LRRK2 is not a Significant Cause of Parkinson's Disease in French-Canadians

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ABSTRACT: ***Background:*** An old founder mutation (G2019S) was found with high frequency in the North African Arabs (30%) and Ashkenazi Jews (18%). ***Objective:*** Demonstrate if mutations in the LRRK2 gene are a significant cause of Parkinson's disease (PD) in the French-Canadian founder population. ***Methods:*** Cases were recruited through a designated movement disorder clinic in Quebec City. Every index case had to meet the Ward and Gibb criteria for PD. Controls consisted of a non-disease group of similar age and ethnicity as the cases. Exons 31 and 41 of LRRK2 were amplified by PCR with intronic primers in all 125 PD cases and directly sequenced on an ABI 3700 sequencer. Six single nucleotide polymorphism were typed in 125 PD cases and 95 normal controls. Associations between unrelated cases and matched controls were analyzed. Single marker analysis and haplotype association tests were performed. ***Results:*** Sequencing analysis did not reveal any reported or novel mutations in exons 31 and 41 of LRRK2. The G2019S mutation as well as mutations affecting amino acid 1441 were absent in the 125 patients. The case-control association study performed to detect the presence of a common variant in LRRK2 did not provide any positive signal. Single-marker and haplotype analyses systematically gave non-significant P values. ***Conclusions:*** We performed a case-control association study in 125 French-Canadian (FC) patients with PD and 95 FC controls and found that common variants in LRRK2 are unlikely to be a significant cause of late-onset PD in this founder population.

RÉSUMÉ: LRRK2 n'est pas une cause significative de la maladie de Parkinson chez les Canadiens français. ***Contexte :*** La fréquence d'une mutation fondatrice ancienne (G2019S) est élevée chez les Arabes de l'Afrique du Nord (30%) et chez les Juifs Ashkenazi (18%). ***Objectif :*** Déterminer si des mutations dans le gène LRRK2 sont une cause importante de la maladie de Parkinson (MP) dans la population canadienne-française. ***Méthodes :*** Les cas ont été recrutés dans une clinique de troubles du mouvement à Québec. Chaque cas index devait satisfaire aux critères de la MP de Ward et Gibb. Le groupe témoin était composé de sujets sains du même âge et de la même origine ethnique que les cas. Les exons 31 et 41 du gène LRRK2 ont été amplifiés par PCR au moyen d'amorces introniques chez les 125 patients atteints de MP et séquencés directement au moyen d'un séquenceur ABI 3700. Six polymorphismes d'un seul nucléotide (SNPs) ont été analysés chez les 125 patients et chez 95 témoins normaux. Un test d'association a été effectué pour chaque marqueur et également pour les haplotypes, entre les cas non apparentés et les témoins appariés. ***Résultats :*** L'analyse du séquençage n'a pas révélé la présence de mutations déjà connues ou de nouvelles mutations dans les exons 31 et 41 du gène LRRK2. La mutation G2019S ainsi que les mutations de l'acide aminé 1441 n'étaient pas présentes chez les 125 patients. L'étude cas-témoins pour détecter la présence d'une variation fréquente du gène LRRK2 était négative. Les valeurs de P des analyses de marqueurs uniques et d'haplotypes ont toutes été non significatives. ***Conclusions :*** Notre étude cas-témoins chez 125 patients d'origine canadienne-française atteints de MP et chez 95 témoins de la même origine ethnique démontre qu'il est peu probable qu'une variation fréquente du gène LRRK2 soit une cause significative de la MP à début tardif dans cette population fondatrice.

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The minimum criteria for the diagnosis of Parkinson's disease (PD) includes the presence of bradykinesia and at least one of the other three primary features: truncal and limb muscle rigidity, resting or postural tremor, and postural instability or gait disorder. At death the brain of patients with PD is characterized by degeneration of the dopamine-containing cells in the substantia nigra and depletion of the dopamine content in terminal areas in the basal ganglia. The cause of PD remains unknown, though evidence points toward a multifactorial etiology, most likely involving a genetic susceptibility to the effects of environmental agents or trauma. A number of

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environmental risk factors have been implicated in the etiology of PD, yet none have been unequivocally identified as causal agent. Several inherited forms of PD are recognized, and these may have substantial genotypic and phenotypic heterogeneity:[1] PARK1 (*α-synuclein*, 4q21-23); PARK2 (*parkin*, 6q25.2-27); PARK3 (2p13); PARK4 (*α-synuclein*, 4q21); PARK5 (*UCHL-1*, 4p14); PARK6 (*pink-1*, 1p36); PARK7 (*DJ-1*, 1p36); PARK8 (*LRRK2*, 12q12); PARK9 (*ATP13A2*, 1p36); PARK10 (1p); PARK11 (2q36); PARK12 (Xq21-q25). One of the most recently identified of these, *LRRK2*, has been shown to have differing mutations of varying frequency among different ethnic groups. The high frequency of the G2019S mutation in North African Arabs (30%) and Ashkenazi Jewish PD subjects (18%) supports the presence of an ancient founder mutation in these populations.[2,3] A significant proportion of mutations in *LRRK2* reported to date are located in exons 31 and 41 of *LRRK2*, particularly mutations affecting amino acids 1441 and 2019 (R1441G, R1441C, R1441H, G2019S, and I2020T).[4-7] We screened these two exons in a sample of French Canadian (FC) PD patients. A case-control association study was also performed to evaluate whether any other common genetic variations within *LRRK2* were associated with PD in the FC founder population.

Methods

Cases were recruited through a designated movement disorder clinic in Quebec City (Quebec, Canada), located at the CHAUQ (Enfant-Jésus). Every index case was seen by a neurologist specialized in movement disorders and had to meet the Ward and Gibb criteria for PD.[8] Additionally, they had to be dopa-responsive. A detailed standardized clinical assessment form was completed for each subject. All cases signed a consent form approved by the ethics committee of the CHAUQ prior to being enrolled in the study. A structured questionnaire was used during the interview to assess environmental, medical, lifestyle, and familial risk factors. Controls consist of a non-disease group of similar age and same ethnicity as the cases. Patients and controls were collected from the same base population.

Upon receipt of informed consent, blood samples were obtained from 125 affected individuals and 95 controls. DNA was extracted from peripheral blood by standard methods. Exons 31 and 41 of *LRRK2* were amplified by PCR with intronic primers in all affected individuals and directly sequenced on an ABI 3700 sequencer, according to the manufacturer's recommended protocol (Applied Biosystems, Foster City, CA). Six single nucleotide polymorphisms (SNPs) were typed in all affected individuals and non-disease controls. The SNPs were selected using Applied Biosystems SNPbrowser™ Software and the HapMap project Linkage Disequilibrium (LD) map included within the software. At least 3 SNPs per Linkage Disequilibrium Unit (LDU) were chosen complete coverage of *LRRK2*. Due to the high LD of this locus, only 6 SNPs were sufficient for the purpose of this association study (Table 1). Associations between unrelated cases and matched controls were analyzed by the program COCAPHASE included in the software package UNPHASED.[9] This software is based on likelihood ratio tests in a log-linear model. Single marker analysis and haplotype association tests were performed. Haplotype sliding windows containing from 2 to 6 SNPs were analyzed (Table 2).

Results

A summary of the clinical data can be found in Table 3. Sequencing analysis did not reveal any reported or novel mutations in exons 31 and 41 of *LRRK2*. Neither non-reported synonymous coding SNPs nor non-synonymous coding SNPs were found. The G2019S mutation as well as mutations affecting amino acid 1441 were absent in all 125 affected individuals. The case-control association study on 125 affected individuals and 95 controls did not provide any positive signal that would suggest the presence of a common variant of *LRRK2* in the FC founder population. As shown in Table 2, single-marker and haplotype analyses systematically gave non-significant P values.

Table 1: SNPs selected in the LRRK2 gene. CEU MAF: allele frequency determined in the HapMap project using 60 CEPH Utah from Northern and Western Europe. CEU LDU: Linkage disequilibrium units based on 60 CEPH Utah from Northern and Western Europe.

SNP ID	Position	CEU MAF	CEU LDU	SNP type
rs10878245	38918058	40	964.3	Synonymous coding
rs7132171	38936016	43	964.58	Intron
rs11175784	38945801	49	964.85	Intron
rs10878368	39001052	38	965.11	Intron
rs4768232	39023507	12	965.48	Intron
rs3886747	39048218	33	965.55	Untranslated

Table 2: P values of single-marker and two to six-marker haplotype sliding window association tests. Window n: n-marker haplotype sliding window.

	SNP ID	Single marker	window 2	window 3	window 4	window 5	window 6
1	rs10878245	0.053	0.063	0.1	0.15	0.19	0.26
2	rs7132171	0.51	0.2	0.31	0.49	0.54	
3	rs11175784	0.2	0.35	0.32	0.86		
4	rs10878368	0.19	0.34	0.84			
5	rs4768232	0.44	0.62				
6	rs3886747	0.87					

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Table 3: Characteristics of the PD population recruited

Clinical data	No of affected / 125 (%)
Male sex	89 (71%)
Originate from Eastern Quebec	125 (100%)
French-Canadian ancestry	125 (100%)
Age of onset < 40	11 (9%)
Positive family history (first degree relative)	13 (10%)
Positive family history (overall)	28 (22%)
Age of onset, mean (range)	55.3 (30-79)
Duration of symptoms, mean	8.0
Hoen & Yahr stage at assessment, mean	1.90

Discussion

The Quebec population contains about six million FC who are descendants of around 8500 permanent French settlers who colonized “Nouvelle-France” between 1608 and 1759. For socio-economic, religious, and linguistic reasons the descendants of the initial settlers did not mix with other immigrants for over three centuries. There was sustained demographic growth, with doubling of the population every 25-30 years, thereby giving rise to a founder population undergoing rapid expansion. The relative contribution of each founder to the FC population varies depending on when they arrived in North America, in that the 3380 founders who settled before 1680 contributed approximately 70% of the present gene pool. From the above, we can conclude that a large segment of the FC population shares a relatively homogeneous genetic background, thereby reducing the possible number of disease producing susceptibility alleles present. The genetic risk factors involved in PD have been examined in the FC population mainly through the work of Andre Barbeau. In a survey of 300 consecutive FC cases with PD and 300 age-matched controls, 13% of PD patients and 5% of controls had one or more first degree relatives with either probable PD or essential tremor. In an additional survey of 135 cases of PD (onset before 40 years) and 30 controls, 45% of PD patients and 3% of controls had one or more first degree relatives with either probable PD or essential tremor.[10] These findings support the presence of important genetic factors for PD in this population, and our study is the first molecular study to be performed in FCs with this disease. In light of the characteristics of the FC population, we had anticipated that, as for the North African Arab and Ashkenazi Jewish populations, a significant proportion of PD could be related to mutations in *LRRK2*. To the contrary, we have observed no contribution of this gene to PD in the FC population. Sequencing of exons 31 and 41 of *LRRK2* demonstrated that none of our patients carried the common mutations. Our data show that G2019S is unlikely to be a common cause of PD in this founder population. However, a larger sample would be needed to assess the exact frequency of G2019S in this population. We are also aware that the association results presented here should be taken with caution due to the small sample size of the study. A larger sample of FC patients would be needed to completely exclude the hypothesis of a common variant within *LRRK2* predisposing to PD in the FC population. Similarly to our study, genetic analysis of the Nurr1 gene and the neurofilament M gene in Canadian and FC patients with PD failed to identify common genetic variants predisposing to PD. These data, together with ours tend to confirm that PD is genetically extremely heterogeneous even in a sample originating from an isolate population.[11,12]

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