SNPs) in the α-synuclein *(SNCA)* gene are associated with differential risk and age at onset (AAO) of both idiopathic and Leucine-rich repeat kinase 2 (LRRK2)-associated Parkinson's disease (PD). Yet potential combinatory or synergistic effects among several modulatory SNPs for PD risk or AAO remain largely underexplored.

Objectives—The mechanistic target of rapamycin *(mTOR)* signaling pathway is functionally impaired in PD. Here we explored whether SNPs in the *mTOR* pathway, alone or by epistatic interaction with known susceptibility factors, can modulate PD risk and AAO.

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InternationalParkinson's Disease Genomics Consortium members are listed in the Appendix.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Methods—Based on functional relevance, we selected a total of 64 SNPs mapping to a total of 57 genes from the *mTOR* pathway and genotyped a discovery series cohort encompassing 898 PD patients and 921 controls. As a replication series, we screened 4170 PD and 3014 controls available from the International Parkinson's Disease Genomics Consortium.

Results—In the discovery series cohort, we found a 4-loci interaction involving *STK11* rs8111699, *FCHSD1* rs456998, *GSK3B* rs1732170, and *SNCA* rs356219, which was associated with an increased risk of PD (odds ratio = 2.59, $P < .001$). In addition, we also found a 3-loci epistatic combination of *RPTOR* rs11868112 and *RPS6KA2* rs6456121 with *SNCA* rs356219, which was associated (odds ratio = 2.89; $P < .0001$) with differential AAO. The latter was further validated (odds ratio = 1.56; $P = 0.046–0.047$) in the International Parkinson's Disease Genomics Consortium cohort.

Conclusions—These findings indicate that genetic variability in the *mTOR* pathway contributes to *SNCA* effects in a nonlinear epistatic manner to modulate differential AAO in PD, unraveling the contribution of this cascade in the pathogenesis of the disease.

Keywords

age at onset; alpha-synuclein; epistasis; *mTOR*; Parkinson's disease; SNP

Parkinson's disease (PD) is a neurodegenerative disease that is characterized by α-synuclein (SNCA) aggregates and neural loss in several brain stem nuclei. Mainly, dopaminergic neurons loss in the substantia nigra leads to the related cardinal motor symptoms of bradykinesia, resting tremor, and rigidity.[1] Intriguingly, the cause of this neural loss is still unknown. Whereas genetic mutations in PD causative genes represent 5% to 10% of total PD patients, the vast majority of cases are idiopathic PD (IPD).[2] The age at onset (AAO) of the motor symptoms and the progression of PD are variable,[3,4] and their modulatory factors remain largely unknown. Yet single nucleotide polymorphisms (SNPs) in the *SNCA* and the microtubule-associated protein Tau *(MAPT)* genes have shown top-hit association signals with PD risk in genomewide association studies[5,6] and in candidate gene studies.[7,8] For instance, the SNP rs356219 in the 3' untranslated region (3'-UTR) of *SNCA* is a haplotype-tag SNP associated with a higher risk of PD[9,10] and related to increased SNCA protein expression.[11,12] This tag SNP also modulates AAO in both IPD and leucine-rich repeat kinase 2 (LRRK2)- associated PD (L2PD).[13,14] Although IPD genetic risk factors such as the glucocerebrosidase gene and others have been identified,[15–18] the overall heritability of IPD as a complex multifactorial disorder remains unclear.

An extended hypothesis in IPD is that multiple genetic susceptibility factors along with environmental cues[19] and their potential interactions modulate disease presentation. In this sense, the genetic contribution to sporadic diseases such as PD can be additive when the effect of multiple genes is exerted in a linear fashion or nonadditive when the genetic effect is nonlinear and can include either dominance (intralocus interaction) or epistatic (interlocus interaction) effects. The latter, epistasis, is considered the complex effect of one gene upon other genes,[20] and it is also suspected as an important genetic component of complex diseases such as IPD.[21] Still, the potential epistatic effects among different susceptibility risk

factors for IPD, despite their plausibility, remain largely neglected and underexplored in this disease.[22]

In this context, the signaling cascades regulating the neurodegenerative process in PD are not well established, but several pathways have been proposed.[23] Recent studies have reported that deregulation of the mechanistic target of rapamycin *(mTOR)* pathway occurs in PD.[24–27] For instance, *mTOR* and Akt activities are impaired in nigral neurons in human postmortem PD brains. Interestingly, restitution of *mTOR* or Akt function in cellular and animal models of PD prevents neuron cell death, pointing out a relevant contribution of this signaling cascade to PD pathophysiology.[24,25,28] In basal conditions, the *mTOR* signaling integrates basic cellular functions such as growth, proliferation, and survival. Specifically in the brain, *mTOR* has a key role in neural development, neuron survival, synaptic plasticity, and memory formation.[29] The central hub of the pathway is *mTOR*, a serine/threonine kinase that is present in 2 different multiprotein complexes, *mTORC1* and mTORC2. When the multiprotein complex contains *mTOR* and Raptor, among other interactors, it is called mTORC1 and controls protein translation and autophagy.[30,31] When *mTOR* multiprotein complex binds to Rictor, it is called mTORC2 and regulates actin polymerization and Akt trophic activity.[30,32]

Based on this biochemical evidence, we performed an *mTOR* genetic pathway candidate approach to explore whether common genetic variability in genes from the *mTOR* signaling cascade could modulate the risk or the AAO of PD. To this end, we genotyped 64 SNPs mapping to 57 genes of the *mTOR* pathway in a discovery series cohort of 898 PD patients and 921 healthy controls (total N = 1819). By classical allelic and genotypic association analyses, we investigated whether individual *mTOR* SNPs are associated with differential susceptibility and AAO of PD. By using the multifactor dimensionality reduction (MDR) method,[33–35] we assessed potential multilocus epistatic interactions of SNPs in *mTOR* genes and classical PD-associated SNPs in *SNCA* and *MAPT* in modulating PD risk and AAO. Furthermore, we performed a validation analysis in a replication series cohort of 4170 PD and 3014 controls (total N = 7184 subjects) available from The International Parkinson's Disease Genomics Consortium (IPDGC). Our study uncovers for the first time novel higher order genetic interactions influencing PD thus exploring a novel field in PD genetic research.

Methods

Cohort of Study and Data Collection

Discovery Series—This study included 1819 subjects (898 PD cases and 921 unrelated healthy controls) of Spanish ancestry. The PD population ratio consisted of 76/100 women/men (43.31/56.69%), with age at PD onset of 55.30± 13.12 (mean ± standard deviation) years in men and 57.75 ± 13.12 years in women and age at sample collection of 64.37 ± 12.25 years in men and 67.52 ± 11.31 years in women. Sex-matched, age-matched, and demographic-matched controls ratio consisted of 71/100 women/men (41.69/58.31%) with age at sample collection of 53.79 ± 12.16 years in men and 56.83 ± 14.30 years in women. In addition, we also genotyped an independent set of 127 L2PD patients carrying the *LRRK2* p.G2019S mutation. This cohort consisted of a ratio of 84/100 men/women, with

age at PD onset of 55.64 ± 14.20 years in men and 58.85 ± 13.56 years in women and age at sample collection of 58.30± 16.66 years in men and 62.22 ± 16.99 years in women.

All PD participants were residents in the northeastern region of the Iberian Peninsula (Catalonia) with European origin and were recruited at the Movement Disorders Unit of the Hospital Clinic de Barcelona. Patients had a clinical diagnosis of definite PD according to Unified Parkinson's Disease Rating Scale (UPDRS) criteria[36] except that family history was not used as exclusion criterion or a neuropathological diagnosis of definite PD according to proposed criteria.[37] The control individuals (total N = 921) encompassed 75 healthy spouses of the patients recruited at the Hospital Clinic de Barcelona by expert neurologists specialized in movement disorders. The remaining 846 controls were population-based controls collected at the Spanish National DNA Bank of Salamanca. This DNA bank hosts a collection of DNA samples and their associated clinical, genealogical, and lifestyle data that are representative of the healthy Spanish population. Specifically, the population-based controls used in the current study passed a questionnaire on health status supervised by a physician and reported no signs, symptoms, or familial history of PD or of other neurological diseases. At recruitment, informed written consent was obtained and whole blood samples were obtained from each participant. Genomic DNA was isolated from peripheral blood lymphocytes as previously described[38] and stored at −80°C until use. The study was approved by the ethics committee of the Hospital Clinic de Barcelona.

Replication Series—As a replication series, we used a large cohort consisting of 7184 subjects (4170 PD patients and 3014 controls) of Spanish ancestry from the IPDGC. The PD population consisted of 1861/2309 women/men (44.62/55.37%), with age at PD onset of 59.74 ± 12.69 years in men and 61.69 ± 12.57 years in women. Sex-matched, age-matched, and demographic-matched controls consisted of 1655/1359 women/men (54.92/45.08%) with age at sample collection of 62.51 ± 15.56 years in men and 62.68 ± 11.50 years in women.

SNP Selection Criteria and Genotyping

A total of 64 SNPs from 57 genes in the *mTOR* pathway and SNPs from genes involved in PD *(SNCA, MAPT, LRRK2,* or *PRKN)* were selected on the basis of the following criteria: (i) a minor allele frequency above 0.1 based on data from the HapMap project or in 1000Genomes[39] and (ii) a published (Pubmed) association or functional deregulation of the SNP in human disease specifically including neurological and psychiatric disorders (Table S1). All of the SNPs were genotyped using TaqMan OpenArray Genotyping Plates (Madrid, Spain), Custom Format 64 QuantStudio TM 12K Flex (Madrid, Spain), in the Genomics Core facility (Universitat Pompeu Fabra, Parc de Recerca Biomedicaède Barcelona, Barcelona, Catalonia, Spain). SNPs that were not in Hardy-Weinberg equilibrium or did not surpass a genotyping call-rate threshold of 0.95 in all studied samples were filtered out. This quality control reduced the list to 54 SNPs, including 52-*mTOR* related genes along with *SNCA* and *MAPT* SNPs.

For the replication cohort, samples were genotyped using the customized NeuroChip Array v.1.0 or v.1.1 (Illumina, San Diego, California, USA).[40] Quality control analysis was

performed as previously described.[5] Individuals related at the level of cousins or closer (sharing proportionally more than 18.5% of alleles) were dropped from the following analysis. Samples were clustered using principal component analysis to ensure European ancestry as compared to the HapMap3 Utah residents with northern and western european ancestry from the CEPH collection/Toscani in Italia (CEU/TSI) populations.[39]

Statistical Analyses

Hardy-Weinberg Equilibrium—We assessed each SNP for Hardy-Weinberg equilibrium separately in cases and controls using a Fisher exact test.

Allelic Association Analysis—We calculated the differences of SNP allelic frequencies between cases and controls using the Expectation- Maximization algorithm as implemented in the statistical package UNPHASED version 3.1.7,[41] adjusting by potential confounders, including gender and age. We used a threshold of .05 for statistical significance. We corrected all *P* values for multiple testing by using the Benjamini and Hochberg method[42] (n = 54 tests).

Genotypic Association Analysis—Statistically significant SNPs detected in the allelic analysis were further analyzed at the genotypic level under the different possible models of inheritance as computed in the software SNPstats (http://bioinfo.iconcologia.net/SNPstats), [43] considering gender and age as covariates and adjusting *P* values by the Benjamini and Hochberg method.[42] As implemented in SNPstats, among all possible models of inheritance for each SNP, the model best fitting the data was defined automatically as the model with the lowest Akaike information value and therefore minimized expected entropy.

Epistatic Association Analysis—We used the MDR software version 3.04 (http://www.multifactordimensionalityreduction.org/) to detect high-order SNP interactions associated with the risk or the AAO of PD. The MDR method is based on a data-mining strategy for detecting combinations of discrete attributes, such as SNPs, or those that are predictive of an outcome, such as case or control status.[34] PD risk was considered a discrete outcome, whereas PD AAO was analyzed as continuous (quantitative) outcome. The MDR analyses were performed using 10-fold cross-validation, and the best model was selected based on balanced accuracy (for PD risk) or the build-in Student *t*-test (for PD AAO) and cross-validation consistency (CVC) scores. The CVC is the number of times a particular SNP combination is identified out of the 10 cross-validations. Statistical significance was evaluated by performing a 1000-time permutation test using the option “explicit test for epistasis,” which specifically tests for interactions and provides multiple testing correction. [44] We used a threshold of .05 for significance.

Results

After filtering out SNPs that were not in Hardy- Weinberg equilibrium or SNPs that did not surpass the quality threshold of unambiguous genotypes above 0.95 in all studied samples, we obtained a set of 54 SNPs (Table S1) that were further analyzed for single or multiple associations with risk or AAO of PD in the discovery series.

Single Marker Association of *SNCA* and *MAPT* With the Risk of IPD

In the discovery series, we first performed allelic association analysis of single markers with the overall PD susceptibility. After adjusting by gender, age, and multiple testing (n = 54 tests), we found a statistically significant allelic association of SNP rs356219 in the *SNCA* gene with a differential PD risk in which the G risk allele had a frequency of 0.41 in cases and 0.33 in controls (odds ratio, OR [95% confidence interval, CI] = 1.35 [1.16–1.57], adjusted $P = .0054$). We also confirmed the previously described (45) significant association of SNP rs1800547 in the *MAPT* gene with PD risk (OR [95% CI] = 0.75 [0.64–0.88], adjusted $P = .01$; Table S2). At the genotypic level, we also detected significant association of *SNCA* rs356219 (OR [95% CI] = 1.36 [1.18–1.56], adjusted $P = .0027$) and also of *MAPT* rs1900547 (OR [95% CI] = 1.33 [1.15–1.54], adjusted $P = .0027$), both under a log-additive model of inheritance (Table 1). These data are in agreement with previous findings from our group[7,8] and from others.[13,45,46] On the contrary, we did not find a significant association with PD risk for any of the other 52 *mTOR* genetic markers, but top signals that did not reach statistical significance included *DDIT4L* rs1053227 (unadjusted $P = .01$) and *EIF4EBP1* rs6605631 (unadjusted $P = .01$; Table 1).

Association of *SNCA* and *mTOR* Interactions With the Risk of IPD

Subsequently, in the discovery series we explored possible gene-gene interactions involving more than 2 loci by MDR analysis. Using this approach, we found that SNP rs356219 in *SNCA* synergistically interacts with *mTOR* markers, modulating the risk of PD, including the SNPs rs8111699 in *SKT11,* rs456998 in *FCHSD1,* and rs1732170 in *GSK3B* (OR [95% CI] = 2.59 [2.14–3.13], explicit epistasis test $P < .001$; Table 2 and Supporting Information Fig. 1). In addition, the CVC for this 4-marker model was 10, and both the training and testing balanced accuracies were around 60% (precision, specificity, and sensitivity; Table S3). However, we could not replicate these results in the IPDGC cohort (Table S4), and thus they are likely to be attributable to a main effect of the *SNCA* marker.

Single-Marker Association of *SNCA* With the AAO of IPD

We further performed a single-marker association analysis of the studied SNPs with the AAO of IPD in the discovery series. We analyzed whether those SNPs significantly associated with PD risk could also modulate the AAO at a single-marker level. For this reason, only the SNP in *MAPT* and *SNCA* were subjected to the analysis. In the single-marker genotypic analysis, adjusting by gender, age, and multiple testing (n = 2 tests), we found that SNP rs356219 in *SNCA* was the only marker associated with differential AAO of PD in our sample. Specifically, we observed a mean IPD AAO ± Standard Error of the Mean (S.E.M). of 55.6 ± 0.8 years for GG carriers, 55.8 ± 0.5 for AG, and 58.0 ± 0.5 for AA (adjusted $P = .0034$) following a log-additive model, that is, an overall IPD AAO difference of approximately 2.5 years attributable to this SNP. These data are consistent with previous results reported in IPD[13,47] or in L2PD,[7,14] although in the latter, *SNCA* rs356219 seemed to have a stronger effect on the L2PD AAO with a difference of up to 11 years (58 years for GG carriers vs 69 years for AA).[14] In addition, we did not find an association of *MAPT* rs1800547 with the AAO of PD (Table 3).

SNCA and *mTOR* Epistatic Interactions Modify the AAO of IPD

We then explored whether epistatic combinations of *mTOR* SNPs were associated with the AAO of idiopathic PD in the discovery series. Using the MDR analysis, we found that *SNCA* SNP rs356219 interacts with rs11868112 in *RPTOR* and rs6456121 in *RPS6KA2* in modulating the AAO of IPD with the maximum CVC of 10/10 (OR [95% CI] = 2.89 [2.90–4.00], explicit epistasis test $P < .001$; Table 4 and Supporting Information Fig. 2). Interestingly, in the IPDGC replication series, we confirmed the epistatic interaction of this 3-loci and their association with differential AAO of IPD with the maximum CVC of 10/10, although with a slighter effect magnitude (OR [95% CI] = 1.56 [1.34–1.81], explicit epistasis test $P = .046$-.047; Table 5 and Supporting Information Fig. 2), thus validating findings from the discovery series.

We also explored whether this 3-loci epistatic interaction modulates AAO in our cohort of 127 L2PD patients whose mean AAO was 56.74 years, similar to the mean AAO of 56.66 years observed in IPD. Despite the relative limited number of participants, we exploratively performed a forced MDR analysis for this 3-loci interaction and observed a similar association trend in L2PD as in IPD that did not reach statistical significance (OR [95% CI] = 3.99 [1.89–8.40], explicit epistasis test $P = .1170$-.1180; Table S5). However, when we oversampled this L2PD cohort to the number of IPD patients (N = 748), we could again observe the epistatic association of these 3 SNPs with L2PD AAO, thus suggesting a similar effect in L2PD as in IPD, which should be further validated in larger L2PD cohorts (data not shown; OR [95% CI] = 4.68 [2.56–8.58], explicit epistasis test $P < .001$).

In summary, these results indicate that there is a higher order complex epistatic effect of *SNCA* rs356219 with markers *RPTOR* rs11868112 and *RPS6KA2* rs6456121 from the *mTOR* pathway in modulating the AAO of IPD. This finding illustrates the proof of principle that epistatic interactions acting on known PD genetic risk factors can further modulate disease presentation.

Discussion

Here, we report for the first time that common genetic variability in the *mTOR* genetic pathway synergistically interacts with known PD risk SNPs in *SNCA* to modulate the AAO of IPD. The association of genetic polymorphisms such as *SNCA* rs356219 or of *MAPT* rs1800547 with the risk of PD was previously reported in our cohort[7,48] and in other cohorts[45,46] as well as by genomewide association studies.[5,6] Here we also observed significant association of these markers with PD risk. At the multilocus level for the *MAPT* polymorphism, we did not find any epistatic interaction with other SNPs. However, for *SNCA,* our data set established the grounds to perform further epistatic analyses involving the candidate *mTOR* pathway. Indeed, at a multilocus level, we did detect a higher order loci epistatic interaction comprising *SNCA* rs356219, *SKT11* rs8111699, *FCHSD1* rs456998, and *GSK3B* rs1732170, which was associated with a differential risk for PD in the discovery series. However, we did not replicate this finding in the IPDGC cohort. This negative result suggests that the SNCA SNP could be driving the main effect in PD risk or, alternatively, that other factors such as genetic heterogeneity, linkage disequilibrium differences, population allele frequency difference, or even additional environmental effects could be

affecting the power to corroborate epistatic associations in populations other than the discovery series.[49]

As for the AAO of IPD, we found a significant association of *SNCA* rs356219 located in the 3'UTR with a 2.4 mean AAO difference of IPD with 55.6 years for carriers of the GG risk genotype and 58.0 years for AA carriers, but no association for *MAPT.* These findings are largely consistent with the most recent meta-analysis of PD AAO to date.[50] Previously, the *SNCA* rs356219 was shown to modulate the AAO of IPD in the German population (3 years difference; 55.7 years for GG vs 58.7 years for AA)[13] and also in another Northern Spain cohort genotyping of the *SNCA* 3'UTR neighboring marker rs356165, which is in absolute linkage disequilibrium with rs356219 (3.5 years difference; 60.1 years for GG vs 56.6 for AA).[47] The most recent meta-analysis of PD AAO to date has reported a similar effect for the *SNCA* 3'UTR rs356203 marker, yet with a slighter AAO effect of 0.6 years difference, probably because of the larger diversity of populations screened in this study.[50] In addition, in our Spanish sample we also found that *SNCA* rs356219 specifically influences the AAO of monogenic L2PD (11 years difference; 58 years for GG vs 69 for AA),[14] overall suggesting a greater effect of *SNCA* rs356165 on AAO in monogenic L2PD than in IPD. At the multilocus level, we found a 3-loci high-order epistatic interaction involving *SNCA* rs356219, *RPTOR* rs11868112, and *RPS6KA2* rs6456121, which was associated with IPD AAO. Indeed, the markers *RPTOR* rs11868112 and *RPS6KA2* rs6456121 were not individually associated with IPD AAO but only in conjunction with *SNCA* rs356165 and in an epistatic manner. Most important, we validated the association of this epistatic interaction with the AAO of IPD in the IPDGC replication series.[49,51] Overall, these findings indicate that common genetic variability in genes from the *mTOR* pathway, by genetic epistatic interaction with *SNCA*, can modulate the pathophysiology of PD and contribute to the AAO of IPD by influencing, either potentiating or diminishing, the effects of well-known risk factors of disease.

SNCA implications in the PD pathogenesis has been widely described at the genetic but also at the protein level (SNCA). In fact, the variant rs356219 of *SNCA* has been associated with enhanced transcription of *SNCA* and increased levels of SNCA.[11,12] Furthermore, we have found that *SNCA, RPTOR,* and *RPS6KA2* interact epistatically in modulating PD AAO. At the biological and biochemical levels, epistasis can be related with the presence of a physical interaction between proteins that participate in the same cellular pathway.[20,21] The *RPTOR* gene codifies for the Raptor protein that is the main component of the mTORC1 complex. This complex regulates important cellular functions such as protein synthesis and autophagy.[52] The third interacting marker is located in the *RPS6KA2* gene, which encodes ribosomal S6 kinase RSK3. This protein is a serine/threonine kinase that stimulates *mTOR* signaling activation and modulates protein synthesis initiation via the phosphorylation of the eukaryotic translation initiation factor B.[52] Increased protein synthesis and reduced autophagy in PD have been both related to SNCA accumulation and oligomerization and, thus, to dopaminergic neurodegeneration.[53–55] Hence, dysfunctional activation of the *mTOR* pathway can contribute to SNCA aggregation and the spreading of PD pathology in the brain.

Given the large number of genetic polymorphisms in genes from the *mTOR* pathway, in the order of thousands, the number of SNPs screened in our study is limited and could be scaled, most specially in selected promising candidates pinpointed in this study. In fact, it is conceivable that the genetic interactions identified here involve physical and functional interactions between the different proteins, a hypothesis that should be tested in further studies. These results also should be validated in other genetically similar populations, to dismiss not only the possibility of false positive association but also any potential population-specific effects. Overall, we found that genetic variability in the *mTOR* pathway interacts with *SNCA* risk variants modulating the effect of *SNCA* rs356219 and determining AAO of IPD. Our findings indicate that the individual effects of classical susceptibility loci associated with PD risk such as *SNCA* could be additionally influenced by genetic variability in other genes, as here shown with loci from the *mTOR* pathway, thus further contributing to elucidating the genetic contribution to IPD as a complex disease. Moreover, a relevant implication of our findings is that classical association of individual markers reported in PD, especially in large genomewide association studies, could be revisited in light of potential epistatic complex interactions among markers that until the present have been overlooked and poorly explored in the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1.

Genotypic association of SNPs in *SNCA* and *MAPT* with PD risk adjusted by sex, age, and multiple testing adjustments of *P* values

			PD			Control						
Gene	**SNP**	**Alleles, M/m**	**Freq. 11**	**Freq. 12**	**Freq. 22**	**Freq. 11**	**Freq. 12**	**Freq. 22**	**OR (95% CI)**	**Model**	**Genotypic *P* value**	**Adj. Gen. *P* value**
SNCA	rs356219	A (1)/G (2)	0.36	0.47	0.17	0.45	0.44	0.11	1.36 (1.18–1.56)	Log-additive	**<.0001**	**<.0027**
MAPT	rs1800547	A (1)/G (2)	0.56	0.38	0.06	0.49	0.41	0.11	1.33 (1.15–1.54)	Log-additive	**.0001**	**.0027**
DDIT4L	rs1053227	G (1)/A (2)	0.33	0.49	0.18	0.37	0.49	0.14	0.84 (0.73–0.96)	Log-additive	**.0100**	1350
EIF4EBP1	rs6605631	T (1)/C (2)	0.69	0.29	0.02	0.66	0.29	0.05	1.98 (1.16–3.38)	Recessive	**.0100**	.1350
LPIN1	rs7595221	A (1)/G (2)	0.29	0.48	0.23	0.25	0.53	0.22	1.23 (1.02–1.49)	Overdominant	**.0270**	.2916
PRKCA	rs887797	G (1)/A (2)	0.48	0.40	0.12	0.43	0.45	0.12	1.22 (1.01–1.48)	Overdominant	**.0360**	.2922

Statistically significant *P* values are bold. Genotypic test calculated in SNPstats software with sex and age adjustment of *P* values. *P* values were adjusted for 54 multiple testing by using FDR correction. The genotypic test was chosen as the genetic test model with lower Akaike information/lower *P* value. N = 898 PD cases; 921 controls (N = 1,819). CI, confidence interval; Freq., frequency; M, major allele; *MAPT,* microtubule-associated protein Tau; m, minor allele; OR, odds ratio; *SNCA,* α-synuclein; SNP, single nucleotide polymorphism.

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TABLE 2.

SNP rs356219 in *SNCA* interacts with rs8111699 in *SKT11*, rs456998 in *FCHSD1*, and rs1732170 in *GSK3B* in modulating PD risk

Gene	SNP	Bal. acc. CV training	Bal. acc. CV testing	CVC	OR (95% CI)	Association *P* value[a]	Epistatic association *P* value[b]
SNCA	rs356219	0.5378	0.5245	7/10	1.37 (1.13–1.66)	.306–.307	.925–.926
STK11 *GSK3B*	rs8111699 rs1732170	0.5529	0.5283	5/10	1.52 (1.26–1.83)	.214–.215	.874–.875
SNCA *STK11* *GSK3B*	rs356219 rs8111699 rs1732170	0.5729	0.5247	5/10	1.79 (1.48–2.16)	.300–.301	.924–.925
SNCA *STK11* *FCHSD1* *GSK3B*	rs356219 rs8111699 rs456998 rs1732170	0.6181	0.5875	10/10	2.59 (2.14–3.13)	**<.001**	**<.001**

Statistically significant *P* values are bold. Test of association for SNP combinations with PD risk. N = 898 PD cases and N = 921 unrelated controls. Random seed = 10; CVC = 10. CI, confidence interval; CV, cross-validation; CVC, cross-validation count; OR, odds ratio; *SNCA, α*-synuclein; SNP, single nucleotide polymorphism.

[a]Normal *P* value.

[b]*P* value of explicit test of epistasis obtained with 1000 permutations.

TABLE 3.

Genotypic association tests of *SNCA* rs356219 and *MAPT* rs1800547 SNPs with AAO of Parkinson's disease with sex, age, and multiple testing adjustments of *P* values

Gene	SNP	Alleles, M/m	Freq. 11	Freq. 12	Freq. 22	AAO 11, y	AAO 12, y	AAO 22, y	Difference, y	Model	*P* value	Adjusted *P* value
SNCA	rs356219	A (1)/G (2)	0.36	0.47	0.17	58.03 ± 0.5	55.84 ± 0.48	55.64 ± 0.85	−1.35 (−2.19 to −0.51)	Log-additive	**.0017**	**.0034**
MAPT	rs1800547	A (1)/G (2)	0.56	0.38	0.06	56.46 ± 0.61	56.15 ± 0.75	58.36 ± 1.73	0.30 (−0.66 to 1.26)	Log-additive	.54	.54

Statistically significant *P* values are bold. Genotypic test calculated in the SNPstats software with sex and age adjustment of *P* values. The model of inheritance best fitting the data was automatically selected as the model with the lowest Akaike information value. Two SNPs were considered for *P* value multiple test adjustment. N = 748 PD cases with AAO available. AAO, age at onset; Freq., frequency; M, major allele; *MAPT,* microtubule-associated protein Tau; m, minor allele; *SNCA,* α-synuclein; SNP, single nucleotide polymorphism; y, years.

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TABLE 4.

SNP rs356219 in *SNCA* interacts with rs11868112 in *RPTOR* and rs6456121 in *RPS6KA2* in modulating the AAO of idiopathic PD

Gene	SNP	*t* statistic CV training	*t* statistic CV testing	CVC	OR (95% CI)	Association *P* value[a]	Epistatic association *P* value[b]
SNCA	rs356219	2.6757	1.0612	5/10	1.31 (0.96–1.79)	.477–.478	.640–.641
RPTOR *RPS6KA2*	rs11868112 rs6456121	4.1806	3.1885	8/10	1.63 (1.20–2.21)	**.0260–.0270**	**.0460–.0470**
RPTOR *SNCA* *RPS6KA2*	rs11868112 rs356219 rs6456121	7.6501	6.0162	10/10	2.89 (2.90–4.00)	**<.001**	**<.001**

Statistically significant *P* values are bold. Test of association for SNP combinations with PD AAO. AAO was considered a continuous variable. N = 748 PD cases; random seed = 10; CVC = 10. OR were obtained generating a dichotomous dataset comparing early PD AAO (before 57 years) and late PD AAO (after 58 years) based on the average of AAO found with the multifactor dimensionality reduction (56.66 years). AAO, age at onset; CI, confidence interval; CV, cross-validation; CVC, cross-validation count; OR, odds ratio; PD, Parkinson's disease; *SNCA, α*-synuclein; SNP, single nucleotide polymorphism.

[a]Normal *P* value.

[b]*P* value of explicit test of epistasis obtained with 1000 permutations.

TABLE 5.

SNP rs356219 in *SNCA* interacts with rs11868112 in *RPTOR* and rs6456121 in *RPS6KA2* in modulating the AAO of idiooathic PD in the International Parkinson's Disease Genomics Consortium cohort

Gene	SNP	*t* statistic CV training	*t* statistic CV testing	CVC	OR (95% CI)	Association *P* value[a]	Epistatic association *P* value[b]
RPTOR	rs11868112						
SNCA	rs356219	5.7190	4.2753	10/10	1.56(1.34–1.81)	**0.001–0.002**	**0.046–0.047**
RPS6KA2	rs6456121						

Statistically significant *P* values are bold. Forced Interaction of SNPs rs11868112 in *RPTOR,* rs356219 in *SNCA,* and rs6456121 in *RPS6KA2* with PD AAO in the International Parkinson's Disease Genomics Consortium cohort. AAO was considered a continuous variable. N = 2798 PD cases; random seed = 10; CVC = 10. OR was obtained generating a dichotomous dataset comparing early PD AAO (before 60 years) and late PD AAO (after 61 years) based on the average of AAO found with the multifactor dimensionality reduction (60.86 years). AAO, age at onset; CI, confidence interval; CV, cross-validation; CVC, cross-validation count; OR, odds ratio; PD, Parkinson's disease; *SNCA, α*-synuclein; SNP, single nucleotide polymorphism.

[a]Normal *P* value.

[b]*P* value of explicit test of epistasis obtained with 1000 permutations.