Investigating RIC3 Variants in Parkinson's Disease: No Association Found in French-Canadians and the French Population

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Abstract

Variants in the *RIC3* gene have recently been suggested as a novel cause of young onset, autosomal dominant Parkinson's disease (PD). In the current study, the entire coding regions and exon intron boundaries of *RIC3* were sequenced in a French-Canadian and French case-control sample series of 535 PD patients and 527 unaffected controls. The effect of single variants and the combined effect of variants were calculated. Sequence Kernel association tests (SKAT, SKAT-O) were done on the entire gene level, and on the different domains and exons of *RIC3*. A total of 28 common and rare variants were identified in patients and controls. No significant association was found between any variant and haplotype in *RIC3* and PD, and there was no over-representation of *RIC3* variants at the entire gene, domain, or exon levels in patients vs. controls. Our results do not support a role for *RIC3* mutations as a common cause of PD in the French Canadian and French populations.

Keywords: *RIC3*; Parkinson's disease; Genetics

1. Introduction

During the past two decades, since the discovery of mutations in *SNCA* as the cause of Parkinson's disease in a Greek family,[1] numerous genetic variants in more than 40 genes are known or suspected to be involved in Parkinson's disease (PD). These include mutations that lead to autosomal dominant or recessive PD, with either full or reduced penetrance, and variants with milder effect on the risk for PD. Only a few genes are reliably associated with autosomal dominant PD, including *SNCA*, *LRRK2* and *VPS35*.[2-4] Mutations in *GBA* may be considered either as

autosomal dominant with reduced penetrance or as risk factors. [5] Heterozygous mutations in other genes such as *DNAJC13*,[6] *SMPD1*,[7] *CHCHD2*,[8] and *TMEM230*[9] may also lead to PD, however more studies are necessary to determine if these are indeed PD-associated genes.

Recently, a whole exome sequencing (WES) study was performed on a large family of Indian origin with autosomal dominant PD. The family included 10 affected individuals from three generations, and the WES suggested that a mutation in *RIC3* (p.P57T) may be the cause of PD in this family.[10] An additional mutation, p.V168L, was identified in a single patient with young onset PD. *RIC3* encodes the RIC3 (Resistant To Inhibitor Of Cholinesterase 3) -acetylcholine receptor chaperone, which promotes the proper folding and assembly of neuronal nicotinic acetylcholine receptors (nAChRs).[11] The association between smoking and reduced risk for PD[12, 13] was hypothesized to involve nAChRs and their potential influence on dopamine production and secretion.[14] Furthermore, variants in genes encoding subunits of nAChRs were demonstrated to affect smoking behavior in PD[15, 16] and in the general population.[17] Therefore, it is possible that the association between *RIC3* and PD is due to RIC3 modulation of nAChRs. However, thus far a role for *RIC3* in PD was not replicated in other populations.

In the current study we sequenced the entire coding sequence and exon-intron boundaries of *RIC3* to examine whether variants in this gene are associated with PD in French-Canadian and French patients and controls.

2. Methods

2.1 Population

The study population included 535 unrelated, consecutively-recruited PD patients from clinics in Québec, Canada and Montpellier, France and 527 ethnically-matched controls. The average age of patients was 65.6 ± 9.9 years (data on age was not available for 14 patients, male to female ratio of 1.8). The control population was composed of 157 elderly controls (average age 65.4 ± 7.2

years) and 352 young controls (average age 37.7 ± 7.7 years). There was no data on age on 18 controls samples. Since there was no difference in frequencies of *RIC3* variants between elderly and young controls (see results), they could be combined for analysis (average age 46.3 ± 14.8 years, male to female ratio 1.4). All participants provided informed consent, and the procedures were approved by the institutional review boards.

2.2 Sequencing

Sequencing was performed using primers previously described[10] to amplify the entire coding regions and exon-intron boundaries of the *RIC3* gene. PCR products were sequenced on 3730XL DNAnalyzer instruments (Applied Biosystems), and chromatograms were viewed using MutationSurveyor v4.0.4 software (SoftGenetics, Pennsylvania, USA).

2.3 Statistical analysis

To examine whether there are differences in *RIC3* variant frequencies between elderly and young controls, fisher exact test was used (SPSS v.23, IBM Inc.). Binary logistic regression adjusted for age and sex was performed to examine the association between *RIC3* variants and disease status (PLINK 1.07).[18] To further examine the potential combined effect of *RIC3* variants on risk for PD, SKAT (Sequence Kernel association test)[19] and SKAT-O (optimal SKAT)[20] were performed using R. These analyses were performed on the entire gene, as well as on each domain and each exon of the gene, to examine whether accumulation of variants in specific domains or exons could be associated with PD.

3. Results

A total of 28 different variants in *RIC3* were observed in our French-Canadian and French case-control series, including 20 nonsynonymous, 3 synonymous, and 5 intronic variants (Table 1). There were no differences between the elderly and young controls in the frequencies of rare or

common variants, which allowed us to combine them into a single control group. The *RIC3* variants reported to cause PD, p.P57T and p.V168L,[10] were not observed in our cohort. Binary logistic regression, with age and sex as covariates for adjustment, demonstrated that none of the variants was specifically associated with PD (Table 1). Two variants were found to be in high linkage disequilibrium (LD), p.C130Y (rs55990541) and p.D351N (rs11826236, D' = 0.97, r^2 = 0.95), however no other variants appeared to be in LD. To examine whether specific *RIC3* haplotypes may be associated with PD, logistic regression with age and sex as covariates was performed on the inferred haplotypes. Four commonly shared haplotypes were identified (Supplementary Table 1), none of which were associated with PD risk (p = 0.3155-0.9968). To further examine whether accumulation of mutations at the gene level, at specific domains or exons, may be associated with PD, SKAT and SKAT-O analyses were performed. *RIC3* includes three domains (luminal, helical and cytoplasmic) and six exons. There was no association between *RIC3* variants at the gene level, domain levels or exon levels (Table 2). In addition, there was no difference in frequencies of variants predicted to be damaging by either Polyphen2[21] or SIFT[22] (62 (15.8%) in patients, 58 (15.3%) in controls, p=0.64, Table 2).

4. Discussion

Despite the well-segregating variant observed in the original family,[10] none of the variants identified in our French-Canadian and French cohort showed clear association with PD. One variant, p.P101S, was observed in more patients than controls, though at a non-significant *p*-value, probably due to random distribution difference. The current study cannot rule out a role for *RIC3* in PD, since it is possible that disease causing variants in *RIC3* occur only in specific populations, such as the South-Asian population of the original study. Similar examples had already been demonstrated in PD. Mutations in *VPS35* occur only in specific populations, such as Swiss,

Austrian, Tunisian and Yemenite Jews,[23, 24] but not in other populations.[25-27] Likewise, an association with PD of mutations in *SMPD1* was reported in the Ashkenazi-Jewish (AJ) population,[7] which was replicated in an independent study of an additional AJ population,[28] but only few other studies confirmed this association in other populations.[29, 30] It is therefore possible that specific gene variants which may lead to PD can be restricted to specific populations. Furthermore, it is possible that *RIC3* mutations lead to young or early onset PD, hence cohorts of young or early onset PD should be further investigated.

There is a strong link between reduced risk for PD and cigarette smoking that is not easily explained by genetic association.[31] Nicotine acts as an agonist for several nAChR subunits,[32] and it is hypothesized that the activation of these receptors by nicotine may be responsible for the observed protective effect. This can occur, for example, due to increased dopamine secretion stimulated by the activation of nAChRs. Since RIC3 is a chaperone protein necessary for expression and localization of the α7 nAChR subunit,[33] as well as other subunits, it is possible that its loss of function may lead to reduced function of nAChRs, and potentially reduced dopaminergic secretion. However, it is important to note that while the association between PD and smoking is very strong and well established, it is possible that this association is due to confounders such as genetic variants that affect both smoking behavior and PD, or due to reverse causality (i.e., PD patients tend to smoke less even in early stages of the disease prior to the motor symptoms). Overall, our results suggest that *RIC3* mutations are not a common cause of PD in French-Canadian and French patients, and that further study will be required to examine these hypotheses and to determine the relationship between *RIC3*, PD, and function of the cholinergic system.

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Table 1. ***RIC3*** **Variants in 535 Parkinson's disease patients and 527 controls**

rs Number	Amino Acid	Freq. ExAC	Polyphen2 score	SIFT score	Patients, n=535 (AF)	Controls, n=527, (AF)	*p* value	OR	95% CI
Common Variants									
rs10839976	p.L118L	0.2691	NA	N/A	226 (0.217)	215 (0.212)	0.4165	1.112	0.8602 - 1.439
rs55990541	p.C130Y	0.0738	0.041	1	51 (0.048)	46 (0.045)	0.5091	1.184	0.7175 - 1.953
rs73411617	p.P135S	0.04066	0.005	0.45	33 (0.032)	40 (0.039)	0.2775	0.7446	0.4373 - 1.268
rs79313028	intronic	0.01281	NA	NA	2 (0.002)	3 (0.003)	0.5802	2.232	0.1298 - 38.39
rs11826236	p.D351N	0.07947	0.846	0.38	47 (0.044)	43 (0.042)	0.3813	1.264	0.7483 - 2.134
Rare Variants									
rs6578936	splicing	0.00654	NA	NA	0	1 (0.001)			
	p.V6A		0.03	0.11	1 (0.001)	0			
	p.A10S		0.997	0.08	0	2 (0.002)			
	p.A12V		0	1	1 (0.001)	0			
rs145965152	p.K25R	0.0014	0.041	0.75	2 (0.002)	3 (0.003)	0.8149	0.7787	0.09592 - 6.322
	p.P63L		0.001	0.08	0	1 (0.001)			
	p.S70T		0.449	0.31	1 (0.001)	1 (0.001)	0.8373	1.533	0.02596 - 90.56
rs149313414	p.A73A	0.0005111	NA	N/A	0	1 (0.001)			
	p.A86A		NA	N/A	1 (0.001)	0			
rs144806410	p.P101S	0.002737	1	0	5 (0.005)	2 (0.002)	0.9605	0.9555	0.1575 - 5.796
rs80168649	p.G121A	0.007348	1	0.17	3 (0.003)	3 (0.003)	0.5889	0.5656	0.07158 - 4.469
	p.T138S		0.001	0.68	1 (0.001)	0			
rs111370836	intronic	0.00771	NA	NA	0	2 (0.002)			
rs144870134	p.R191Q	0.001499	0.266	0.16	0	1 (0.001)			
rs139685245	p.V196F	0.002661	1	0.02	1 (0.001)	0			
rs773259414	p.R205K	0.00002471	0.034	0.83	0	1 (0.001)			
	p.P216S		0.707	0.28	1 (0.001)	0			
rs11041753	intronic		NA	NA	7 (0.007)	6 (0.006)	0.2586	0.5053	0.1546 - 1.652
rs765540849	intronic		NA	NA	0	1 (0.001)			
rs747142587	p.A257S	0.000008238	0.997	0.17	0	1 (0.001)			
	p.S279R		0	0.48	1 (0.001)	0			
rs749020968	p.P281L	0.000008237	0.058	0.18	2 (0.002)	0			
rs116932252	p.D311N	0.003748	1	0	6 (0.006)	7 (0.007)	0.8239	0.854	0.2128 - 3.428

Freq. ExAC, frequency in the ExAC database; AF, allele frequency; OR, odds ratio; CI, confidence interval
Deleteriousness threshold values: Polyphen2 – greater than 0.86, SIFT – less than 0.05

Table 2. Sequence Kernel association test (SKAT)[a] analyses of *RIC3* at the entire gene level, domain level, exon level and function of variant level.

Level of comparison	Cases with Allele	Controls with Allele	Marker (n)	*p* Value
Entire *RIC3* Gene	392	380	25	0.8405
Domains				
Lumenal	6	8	8	0.4575
Helical	1	0	1	0.5173
Cytoplasmic	372	357	13	0.7918
Exons				
Exon 1	4	5	4	0.4146
Exon 2	7	5	5	0.5569
Exon 3	314	304	5	1.000
Exon 4	0	0	0	NA
Exon 5	2	2	3	0.6162
Exon 6	56	51	5	0.4462
intronic	9	13	3	0.5315
Type of mutation				
Non-Synonymous/Splicing	155	151	20	0.8157
Synonymous	237	229	5	0.5964
Functional prediction				
Damaging	62	58	7	0.6415
Tolerated	330	322	18	0.8630

[a] SKAT-O was also performed with very similar results (data not shown).