Genetic variants of α-synuclein are not associated with essential tremor

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Abstract

Background—Given the overlap between Parkinson disease and essential tremor, we examined genetic variants in α-synuclein (*SNCA*) as risk determinants for essential tremor.

Methods—Samples from 661 essential tremor subjects and 1,316 control subjects from four participating North American sites were included in this study. Parkinson disease samples (n=427) were compared against controls for two cohorts. Twenty variants were selected for association analysis within the *SNCA* locus. Individual logistic regression analyses against essential tremor diagnosis and then combined using meta-analysis was run for each variant.

Results—Our results do not show a significant association between variants in the *SNCA* locus and risk of essential tremor, while the established association of *SNCA* variants with Parkinson disease risk was observed.

Conclusions—While genetic factors are likely to play a large role in essential tremor pathogenesis our results do not support a role for common *SNCA* genetic variants in risk for essential tremor.

Keywords

tremor; essential tremor; association studies in genetics; Parkinson's disease; parkinsonism; synuclein

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Introduction

Clinical phenotyping and neuropathological data suggest overlaps between essential tremor (ET) and Parkinson disease (PD)[1-6]. There is considerable evidence that genetics plays a large role in ET pathogenesis[7-9]. A few cases of ET-like phenotypes are reported with PD-causative mutations[10-14]. However, of the three familial loci nominated for ET[15-17] (www.ncbi.nlm.nih.gov/sites/omim) none correspond to PARK loci or PD genes (www.PDgene.org). A genomewide ET association study recently nominated association at the *LINGO1* locus[18]. Subsequent studies also suggested a role for *LINGO1* variants in PD risk, although the findings in ET and PD remain equivocal[19-27].

Mutations in the gene encoding α-synuclein (*SNCA*) cause monogenic PD, with altered α-synuclein biology likely playing a role in PD pathogenesis[28]. α-Synuclein-positive Lewy bodies are reported in a subset of ET cases in some neuropathological series[1, 29, 30]. Early ET studies focused on *SNCA* locus 5′-end genetic variation[31, 32], reporting significant association of Rep1 alleles with PD (n=100; p=0.02), and ET (n=46; p=0.02)[31], a finding not replicated in a larger study (ET n=106)[32]. This study also examined intron 1 single nucleotide polymorphisms (SNPs): neither SNP nor haplotype analyses showed significant association with ET risk[32]. Early Rep1 PD studies also yielded mixed results; however, a recent large meta-analysis established a role for *SNCA* variation in PD risk[33].

Given this body of evidence we extensively examined SNPs in conserved regions throughout the *SNCA* locus in four large ET patient-control series.

Subjects and Methods

Human Subjects

Four independent North American ET case-controls series (Emory University, Mayo Clinic Florida, Columbia University, USA and University of Saskatchewan, Canada) were examined (Table 1). A total of 661 ET subjects and 1,316 control subjects were included in this study. PD samples (n=427) from Mayo Clinic were run as association controls. The institutional review boards approved all work. All participants provided written informed consent. There are no known related samples within or between the diagnosis groups or the cohorts. All subjects self-identified ethnicity.

For all samples meeting consent and family relationships screening criteria, an ET research diagnosis was determined using direct examination by a movement disorders neurologist (CMT, AR, ZKW, RJU, EL), review of videotaped examination by a movement disorders neurologist (EL), or review of longitudinal movement disorders clinical notes with examination, medication response data, handwriting, and research interview data (CMT). Cases were given a research diagnosis of ET using published criteria[34, 35] and were assigned possible, probable or definite ET status[17, 35]. All cases with both ET and PD diagnoses were excluded. PD was determined using UK Brain Bank criteria[36]. ET cases with reported history or reported exam evidence of dystonia, reported family history of dystonia, or reported medical history of other significant neurological diagnoses were excluded. All remaining definite and probable ET cases were then analyzed. Control subjects were obtained through review of all available neurology clinical research control samples at each center meeting screening criteria above. Control subjects were excluded for: any reported personal or family history of tremor, ET, PD or dystonia; any other significant neurological diagnoses.

Genetic Analysis

DNA was extracted from whole blood samples (or, rarely, buccal brush) using standard protocols. All samples were coded by randomly assigned unique identifier. Conserved regions (conservation score > 200) were identified across *SNCA* (coding regions ±10Kb) using the phastConst software embedded in UCSC Genome Browser (http://genome.ucsc.edu), based on the NCBI March 2006 assembly[37]. We identified 20 SNPs with a minor allele frequency >1% which were selected for analysis. Genotyping was performed on a Sequenom MassArray iPLEX platform (San Diego, CA) (primer sequences, Supplemental Table 1).

Statistical Analysis

Exact tests for Hardy-Weinberg equilibrium (HWE) were performed separately for ET samples and controls. SNPs with HWE *P*-values<.01 or a less than 95% SNP call rate in either ET samples or controls for each cohort were excluded from further analyses involving that cohort. Association tests were performed separately for each of the four sets of ET samples and controls. For each cohort, individual logistic regression analyses were run for each of the 20 SNPs, with ET status modeled as a function of SNP allele count (0, 1, or 2) and race (Caucasian or African-American). The four cohorts of subjects were then combined in a meta-analysis by running a logistic regression for each SNP that modeled ET status as a function of SNP allele count, race, and cohort. All statistical association tests were performed using R software (http://www.r-project.org/).

Power analyses were carried out for each SNP based on average minor allele frequency and number of available samples using Quanto software (hydra.usc.edu/gxe/) (Supplemental Table 2). An additive genetic model was assumed in all calculations. With the available samples, a moderate association with a genotype relative risk (GRR) of 1.3 or greater would be detectable with >80% power in 15 out of 20 SNPs. Larger effects (GRR ≥1.5) would be detectable with >80% power in 17/20 SNPs and with >60% power in all SNPs.

Results

Only four SNPs were excluded from analysis in one or more cohorts due to HWE failure, but the inclusion or exclusion of these SNPs in the analysis had no substantial effect on the observed results. The established PD risk association was observed in the Mayo Clinic series (Supplemental Table 3), however, no significant associations were observed between ET and any of the 20 SNPs tested in any of the four cohorts or in the meta-analysis combining the four cohorts (Table 2, 0.24 < *P* < 1). Allele frequencies and odds ratios from the logistic regression association tests are presented in Table 2, with odds ratios and *P*-values from the meta-analysis in rightmost columns. Similar results (not shown) were obtained restricting the analysis to over age 65 controls. For SNPs with minor allele frequencies > 0.25 an effect with genotype relative risk ≥1.3 (≥1.5) would have been detectable in our ET meta-analysis with >93% (≥99%) power; subtle effects with genotype relative risk ~1.2 were detectable with ≥70% power. All SNPs associated with PD in the Mayo Clinic series had minor allele frequencies > 0.25 (Supplemental Table 2).

Discussion

Although a wide range of data suggests ET and PD could share some common etiologies, no significant genetic link has been demonstrated. Genetic associations may support hypotheses on disease mechanism and relationships between disorders. We therefore investigated whether genetic variation at the *SNCA* locus is a risk for ET, using North American ET case-control collections (Table 1), and SNPs associated with PD risk (Supplemental Table

3). We examined SNPs in conserved regions throughout the *SNCA* locus, extending genetic data compared to prior ET studies focusing on the locus 5′-end. Our study contained three SNPs examined by Pigullo and colleagues[32] (Table 2).We examined four independent ET patient-control series, a collaborative effort providing significantly larger ET and control datasets compared to prior studies. Overall our results did not show any association with ET risk (Table 2). Of note, no association with *SNCA* variants was reported by Stefansson et al[18] in their genomewide association study. They employed the Illumina HumanHap300 SNP array which carries approximately 20 variants (only two of which overlap with the present study) across the *SNCA* locus, thus it would be of interest to examine the raw p-values for *SNCA* and other PD-related loci.

ET is often reported to precede PD onset, and to occur at higher than expected frequencies in relatives of PD patients[2, 3, 5]. ET patients can develop parkinsonian features including rest tremor and mild motor tone changes that do not fulfill diagnostic criteria for PD[4, 5, 38]. Overlaps between ET and PD features could argue for similar underlying neuropathology between the two disorders; however, the extent of motor and non-motor findings outside of kinetic tremor in ET is disputed. Much like tremor-predominant versus primary gait impairment PD, the clinical entity of ET is sometimes subdivided, for example by medication responsiveness or family history, but it is unknown whether these subdivisions correlate with pathology. Disputed ET subsets, such as ET with mild parkinsonian findings, are simply included under ET[5]. Failure to replicate genetic associations may thus be due to inclusion of multiple, distinct ET forms within a single cohort. For example, a sub-group of ET patients with more of a parkinsonian presentation may be influenced by *SNCA* variation. Stratifying *SNCA* or other genetic analyses by phenotypic variables will require increasing the numbers of prospectively gathered ET samples with standardized detailed phenotypic information. Finally, even assuming a genetically homogeneous ET cohort, results may be compromised by false negative controls. ET is common, onset age range is large, and disease risk increases with age; thus, controls may represent pre-symptomatic ET, even when restricted to older ages.

Despite the challenges inherent in ET studies, molecular genetics represents an important approach to uncovering the pathogenic mechanisms behind ET[9]. Understanding genetic risks in ET will play a significant part in designing therapeutic strategies aimed at prevention and cure of this prevalent movement disorder. We have formed a multi-center collaboration to advance work in the field. Further studies on large, ethnically diverse and prospectively phenotyped populations are necessary to generate the genetic findings that will enlighten the field of ET research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Appendix

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Table 1

ET subject and control series demographics.

	Mayo		Canada		Emory		Columbia	
	ET	**control**	**ET**	**control**	**ET**	**control**	**ET**	**control**
n	135	427	201	313	118	268	193	282
age	72.8+10.7 (43, 93)	72.1+10.9 (33, 92)	70.0+13.3 (18, 95)	67.8+13.2 (22, 95)	69.6+12.3 (18, 96)	76.0+6.9 (65, 101)	69.9+13.7 (22, 91)	67.5+11.8 (20, 92)
Age at onset	50.1+20.2 (5, 88)	N/A	54.3+18.5 (14, 87)	N/A	46.0+21.7 (1, 83)	N/A	44.5+23.2 (1, 89)	N/A
Gender (male)	66	228	75	90	51	102	90	118
Caucasian	135	427	201	313	112	218	188	258
African-American	-	-	-	-	6	50	5	24

The sample mean ± SD (minimum, maximum) is given for age and age at onset. Ages are given in years. All other data are total counts (n). Total sample sizes given for each series do not account for genotyping failure, which occurred in <5% of samples.

Table 2

Minor allele frequencies (MAF) and odds ratios (OR) from association tests of ET and 20 SNPs throughout the SNCA locus.

Chr4 (bp)†	SNP	Mayo 135 cases 423 controls		Canada 201 cases 313 controls		Emory 118 cases 267 controls		Columbia 193 cases 282 controls		Combined samples	
		MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	Combined OR (95% CI)	P-value*
90637010	rs356218	0.311 0.319	0.96 (0.72-1.3)	0.308 0.329	0.91 (0.69-1.2)	0.326 0.315	0.99 (0.70-1.4)	0.396 0.328	1.29 (0.99-1.7)	1.04 (0.90-1.2)	0.621
90653134	rs17180453	0.074 0.079	0.93 (0.56-1.6)	0.075 0.085	0.87 (0.55-1.4)	0.055 0.071	0.66 (0.34-1.3)	0.093 0.087	1.02 (0.65-1.6)	0.89 (0.69-1.1)	0.363
90657491	rs3775423	0.111 0.073	1.56 (0.99-2.5)	0.075 0.083	0.89 (0.56-1.4)	- -	-	- -	-	1.08 (0.92-1.3)	0.35
90678541	rs2736990Φ	0.467 0.462	1.02 (0.78-1.3)	0.463 0.447	1.06 (0.83-1.4)	0.479 0.507	1.02 (0.75-1.4)	- -	-	1.03 (0.90-1.2)	0.654
90678798	rs2572324	0.289 0.279	1.05 (0.78-1.4)	0.286 0.289	0.98 (0.74-1.3)	0.275 0.26	0.93 (0.65-1.3)	0.298 0.27	1.09 (0.82-1.4)	1.02 (0.88-1.2)	0.808
90687507	rs3796661	0.022 0.027	0.83 (0.35-2.0)	0.02 0.032	0.63 (0.28-1.4)	0.03 0.028	1.37 (0.55-3.5)	0.036 0.055	0.69 (0.36-1.3)	0.79 (0.53-1.2)	0.241
90707947	rs2737033	0.278 0.252	1.14 (0.84-1.5)	0.251 0.278	0.87 (0.65-1.2)	0.28 0.258	0.99 (0.70-1.4)	0.256 0.241	1.03 (0.77-1.4)	1 (0.86-1.2)	0.998
90709741	rs3775439Φ	0.144 0.144	1 (0.67-1.5)	- -	-	0.148 0.182	1.14 (0.73-1.8)	0.148 0.186	0.9 (0.63-1.3)	0.99 (0.79-1.2)	0.926
90712629	rs10014396	0.126 0.125	1.01 (0.66-1.5)	- -	-	0.114 0.152	0.89 (0.55-1.4)	0.098 0.138	0.77 (0.50-1.2)	0.88 (0.68-1.1)	0.328
90716177	rs9995651	0.067 0.04	1.76 (0.96-3.2)	0.037 0.038	0.97 (0.50-1.9)	0.047 0.075	0.82 (0.40-1.7)	0.021 0.048	0.58 (0.25-1.3)	1.01 (0.71-1.4)	0.966
90721637	rs2583959	0.278 0.243	1.19 (0.88-1.6)	0.241 0.276	0.83 (0.62-1.1)	0.271 0.243	0.98 (0.69-1.4)	0.251 0.23	1.05 (0.78-1.4)	1 (0.86-1.2)	0.993
90745707	rs2737012	0.281 0.241	1.23 (0.90-1.7)	0.241 0.275	0.84 (0.62-1.1)	0.271 0.242	0.99 (0.70-1.4)	0.251 0.23	1.05 (0.78-1.4)	1.01 (0.87-1.2)	0.884

Chr4 (bp)[†]	SNP	Mayo 135 cases 423 controls		Canada 201 cases 313 controls		Emory 118 cases 267 controls		Columbia 193 cases 282 controls		Combined samples	
		MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	MAF: cases controls	OR (95% CI)	Combined OR (95% CI)	P-value*
90757309	rs1372519[‡]	0.193 / 0.23	0.81 (0.58-1.1)	0.228 / 0.208	1.12 (0.83-1.5)	0.195 / 0.23	0.83 (0.56-1.2)	0.263 / 0.223	1.25 (0.92-1.7)	1.01 (0.86-1.2)	0.926
90757394	rs3756063[‡]	0.474 / 0.474	1 (0.76-1.3)	0.468 / 0.484	0.93 (0.72-1.2)	0.466 / 0.472	0.88 (0.64-1.2)	0.513 / 0.456	1.2 (0.92-1.5)	1.01 (0.88-1.2)	0.937
90757505	rs1372520[‡]	0.193 / 0.23	0.81 (0.58-1.1)	0.226 / 0.208	1.11 (0.82-1.5)	0.195 / 0.226	0.85 (0.57-1.2)	0.263 / 0.22	1.26 (0.93-1.7)	1.01 (0.86-1.2)	0.871
90757735	rs2619361	0.281 / 0.243	1.21 (0.89-1.7)	0.241 / 0.276	0.83 (0.62-1.1)	0.271 / 0.242	0.99 (0.70-1.4)	0.251 / 0.232	1.04 (0.77-1.4)	1 (0.86-1.2)	0.953
90757845	rs2619362	0.281 / 0.243	1.21 (0.89-1.7)	0.241 / 0.271	0.85 (0.63-1.1)	0.275 / 0.242	1.01 (0.72-1.4)	0.251 / 0.234	1.03 (0.77-1.4)	1.01 (0.87-1.2)	0.866
90758389	rs2301135	0.477 / 0.488	0.96 (0.73-1.3)	0.47 / 0.485	0.94 (0.72-1.2)	0.466 / 0.479	0.86 (0.63-1.2)	0.518 / 0.457	1.21 (0.94-1.6)	1 (0.87-1.1)	0.957
90759047	rs2619363	0.278 / 0.243	1.19 (0.88-1.6)	0.239 / 0.275	0.82 (0.62-1.1)	0.271 / 0.238	1.01 (0.71-1.4)	0.249 / 0.232	1.02 (0.76-1.4)	1 (0.85-1.2)	0.979
90760828	rs2583988	0.272 / 0.242	1.17 (0.86-1.6)	0.241 / 0.272	0.85 (0.63-1.1)	0.276 / 0.241	1.03 (0.72-1.5)	0.249 / 0.245	0.97 (0.72-1.3)	0.99 (0.85-1.2)	0.888

Entries of "-" indicate that a SNP was excluded from analysis for a particular cohort due to HWE failure in either ET samples or controls. Chr4 (bp) = Chromosome 4 base pair.

*
P-value given for combined series analysis

[†]
Chromosomal positions based on the February 2009 (GRCH37/hg19) genome assembly

[‡]
SNPs that overlap with Pigullo et al36

[Φ]
SNPs that are present on the Illumina HumanHap300 array16