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Negative results

RIC3 variants are not associated with Parkinson's disease in French-Canadians and French

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ABSTRACT

Variants in the *RIC3* gene have recently been suggested as a novel cause of Parkinson's disease (PD). Herein, the entire *RIC3* gene was sequenced in a French-Canadian and French 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 versus controls. Our results do not support a role for *RIC3* mutations as a common cause of PD in the French-Canadian and French populations.

1. Introduction

Recently, a whole exome sequencing study was performed on a large family of Indian origin with autosomal dominant PD. The family included 10 affected individuals from 3 generations, and the whole exome sequencing suggested that a mutation in *RIC3* (p.P57T) may be the cause of PD in this family (Sudhaman et al., 2016). 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; Millar, 2008). In the present 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. See Supplementary Material for detailed introduction, methods, results, discussion, acknowledgements, and full reference list.

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 were 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). 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 (Sudhaman et al., 2016) to amplify the entire coding regions and exon-intron boundaries of the *RIC3* gene.

2.3. Statistical analysis

Binary logistic regression adjusted for age and sex was performed to examine the association between *RIC3* variants and

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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

Key: AF, allele frequency; CI, confidence interval; Freq. ExAC, frequency in the ExAC database; OR, odds ratio.
Deleteriousness threshold values: polyphen2—greater than 0.86, SIFT—less than 0.05.

disease status (PLINK 1.07). To further examine the potential combined effect of *RIC3* variants on risk for PD, SKAT (Sequence Kernel association test) and SKAT-O (optimal SKAT) 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 (Table 1). The *RIC3* variants reported to cause PD, p.P57T, and p.V168L (Sudhaman et al., 2016) were not observed in our cohort. Binary logistic regression, with age and sex as covariates for adjustment, demonstrated that none of the variants were specifically associated with PD (Table 1). 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 ($p = 0.3155–0.9968$). *RIC3* includes 3 domains (luminal, helical, and cytoplasmic) and 6 exons. There was no association between *RIC3* variants at the gene level, domain levels, or exon levels analyzed using SKAT and SKAT-O (Table 2). In addition, there was no difference in frequencies of variants predicted to be damaging by either Polyphen2 (Adzhubei et al., 2010) or SIFT (Kumar et al., 2009; 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 (Sudhaman et al., 2016), 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 nonsignificant *p*-value, probably due to random distribution difference. The present 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. An association with PD of mutations in *SMPD1*

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				
Nonsynonymous/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).

was reported in the Ashkenazi-Jewish population (Gan-Or et al., 2013), which was replicated in an independent study of an additional Ashkenazi-Jewish population, but only few other studies confirmed this association in other populations. 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.

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.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2017.01.005.

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