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Genetic Association Between α-Synuclein and Idiopathic Parkinson's Disease

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Point mutations and copy number variations in *SNCA*, the gene encoding α-synuclein, cause familial Parkinson's disease (PD). A dinucleotide polymorphism (REP1) in the *SNCA* promoter may be a risk factor for common forms of PD. We studied 1,802 PD patients and 2,129 controls from the NeuroGenetics Research Consortium, using uniform, standardized protocols for diagnosis, subject recruitment, data collection, genotyping, and data analysis. Three common REP1 alleles (257, 259, and 261 bp, with control frequencies of 0.28, 0.65, and 0.06) and several rare alleles (combined frequency <0.01) were detected. We confirmed association of REP1 with PD risk [odds ratio (OR) = 0.86, $P = 0.006$ for 257-carriers; OR = 1.25, $P = 0.022$ for 261-carriers]. Using a normalization procedure, we showed that the 257 and 261 alleles are both independently associated with PD risk (for 257, $P = 0.002$ in overall data, 0.003 in non-familial PD, 0.001 in early-onset PD; for 261, $P = 0.056$ in overall data, 0.024 in non-familial PD, 0.052 in early-onset PD). The 257-associated risk was consistent with a dominant model [hazard ratio (HR) = 0.99, $P = 0.91$ for 257/257 vs. 257/X where X denotes all other common alleles; HR = 1.16, $P = 0.004$ for X/X vs. 257/X]. The 261-associated risk was consistent with a recessive model (HR = 1.89, $P = 0.026$ for 261/261 vs. 261/X; HR = 0.95, $P = 0.42$ for X/X vs. 261/X). Genotype-specific mean onset ages (±SD) ranged from 54.8 ± 12.1 for 261/261 to 59.4 ± 11.5 for 257/257, displaying a trend of decreasing onset age with increasing allele size ($P = 0.055$). Genetic variation in *SNCA* and its regulatory regions play an important role in both familial and sporadic PD.

KEY WORDS: **REP1; risk; age at onset; relative predispositional effects; mode of inheritance**

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INTRODUCTION

SNCA encodes the α-synuclein protein, aggregates of which constitute the primary component of Lewy bodies [Spillantini et al., 1998], the pathological hallmark of Parkinson's disease (PD). Point mutations [Polymeropoulos et al., 1997] and multiplications [Singleton et al., 2003] of *SNCA* cause autosomal dominant PD. Genomic multiplication of *SNCA* results in increased gene expression [Farrer et al., 2004; Miller et al., 2004], suggesting that excess amounts of normal α-synuclein can cause PD. This led to the hypothesis that less dramatic variation in *SNCA* expression, controlled via the promoter and other regulatory regions, might influence the risk for common, non-Mendelian forms of PD.

REP1 is a mixed dinucleotide repeat polymorphism in the *SNCA* promoter. REP1 has three common alleles in Caucasian populations, measured by length as 257, 259, and 261 bp in this study (although all studies detect these three common alleles, allele designations may differ across studies). Allele length variability within REP1 is associated with altered expression of *SNCA* [Chiba-Falek and Nussbaum, 2001; Chiba-Falek et al., 2003]. Thus, REP1 may be associated with susceptibility to PD via modulation of *SNCA* expression. Numerous studies

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have examined genetic association between REP1 allele length and susceptibility to PD [Farrer et al., 2001; Spadafora et al., 2003; Pals et al., 2004; Ross et al., 2007]. The individual studies were small and the results were inconsistent. A meta-analysis found significant evidence for a negative association with the shortest of the three common alleles (denoted as allele 0 in the meta-analysis, equivalent to 257 bp here) [Mellick et al., 2005]. A large follow-up collaborative study, with published and unpublished data, including data from the previous meta-analysis, also found significant evidence for association between REP1 and PD risk, but their primary finding was a positive association with the longest of the three common alleles (denoted as 263 in the collaborative study, equivalent to 261 here) [Maraganore et al., 2006]. The primary aim of this study was to replicate the association of REP1 with PD in an independent study with entirely new, previously unpublished data. In addition, we explored some of the unresolved issues about the REP1 main effect, including the relative predispositional and protective effects of the three common alleles, their mode of inheritance with respect to PD risk, and their possible influence on age at onset.

MATERIALS AND METHODS

Study Subjects

This study was approved by the Institutional Review Board of all participating institutions. PD patients (N = 1,802) and controls (N = 2,129) were recruited by the neurology clinics of the NeuroGenetics Research Consortium (NGRC) in Oregon, Washington, New York, and Georgia (Table I). Uniform and standardized methods were used across all sites for diagnosis, subject selection (exclusion/inclusion criteria for cases and controls), and data acquisition. Patients were enrolled sequentially and carried a clinical diagnosis of PD by a movement disorder neurologist using United Kingdom Brain Bank criteria [Gibb and Lees, 1988]. Age at onset was defined as the age when the first symptom of PD was noticed (tremor, rigidity or bradykinesia). Reported onset age is highly reliable [Richards et al., 1994; Reider et al., 2003]. Family history was obtained using a standardized self-administered questionnaire. Family history was considered positive if the patient had at least one first- or second-degree relative with PD. Controls were genetically unrelated to patients, consisted of spouses and community volunteers, and were free of neurodegenerative disease by self-report (78%) or neurological exam (22%). Approximately 85% of patients and 85% of controls who were invited to participate agreed and were enrolled. Ethnicity and race were defined according to NIH guidelines and were presented to subjects for self-assignment. The following subjects were excluded to minimize heterogeneity: patients with onset age <21 years, controls <21 years old at blood draw, carriers of known pathogenic mutations in *PRKN* (homozygotes or compound heterozygotes), *LRRK2* or *SCA2*, and those who were non-Caucasian.

Genotyping

Genomic DNA was extracted from peripheral blood using standard methods. REP1 was PCR-amplified using fluorescently labeled primers, as described by Kruger et al. [1999] (5′ FAM-GCA ATA GAG TAG ACA AAA GGA TGG-3′ and 5′ CTA CAT GAC TGG CCC AAG ATT AA-3′). REP1 dinucleotide repeat length was determined by electrophoresis of PCR products, using an ABI PRISM 3100 Genetic Analyzer and Genotyper version 3.7 software (Applied Biosystems, Foster City, CA). Genotyping and allele size calling were standardized and carried out at the Genotyping Core Facility at the Wadsworth Center (New York).

Data Analysis

Genotype frequencies (Table I) were in Hardy–Weinberg equilibrium. Allele frequencies were estimated by allele counting (Table I). Rare alleles (frequency ≤ 0.002) were excluded from analysis. Data from the four states were pooled after it was determined that they did not differ significantly in the frequency distribution of REP1 genotypes. Data analysis was carried out twice: once treating REP1 as a 3-allele, 6-genotype polymorphism, and a second time collapsing it into a 2-allele, 3-genotype system according to the previously published collaborative study for direct comparability [Maraganore et al., 2006]. Data were adjusted for age, sex, and site; and where noted (Table IV), for age, sex, site, *MAPT* H1/H1 diplotype [Zabetian et al., 2007], smoking and coffee [Powers et al., 2008]. Stratified analyses were performed by age (age at onset in patients and age at blood draw for controls, ≤50 years or >50 years) and family history (with or without PD in at least one first- or second-degree relative). Statistical analyses were carried out using SPSS version 15.0.

The replication study was modeled after the published collaborative study [Maraganore et al., 2006]. Briefly, alleles and genotypes were collapsed so that allelic comparisons were based on the presence or absence of each allele, and genotypic comparisons were based on the presence/absence of two, one or zero copies of a given allele (see Table II). Logistic regression was used to estimate odds ratios (ORs), 95% confidence intervals (CIs) and statistical significance (*P*-value).

Collapsing genotypes results in overlap because the reference group for one allele includes the other and vice versa (i.e., tests are not independent because 257 is compared to 259 + 261 and 261 is compared to 259 + 257). To alleviate this overlap and test effects of 257 and 261 on risk independently, we tested allelic and genotypic association without collapsing the data. To test for allelic association, we used the relative predispositional effects (RPE) method [Payami et al., 1989]. This was done by first performing a χ^2-test as a global test of the three alleles, followed by removal of the allele contributing the most to the χ^2, applying a normalization procedure, and re-testing to identify additional independent disease associations. The normalization procedure alleviates the concern that the increase or decrease in the frequency of one allele may be responsible for the changes in the frequency of the other alleles. The original RPE method was modified to a test of two populations (cases vs. controls). To test genotypic associations, we set 259/259 as the reference genotype, and tested the other five genotypes against it using logistic regression to calculate ORs, and the Cox proportional hazards model to calculate hazard ratios (HR). For the Cox model, age was used as time, patients' age at onset was the time of event, and controls' age at blood draw was the time when censored.

To test association of REP1 with onset age, we compared the genotype-specific means [±standard deviation (SD)] using analysis of variance (ANOVA). We also plotted and tested genotype-specific age at onset distributions using Kaplan–Meier survival analysis and log rank statistics. For both methods (ANOVA and Kaplan–Meier), we used the standard analysis, as well as a test for linear trend between genotype and age at onset. For the trend analysis, the 257/261 genotype was excluded, due to the opposing effects of the two alleles on risk.

To assess mode of inheritance for risk associated with 257 and 261, we examined the relative OR and HR for the heterozygotes compared to homozygotes and non-carriers, expecting homozygotes = heterozygotes > non-carriers for dominant, homozygotes > heterozygotes = non-carriers for recessive, and homozygotes > heterozygotes > non-carriers for an additive model.

TABLE I. Clinical and Demographic Characteristics, and *SNCA* REP1 Genotype and Allele Frequencies in PD Patients and Control Subjects

	Overall		Oregon		Washington		New York		Georgia	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Subject characteristics[a]										
N	1802	2129	601	1220	665	560	393	271	143	78
Age[b]	68.0 ± 10.6 (29–96)	67.7 ± 18.2 (20–109)	68.1 ± 10.3 (30–91)	68.9 ± 21.9 (20–109)	67.8 ± 10.9 (29–95)	66.0 ± 12.4 (26–99)	68.4 ± 10.8 (35–95)	65.5 ± 10.7 (34–93)	67.0 ± 10.2 (39–96)	68.5 ± 8.0 (50–83)
Age at onset[c]	58.6 ± 11.7 (24–93)	—	58.0 ± 11.7 (24–87)	—	58.5 ± 11.8 (25–89)	—	59.7 ± 11.6 (29–93)	—	58.3 ± 11.3 (30–92)	—
% Early onset[d]	25.9	—	26.5	—	27.1	—	24.0	—	23.4	—
% Male	67.6	37.4	65.1	38.4	74.4	36.3	61.6	37.3	62.9	30.8
% Pos. family history[e]	22.8	—	30.1	—	19.4	—	15.8	—	26.6	—
Genotype counts (frequency)										
255/257	3 (0.002)	1 (<0.001)	1 (0.002)	1 (0.001)	2 (0.003)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
255/259	3 (0.002)	1 (<0.001)	0 (0.000)	0 (0.000)	3 (0.005)	0 (0.000)	0 (0.000)	1 (0.004)	0 (0.000)	0 (0.000)
255/261	2 (0.001)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.002)	0 (0.000)	1 (0.003)	0 (0.000)	0 (0.000)	0 (0.000)
257/257	118 (0.066)	170 (0.081)	39 (0.065)	97 (0.082)	39 (0.059)	45 (0.082)	30 (0.077)	19 (0.070)	10 (0.070)	9 (0.115)
257/259	612 (0.342)	775 (0.371)	198 (0.331)	423 (0.356)	238 (0.362)	215 (0.390)	129 (0.329)	113 (0.419)	47 (0.329)	24 (0.308)
257/261	55 (0.031)	70 (0.034)	19 (0.032)	43 (0.036)	25 (0.038)	12 (0.022)	9 (0.023)	11 (0.041)	2 (0.014)	4 (0.051)
257/263	1 (0.001)	1 (<0.001)	0 (0.000)	0 (0.000)	1 (0.002)	0 (0.000)	0 (0.000)	1 (0.004)	0 (0.000)	0 (0.000)
259/259	788 (0.440)	879 (0.421)	264 (0.441)	510 (0.429)	287 (0.436)	236 (0.428)	166 (0.423)	99 (0.367)	71 (0.497)	34 (0.436)
259/261	193 (0.108)	178 (0.085)	68 (0.114)	105 (0.088)	61 (0.093)	42 (0.076)	51 (0.130)	26 (0.096)	13 (0.091)	5 (0.064)
259/263	2 (0.001)	2 (0.001)	2 (0.003)	1 (0.001)	0 (0.000)	1 (0.002)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
259/265	1 (0.001)	1 (<0.001)	1 (0.002)	1 (0.001)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
261/261	13 (0.007)	7 (0.003)	6 (0.010)	6 (0.005)	1 (0.002)	0 (0.000)	6 (0.015)	0 (0.000)	0 (0.000)	1 (0.013)
261/263	0 (0.000)	2 (0.001)	0 (0.000)	1 (0.001)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.013)
Allele counts (frequency)										
255	8 (0.002)	2 (<0.001)	1 (0.001)	1 (<0.001)	6 (0.005)	0 (0.000)	1 (0.001)	1 (0.002)	0 (0.000)	0 (0.000)
257	907 (0.253)	1187 (0.284)	296 (0.247)	661 (0.278)	344 (0.261)	317 (0.288)	198 (0.253)	163 (0.302)	69 (0.241)	46 (0.295)
259	2387 (0.666)	2715 (0.65)	797 (0.666)	1550 (0.652)	876 (0.666)	730 (0.662)	512 (0.653)	338 (0.626)	202 (0.706)	97 (0.622)
261	276 (0.077)	264 (0.063)	99 (0.083)	161 (0.068)	89 (0.068)	54 (0.049)	73 (0.093)	37 (0.069)	15 (0.052)	12 (0.077)
263	3 (0.001)	5 (0.001)	2 (0.002)	2 (0.001)	1 (0.001)	1 (0.001)	0 (0.000)	1 (0.002)	0 (0.000)	1 (0.006)
265	1 (<0.001)	1 (<0.001)	1 (0.001)	1 (<0.001)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)

[a]All subjects self-identified as Caucasian, Non-Hispanic.
[b]Age defined as age at blood draw, mean ± SD (range).
[c]Mean age at onset ± SD (range).
[d]Early onset PD defined as onset at or before age 50.
[e]Positive family history defined as having at least one first- or second-degree relative with PD.

TABLE II. Replication Study: Association of *SNCA* REP1 With Risk of PD[a]

Study	Patients	Controls	Trend OR (95% CI)	Trend P	Dominant OR (95% CI)	Dominant P	Recessive OR (95% CI)	Recessive P	Unrestricted (1) OR (95% CI)	Unrestricted (1) P	Unrestricted (2) OR (95% CI)	Unrestricted (2) P
Genotypes defined by the 261-bp allele												
Present	1779	2079	1.25 (1.03–1.51)	0.022	1.23 (1.01–1.51)	0.042	2.51 (0.94–6.70)	0.07	1.20 (0.98–1.47)	0.09	2.57 (0.96–6.87)	0.06
Published[a]	2686	2454	1.43 (1.22–1.69)	<0.001	1.44 (1.21–1.70)	<0.001	2.46 (0.95–6.37)	0.06	1.41 (1.19–1.68)	<0.001	2.57 (0.99–6.67)	0.05
Genotypes defined by the 257-bp allele												
Present	1779	2079	0.86 (0.77–0.96)	0.006	0.83 (0.72–0.95)	0.006	0.83 (0.64–1.07)	0.16	0.84 (0.73–0.97)	0.017	0.77 (0.58–1.00)	0.052
Published[a]	2686	2454	0.86 (0.79–0.94)	0.002	0.85 (0.76–0.96)	0.01	0.76 (0.61–0.96)	0.02	0.88 (0.78–0.99)	0.04	0.72 (0.57–0.92)	0.01

Trend (A): Linear trend for 0 (X/X), 1 (261/X), or 2 261 alleles (261/261) where X = 257 or 259; (B): Linear trend for 0 (X/X), 1 (257/X), or 2 257 alleles (257/257) where X = 259 or 261. Dominant model (A): (261/261 + 261/X) versus X/X where X = 257 or 259; (B): (257/257 + 257/X) versus X/X where X = 259 or 261. Recessive model (A): 261/261 versus (261/X + X/X) where X = 257 or 259; (B): 257/257 versus (257/X + X/X), where X = 259 or 261. Unrestricted (1) (A): 261/X versus X/X where X = 257 or 259; (B) 257/X versus X/X where X = 259 or 261. Unrestricted (2) (A): 261/261 versus X/X where X = 257 or 259; (B) 257/257 versus X/X where X = 259 or 261.

[a]This particular analysis was modeled after the published collaborative study [Maraganore et al., 2006] to allow direct comparability (i.e., the 3-allele, 6-genotype REP1 polymorphism was collapsed into a 2-allele, 3-genotype system, see methods). Alleles 257, 259, and 261 in this study are equivalent to alleles 259, 261, and 263, respectively, in the collaborative analysis. Analyses were adjusted for age, sex and site, but not for *MAPT* H1H1, smoking or coffee, in order to be comparable to the published study.

RESULTS

Replication Study

REP1 allele frequencies in cases and controls were nearly identical to those reported in the collaborative study (Table II). When each allele was compared to the other two alleles combined, patients had a lower frequency of the 257 allele ($P = 0.002$) and a higher frequency of the 261 allele ($P = 0.016$) than controls. Results of genotypic tests (Table II) were also similar to the collaborative study. A notable difference was the stronger statistical significance for the 261-bearing genotypes in the collaborative study. Allele 261 is rare (frequency in both studies is 0.06 in controls, 0.08 in patients). Thus, we suspect that the larger sample size of the collaborative study allowed this association to reach higher significance for 261. Both studies suggest reduced risk for genotypes defined by the 257 allele and increased risk for genotypes defined by the 261 allele. The analyses for 261 (panel A) and 257 (panel B) are not independent because the reference group for 261 includes 257 and the reference group for 257 includes 261. Therefore, despite designations for dominant and recessive models, mode of inheritance cannot be deduced from these results.

Testing Independence of 257 and 261 as Risk Factors

We performed allelic and genotypic association studies without collapsing alleles or genotypes. The global allelic association test was significant ($P = 0.002$, Table IIIA). The largest deviation was that of 257, representing the strongest statistical effect. After removing 257, and normalizing the frequencies, 261 nearly reached significance ($P = 0.056$, Table IIIA). Stratified analyses showed stronger association for 261 than 257 in non-familial PD ($P = 0.003$, Table IIIC), and in subjects younger than 50 years ($P = 0.001$, Table IIID). The genotypic association test was significant overall ($P = 0.031$ for OR, $P = 0.010$ for HR, Table IV). Using logistic regression (OR), individual genotypes did not reach statistical significance, but the pattern suggested risk reduction in the presence of 257, and risk increase in the presence of 261. The Cox model (HR), which is more sensitive to age and age at onset, yielded similar results and achieved significance for 257/259 and for 261/261. Stratified analyses showed a stronger 261/261 effect in non-familial PD. Taken together, the allelic and genotypic analyses suggest that 257 and 261 are both independently associated with PD. Our data suggest that 257 is associated with reduced risk, 259 is neutral, and 261 is associated with increased risk of PD.

Age at Onset

In the overall data, the genotype-specific mean onset ages ranged from 54.8 ± 12.1 for 261/261 to 59.4 ± 11.5 for 257/257 (Table IV). The pattern was consistent with decreasing onset age with increasing allele size (trend test $P = 0.055$). When plotted as age at onset distributions, however, the Kaplan–Meier curves were indistinguishable, except for 261/261 (Fig. 1). Despite its clear separation from other genotypes, the 261/261 curve was not significantly different from the others. Note that the effect sizes are relatively small, with the mean difference between the extremes being less than 5 years. Also, the 261/261 genotype, which shows the most dramatic effect, has the smallest sample size, which reduces statistical power.

Tests of Mode of Inheritance

To examine mode of inheritance, OR and HR were calculated using the heterozygous genotype as the reference

TABLE III. Tests of Allelic Association of *SNCA* REP1 With PD, Overall and Stratified by Family History and Age

	Round 1				Round 2			
REP1 allele	N patient chromosomes	N control chromosomes	χ^2	*P*	N patient chromosomes	N control chromosomes	χ^2	*P*
A. Overall								
257	907	1187	6.77		—	—	—	
259	2387	2715	0.83		2387	2715	0.35	
261	276	264	5.35		276	264	3.32	
Total	3570	4166	12.95	0.002	2663	2979	3.67	0.056
B. Familial PD[a]								
257	201	1187	3.41					
259	554	2715	0.97					
261	57	264	0.49					
Total	812	4166	4.86	0.09				
C. Non-Familial PD[a]								
257	706	1187	5.08		706	1187	3.58	
259	1833	2715	0.42		1833	2715	1.49	
261	219	264	6.12		—	—	—	
Total	2758	4166	11.62	0.003	2539	3902	5.06	0.024
D. Age $\leq$ 50[b]								
257	207	208	4.47		207	208	2.76	
259	627	504	0.07		627	504	1.01	
261	83	37	9.67		—	—	—	
Total	917	749	14.22	0.001	834	712	3.77	0.052
E. Age > 50[b]								
257	697	979	2.80					
259	1753	2211	0.60					
261	193	227	0.93					
Total	2643	3417	4.33	0.115				

Allelic associations were tested using the Relative Predispositional Effects method [Payami et al., 1989]. In Round 1, a χ^2-test for heterogeneity was used to compare the overall allele frequencies in patients versus controls. The resulting χ^2 and *P*-value, shown in the Total row, suggest that the REP1 allele frequency distribution in patients and controls differed significantly in the overall sample (panel A), in non-familial PD (panel C) and in younger subjects (panel D). To determine which allele contributed the most to the deviation, the contribution of each allele to the total χ^2 was determined as the sum of χ^2 for the two cells in each row (i.e., patients vs. expected and controls vs. expected for each allele), as noted in the χ^2 column. In the overall data (panel A), allele 257 had the largest contribution to the total χ^2, whereas in non-familial PD and young subjects (panels C,D) 261 had the strongest effect. For round 2, the allele responsible for the largest deviation was removed and the χ^2-test was repeated. The test was still significant in non-familial PD and nearly significant in the overall data and in subjects $\leq$50 years old. These data demonstrate that the reduction in the frequency of 257, and the increase in the frequency of 261 in PD are not events in which one allele frequency change is a consequence of the other, rather, each signifies an independent disease association.
[a]Familial and non-familial PD were compared to all controls (family history unavailable for controls).
[b]Age represents age at onset for patients and age at blood draw for controls.

(Table V and Fig. 2). The patterns were similar when genotypes were treated individually or collapsed. The risk for 257 heterozygotes was similar to 257 homozygotes and significantly different from non-257-carriers, suggesting a dominant effect for 257 (Table VA,B and Fig. 2A,B). The risk for 261 heterozygotes was ~2-fold lower than for 261 homozygotes which argues against a strict dominant effect, and risk for 261 heterozygotes was not significantly different from those who lacked 261, which is consistent with a recessive model. However, a non-significant trend suggested 261 heterozygotes might be at slightly higher risk than non-carriers (Table VC,D and Fig. 1A,C).

DISCUSSION

The present study adds to the accumulating evidence that genetic variation in α-synuclein plays an important role in the etiology of PD, not only in the rare Mendelian forms but also in the common sporadic forms of the disease. It has been well established that rare non-synonymous mutations in *SNCA* and multiplications of the entire gene cause autosomal dominant PD [Polymeropoulos et al., 1997; Singleton et al., 2003]. Association of *SNCA* polymorphisms with common forms of PD, however, has been more difficult to establish, because their effect size on disease risk is much smaller than that of causative mutations, and also because of inherent confounders such as disease heterogeneity, which are more serious in association studies than in linkage studies. A large and growing body of evidence now points to polymorphisms in *SNCA* and its regulatory regions as being associated with susceptibility to common non-Mendelian forms of PD [Pals et al., 2004; Mellick et al., 2005; Mueller et al., 2005; Maraganore et al., 2006; Ross et al., 2007; Winkler et al., 2007]. The REP1 polymorphism, in particular, has a direct functional relevance to PD via its effect on gene expression [Chiba-Falek and Nussbaum, 2001; Chiba-Falek et al., 2003]. Here we confirmed a genetic association between REP1 and PD risk, and demonstrated a trend of increasing risk, and decreasing age at onset, with increasing allele size. The magnitude of the effect is modest, which is not surprising, considering that the majority of susceptibility alleles for common disorders have small main effects with ORs averaging around 1.25 [Topol et al., 2007]. A small main effect may decrease the value of REP1 as a predictive marker, but it does not detract from its significance for understanding disease etiology.

Our study complements prior studies of PD and REP1 in several ways. (i) The present study was the largest single study of REP1 and PD to date. The two large studies published earlier, a meta-analysis and a collaborative study, pooled many studies, and included overlapping and previously published data. Our study consisted entirely of unpublished data,

TABLE IV. Tests of Genotypic Association of *SNCA* REP1 With PD Risk and Age at Onset

	N patients	N controls	OR (95% CI)[a]	P	HR (95% CI)[b]	P	AAO (mean ± SD, range)[c]
Overall							
Overall	1779	2079		0.031		0.010	
257/257	118	170	0.80 (0.61–1.05)	0.10	0.87 (0.72–1.05)	0.15	59.4 ± 11.5 (35–83)
257/259	612	775	0.87 (0.74–1.01)	0.07	0.88 (0.79–0.98)	0.021	59.2 ± 11.5 (25–93)
257/261	55	70	0.92 (0.62–1.36)	0.67	0.83 (0.63–1.10)	0.20	58.9 ± 12.4 (25–84)
259/259	788	879	Reference		Reference		58.3 ± 11.7 (24–92)
259/261	193	178	1.18 (0.92–1.50)	0.19	1.05 (0.89–1.23)	0.56	58.1 ± 12.3 (26–87)
261/261	13	7	2.38 (0.89–6.36)	0.08	1.87 (1.08–3.25)	0.025	54.8 ± 12.1 (30–75)
Familial[d]							
Overall	404	2079		0.22		0.24	
257/257	26	170	0.71 (0.45–1.12)	0.14	0.76 (0.50–1.14)	0.18	56.8 ± 12.6 (35–76)
257/259	138	775	0.86 (0.67–1.11)	0.24	0.85 (0.68–1.06)	0.14	57.8 ± 12.0 (25–83)
257/261	9	70	0.58 (0.28–1.20)	0.14	0.56 (0.28–1.09)	0.09	55.7 ± 8.6 (40–65)
259/259	185	879	Reference		reference		56.5 ± 12.3 (24–84)
259/261	44	178	1.19 (0.82–1.75)	0.36	1.08 (0.78–1.50)	0.64	56.4 ± 11.5 (35–84)
261/261	2	7	1.37 (0.27–6.88)	0.70	1.21 (0.30–4.90)	0.79	50.0 ± 0.0
Non-familial[d]							
Overall	1375	2079		0.06		0.024	
257/257	92	170	0.84 (0.62–1.12)	0.23	0.90 (0.72–1.12)	0.32	60.2 ± 11.1 (35–83)
257/259	474	775	0.87 (0.73–1.03)	0.10	0.89 (0.79–1.00)	0.05	59.7 ± 11.3 (25–93)
257/261	46	70	1.01 (0.66–1.54)	0.97	0.87 (0.65–1.18)	0.38	59.6 ± 13.0 (25–84)
259/259	603	879	Reference		Reference		58.8 ± 11.4 (25–92)
259/261	149	178	1.16 (0.89–1.51)	0.26	1.06 (0.88–1.26)	0.55	58.7 ± 12.5 (26–87)
261/261	11	7	2.73 (0.97–7.67)	0.06	2.10 (1.16–3.83)	0.015	55.7 ± 13.0 (30–75)
Age ≤ 50[e]							
Overall	457	374		0.25		0.71	43.2 ± 5.9 (24–50)
257/257	26	33	0.47 (0.18–1.23)	0.12	1.00 (0.66–1.50)	0.99	43.2 ± 5.4 (35–50)
257/259	139	136	0.86 (0.50–1.48)	0.59	1.00 (0.81–1.24)	1.00	43.1 ± 6.1 (25–50)
257/261	14	6	3.36 (0.69–16.20)	0.13	1.45 (0.84–2.50)	0.18	42.3 ± 6.2 (25–49)
259/259	216	168	Reference		Reference		43.4 ± 5.9 (24–50)
259/261	56	31	1.09 (0.47–2.52)	0.85	1.17 (0.87–1.58)	0.29	43.0 ± 5.6 (26–50)
261/261	6	0	—	—	0.92 (0.41–2.08)	0.84	44.8 ± 7.8 (30–50)
Age > 50[e]							
Overall	1317	1705		0.48		0.47	64.0 ± 7.8 (51–93)
257/257	92	137	0.88 (0.65–1.20)	0.42	0.92 (0.74–1.15)	0.48	64.0 ± 8.0 (51–83)
257/259	470	639	0.90 (0.75–1.07)	0.23	0.93 (0.82–1.05)	0.22	64.0 ± 7.8 (51–93)
257/261	41	64	0.87 (0.56–1.36)	0.55	0.82 (0.60–1.13)	0.23	64.6 ± 8.1 (51–84)
259/259	570	711	Reference		Reference		63.9 ± 7.7 (51–92)
259/261	137	147	1.13 (0.85–1.49)	0.40	1.01 (0.84–1.22)	0.88	64.3 ± 8.2 (51–87)
261/261	7	7	1.67 (0.55–5.07)	0.37	1.54 (0.73–3.24)	0.26	63.4 ± 7.3 (55–75)

OR, odds ratio; HR, hazard ratio; CI, confidence interval; AAO, age at onset; SD, standard deviation.
[a]OR adjusted for age, sex, and site (results shown). Additional adjustments for coffee, smoking and *MAPT* H1/H1 produced similar results.
[b]HR adjusted for sex and site (results shown). Additional adjustments for coffee, smoking and *MAPT* H1/H1 produced similar results.
[c]Test of linear trend for age at onset $P = 0.055$ for overall data, $P = 0.087$ for non-familial PD. ANOVA $P = 0.45$ overall, $P = 0.62$ for non-familial PD.
[d]Familial and non-familial PD were compared to all controls (family history unavailable for controls).
[e]Age represents age at onset for patients and age for controls.

thus constitutes an independent replication. (ii) We used standardized study protocols, implemented uniformly across seven NGRC neurology clinics for subject recruitment and characterization, and performed REP1 genotyping in one laboratory. These measures minimized intra-study variability that may exist when using pooled samples. (iii) Some studies have collapsed alleles and genotypes, rendering REP1 from a 3-allele, 6-genotype polymorphism into a 2-allele, 3-genotype system [Maraganore et al., 2006]. Collapsing the alleles and genotypes makes the study more powerful because it creates a wider separation in risk estimates and increases the sample size in each cell, but it does not distinguish the independent effects of each allele. We analyzed the data both ways; with genotypes collapsed for consistency with the collaborative study for replication, and not collapsed to explore the patterns of association in more detail. (iv) We performed formal tests to assess the individual effects of the three common REP1 alleles on risk. (v) We tested mode of inheritance of REP1-associated risk. (vi) In exploring possible REP1 effect on age at onset, we found a trend toward a linear inverse relationship between allele size and onset age. Results from the collapsed data were consistent with prior findings, confirming association of REP1 with PD. The pattern that emerged from non-collapsed data was analogous to the association of *APOE* with Alzheimer's disease (AD), where allele ε2 is associated with decreased risk, ε3 is neutral, and ε4 is associated with increased risk of AD. Here we showed that allele 257 is associated with decreased risk of PD, 259 is neutral, and 261 is associated with increased risk. The effect of REP1 on PD risk, however, is several-fold smaller than the *APOE* effect on AD.

Prior studies did not consistently find the same allelic association with PD. The meta-analysis reported the negative association with 257 as the main finding (designated allele 0 in their study) [Mellick et al., 2005], while the collaborative study found the positive association with 261 more prominent (designated as allele 263 in their study) [Maraganore et al., 2006]. We showed that 257 and 261 are both associated with PD risk; 257 is protective and 261 is predisposing. In our data the

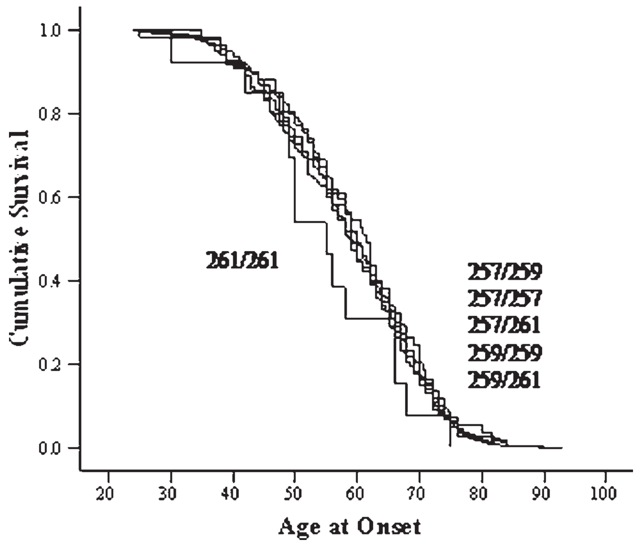

Fig. 1. Age at onset distributions for *SNCA* REP1 genotypes Kaplan–Meier survival analysis was used to plot the age at onset distributions for the six genotypes. All distributions were indistinguishable except 261/261. The overall significance, tested using log rank statistics, was $P = 0.62$, and $P = 0.21$ for linear trend. 261/261 included only 13 patients, thus even when 261/261 was compared individually to 259/259 ($P = 0.30$) or to all other genotypes combined ($P = 0.23$) the difference did not reach statistical significance. Results were similar and not significant when only non-familial PD was analyzed.

magnitude of the effect was larger for 261 (138% increase in risk) than for 257 (13–20% decrease in risk). However, 257 emerged as statistically more significant because (i) the allele frequency of 257 was higher than 261 (0.28 vs. 0.06) and (ii) the 257 effect was dominant (i.e., both 257 heterozygotes and homozygotes, who represented nearly 40% of all subjects, contributed to the 257 effect, whereas the 261 effect was driven by only 0.5% of subjects who were 261/261 homozygous). This may explain why the meta-analysis detected the 257 effect more readily, while in the collaborative study, which had a larger sample size and more power, the 261 effect was more prominent. It may be noteworthy that we found the effect of 261 on risk to be stronger and more prominent than 257 in non-familial PD and in younger subjects. Moreover, we found suggestive evidence that the 261/261 genotype is associated with earlier onset age than other genotypes.

In families with an *SNCA* multiplication, where PD manifests as an autosomal dominant disease, age at onset is inversely associated with *SNCA* copy number [Chartier-Harlin et al., 2004; Fuchs et al., 2007]. This would suggest that REP1 and other promoter polymorphisms that modulate gene expression should also have an effect on age at onset, although the effect may not be as dramatic as gene multiplication and therefore more difficult to detect. One study has reported earlier disease onset in patients with the long REP1 allele (denoted 261 here) [Hadjigeorgiou et al., 2005], but others were unable to replicate [Maraganore et al., 2006; Ross et al., 2007; Winkler et al., 2007]. We did find evidence for association of REP1 with age at onset, although the effect was relatively small and marginally significant. The trend, however, suggested decreasing age at onset with increasing allele size, which fits the pattern that would be predicted from the association of REP1 with gene expression and with PD risk. Longer REP1 alleles are associated with increased gene expression, higher PD risk, and earlier disease onset, as the present data suggest.

Polymorphisms in α-synuclein may have a greater impact on PD susceptibility than is reflected by the modest main effect of REP1. Several *SNCA* haplotype and polymorphisms have been implicated in PD, including single nucleotide polymorphisms (SNPs) in the 5′ UTR/promoter region, and the 3′ UTR [Pals et al., 2004; Mueller et al., 2005; Ross et al., 2007; Winkler et al., 2007]. A recent study reported on SNPs in the promoter that may be independently associated with PD [Winkler et al., 2007], which would imply their effects on PD risk may be cumulative to the REP1 effect. A logical next step would be a global analysis of *SNCA* polymorphisms to delineate the full spectrum of *SNCA* association with PD.

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TABLE V. Exploring Mode of Inheritance of *SNCA* REP1-Associated PD Risk

	N patients	N controls	OR (95% CI)[a]	P	HR (95% CI)[b]	P
A. Individual genotypes: 257						
257/257	118	170	0.92 (0.70–1.22)	0.56	0.98 (0.81–1.20)	0.88
257/259	612	775	Reference		Reference	
259/259	788	879	1.16 (0.99–1.35)	0.07	1.13 (1.02–1.26)	0.022
B. Collapsed genotypes: 257						
257/257	118	170	0.92 (0.70–1.21)	0.54	0.99 (0.81–1.20)	0.91
257/X	667	845	Reference		Reference	
X/X	994	1064	1.19 (1.03–1.38)	0.016	1.16 (1.05–1.28)	0.004
C. Individual genotypes: 261						
261/261	13	7	2.01 (0.73–5.52)	0.18	1.77 (1.01–3.11)	0.048
259/261	193	178	Reference		Reference	
259/259	788	879	0.85 (0.67–1.09)	0.21	0.96 (0.82–1.12)	0.60
D. Collapsed genotypes: 261						
261/261	13	7	2.15 (0.79–5.83)	0.13	1.89 (1.08–3.30)	0.026
261/X	248	248	Reference		Reference	
X/X	1518	1824	0.84 (0.68–1.03)	0.09	0.95 (0.83–1.08)	0.42

OR, odds ratio; HR, hazard ratio; CI, confidence interval.
[a]OR adjusted for age, sex, and site.
[b]HR adjusted for sex and site.

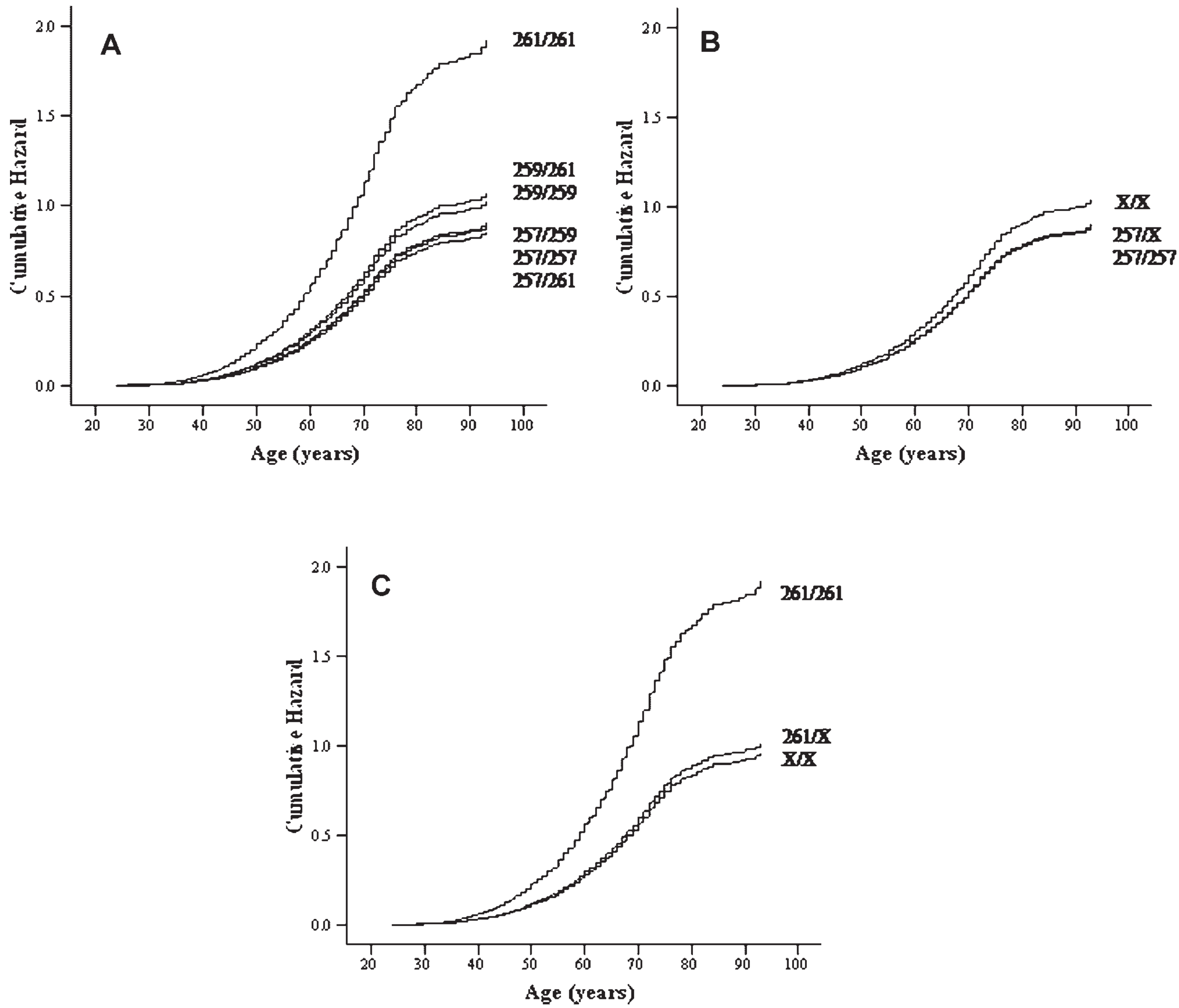

Fig. 2. Age-specific hazard ratios for *SNCA* REP1 genotypes. Cox proportional hazard models were used to calculate and plot age-specific HR for REP1 genotypes, using age at onset for patients as the time of event and age for controls as the time censored. **A**: Each genotype was plotted separately and compared to 259/259, which was used as the baseline reference for HR calculations. Overall significance was $P = 0.01$. The HR was significant for 257/259 (HR = 0.88, $P = 0.021$) and for 261/261 genotypes (HR = 1.87, $P = 0.025$). Note that the 257-bearing genotypes (257/257, 257/259 and 257/261) clustered at the lowest risk, 259/259 and 259/261 clustered at slightly higher risk, and 261/261 had substantially higher risk. **B**: Genotypes were combined into three classes, 257/257, 257/X and X/X where X denotes 259 or 261. Compared to X/X, HR = 0.87 ($P = 0.004$) for 257/X, and HR = 0.86 ($P = 0.11$) for 257/257. 257/X did not differ significantly from 257/257 (HR = 0.99, $P = 0.92$). **C**: Genotypes were combined into three classes, 261/261, 261/X and X/X, where X denotes 257 or 259. Compared to X/X, HR = 1.06 ($P = 0.42$) for 261 heterozygotes, and HR = 2.00 ($P = 0.013$) for 261/261. 261/261 had significantly higher risk than 261/X (HR = 1.90, $P = 0.027$).

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