Collaborative Analysis of α-Synuclein Gene Promoter Variability and Parkinson Disease

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Context Identification and replication of susceptibility genes for Parkinson disease at the population level have been hampered by small studies with potential biases. α-Synuclein (*SNCA*) has been one of the most promising susceptibility genes, but large-scale studies have been lacking.

Objective To determine whether allele-length variability in the dinucleotide repeat sequence (REP1) of the *SNCA* gene promoter is associated with Parkinson disease susceptibility, whether *SNCA* promoter haplotypes are associated with Parkinson disease, and whether REP1 variability modifies age at onset.

Design, Setting, and Participants We performed a collaborative analysis of individual-level data on *SNCA* REP1 and flanking markers in patients with Parkinson disease and controls. Study site recruitment, data collection, and analyses were performed between April 5, 2004, and December 31, 2005. Eighteen participating sites of a global genetics consortium provided clinical data. Genotyping was performed for *SNCA* REP1, –770, and –116 markers at individual sites; however, each site also provided 20 DNA samples for regenotyping centrally.

Main Outcome Measures Measures included estimations of Hardy-Weinberg equilibrium in controls; a test of heterogeneity; analyses for association of single variants or haplotypes; and survival analyses for age at onset.

Results Of the 18 sites, 11 met stringent criteria for concordance with Hardy-Weinberg equilibrium and low genotyping error rate. These 11 sites provided complete data for 2692 cases and 2652 controls. There was no heterogeneity across studies ($P>.60$). The *SNCA* REP1 alleles differed in frequency for cases and controls ($P<.001$). Genotypes defined by the 263 base-pair allele were associated with Parkinson disease (odds ratio, 1.43; 95% confidence interval, 1.22-1.69; $P<.001$ for trend). Multilocus haplotypes differed in frequency for cases and controls (global score statistic, $P<.001$). Two-loci haplotypes were associated with Parkinson disease only when they included REP1 as one of the loci. However, genotypes defined by REP1 alleles did not modify age at onset ($P=.55$).

Conclusion This large-scale collaborative analysis demonstrates that *SNCA* REP1 allele-length variability is associated with an increased risk of Parkinson disease.

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PARKINSON DISEASE IS A COMMON neurological condition associated with increased morbidity and reduced survival.[1-3] The origin of Parkinson disease remains largely elusive, but genetic factors may be important.[4,5] One of the most promising leads in the genetics of Parkinson disease is the potential role of the α-synuclein (*SNCA*) gene. Linkage studies have revealed several *SNCA* mutations

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that cause Parkinson disease, likely via amino-acid substitutions and configurational changes in the encoded protein.[6-8] α-Synuclein locus multiplication also causes Parkinson disease, likely via gene overexpression.[9-13]

Although *SNCA* gene mutations are rare, accounting for less than 1% of Parkinson disease in the general population, abnormal aggregation of the SNCA protein is present in all patients with Parkinson disease. The SNCA protein is the principal component of Lewy bodies, the pathological hallmark of Parkinson disease.[14] Common *SNCA* variants may be associated with the risk of Parkinson disease in the general population, apart from the rare causal mutations. Sequencing of the *SNCA* gene has revealed common variants including a dinucleotide repeat sequence (REP1) within the promoter. Some studies have found that certain alleles are associated with an increased risk for Parkinson disease.[15-17]

Functional biological data are in concordance with this observation. The *SNCA* REP1 locus is a major promoter of normal gene expression in transient transfection assays in HEK293 cells using a luciferase reporter construct.[18] α-Synuclein gene expression varies significantly over a 3-fold range across the different REP1 alleles, suggesting that the association of specific genotypes with an increased risk for Parkinson disease may be explained by an increase in *SNCA* transcription.[19,20] The recent discovery of *SNCA* gene triplication as a rare cause of Parkinson disease suggests that polymorphism within the gene promoter may confer susceptibility via the same mechanism of gene overexpression.[9] Functional analysis of brain tissue from *SNCA* triplication families confirmed this hypothesis.[10] In addition, families with *SNCA* duplication demonstrated the association of gene dosage with age at onset, rate of progression, and severity of disease.[11] In a yeast model, a 2-fold difference in expression of the *SNCA* gene was sufficient to cause a catastrophic change in SNCA protein localization and resulted in cytoplasmic inclusions and cell death.[21]

Nevertheless, whereas several studies observed an association of *SNCA* REP1 variability with Parkinson disease,[15-17,22-25] others have observed no association.[26-28] Moreover, in contrast to studies in whites, a meta-analysis of 2 Japanese studies reported an inverse association between the risk allele and Parkinson disease.[29,30] Nonreplication may in part reflect differences across populations in allele frequencies and in the causes of Parkinson disease. Alternatively, methodological limitations may be responsible because all of the studies had modest sample sizes and the sampling and measurements were not standardized.[31]

We considered that a collaborative analysis of individual participants' data (published and unpublished) may resolve the observed discrepant results.[32,33] Confirmation of the association of *SNCA* promoter variability with Parkinson disease would boost confidence in *SNCA* gene overexpression as a common mechanism of Parkinson disease susceptibility and pathogenesis and as a plausible target for new pharmacological therapies aimed at lowering *SNCA* expression (eg, RNA interference).[34] Therefore, we established a global genetics consortium to determine whether *SNCA* REP1 allele-length variability is associated with Parkinson disease susceptibility, whether extended *SNCA* promoter variability (haplotype analyses, including REP1 and flanking markers) is associated with Parkinson disease, and whether REP1 variability modifies age at onset.

METHODS

The Genetic Epidemiology of Parkinson Disease Consortium included 3 cores (coordinating, statistical, and laboratory) and several global sites. All participating sites collected and shared biospecimens and data via the written informed consent of study participants and via the approval of institutional review boards. This included permission to publish the results of the study in a medical journal.

Coordinating Core

Following a notice of grant award from the Michael J. Fox Foundation (dated April 5, 2004; in response to a request for applications to create global genetics consortia for Parkinson disease), the coordinating core invited the corresponding authors of published genetic association studies of *SNCA* REP1 and Parkinson disease to serve as global site principal investigators. These publications were identified via PubMed searches using the terms *synuclein* and *Parkinson*, as of April 5, 2004. The coordinating core also invited additional investigators who had previously participated in a collaborative analysis of the *UCHL1* gene S18Y variant and Parkinson disease,[35] or investigators who had otherwise collaborated with members of the consortium, to provide unpublished data or to conduct new studies. Between April 5, 2004, and March 31, 2005, the global site principal investigators were asked to contribute information for the study using a formatted Excel spreadsheet including the following variables: sources of participants, sample size, median age at study start and range, sex distribution, and ethnicity (for cases and controls) and median age at onset and range, distribution of familial Parkinson disease cases, and diagnostic criteria for Parkinson disease used (for cases). For all sites, race and ethnicity was self-reported by the study participants. We collected these data because a primary aim of the study was to determine whether *SNCA* promoter variability was associated with Parkinson disease across populations and because a secondary aim was to determine whether there were population differences (stratified analyses). The global site principal investigators were also asked to contribute individual level clinical and genetic data (published and unpublished) using a second formatted Excel spreadsheet including the following variables: laboratory identification numbers, affected status, sex, ethnicity, age at study (for all participants); age at onset and family history of Parkinson disease (for cases); and *SNCA* REP1 genotypes and –770 and –116 base-pair (bp) single-nucleotide

polymorphism (SNP) genotypes (for all participants). These SNPs were selected because data were available for multiple sites and because they were possibly informative of functional promoter variability. The individual level data were then forwarded to the statistical core.

Statistical Core

Data management and statistical analyses were performed during the period April 5, 2004, through December 31, 2005. Each global site provided data for individuals identified by study-specific laboratory identification numbers only. The data included *SNCA* REP1, –770, and –116 genotypes, and also clinical and demographic information. The data were archived in a SAS database maintained by the statistical core. Logical checks were performed on the data and inconsistencies were corrected through queries to the sites.

Each site also provided the statistical core with a random list of 20 laboratory identification numbers and the corresponding REP1 genotypes, for consideration of regenotyping in the laboratory core. This number of samples was chosen for regenotyping because it was compatible with resources and feasible for all sites. The statistical core checked whether these 20 genotypes fulfilled the heterozygosity criterion. Specifically, we required the proposed 20 samples to achieve at least 50% heterozygosity to ensure a mixture of genotypes. If they did, the samples were shipped to the laboratory core for regenotyping. If they did not, a revised list was requested until a list fulfilling the heterozygosity criterion was specified; all 18 study sites fulfilled the criterion.

Laboratory Core

Study-specific genotyping was performed by the laboratory core during the period October 1, 2004, through March 31, 2005. Upon the approval of the statistical core (heterozygosity checks), each global site transferred a minimum of 20 aliquots (200 ng each) of DNA to the laboratory core for regenotyping. The DNA samples were coded with study-specific laboratory identification numbers, and regenotyping was blinded to the genotypes originally determined by the global sites. The REP1 genotyping method used by the laboratory core was described elsewhere.[16] For some of the global sites, genotyping data was not available for the *SNCA* REP1 or for the –770 or –116 variants. For these 5 global sites, the genotyping of all available samples was performed by the laboratory core, using methods described elsewhere.[16,17]

Briefly, we genotyped *SNCA* REP1 allele-length variants as follows: 15 ng of genomic DNA was polymerase chain reaction (PCR)–amplified using fluorescently labeled forward and reverse primers Fam5′-CCT GGC ATA TTT GAT TGC AA-3′ and 5′GAC TGG CCC AAG ATT AAC CA-3′ designed to amplify the dinucleotide repeat polymorphism (REP1; *D4S3481*) in the *SNCA* promoter. Genotyping was performed on an ABI 3730 and allelic sizes assessed using GeneMapper version 4.0 software (Applied Biosytems, Inc, Foster City, Calif). Genotyping of the 2 promoter SNP variants was carried out using TaqMan fluorogenic 5′ nuclease assay (Applied Biosystems). The final volume of the PCR reaction was 2 µL, containing 2 ng of genomic DNA and 1 µL TaqMan Universal PCR Master Mix, with 0.1 µL of 20× Assay Mix C_15755957_10 for the –116 polymorphism (rs2301135) and C_16036895_10 for the –770 polymorphism (rs2619363) (Applied Biosystems, Inc). Polymerase chain reaction master mix was dispensed into 384 well plates using a Cartesian dispensing platform (Genomic Solutions, Ann Arbor, Mich) and the PCR thermal cycle conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes to activate the DNA polymerase, followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. End point fluorescent readings were detected on an ABI 7900 and genotype analysis was performed using SDS version 2.2 software (Applied Biosystems).

When genotyping variable length markers such as REP1, a common reference is necessary for defining the allele length. Thus, published data may not be comparable due to differences in allele calling. Our central regenotyping allowed us to standardize REP1 allele length calls for each Global Site according to the Laboratory Core determinations. Because published genotyping data from nonparticipating sites could not be standardized, those data were excluded.

Analysis

We conducted an analysis of the assembled individual-level data to determine whether *SNCA* REP1 variability conferred Parkinson disease susceptibility. All tests were considered significant at $P<.05$. We used 2 tests of heterogeneity of the odds ratios (ORs) from the studies, Cochran's Q and I^2 statistics, and assessed the goodness-of-fit of Hardy-Weinberg equilibrium for controls in each study.[36,37] We set a priori that only studies fulfilling Hardy-Weinberg equilibrium and with at least 90% REP1 interlaboratory agreement would be included in the main analyses (arbitrary threshold). However, we also performed sensitivity analyses including all studies.

Because not all studies had data available for adjustment variables, we first performed analyses using unadjusted data. We tested for association of the REP1 alleles with Parkinson disease using a standard χ^2 test. We also tested for association of the genotypes with Parkinson disease in each site using logistic regression models and overall using a random-effects method.[38] The attributable fraction in the population of REP1 variability was calculated with the formula: frequency × (OR –1)/[1 + frequency × (OR –1)]. To test for statistical association of the genotypes with Parkinson disease while accounting for differences in sex and age at study between cases and controls, we used logistic regression mixed models where site was a random effect and genotype was a fixed effect.

Likelihood ratio tests were calculated by dividing the likelihood of a regression model including and excluding the gene, adjusting both models for

sex and age at examination. This statistic was compared with a χ^2 distribution with the appropriate degrees of freedom to produce a *P* value. We decided a priori that rare alleles (frequencies <0.1%) were to be excluded from the analyses. Specifically, we considered only the REP1 alleles 259 bp, 261 bp, and 263 bp because the other alleles were rare. We performed analyses for strata defined by family history (≥1 first-degree relatives with Parkinson disease), age at study (using a median cutoff), and sex using the same methods detailed above. We also examined whether the results of small studies differed from those of larger ones,[39] and further assessed bias by excluding the data from the first published study that might be considered as hypothesis generating.[31]

We conducted multiple locus analyses of the *SNCA* REP1, –770 and –116 variants to determine whether core promoter variability is associated with an increased risk for Parkinson disease using score tests for the association between Parkinson disease and inferred haplotypes, as previously described.[40]

Finally, we explored the possibility that *SNCA* REP1 genotypes were associated with Parkinson disease age at onset. We performed age at onset survival analyses for cases only. Kaplan-Meier survival plots were generated to describe the survivorship functions, and likelihood ratio tests from Cox proportional hazards models were used to test for association. Analyses were performed for cases overall and stratified for sex and family history of Parkinson disease.

All statistical analyses were performed in SAS version 9.1 (SAS Institute Inc, Cary, NC) or S-Plus version 7 (Insightful Corp, Seattle, Wash). All *P* values were 2-tailed.

RESULTS

Participating Sites

All methods (including study site recruitment, data collection, and analyses) were completed between April 5, 2004, and December 31, 2005. We initially identified 9 teams that had published *SNCA* REP1 data. We identified another 6 teams with unpublished REP1 data and also 5 teams that were willing to participate in a collaborative study but had no REP1 data yet. Of these 20 teams, 2 declined participation[27,30] because one corresponding author was lost to contact and because the other no longer had access to individual level data.

Eighteen global sites agreed to participate in the study (TABLE 1). The sites either had published (n=7) or unpublished (n=6) *SNCA* REP1 data available or were able to provide DNA to the laboratory core for *SNCA* REP1 genotyping (n=5). Seven of the 18 global sites were excluded from the main analyses: 1 site provided no controls, 3 sites had REP1 genotypes deviating significantly from Hardy-Weinberg equilibrium in controls, and 3 sites had interlaboratory reliability for the REP1 genotyping below 90%, which resulted in a loss of 1412 cases and 1136 controls. Of the 11 sites included in analyses, there were 1129 cases and 652 controls represented by published studies (4 sites). There were 1563 cases and 2000 controls represented by the unpublished studies (7 sites). In total, the 11 sites included contributed complete data for 2692 Parkinson disease cases and 2652 unrelated controls. Data were missing for at least 1 of the adjustment variables for 6 cases (0.2%) and 198 controls (7.5%). These individuals were included in the unadjusted analyses and excluded from the adjusted analyses.

Association of Parkinson Disease With REP1 Alleles

There was no heterogeneity of the ORs across the 11 sites. The allele frequencies were significantly different for the Parkinson disease cases vs the controls (*P*<.001). TABLE 2 summarizes the allele distributions for the 11 studies.

TABLE 3 and FIGURE 1 summarize the results of logistic mixed models for the 263 bp allele, using sites as random effects, and including age and sex. For the trend model (linear trend in log-odds for 0, 1, and 2 alleles), the 263 bp genotypes were associated with an increased risk for Parkinson disease (OR, 1.43; 95% confidence interval [CI], 1.22-1.69; *P*<.001). The association was significant also using alternative genetic models and in subgroup analyses.

TABLE 4 and FIGURE 2 summarize the results of logistic mixed models for the 259 bp allele. Overall, we found a significant association between 259 bp genotypes and a reduced risk of Parkinson disease (OR, 0.86; 95% CI, 0.79-0.94; *P*=.002 for trend). There was a trend of increasing risk with increasing bp length (data not shown; *P*<.001).

Bias Diagnostics and Sensitivity Analyses

The ORs for the 263 bp allele were similar in published studies (OR, 1.40; 95% CI, 1.01-1.94) and unpublished studies (OR, 1.43; 95% CI, 1.19-1.73). The log ORs were not significantly associated with the sample size of the study (regression coefficient *P*=.78 for 263 bp and *P*=.99 for 259 bp analyses).

We observed the same overall findings in analyses including the 6 sites that did not meet Hardy-Weinberg equilibrium or genotyping reliability criteria. Specifically, the distribution of alleles and of genotypes defined by either the 263 bp allele or by the 259 bp allele remained significantly different for the 3730 cases and the 3550 controls (allele distribution, *P*<.001; 263 bp, *P*<.001 for trend; and 259 bp, *P*<.001 for trend).

Haplotype Analyses

For the 11 sites combined, the frequency of haplotypes defined by the REP1, –770, and –116 loci was significantly different in cases and controls (global score statistic, *P*<.001). For haplotypes defined by only 2 of the 3 loci, the frequency of haplotypes differed significantly in cases and controls only when REP1 was 1 of the 2 loci considered. Specifically, when considering the REP1 and –770 or REP1 and –116 loci, the global score statistic *P* values were both <.001. However, when considering the –770 and –116 loci

only, the global score statistic *P* value was .15. Therefore, variability at the REP1 locus was driving the haplotype associations.

Age at Onset of Parkinson Disease Analyses

Dinucleotide repeat sequence genotypes had no effect on the age at onset of Parkinson disease overall (*P*=.55). The median (range) age at onset for genotypes defined by the 3 common alleles were as follows: 259/259, 61.0 years (22.0-81.9 years); 259/261, 60.2 years (28.0-88.0 years); 259/263, 58.1 years (28.0-86.9 years); 261/261, 61.0 years (22.0-88.0 years); 261/263, 60.0 years (26.0-88.0 years); 263/263, 59.7 years (42.0-79.0 years). Similarly, REP1 genotypes had no effect on age at onset of Parkinson disease for strata defined by women (*P*=.81), men (*P*=.54), familial (*P*=.63), or sporadic (*P*=.37) Parkinson disease.

COMMENT

Our large-scale collaborative analysis documents that variability in the length of a dinucleoptide repeat sequence (REP1) within the *SNCA* promoter is associated with Parkinson disease susceptibility. Genotypes that included the 263 bp allele were associated with an increased risk for Parkinson disease, while genotypes that included the 259 bp allele were associated with a reduced risk for Parkinson disease. Haplotypes

Table 1. Characteristics of the 18 Studies Included in the Collaborative Reanalysis*

		Cases†						Controls			
Investigator	Location	No.‡	Age at Examination, (Range) y	Male Sex, %	Age at Onset (Range), y	Familial Parkinson Disease, %	Diagnostic Criteria	Source	No.‡	Age at Examination (Range), y	Male Sex, %
Aasly	Trondheim, Norway	401	70 (41-92)	61.60	60 (25-88)	28.79	Gelb[41]	Blood bank, spouses, community, cataracts	545	62 (46-96)	55.96
Ashizawa	Houston, Tex	100	65 (32-84)	59.00	53 (28-80)	61.00	Gelb[41]	Spouses of other genetic studies	100	60 (22-75)	49.00
Chartier-Harlin	Lille, France	118	67 (38-88)	47.46	60 (29-84)	100	UKPDBB[42]	Clinic, community	104	67 (41-89)	50.00
Checkoway	Seattle, Wash	160	68 (40-88)	60.00	. . .	13.75	CAPIT[43]	Community	240	72 (45-82)	56.25
Elbaz	Paris, France	200	69 (37-76)	57.50	65 (35-75)	8.72	Bower[44]	Community	444	69 (36-79)	59.46
Ferrarese	Monza, Italy	114	67 (44-86)	60.53	58 (37-77)	19.30	Gelb[41]	Spouses, healthy blood donors	112	66 (48-77)	71.43
Hadjigeorgiou	Larissa, Greece	152	72 (40-95)	59.87	65 (32-88)	9.87	Bower[44]	Community	120	69 (32-89)	53.33
Hattori	Tokyo, Japan	328	66 (33-92)	43.60	59 (27-88)	4.59	. . .	. . .	0	. . .	. . .
Kawakami	Hiroshima, Japan	204	69 (48-88)	40.69	61.5 (32-80)	0.49	Bower[44]	Spouses, community	250	61 (30-100)	48.0
Lynch	Dublin, Ireland	221	61 (21-87)	53.85	50 (17-74)	14.03	UKPDBB[42]	Spouses, community	426	63 (18-99)	38.26
Maraganore	Rochester, Minn	678	68 (31-99)	62.68	63 (28-88)	18.28	Bower[44]	Spouses, community	182	72 (37-94)	37.36
Mellick	Brisbane, Australia	373	68 (33-89)	55.50	61 (26-86)	12.33	Bower[44]	Spouses, community	375	66 (20-89)	30.13
Pappetropoulos	Miami, Fla	77	70 (29-87)	48.05	62 (27-86)	32.47	UKPDBB[42]	Spouses, community	19	64 (55-80)	52.63
Parsian	Little Rock, Ark	217	69 (27-89)	58.99	60 (22-85)	38.25	UKPDBB[42]	Spouses	86	65 (42-84)	33.72
Quattrone	Catanzaro, Italy	190	68 (43-88)	57.89	58 (34-84)	0.00	UKPDBB[42]	Community	181	74 (56-93)	44.75
Riess	Tübingen, Germany	163	68 (38-91)	56.52	56 (22-87)	2.45	UKPDBB[42]	Healthy blood donors MEMO Study	738	73 (65-83)	51.30
Tan	Singapore	247	65 (27-91)	58.30	60 (27-85)	0.40	UKPDBB[42]	Community	251	64 (26-93)	55.78
Van Broeckhoven	Antwerp, Belgium	186	66 (40-87)	58.60	57 (28-75)	0.00	Pals[45]	Spouses, community	188	64 (28-89)	55.32

*Elipses indicate that data were not available.
†All the sources for genetic testing were from a clinical setting except in Seattle, Wash, and Paris, France, which were from a community setting. All the participants were white, except those from Singapore and Japan.
‡Indicates number of participants.

Table 2. Allele Distributions for the 11 Studies That Met Hardy-Weinberg Equilibrium and Genotyping Reliability Criteria

Investigator, Location	Total No. of Alleles*	263 bp Alleles*		259 bp Alleles*		261 bp Alleles*		Overall
		No. (%)	*P* Value†	No. (%)	*P* Value†	No. (%)	*P* Value†	*P* Value‡
Overall								
Case	5384	443 (0.08)	<.001	1294 (0.24)	.002	3647 (0.68)	.77	<.001
Control	5304	312 (0.06)		1413 (0.27)		3579 (0.68)		
Aasly, Trondheim, Norway								
Case	780	79 (0.10)	.03	185 (0.24)	.64	516 (0.66)	.08	.06
Control	1066	77 (0.07)		243 (0.23)		746 (0.70)		
Ashizawa, Houston, Tex								
Case	198	18 (0.10)	.01	43 (0.22)	.22	137 (0.69)	.86	.03
Control	200	6 (0.03)		54 (0.27)		140 (0.70)		
Checkoway, Seattle, Wash								
Case	312	29 (0.10)	.11	82 (0.26)	.44	201 (0.64)	.11	.17
Control	478	30 (0.063)		114 (0.24)		334 (0.70)		
Elbaz, Paris, France								
Case	400	22 (0.06)	.81	115 (0.29)	.89	263 (0.66)	.81	.96
Control	888	46 (0.05)		252 (0.28)		590 (0.66)		
Ferrarese, Monza, Italy								
Case	226	18 (0.08)	.27	68 (0.30)	.50	140 (0.62)	.23	.37
Control	224	12 (0.05)		61 (0.27)		151 (0.67)		
Hadjigeorgiou, Larissa, Greece								
Case	300	15 (0.05)	.38	85 (0.28)	.08	200 (0.67)	.19	.18
Control	232	8 (0.03)		82 (0.35)		142 (0.61)		
Maraganore, Rochester, Minn								
Case	1356	107 (0.08)	.52	313 (0.23)	.005	936 (0.69)	.03	.02
Control	364	25 (0.07)		110 (0.30)		229 (0.63)		
Mellick, Brisbane, Australia								
Case	736	60 (0.08)	.31	163 (0.22)	.05	513 (0.70)	.22	.12
Control	740	50 (0.07)		196 (0.27)		494 (0.67)		
Quattrone, Catanzaro, Italy								
Case	378	27 (0.07)	.62	99 (0.26)	.30	252 (0.67)	.47	.55
Control	354	22 (0.06)		105 (0.30)		227 (0.64)		
Riess, Tübingen, Germany								
Case	326	35 (0.11)	.006	66 (0.20)	.41	225 (0.69)	.38	.02
Control	386	20 (0.05)		88 (0.23)		278 (0.72)		
Van Broeckhoven, Antwerpen, Belgium								
Case	372	33 (0.09)	.01	75 (0.20)	.005	264 (0.71)	.21	.002
Control	372	16 (0.04)		108 (0.29)		248 (0.67)		

Abbreviation: bp, base pair.
*Number of alleles (2 per participant); the number of participants are half the number of alleles indicated; allele frequencies.
†*P* values comparing frequencies in cases and controls for a given allele vs the other 2 alleles combined. The *P* values were obtained from χ^2 distribution with 1 degree of freedom.
‡Overall *P* value comparing frequencies in cases and controls for all alleles. The *P* values were obtained from a χ^2 distribution with 2 degrees of freedom

Table 3. Results of Logistic Regression Mixed Models for Genotypes Defined by the 263 Base Pair vs Others*

Sample or Stratum	No. of Cases/ Controls	Trend OR (95% CI)†	*P* Value	Dominant 263/263 or 263/X vs X/X OR (95% CI)†	*P* Value	Recessive 263/263 vs 263/X or X/X OR (95% CI)†	*P* Value	Unrestricted 263/X vs X/X OR (95% CI)†	*P* Value	Unrestricted 263/263 vs X/X OR (95% CI)†	*P* Value
All	2686/2454	1.43 (1.22-1.69)	<.001	1.44 (1.21-1.70)	<.001	2.46 (0.95-6.37)	.06	1.41 (1.19-1.68)	<.001	2.57 (0.99-6.67)	.05
Negative family history	2241/676	1.33 (1.03-1.72)	.03	1.29 (0.99-1.66)	.06	. . .	. . .	. . .	. . .	. . .	. . .
Positive family history	413/38	1.67 (0.51-5.50)	.40	1.66 (0.50-5.54)	.41	. . .	. . .	. . .	. . .	. . .	. . .
Age, y											
≤68	1361/1317	1.47 (1.17-1.84)	.001	1.49 (1.18-1.90)	.001	1.65 (0.48-5.68)	.43	1.49 (1.17-1.90)	.001	1.74 (0.51-6.00)	.38
>68	1325/1137	1.31 (1.03-1.66)	.03	1.30 (1.01-1.66)	.04	3.08 (0.65-14.46)	.16	. . .	. . .	. . .	. . .
Women	1083/1205	1.33 (1.06-1.67)	.01	1.35 (1.06-1.72)	.01	1.65 (0.53-5.08)	.39	1.34 (1.05-1.71)	.02	1.72 (0.56-5.32)	.35
Men	1603/1249	1.54 (1.22-1.95)	<.001	1.52 (1.20-1.97)	.001	6.23 (0.78-49.70)	.08	1.48 (1.16-1.89)	.002	6.53 (0.82-52.19)	.08

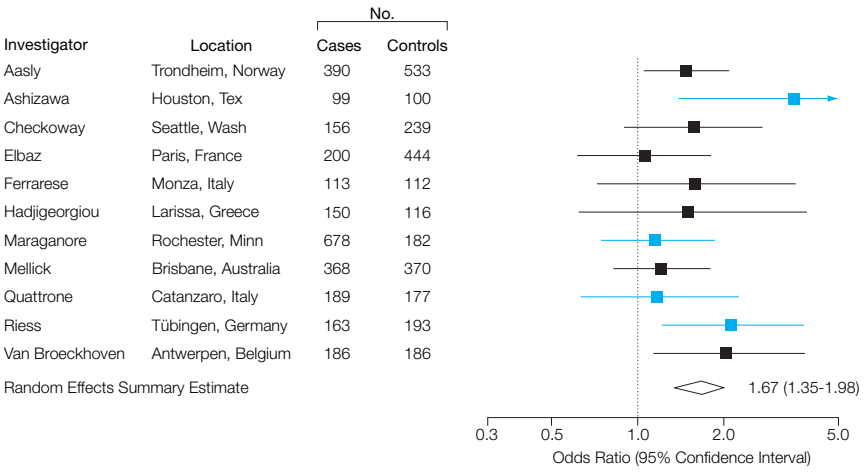
Abbreviations: CI, confidence interval; OR, odds ratio; X, alternate REP1 alleles; ellipses indicate that the model did not converge to final estimates.
*Restricted to 11 sites meeting Hardy-Weinberg equilibrium and genotyping reliability criteria (8 sites had data for positive family history); sites treated as random effect in logistic regression mixed effects models; models adjusted for age (continuous) and sex as appropriate; all available participants with nonmissing data were used where possible.
†Assuming a linear trend in log odds for 0, 1, and 2 alleles

defined by *SNCA* REP1 and 2 SNPs flanking the core promoter at the –770 and –116 positions were associated with Parkinson disease, but REP1 variability was driving this association. Although genetic association studies are prone to false-positive findings, we think this is an unlikely explanation of our findings because (1) we only tested 3 hypotheses; (2) our sample size was large and the alleles were common (sufficient power); (3) we used rigorous quality measures in our data sharing, genotyping, and analyses; (4) our findings were generalizable to multiple populations worldwide; and (5) our findings had biological plausibility.[46-48] In summary, our findings further highlight the importance of *SNCA* as a susceptibility gene for Parkinson disease.

Previous studies suggested that *SNCA* REP1 allele length variability conferred an increased risk for Parkinson disease via a mechanism of gene overexpression.[18-20] If so, interventions targeting *SNCA* expression may reduce the risk for developing Parkinson disease (primary prevention). However, there is no evidence to date that *SNCA* gene variability modifies prognostic outcomes for Parkinson disease, and it is therefore uncertain whether therapies to reduce *SNCA* expression would slow the progression of Parkinson disease (secondary prevention). We observed no modifying effect for *SNCA* REP1 genotypes on age at onset of Parkinson disease. This was unexpected, because gene multiplication carriers develop Parkinson disease at a younger age proportionate to the number of gene copies (overexpression),[10] and because a small study recently reported an association of REP1 and age at onset of Parkinson disease in individuals from Greece.[25] It is possible therefore that the association of *SNCA* REP1 variability with Parkinson disease is through a mechanism other than gene overexpression or that the modest degree of overexpression is sufficient to increase risk but not to modify disease characteristics or outcomes. Longitudinal studies of Parkinson disease cohorts are required to determine whether *SNCA* is also a modifier gene for Parkinson disease.

There are several strengths and also weaknesses inherent to collaborative analyses of genetic association studies.[35] A strength of our study is that we included published and unpublished data from several diverse sites worldwide. The combined sample size for our analyses was substantial, and to our knowledge this represents the largest case-control study of Parkinson disease to date. We excluded studies that

Figure 1. Results of Logistic Mixed Models for the 263 Base Pair Allele



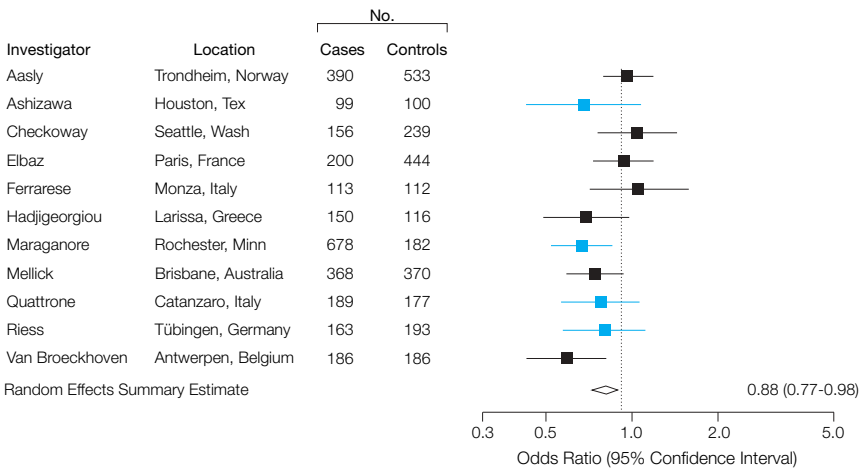
Odds ratios estimates with 95% confidence intervals for the *SNCA* REP1 genotypes defined by the 263 base pair allele length. The confidence interval plot assumes log additive (trend) effects in logistic regressions, and the estimates are unadjusted for age and sex because not all studies had data for adjustment variables. The data are presented separately for each of the 11 studies meeting Hardy-Weinberg equilibrium and genotyping reliability criteria. Blue squares indicate published data and black squares indicate unpublished data. The summary estimate (treating the studies as random effects) is indicated by the diamond.

Table 4. Results of Logistic Regression Mixed Models for Genotypes Defined by the 259 Base Pair vs Others*

Sample or Stratum†	No. of Cases/ Controls	Trend‡ OR (95% CI)†	*P* Value	Dominant 259/259 or 259/X vs X/X OR (95% CI)	*P* Value	Recessive 259/259 vs 259/X or X/X OR (95% CI)	*P* Value	Unrestricted 259/X vs X/X OR (95% CI)	*P* Value	Unrestricted 259/259 vs X/X OR (95% CI)	*P* Value
All	2686/2454	0.86 (0.79-0.94)	.002	0.85 (0.76-0.96)	.01	0.76 (0.61-0.96)	.02	0.88 (0.78-0.99)	0.04	0.72 (0.57-0.92)	.01
Negative family history	2241/676	0.98 (0.85-1.13)	.75	1.00 (0.83-1.19)	.98	0.89 (0.64-1.24)	.49	1.02 (0.84-1.23)	0.84	0.90 (0.64-1.26)	.53
Positive family history	413/38	0.65 (0.40-1.06)	.08	0.73 (0.40-1.32)	.30	0.25 (0.07-0.85)	.03	0.84 (0.46-1.54)	0.58	0.23 (0.08-0.81)	.02
Age, y											
≤68	1361/1317	0.89 (0.78-1.01)	.07	0.86 (0.73-1.01)	.06	0.87 (0.63-1.21)	.42	0.86 (0.73-1.02)	0.09	0.82 (0.59-1.15)	.26
>68	1325/1137	. . .	. . .	. . .	. . .	0.68 (0.49-0.94)	.02	. . .	. . .	. . .	. . .
Women	1083/1205	0.88 (0.77-1.01)	.07	0.90 (0.76-1.07)	.24	0.68 (0.48-0.96)	.03	0.95 (0.80-1.14)	0.62	0.67 (0.47-0.95)	.02
Men	1603/1249	0.85 (0.75-0.97)	.01	0.81 (0.69-0.95)	.01	0.84 (0.62-1.16)	.29	0.82 (0.69-0.97)	0.02	0.78 (0.56-1.07)	.13

Abbreviations: CI, confidence interval; OR, odds ratio; X, alternate REP1 alleles; ellipses indicate that the model did not converge to final estimates.
*Restricted to 11 sites meeting Hardy-Weinberg equilibrium and genotyping reliability criteria (8 sites had data for positive family history); sites treated as random effect in logistic regression mixed effects models; models adjusted for age (continuous) and sex as appropriate; all available participants with nonmissing data were used where possible.
†Assuming a linear trend in log-odds for 0, 1, and 2 alleles.

Figure 2. Results of Logistic Mixed Models for the 259 Base Pair Allele



Odds ratios estimates with 95% confidence intervals for the *SNCA* REP1 genotypes defined by the 259 base pair allele length. The confidence interval plot assumes log additive (trend) effects in logistic regressions and the estimates are unadjusted for age and sex, because not all studies had data for adjustment variables. The data are presented separately for each of the 11 studies meeting Hardy-Weinberg equilibrium and genotyping reliability criteria. Blue squares indicate published data and black squares indicate unpublished data. The summary estimate (treating the studies as random effects) is indicated by the diamond.

did not fulfill Hardy-Weinberg equilibrium expectations in controls. The Hardy-Weinberg equilibrium can be a surrogate measure of genotyping accuracy and Hardy-Weinberg equilibrium deviations may be an unrecognized source of failed replications of postulated gene-disease associations.[49,50] For each site, we also assessed the reliability of genotyping compared with the genotyping in a laboratory core, and we included in the analyses only those studies with 90% or greater reliability. For studies with reliable genotyping, we postcoded genotypes so that allele length calling was standardized for all studies according to the allele lengths called by the laboratory core.

The deviations from Hardy-Weinberg equilibrium and the variability of allele length that we observed across multiple sites may have introduced bias in the published literature. In the case of *SNCA* REP1 and Parkinson disease, a meta-analysis of more limited published data reached conclusions that were in part consistent with our findings.[24] The authors of that study were able to perform allele frequency analyses only. The most significant finding of that study was the association between the 259 bp allele and decreased risk of Parkinson disease, but an association between the 263 bp allele and increased risk of Parkinson disease was also reported. By contrast, the most significant finding of this study is the association between genotypes defined by the 263 bp allele and increased risk of Parkinson disease (while an association between genotypes defined by the 259 bp allele and decreased risk of Parkinson disease was reported as a less significant finding).

By contrast to other studies, we were only able to define *SNCA* promoter haplotypes using 3 loci (REP1 and the flanking –770 and –116 SNPs).[16,17,51,52] However, these were the only *SNCA* gene variants that were genotyped in common for 2 or more of the published studies at the time that the collaborative analysis was designed. Extended haplotype analyses may have been more informative. However, we also note that haplotype-tagging SNPs may be in part population-specific, precluding their value for collaborative analyses of data from many centers worldwide. A gene-based analysis rather than a single SNP or haplotype-tagging SNP approach may be more appropriate for replication studies of candidate genes across diverse populations.[53]

The results did not differ significantly in the sensitivity analyses including 6 additional studies that did not fulfill Hardy-Weinberg equilibrium or genotyping reliability criteria. The 3 studies that did not fulfill Hardy-Weinberg equilibrium criteria were not of 1 ethnicity or race. This would argue against population genetic forces as the source of the deviation from expectancy. More likely, laboratory error was the source of the deviation because the genotyping of microsatellite markers can be problematic. Indeed, for 3 additional studies, the reliability of the REP1 genotyping was clearly in question. Even so, although the exclusion of these 6 studies resulted in a nearly 30% reduction in the total sample size, this quality measure had little impact on the results of the study. We cannot exclude the possibility that other site-specific differences may have biased the results of the study. However, we observed no statistically significant heterogeneity of the ORs, and we adjusted our analyses for study (where appropriate). Furthermore, a qualitative assessment suggested that other study differences, such as diagnostic criteria (Table 1), did not seem to associate with the distribution of ORs (greater or smaller than 1, Figure 1 and Figure 2).

In conclusion, our study demonstrates that the *SNCA* gene is not only a rare cause of autosomal dominant Parkinson disease in some families[6-9] but also a susceptibility gene for Parkinson disease at the population level. Based on our results, we estimate that REP1 locus variability may explain approximately 3% of the risk in the general population. This is in the same range as the population effect of other common variants implicated in Parkinson disease.[35] The additive effects of these and other common gene variants may ultimately account for a substantial fraction of the susceptibility to Parkinson disease.[5,54] However, given the small effect sizes implicated, large-scale collaborations with meticulous standard-

ization of methods (including statistical adjustments for multiple possible confounders) would be desirable.[55] Similarly, large-scale collaborations will be required to document interactions of *SNCA* with other genes or environmental factors conferring susceptibility to Parkinson disease.[56,57]

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