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Association of *SNCA* with Parkinson: replication in the Harvard NeuroDiscovery Center Biomarker Study

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Abstract

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Background—Mutations in the α-synuclein gene (*SNCA*) cause autosomal dominant forms of Parkinson's disease, but the substantial risk conferred by this locus to the common sporadic disease has only recently emerged from genome-wide association studies.

Methods—Here we genotyped a prioritized non-coding variant in *SNCA* intron-4 in 344 patients with Parkinson's and 275 controls from the longitudinal Harvard NeuroDiscovery Center Biomarker Study.

Results—The common minor allele of rs2736990 was associated with elevated disease susceptibility (odds ratio = 1.40, *P* value = 0.0032).

Conclusions—This result increases confidence in the notion that in many clinically well-characterized patients genetic variation in *SNCA* contributes to "sporadic" disease.

Keywords

Parkinson's disease; α-synuclein; GATA transcription factors; biomarker; genome-wide association study

INTRODUCTION

Parkinson's disease (PD) is an aging-dependent, progressive neurodegenerative disease that poses an increasing threat to public health as life expectancy is improving worldwide. α-Synuclein has been correlated with sporadic PD since its discovery as a core constituent of Lewy bodies, but a considerable genetic contribution of non-coding *SNCA* variants to the sporadic disease has only recently emerged from genome-wide association studies. Here we successfully replicated an association between the rs2736990 variant in *SNCA* intron-4 and PD highlighted by Simon-Sanchez et al. 2009 in the Harvard NeuroDiscovery Center Biomarker Study (HBS).

METHODS

Study population

HBS is a Harvard-wide, longitudinal, case-control study designed to accelerate the discovery and validation of molecular diagnostics that track or predict progression of early-stage PD and Alzheimer's disease (AD). Inclusion criteria for cases with PD are age ≥ 21, diagnosis of PD according to UK PD Society Brain Bank (UKPDSBB) criteria or according to movement disorders specialist assessment [1], MMSE score > 21 or next of kin present to provide informed consent, and ability to provide informed consent. Two modifications to UKPDSBB clinical diagnostic criteria were made to allow for more than one affected relative and response to dopamine replacement therapy. Exclusion criteria for cases with PD in HBS: diagnosis of a blood or bleeding disorder, known hematocrit < 30, or known active ulcer or active colitis. Inclusion criteria for healthy controls were non-blood relatives (generally spouses) of cases with PD or patients with AD enrolled in HBS, no current diagnosis or history of a neurological disease, ability to provide informed consent, and age ≥ 21 (≥ 30 for spouses of AD patients). Using this definition, the controls are comparable to the PD cases in that they are drawn from the same source population and could be identified as a case, if they had disease. Exclusion criteria for non-blood relatives of patients with PD: same as for cases. For the current genetic case-control association study nested in HBS, all cases with PD and healthy controls enrolled in HBS at the time of analysis (February 2010) with available DNA specimens were included yielding a total of 375 cases with PD and 275 controls. The Institutional Review Boards of Brigham and Women's Hospital and Massachusetts General Hospital approved all studies.

Genetic association study

Genotyping was performed by TaqMan SNP assay on an ABI7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) for SNP rs2736990 (ACCTTATGAGCTGTTTAGGAAGAAG[A/G]TGTATATGTGTGTAACAGGGAGCAA). The genotyping completion rate was 98% and the concordance rate was 100% for replicate assays included for 10% of randomly selected samples. Genotype frequencies were examined for deviation from Hardy-Weinberg equilibrium using χ^2 tests. Logistic regression was used to estimate statistical significance, odds ratios (ORs), 95% confidence intervals (CIs) under allelic, dominant, and recessive models, while adjusting for age and sex, using SAS software 9.2 for Windows (SAS Institute, Cary, NC, USA). The Cochran-Armitage trend test was used to examine allelic additive effects. The primary analysis included cases meeting UKPDSBB diagnostic criteria. The secondary analysis included all cases based on diagnosis of PD by movement disorders specialist. A *P* value less than 0.05 was considered statistically significant.

RESULTS

344 of 375 patients (91.73%) diagnosed with PD by a neurology board-certified, movement disorders fellowship-trained neurologist met modified UKPDSBB criteria. An overview of baseline clinical characteristics is shown in Table 1. Allele frequency distribution of the rs2736990 polymorphism in *SNCA* is shown in Table 2. Hardy-Weinberg equilibrium was not violated in controls. This SNP was highly present in the general population with a minor allele frequency (G) of 45.9% in controls and 54.5% in cases with PD. We found a significant association between the rs2736990 variant and PD in the HBS population (Table 2). For cases meeting UKPDSBB criteria significant associations were obtained under dominant (OR = 1.60, 95% CI: 1.08–2.36), recessive (OR = 1.54, 95% CI: 1.04–2.28) and allelic models (OR = 1.41, 95% CI: 1.11–1.78) (Table 2). For each minor allele, there was a 40% increase in risk of PD (OR=1.40, 95% CI: 1.12–1.76, *P* = 0.0032) in the carriers. A secondary analysis that included all 375 cases based on diagnosis of PD by movement disorders specialist produced virtually identical results (Table 2). Exploratory analyses of clinical phenotypes of cases with PD carrying two (GG), one (AG), or no risk allele (AA) are shown on the right side of Table 1. Considering the many clinical characteristics explored, none would reach compelling statistical significance after adjustment for multiple testing (although trends observed may justify further exploration in a much larger cohort).

DISCUSSION

α-Synuclein is central to the pathobiology of PD. Simply genetically increasing the expression of the α-synuclein gene (*SNCA)* by 50–100% through locus multiplication unequivocally causes autosomal dominant Parkinson's [2]. Although small, over years these increases in wild-type *SNCA* expression are sufficient to bring death to a majority of vulnerable dopamine neurons. Whereas mutations in *SNCA* have long been linked to rare autosomal dominant forms of PD, a substantial genetic contribution of this gene to sporadic PD has only recently been appreciated [3]. A recent genome-wide association study highlighted an association between the rs2736990 variant in *SNCA* intron-4 and common, sporadic PD [3]. Here we confirmed this association in an independent, clinically well-characterized population. This intronic variant, together with the REP1 *SNCA* promoter polymorphism [4] and other implicated 5′ and 3′ variants [3,5], suggests a genetic role for non-coding variants in *SNCA* in conferring susceptibility to some forms of the common "sporadic" disease. How such polymorphisms enhance susceptibility to PD is unclear. It is possible that rs2736990 or an as yet unidentified linked causal sequence variant may regulate transcription of *SNCA* either directly through a *cis*-acting mechanism or indirectly through interaction with transcriptional enhancers [6] and repressors. Pinpointing the true

PD-associated variants in *SNCA* and their mechanism and clarifying the relation to early mitochondrial dysfunction [7] will be important challenges for future research.

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References

1. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain. 2002; 125:861–70. [PubMed: 11912118]
2. Singleton AB, Farrer M, Johnson J, et al. Alpha-Synuclein locus triplication causes Parkinson's disease. Science. 2003; 302:841. [PubMed: 14593171]
3. Simon-Sanchez J, Schulte C, Bras JM, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009; 41:1308–12. [PubMed: 19915575]
4. Chiba-Falek O, Nussbaum RL. Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (*SNCA*) on transcription in a cell culture luciferase reporter system. Hum Mol Genet. 2001; 10:3101–9. [PubMed: 11751692]
5. Mueller JC, Fuchs J, Hofer A, et al. Multiple regions of alpha-synuclein are associated with Parkinson's disease. Ann Neurol. 2005; 57:535–41. [PubMed: 15786467]
6. Scherzer CR, Grass JA, Liao Z, et al. GATA transcription factors directly regulate the Parkinson's disease-linked gene alpha-synuclein. Proc Natl Acad Sci U S A. 2008; 105:10907–12. [PubMed: 18669654]
7. Zheng B, Liao Z, Locascio JJ, et al. PGC-1α, a potential therapeutic target for early intervention in Parkinson's disease. Sci Transl Med. 2010; 2:52ra73.

HARVARD NEURODISCOVERY CENTER BIOMARKER STUDY

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Table 1

Clinical characteristics of study participants

Disease status	**PD**	**Control**		**PD**			
Genotype				**AA**¶	**AG**¶	**GG**¶	
N	**375**	**275**	***P*#**	**80**	**181**	**114**	***P****
Age (Mean ± SD)	66.42 ± 10.83	68.57 ± 10.42	0.0114	67.23 ± 13.89	66.18 ± 9.97	66.24 ± 9.73	0.4942
Male	244 (65.07%)	94 (34.18%)	<0.0001	56 (70.00%)†	118 (65.19%)	70 (61.40%)	0.4651
Age of onset	61.00 ± 11.51			61.56 ± 14.43	61.07 ± 10.37	60.44 ± 10.88	0.8136
Clinical findings (%)							
Bradykinesia	374 (99.73)			80 (100.00)	181 (100.00)	113 (99.12)	1.0000
Resting tremor	265 (70.67)			61 (76.25)	130 (71.82)	74 (64.91)	0.2006
Rigidity	366 (97.60)			79 (98.75)	176 (97.24)	111 (97.37)	0.7006
Asymmetric onset	283 (75.47)			59 (73.75)	137 (75.69)	87 (76.32)	0.8749
Postural instability	191 (50.93)			41 (51.25)	84 (46.41)	66 (57.89)	0.1335
UPDRS total (Mean ± SD)	32.78 ± 15.70			33.12 ± 15.10	31.51 ± 15.06	34.47 ± 16.96	0.2322
UPDRS subscale 1	1.83 ± 1.70			1.93 ± 1.68	1.70 ± 1.58	1.98 ± 1.89	0.3412
UPDRS subscale 2	9.50 ± 5.84			9.98 ± 6.43	9.20 ± 5.49	9.66 ± 5.96	0.5830
UPDRS subscale 3	19.32 ± 10.09			19.71 ± 9.47	18.31 ± 9.67	20.58 ± 11.01	0.1628
UPDRS subscale 4	2.35 ± 2.18			2.13 ± 2.04	2.46 ± 2.32	2.33 ± 2.03	0.5361
Hoehn and Yahr	2.16 ± 0.75			2.30 ± 0.84	2.06 ± 0.66	2.21 ± 0.81	0.0459
MMSE	28.19 ± 2.46	29.27 ± 1.10	<0.0001	28.00 ± 2.82	28.36 ± 2.24	28.03 ± 2.53	0.2094
Medications (%)							
De novo	50 (13.33)			9 (11.25)	28 (15.47)	13 (11.40)	0.5009
Carbidopa+levodopa	250 (66.67)			53 (66.25)	118 (65.19)	79 (69.30)	0.6640
Ropinirole	53 (14.13)			12 (15.00)	26 (14.36)	15 (13.16)	0.9466
Pramipexole	106 (28.27)			22 (27.50)	48 (26.52)	36 (31.58)	0.5869
Amantadine	37 (9.87)			5 (6.25)	19 (10.50)	13 (11.40)	0.4405
Entacapone	52 (13.87)			9 (11.25)	27 (14.92)	16 (14.04)	0.7188
Trihexyphenidyl	15 (4.00)			2 (2.50)	5 (2.76)	8 (7.02)	0.1341
Carbidopa+levodopa+entacapone	39 (10.40)			9 (11.25)	17 (9.39)	13 (11.40)	0.8155

Disease status	**PD**	**Control**		**PD**			
Genotype				**AA¶**	**AG¶**	**GG¶**	
N	**375**	**275**	***P*#**	**80**	**181**	**114**	***P****
Selegiline	25 (6.67)			3 (3.75)	15 (8.29)	7 (6.14)	0.3843
Rasagiline	32 (8.53)			8 (10.00)	15 (8.29)	9 (7.89)	0.8735
Other PD meds	36 (9.60)			6 (7.50)	21 (11.60)	9 (7.89)	0.4790

#T-test or χ^2 test was used to estimate the significance between cases and controls for numerical or nominal variables, respectively.

¶The right side of the table shows clinical characteristics of cases with PD carrying two (GG), one (AG), or no risk allele (AA).

†In columns 5, 6, and 7, % of cases per genotype are shown in parenthesis.

*ANOVA or χ^2 test was used to explore differences in clinical characteristics within PD cases of distinct genotypes; note that *P* values shown were not adjusted for the multiple clinical characteristics explored.

Table 2

Association between the intron-4 *SNCA* polymorphism rs2736990 and risk of PD

Diagnostic Criteria	N		MAF† (%)		Additive*		Dominant		Recessive		Allelic	
	PD	Control	PD	Control	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
UKPDSBB	344	275	54.51	45.94	1.40 (1.12–1.76)	0.0032	1.60 (1.08–2.36)	0.0183	1.54 (1.04–2.28)	0.0297	1.41 (1.11–1.78)	0.0052
PD Specialist	375	275	54.53	45.94	1.40 (1.12–1.75)	0.0026	1.59 (1.09–2.34)	0.0173	1.55 (1.06–2.28)	0.0245	1.41 (1.14–1.78)	0.0043

Odds ratio (OR) and 95% confidence interval (CI) were calculated for minor allele, adjusted for age and sex.

† Minor allele frequency.

* Linear trend for zero, one or two minor alleles.