ORIGINAL ARTICLE

LRRK2 Screening in a Canadian Parkinson's Disease Cohort

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ABSTRACT: ***Background:*** Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) have become the most common known cause for developing Parkinson's disease. The frequency of mutations described in the literature varies widely depending on the population studied with most reports focusing only on screening for the most common G2019S mutation in exon 41. ***Methods***: In this study seven exons (19, 24, 25, 31, 35, 38, and 41) in LRRK2 where mutations have been reported were screened in 230 unselected Parkinson's disease patients using denaturing high-performance liquid chromatography. ***Results:*** The sequencing of samples with heteroduplex profiles revealed five novel and two known intronic sequence variants. In our cohort, we were unable to detect any of the known mutations in these exons or identify novel mutations within the LRRK2 gene. ***Conclusions:*** Therefore, despite the availability of diagnostic LRRK2 genetic testing it is unlikely to yield a positive result in this population.

RÉSUMÉ: Dépistage de mutations du gène LRRK2 dans une cohorte de patients canadiens atteints de la maladie de Parkinson. ***Contexte :*** Les mutations du gène LRRK2 (leucine-rich repeat kinase 2) sont la cause connue la plus fréquente de la maladie de Parkinson (MP). La fréquence des mutations décrites dans la littérature varie beaucoup, selon la population étudiée. La plupart des articles publiés portent seulement sur le dépistage de la mutation la plus fréquente, G2019S, située dans l'exon 41. ***Méthodes :*** Nous avons fait le dépistage de mutations dans sept exons (19, 24, 25, 31, 35, 38 et 41) du gène LRRK2 où des mutations ont déjà été rapportées, chez 230 patients atteints de la MP choisis au hasard, au moyen de la chromatographie liquide haute performance en conditions dénaturantes. ***Résultats :*** Le séquençage des échantillons ayant un profil hétéroduplex a révélé la présence de cinq nouvelles variantes de séquences introniques et de deux connues. Nous n'avons détecté aucune des mutations connues dans ces exons et nous n'avons pas identifié de nouvelles mutations dans le gène LRRK2. ***Conclusions :*** Même si un test génétique est disponible pour le gène LRRK2, il est peu probable qu'on obtiendra un résultat positif dans cette population.

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In most patients, the cause of Parkinson disease (PD) is unknown, but having a positive family history has long been known to be a strong risk factor for the development of the disease.[1] In the past this had been felt to be secondary to a shared, possibly toxic, environmental cause. It is now clear that autosomal dominant and autosomal recessive forms of PD exist yet these too have been felt to be rare causes of the typical adult onset forms of PD. The leucine-rich repeat kinase 2 gene (LRRK2) is the most recent of a growing list of genes that cause PD and mutations in this gene now represent the single most common reason to develop the disease. Detection of the most common mutation, G2019S in exon 41, in non-autosomal dominant populations of PD patients has been reported with widely varying rates. Typically, the G2019S mutation has been identified in 1-2% of populations screened, however rates as high as 40% are observed in isolated populations.[2-4] European and North America patients reported to date seem to share a common ancestral haplotype indicative of a single founder effect.[5] In families with a strong family history of PD the G2019S mutation in exon 41 occurs in 5-6% of populations.[6,7]

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It has been suggested that testing for G2019S LRRK2 mutation could be important in the management and genetic counseling of patients with PD and commercial testing is available. With the wide variation in mutation rates it will be important to know how common mutations are occurring in other non-selected PD population if mass screening was to be considered. In addition there are other mutations found within the LRRK gene that cause PD although these are not as common as the G2019S mutations.[8,9] However, LRRK2 is a large gene consisting of 51 exons (7642 bp mRNA) and therefore direct sequencing of all the exons in all PD individuals is currently not practical and a better screening method is needed.

We chose denaturing high-performance liquid chromatography (DHPLC) to screen an unselected PD population for possible mutations occurring in the seven exons where most mutations in LRRK2 are found. The advantage of DHPLC is that it can be used as a high throughput screening method to detect sequence variants with great sensitivity and doesn't have the cost associated with having to directly sequence all individual samples.

Methods

This study was approved by the Ottawa Hospital and McGill University Ethics Review Boards. Written and informed consent was obtained from all participants. Blood samples were collected from PD patients and neurologically normal controls (mainly spouses) attending one of two clinics (one in Ottawa, Ontario, one in Montreal, Quebec, Canada) from 2001-2004 as part of an ongoing effort to explore the role of genetics in PD in a Canadian cohort. All participants completed a structured questionnaire, underwent a history and examination by a neurologist with extensive experience in PD (M.P. or D.G.). Diagnosis of PD and exclusionary criteria suggestive of another disease were based on previously published criteria.[10]

Genomic DNA from blood samples of 230 PD patients and 129 normal controls was extracted and processed using the QIAmp DNA Blood Mini Kit (QIAGEN Inc. Mississauga, ON, Canada). All samples were individually amplified in a 50ul PCR containing 50-100ng genomic DNA, 10mM Tris-Cl pH 8.0, 0.2mM dNTPs,1.5mM $MgCl_2$, 10uM forward and reverse primers, and 5U Taq DNA polymerase. PCR in a 96 well microplate was carried out on a thermal cycler (Thermo Electron Corp., Waltham, MA, U.S.A.) with the conditions of 94°C for 3 min, 37 cycles at 94°C for 45 s; 55°C for 30 s; 72°C for 30s; and a final extension at 72 °C for 10 min. Primers were designed to screen the following known LRKK2 mutations: exon 19 - R793M, exon 24 - L1114L, exon 25 - I1122V, exon 31 - R1441C, R1441G, R1441H, exon 35 - Y1699C, exon 38 - M1869T, and exon 41 - G2019S, I2020T. Positive DNA controls, except for LRRK2 exon 19, were kindly donated by M. Farrer. After confirming the presence of the amplicons on a 1.0% agarose gel, all samples were denatured at 94°C for 2 min and slowly cooled to 25°C at a decreasing rate of 1°C per cycle to encourage any possible heteroduplex formation. The initial mutation detection was carried out on a Transgenomics WAVE DHPLC (Transition Technologies, Toronto, ON, Canada). Each of the samples including positive and normal controls were injected separately onto a DNASep column. The temperatures required for the detection of heteroduplex formation of each amplicon were predicted using the Wavemaker version 1.6.2 software. DNA fragments were eluted from the DNASep column at a predetermined elution gradient using Wavemaker in the range of 55.0 to 58.5% Buffer B (0.1M TEAA and 25% acetonitrile) at a flow rate of 0.9ml per minute. The profiles for each sample were visually analyzed and clustered using default values in Wavemaker. The PCR products of any variants detected with the DHPLC were treated with ExoSap-IT (USB Corporation, Cleveland, OH, U.S.A.) at 37 for 20 min before proceeding with BigDye Terminator v3.1 cycle sequencing on an ABI 3130*xl* Genetic Analyzer (Foster City, CA, U.S.A.) using both PCR forward and reverse amplicon primers.

Results

In our PD cohort of 230 patients, 34% had at least one relative with PD, 14% had at least one first degree relative affected and 2% had 3 or more affected relatives. One hundred forty six PD patients were men while 84 patients were women; 63 patients had an age of onset less than or equal to 50 years. The average age at onset of PD was 57.7 years (range 31 to 92 years old). Almost all individuals were of North or Central European ancestry (97%) with 127 (55%) of those people being of French Canadian heritage. The average age for controls when the sample was drawn was 63.5 years old (range 24 to 86 years old).

The initial mutation screening on the DHPLC identified 120 PD and 7 normal control samples with heteroduplex profiles. The sequencing of these samples revealed 5 novel and 2 known intronic sequence variants (Table). We were unable to detect any of the known mutations or identify novel mutations within LRRK2 exons 19, 24, 25, 31, 35, 38, and 41 (Table).

Table: LRRK2 variants excluded or identified in our PD population consisting of 460 chromosomes and 258 control chromosomes

	Nucleotide change[1]	dbSNP rs#	AA change[2]	PD population		Control population	
				variant	frequency	variant	frequency
Intron18	IVS18-27A>G*			1/460	0.22%	0	0
Intron18	IVS18-22C>T*			13/460	2.83%	2/258	0.78%
EXON19	**c.2378G>T**		**R793M**	0	0	nd	nd
Intron19	IVS19+64A>C*			1/460	0.22%	0	0
EXON24	**c.3342A>G**		**L1114L**	0	0	nd	nd
EXON25	**c.3364A>G**		**I1122V**	0	0	nd	nd
EXON31	**c.4321C>T**		**R1441C**	0	0	nd	nd
	c.4321C>G		**R1441G**	0	0	nd	nd
	c.4321G>A		**R1441H**	0	0	nd	nd
EXON35	**c.5096A>G**		**Y1699C**	0	0	nd	nd
Intron35	IVS35+23T>A	rs7307276		90/460	19.56%	nd	nd
Intron37	IVS37-103A>T*			3/460	0.65%	2/258	0.78%
	IVS37-9A>G*			1/460	0.22%	1/258	0.39%
EXON38	**c.5606T>C**		**M1869T**	0	0	nd	nd
Intron38	IVS38+35G>A	rs17484342		11/460	2.39%	2/258	0.78%
EXON41	**c.6055G>A**		**G2019S**	0	0	nd	nd
	c.6059T>C		**I2020T**	0	0	nd	nd

Previously identified coding mutations are highlighted in bold.
(1) Nucleotide position based on GenBank AY792511.1 (2) Amino acid position based on GenPept AAV63975.1; nd: not determined
*novel variants

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Discussion

Testing of PD genes remains technically demanding and to date most studies involving LRRK2 mutations used techniques that only screened for the G2019S mutation in exon 41. This mutation has been suggested to occur at a frequency of 1 to 2% in unselected PD populations in European studies[11] yet very rare in Asian cohorts.[12,13] Most cases of PD caused by any of the LRRK2 mutations are identical to typical late onset disease and therefore clinical criteria cannot help in determining who might be considered for testing. The advantage of our DHPLC method is that it will detect not only the known mutations in the region screened but also novel ones. However, it does have some limitations as homozygous mutations can be missed and it is possible that because the DHPLC conditions were optimized to detect the known mutations in the specific exons, variants in other domains within the exons could have been missed. In our large cohort of mainly Caucasian individuals of which a small majority were of French Canadian descent we were unable to detect any mutations in the seven LRRK2 exons examined. There are other groups that have found a very low incidence of LRRK2 mutations derived from North American populations of PD.[14] These results would question the diagnostic interest to mass screen PD individuals from these populations especially if they are of French Canadian heritage.

The seven variants that were identified were located within intronic sequences proximal to the exon of interest. Two are known variants that have already been identified in non-PD individuals and three were also found in the control population making it unlikely they are involved in any pathologic process. For the variants in intron 18 (IVS18-27A>G) and 19 (IVS19+64A>C) it is possible they could be contributing to alternative splicing or the disruption of regulatory binding elements. However preliminary data (unpublished) indicate that these variants do not disrupt the transcription of LRRK2 mRNA. The growing list of genes that cause PD has already provided important insights into the mechanisms of neuronal degeneration. Early work on the mechanism by which LRRK2 mutations could be causing disease has suggested they may increase the kinase activity of the LRRK2 protein.[15] Protein kinases are good targets for small-molecule drugs and therefore this could become an innovative therapeutic strategy. At this point these discoveries have not generated any new treatment options for patients with PD especially ones that could be directed at specific genetic forms of the disease. Therefore knowing the exact cause of one's PD at this point, doesn't change the management of the disease and the reasoning behind screening for mutations for diagnostic confirmation comes into question.

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