Journal of Parkinson's Disease 12 (2022) 117–128
DOI 10.3233/JPD-212730
IOS Press

Research Report

Axial Impairment Following Deep Brain Stimulation in Parkinson's Disease: A Surgicogenomic Approach

Naomi P. Visanji[a,b,1], Mahdi Ghani[c,1], Eric Yu[d,e], Erfan Ghani Kakhki[c,f], Christine Sato[f], Danielle Moreno[f], Taline Naranian[a], Yu-Yan Poon[a], Maryam Abdollahi[a], Maryam Naghibzadeh[a], Rajasumi Rajalingam[a,m], Andres M. Lozano[g,h], Suneil K. Kalia[g,h,l], Mojgan Hodaie[g,h], Melanie Cohn[h], Marta Statucka[h], Alexandre Boutet[i], Gavin J.B. Elias[g], Jürgen Germann[g], Renato Munhoz[a,j], Anthony E. Lang[a,f,h,j], Ziv Gan-Or[d,e,k], Ekaterina Rogaeva[f,j,*,1] and Alfonso Fasano[a,h,j,l,*,1]

[a]*Edmond J. Safra Program in Parkinson's Disease, Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, UHN, Toronto, Ontario, Canada*
[b]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*
[c]*DisorDATA Analytics, Ottawa, ON, Canada*
[d]*The Neuro (Montreal Neurological Institute-Hospital), McGill University, Montreal, Quebec, Canada*
[e]*The Department of Human Genetics, McGill University, Montreal, Quebec, Canada*
[f]*Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, ON, Canada*
[g]*Department of Neurosurgery, Toronto Western Hospital, University Health Network, Toronto, Ontario, Canada*
[h]*Krembil Brain Institute, Toronto, Ontario, Canada*
[i]*Joint Department of Medical Imaging, University of Toronto, Toronto, Ontario, Canada*
[j]*Division of Neurology, University of Toronto, Toronto, Ontario, Canada*
[k]*The Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada*
[l]*CenteR for Advancing Neurotechnological Innovation to Application (CRANIA), Toronto, ON, Canada*
[m]*Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada*

Accepted 1 September 2021
Pre-press 28 September 2021

Abstract.
Background: Postoperative outcome following deep brain stimulation (DBS) of the subthalamic nucleus is variable, particularly with respect to axial motor improvement. We hypothesized a genetic underpinning to the response to surgical intervention, termed "surgicogenomics".
Objective: We aimed to identify genetic variants associated with clinical heterogeneity in DBS outcome of Parkinson's disease (PD) patients that could then be applied clinically to target selection leading to improved surgical outcome.
Methods: Retrospective clinical data was extracted from 150 patient's charts. Each individual was genotyped using the genome-wide NeuroX array tailored to study neurologic diseases. Genetic data were clustered based on surgical outcome

[1]These authors contributed equally to this work.

*Correspondence to: Ekaterina Rogaeva, PhD, Professor Department of Neurology (Faculty of Medicine) University of Toronto, Tanz Centre for Neurodegenerative Diseases, 60 Leonard Avenue, Toronto, ON M5T 0S8, Canada. Tel.: +1 416 507 6872; Fax: +1 416 603 6435; E-mail: ekaterina.rogaeva@utoronto.ca and Alfonso Fasano, MD, PhD Chair in Neuromodulation and Multidisciplinary Care, UHN and UofT Movement Disorders Centre, Toronto Western Hospital Address: 399 Bathurst St, 7McL410 – Toronto, ON Canada M5T 2S8, Canada. Tel.: +416 603 5800/ Ext. 5961; Fax +1 (416) 603-5004; E-mail: alfonso.fasano@uhn.ca.

assessed by comparing pre- and post-operative scores of levodopa equivalent daily dose and axial impairment at one and five years post-surgery. Allele frequencies were compared between patients with excellent vs. moderate/poor outcomes grouped using *a priori* defined cut-offs. We analyzed common variants, burden of rare coding variants, and PD polygenic risk score.
Results: NeuroX identified 2,917 polymorphic markers at 113 genes mapped to known PD loci. The gene-burden analyses of 202 rare nonsynonymous variants suggested a nominal association of axial impairment with 14 genes (most consistent with *CRHR1*, *IP6K2*, and *PRSS3*). The strongest association with surgical outcome was detected between a reduction in levodopa equivalent daily dose and common variations tagging two linkage disequilibrium blocks with *SH3GL2*.
Conclusion: Once validated in independent populations, our findings may be implemented to improve patient selection for DBS in PD.

Keywords: Parkinson's disease, deep brain stimulation, gait apraxia, postural instability, genotyping

INTRODUCTION

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an increasingly common treatment for severe motor fluctuations and dyskinesias in Parkinson's disease (PD) [1]. STN-DBS provides excellent control of appendicular cardinal signs of PD (e.g., tremor or rigidity); however, postoperative outcome can be variable in terms of axial motor symptoms. In fact, speech, gait and balance can worsen post STN-DBS due to the complex interplay of modifiable (e.g., medication/parameter adjustments) and unmodifiable (e.g., disease progression) factors.

The majority of DBS centers use the same criteria for patient selection (e.g., levodopa responsiveness) [2]. Yet, some studies report excellent control of gait [3], while others report gait impairment after DBS in ∼13% of patients [4]. Over a third of DBS failures can be ascribed to inappropriate indication for surgery [5], and a consensus review has suggested that careful patient selection is key to improving outcome [6]. While some predictors of poor axial outcome are starting to become clear, there is no consensus on the impact of age or disease duration. Furthermore, a limited number of inconclusive studies were seeking long-term predictors of surgical success [7]. Levodopa responsiveness has long been considered a valuable predictor of short-term outcome [8], but longer follow-up studies suggest otherwise [9]. Overall, there is considerable heterogeneity in both an individual's PD phenotype and their response to DBS.

PD-related genetic loci undoubtedly play an important role in phenotypic heterogeneity, including disease progression, development of motor complications, cognitive decline, and psychiatric disturbances [10, 11]. Furthermore, there is heterogeneity in the time to develop postural instability among monogenic forms of parkinsonism (e.g., progression-free survival from postural instability 10 years after disease onset was 97% in *ATP13A2* and 50% in *SNCA* carriers) [12].

Pharmacogenomics established that genes play an important role in the response to pharmaceutical intervention. Here, we test the hypothesis that there is a genetic underpinning to a patient's response to surgical intervention, which we have termed "surgicogenomics". Specifically, we sought to identify genetic predictors of the development of axial signs post STN-DBS in PD. A precedent for the concept of "surgicogenomics" in STN-DBS in PD is supported by a meta-analysis that found carriers of mutations in *LRRK2, GBA*, and *PRKN* had different postoperative outcomes with respect to levodopa equivalent daily dose, activities of daily living, motor complications and cognitive functions [13]).

Specifically, our recent review found evidence of a good short-term outcome following STN-DBS in patients with pathogenic mutations in *PRKN*, *LRRK2*, and *GBA*. However, *GBA*-carriers developed earlier cognitive impairment and had a lesser reduction in medication, demonstrating that genetic background can influence response to STN-DBS [14]. Evidence in monogenic forms of PD gathered by systematic reviews have obvious limitations. Only one study tried to establish the predictive value of a few variants in two genes, including variants tagging a PD-associated *SNCA* haplotype [15].

In this study of 150 deeply phenotyped individuals, we aimed to identify loci associated with outcome after up to five years post STN-DBS, using the genome-wide NeuroX array tailored to study neurologic diseases [16].

MATERIALS AND METHODS

Participants

This study was performed in accordance with University Health Network Research Ethics Board approved protocol (UHN-REB 08-0615-AE). Informed consent was obtained from patients who had undergone STN-DBS under the care of the Edmund

Table 1
Dataset characteristics

Cohort characteristics ($N = 148$)	Mean ± SD (Min–Max)	Missing Data (N)
Male/Female (N)	108/40	0
Age at onset (y)	45 ± 8 (21–63)	0
Age at surgery (y)	58 ± 7 (35–73)	0
disease duration at surgery (y)	11 ± 5 (4–29)	0
1-year follow-up (months)	11 ± 3 (4–21)	0
5-year follow-up (months)	60 ± 7 (36–88)	0
Pre-op UPDRS-III OFF L-dopa	39 ± 12 (11–86)	0
Pre-op UPDRS-III ON L-dopa	15 ± 8 (3–50)	2
L-dopa responsiveness (%)	62 ± 22 (–109–95)	2
1-year UPDRS-III Total OFF L-dopa ON DBS	21 ± 10.1 (2–55)* ($p < 0.05$ vs. Pre-op UPDRS-III OFF L-dopa)	1
DBS efficacy (1-year UPDRS-III Total OFF L-dopa ON DBS vs. Pre-op UPDRS-III Total OFF L-dopa) (%)	42 ± 29 (–82–93)	1
5-year UPDRS-III Total OFF L-dopa ON DBS	26 ± 10.9 (3–55) *($p < 0.05$ vs. Pre-op UPDRS-III OFF L-dopa)	89
DBS efficacy (5-year UPDRS-III Total OFF L-dopa ON DBS vs. Pre-op UPDRS-III Total OFF L-dopa) (%)	34 ± 31 (-67–87)	89
Pre-op LEDD	1489 ± 715 (0–3776)	1
1-year LEDD	798 ± 512 (0–3750) *($p < 0.05$ vs. Pre-op LEDD)	4
1-year LEDD reduction (vs. Pre-op LEDD) (%)	48 ± 72(–775.0%–100.0)	4
5-year LEDD	863 ± 595 (0–4200) *($p < 0.05$ vs. Pre-op LEDD)	1
5-year LEDD reduction (vs. Pre-op LEDD) (%)	41109.6±(–1150.0%–100.0)	0
STN-DBS outcome clusters (N)		
ST Axial UPDRS subscore (Excellent vs. moderate/poor)	46 vs. 102	0
LT Axial UPDRS subscore (Excellent vs. moderate/poor)	20 vs. 124	4
ST LEDD (high vs. moderate/low LEDD reduction)	59 vs. 85	4
LT LEDD (high vs. moderate/low LEDD reduction)	60 vs. 87	1

STN, subthalamic nucleus; DBS, deep brain stimulation; LEDD, levodopa equivalent daily dose (mg/day); UPDRS-III, motor section (part III) of the Unified Parkinson's Disease Rating Scale; ST, short-term (∼1 year); LT, long-term (∼5 years).

J. Safra program in PD at Toronto Western Hospital. We recruited all consecutive patients fulfilling the inclusion criteria (e.g., diagnosis of PD, bilateral stimulation of STN, absence of post-surgical complications, or electrode misplacement) with satisfactory clinical information and adequate follow-up. All patients were operated at Toronto Western Hospital by the same neurosurgical team, in the same operating room, and under the supervision of one of three neurosurgeons (MH, SKK, or AML) in the period 1997–2014. All included subjects met the UK brain bank criteria for PD [17]. To minimize the variability introduced by the inherent error of surgical techniques, using neuroimaging techniques, we confirmed adequate electrode position using activation volume overlap with the STN and/or adjacent Zona Incerta. These data were available for 129 of 150 enrolled participants. Only 2 of 129 individuals were excluded due to poorly placed electrodes, as evidenced by a lack of overlap with either the STN or Zona Incerta. 21 participants lacking in electrode placement data remained in the cohort, as based on our observed 1.6% rate of poor placement, less than one of these 21 participants might be expected to have a poorly placed electrode. Thus, our final, cohort comprised 148 participants, the demographics of which are summarized in Table 1.

Clinical data

Patients were assessed according to a standardized protocol in place at Toronto Western Hospital [18–20]. Briefly, this is inspired by the Core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD) criteria [2]). Patients were assessed in the morning at 9 am in the 'practically defined off state' (e.g., at least 12 h from the last dopaminergic agent, usually the night before) and video-evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS). For the on-medication condition, patients were challenged with the same dose used in the pre-operative phase (total levodopa equivalent doses of the first morning intake +20%, administered as crushed tablets of levodopa/carbidopa dissolved in carbonated water). Retrospective clinical data was extracted from patient

charts, by investigators blinded to the genetic data, at two time points post STN-DBS: short-term (ST) follow up was ∼1 year post STN-DBS (11.4 ± 3.0 months) and long-term (LT) follow up was ∼5 years post STN-DBS (60.5 ± 7.3 months). The clinical data collected included Movement Disorders Society (UPDRS) parts I-IV [21] and levodopa equivalent daily dose (LEDD), which was calculated using standardized methods [22] (Table 1). DBS efficacy was calculated by comparing the percentage change in the UPDRS-III subtotal in the off-medication state pre surgery, with the UPDRS-III subtotal off medication with stimulation on post-surgery. To cluster genetic data, surgical outcome, assessed by comparing pre- and post-operative scores at both ST and LT follow up, were used to create four subgroups (Table 1), cut-offs for which were identified a priori based on the longstanding clinical experience of our center.

The percentage change of LEDD at baseline before STN-DBS surgery was calculated at ST and LT follow up. Compared to baseline, STN-DBS patients were subdivided into two groups: those with a high reduction (> 50%) in LEDD and those with a moderate/low reduction in LEDD (≤50%) (Table 1).

The UPDRS axial subscore was generated using a standardized calculation [23], namely the sum of items 13 (falling), 14 (freezing), and 25 (walking) of the UPDRS part II and items 29 (gait) and 30 (postural stability) of the UPDRS part III. The percentage change in the axial subscore in the off-medication state at baseline before STN-DBS surgery, compared to the off-medication on stimulation state at both ST and LT follow-up was calculated. Based on percentage change in axial subscore, STN-DBS patients were subdivided into two groups: those with an excellent outcome (> 70% reduction in axial subscore compared to baseline) and those with a moderate/poor outcome (≤70% reduction in axial subscore compared to baseline) (Table 1).

In total, our study focused on four different clusters of STN-DBS outcome: ST Axial subscore, LT Axial subscore, ST LEDD and LT LEDD (Table 1). For each of these outcomes, cluster scores were missing for only 2–7 patients. Other traditional outcome measures, such as total UPDRS, were not included in our study due to a lack of available data.

Analyses of NeuroX markers

Genomic DNA was isolated from blood using a QIAGEN kit and genotyped on the NeuroX array (Illumina Inc.) at the Clinical Genomics Centre (Toronto, Canada). NeuroX has the standard exome content of ∼240,000 variants, as well as ∼24,000 custom variants related to neurologic diseases, including ∼1,000 known mutations causing neurodegenerative diseases and ∼10,000 single nucleotide polymorphisms (SNPs) tagging significant loci detected by genome-wide association studies (GWASs). Genotype data obtained by NeuroX was loaded to GenomeStudio (Illumina Inc.), which confirmed a call rate of > 0.96 for all samples, and GenTrain score of > 0.35 for all SNPs. NeuroX markers with GenTrain scores between 0.35–0.70 were visually inspected and those with a cluster separation score < 0.2 were removed [24]. ANNOVAR was used for the functional annotation of NeuroX markers including their associated protein changes and the potential impact of variants on protein function [25], as well as to obtain the frequency of the NeuroX markers in the Genome Aggregation Database (gnomAD v.1). For the association study, we extracted NeuroX markers mapped to both Mendelian PD genes and PD loci implicated by the most recent GWAS [26]. In total, we analyzed 113 PD-related genes (Supplementary Table 1).

NeuroX genotypes were converted to PLINK input files to perform chi-square association tests and obtain *p*-values (adjusted for multiple testing). To evaluate the potential impact of genetic variants on STN-DBS outcome, allele frequencies were compared between patients with excellent vs. moderate/poor outcomes (as defined above). Variants with nominal associations in different outcome clusters ($p < 0.05$) were subjected to the Tagger function of the Haploview program (aggressive tagging; using 2- and 3-marker haplotypes) based on genotype data from the study participants. Tagging SNPs were further analyzed for association with the four outcome clusters.

For each STN-DBS outcome cluster, we assessed the burden of rare coding variants with minor allele frequencies < 0.01 in the gnomAD exome subset (v1) (https://gnomad.broadinstitute.org/). We used the Sequencing Kernel Association Test (SKAT) package, including three tests: 1) SKAT (most powerful when most variants in the target region are non-causal or the effects of causal variants are in different directions); 2) burden (more suitable when most variants have effects on the phenotype in the same direction); and 3) SKATO optimized for both scenarios [27]. The combined *p*-values for all rare variants of each gene are reported.

Polygenic risk score (PRS) was calculated for each participant with PRSice v 2.2.11, without linkage

disequilibrium (LD) clumping or P thresholding. We used the summary statistics of 1,805 SNPs from the latest PD GWAS, which were shown to best differentiate patients and controls [26]. Using principal component analysis with HapMap 3, we restricted this analysis to individuals of European ancestry ($n = 124$), since the original PRS was calculated for the European population. To determine the genotype of missing variants, we performed imputation with the Michigan Imputation Server on the Haplotype Reference Consortium (Version r1.1 2016) using Minimac4 and Eagle v2.4. Out of the 1,805 SNPs, only 1,667 variants were available with an imputation quality (r^2) above 0.8.

RESULTS

Clinical data are summarized in Table 1. Levodopa responsiveness pre-operatively was $62 \pm 22\%$. One year after surgery, STN-DBS efficacy and medication reduction were calculated to be 42% and 48%, respectively (both $p < 0.0001$ vs. pre-operative values).

Annotation of the NeuroX data revealed 5,128 markers located at 113 genes mapped to known PD loci (Supplementary Table 1). Their genotypes were analyzed among the 150 PD patients who had undergone STN-DBS. In total, 2,917 of these markers were polymorphic in our PD cohort, including 373 coding variants, 202 of which were rare non-synonymous variants with a minor allele frequency < 0.01 in the gnomAD exome database. Notably, 15 of them (Table 2) had combined annotation dependent depletion scores > 30 (representing the top 0.1% of deleterious variants in the human genome), including a pathogenic mutation in *LRRK2* (p.G2019S).

Using the SKAT package, we investigated the gene-by-gene joint burden of rare variants on STN-DBS outcome. Nominally significant findings were detected for 14 genes (Table 3), none remained significant after correction for multiple testing. However, three genes showed nominally significant results by all three SKAT tests, including *CRHR1* (NM_001145146.2) and *IP6K2* (NM_001005909.2) for the ST Axial subscore; as well as *PRSS3* for the LT Axial subscore.

The chi-square association test between each polymorphic marker and the four outcome clusters identified 590 nominally significant signals (Fig. 1A, Supplementary Table 2), none of which survived Bonferroni correction ($p < 0.00002$). The strongest association was detected between the LT LEDD outcome and an intronic SNP (rs10810812) in *SH3GL2* ($p = 0.00028$), as well as between ST LEDD outcome and 13 SNPs located in a ~150 Kb genomic region within intron 1 of *SH3GL2* (Chr9:17586101-17735083) ($p = 0.001$). Investigation of the LD-structure of the *SH3GL2* locus (Supplementary Figure 1) showed that the region tagged by 13 SNPs belongs to a single LD-block, which includes the GWAS-significant SNP rs10756907 [26]. In contrast, rs10810812 belongs to a different LD-block, containing another GWAS-significant SNP rs13294100 [26]. To identify independent signals, all nominally significant variations were analyzed using the Haploview Tagger function, which revealed 153 variants tagging separate blocks (100% of alleles at $r^2 >= 0.8$ were captured with a mean max r^2 of 0.96). Among them, rs10810812 in *SH3GL2* showed a significant association of the G-allele with a high LT LEDD outcome even after Bonferroni correction (adjusted $p = 0.043$) (Fig. 1B).

To test for associations between PRS and the four outcome clusters, we used a logistic regression approach with and without adjustment for sex, age at surgery, and disease duration at the time of surgery. No statistically significant associations were found (Table 4).

DISCUSSION

Demand for DBS in PD has increased exponentially in recent years, and this trend is likely to continue with the suggestion that patients should undergo surgery sooner [28]. However, three decades after its first clinical application, the selection of DBS candidates is still far from optimal as it remains reliant upon clinical features that lack the specificity and granularity to capture the highly heterogeneous neurobiological underpinnings of PD [29]. This failure is particularly relevant to two aspects, first the ability of the brain to tolerate the procedure (e.g., clinical deterioration following surgery); and second a lack of accurate predictors of long-term outcome. For example, the assumption that patients over 70 should not undergo STN-DBS, in light of the reported post-surgical axial motor deterioration [30], has been challenged by successful procedures in elderly patients, indicating that age *per se* is not a reliable proxy of brain frailty. As for long-term outcome, recent—not yet replicated—data point to a role for frontal cognitive impairment, indicating that a

Table 2
Characteristics of patients carrying nonsynonymous heterozygous variants with a CADD score > 30 or a stop gain variant

Gene	Variant	SNP ID	CADD	Minor allele frequency		Patient characteristics			STN-DBS outcome			
				PD cohort	gnom AD-exome	ID	sex	age at onset	ST Axial	LT Axial	ST LEDD	LT LEDD
LRRK2	p.G2019S	rs34637584	35	0.003	0.0005	10114	F	47	E	E	E	E
VPS13C	p.R3564H	rs116228685	35	0.003	0.0003	10538	M	52	M	M	M	M
DNAH17	p.G4044S	rs199692490	35	0.003	0.0004	10239	M	39	E	E	E	E
UBAP2	p.R174Q	rs79607078	34	0.003	0.0043	10478	M	42	E	na	M	M
HIP1R	p.A911T	rs141813189	34	0.003	0.0012	10537	M	37	M	E	M	M
NEK1	p.R261H	rs200161705	34	0.007	0.0024	10446	M	51	M	M	M	M
						10739	M	33	M	M	M	M
PRKN	p.R126W	rs34424986	34	0.007	0.0019	10487	M	48	M	M	M	M
						10550	M	44	M	M	E	E
CNTN1	p.R966C	rs150734960	34	0.007	0.0008	10446	M	51	M	M	M	M
						10792	M	34	M	M	M	E
HIP1R	p.R564W	rs140743610	33	0.003	0.001	10681	M	47	M	M	E	M
SIPA1L2	p.P714S	rs200216436	32	0.003	0.0003	10715	M	33	M	M	E	M
SH3GL2	p.A221T	rs760865937	32	0.003	0.0002	10487	M	48	M	M	M	M
BRIP1	p.R264W	rs28997569	32	0.003	0.0008	10313	M	30	M	M	M	M
KCNIP3	p.R39H	rs35516857	32	0.01	0.0022	10487	M	48	M	M	M	M
						10442	F	45	E	M	E	E
						10698	F	46	M	M	M	M
DNAH17	p.F2520V	rs200203879	32	0.003	0.0025	10316	F	61	E	M	E	E
RIT2	p.R182H	rs148544378	31	0.01	0.009	10434	M	43	M	M	M	M
						10338	M	55	M	M	E	M
						10595	M	34	E	E	E	E
VPS13C	p.R3609X	rs138846118	49	0.003	0.0002	10413	M	51	M	M	E	E

CADD, combined annotation dependent depletion; PD, Parkinson's disease; MAF, Minor allele frequency; Sex, F – female, M – male; STN, subthalamic nucleus; DBS, deep brain stimulation; ST, short-term (∼1 year); LT, long-term (∼5 years); Axial, Axial subscore of the Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose (mg/day); STN-DBS outcome, E – excellent, M – moderate/poor; na, not available.

Table 3

Results of the joint burden analysis of rare variants on STN-DBS outcome. The Burden, SKATO, and SKAT test of the SKAT package were performed for the four DBS outcome groups (ST Axial subscore, LT Axial subscore, ST LEDD, and LT LEDD). Three genes (CRHR1, IP6K2, PRSS3) showed nominally significant *p*-values for the excellent outcome by all 3 tests (bold-typed)

Gene	Burden				SKATO				SKAT			
	ST LEDD	ST Axial	LT LEDD	LT Axial	ST LEDD	ST Axial	LT LEDD	LT Axial	ST LEDD	ST Axial	LT LEDD	LT Axial
CHD9	**0.040**	0.820	0.200	0.520	0.063	0.730	0.300	0.520	0.140	0.560	0.630	0.520
CRHR1	0.070	**0.015**	0.370	0.089	0.067	**0.015**	0.370	0.090	0.070	**0.015**	0.370	0.090
HIP1R	0.630	0.460	0.230	0.150	0.750	0.660	0.370	0.080	0.567	0.480	0.400	**0.044**
IP6K2	0.230	**0.009**	0.350	0.480	0.240	**0.009**	0.350	0.480	0.240	**0.009**	0.350	0.480
ITGA8	0.790	0.920	**0.035**	0.480	0.580	0.520	0.060	0.650	0.420	0.370	0.220	0.920
KCNS3	0.230	**0.033**	0.240	0.570	0.330	0.050	0.330	0.700	0.500	0.100	0.500	0.850
LRRK2	0.270	0.160	0.130	0.140	0.420	0.260	0.210	0.060	0.450	0.420	0.320	**0.036**
MAP4K4	0.240	0.350	0.783	0.140	0.330	0.460	0.460	0.060	0.500	0.640	0.340	**0.043**
MED12L	0.240	0.550	0.783	0.140	0.330	0.350	0.460	0.060	0.500	0.260	0.340	**0.043**
PAM	0.440	0.830	0.673	0.220	0.570	0.054	0.850	0.340	0.390	**0.034**	0.780	0.900
PRSS3	0.150	0.920	0.150	**0.008**	0.150	0.920	0.150	**0.008**	0.150	0.920	0.150	**0.008**
RIMS1	0.090	0.550	0.086	0.140	0.130	0.350	0.130	0.060	0.230	0.260	0.230	**0.043**
SMPD1	0.690	0.051	0.710	0.160	0.560	0.087	0.660	0.090	0.380	0.130	0.460	**0.044**
TMEM175	0.240	**0.033**	0.240	0.140	0.330	0.050	0.330	0.060	0.500	0.100	0.510	**0.043**

DBS, deep brain stimulation; STN, subthalamic nucleus; Axial, Axial subscore of the Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose (mg/day); ST, short-term (∼1 year); LT, long-term (∼5 years).

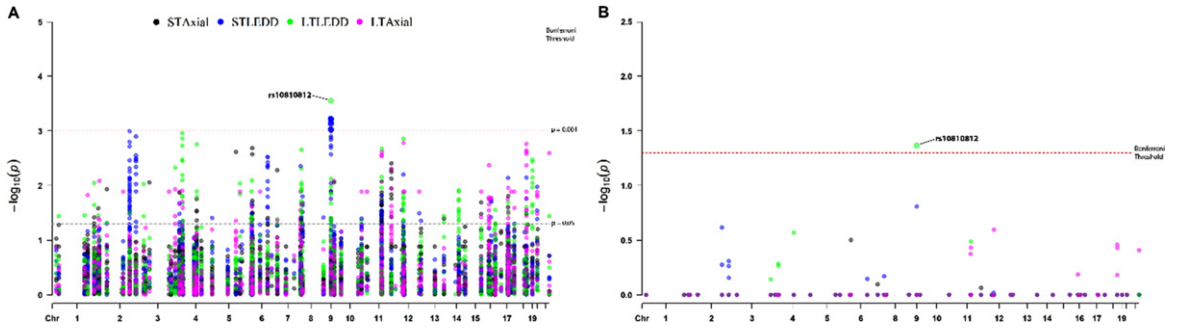

Fig. 1. Manhattan plot. A) The association study between the four STN-DBS outcome clusters and 2917 polymorphic NeuroX markers (mapped to known PD loci) identified 590 nominally significant signals. The strongest nominal association (uncorrected $p < 0.00028$) was detected between the LT LEDD outcome and rs10810812 in SH3GL2, as well as between the ST LEDD outcome and 13 SNPs located in a ~150Kb genomic region (Chr9:17586101-17735083). B) To reveal the independent association signals, 153 tagging variants selected by Haploview were tested for association with STN-DBS outcome clusters. Among these tagging variants, the rs10810812 SNP in *SH3GL2* showed a significant association with LT LEDD outcome after Bonferroni correction (adjusted $p = 0.043$). DBS, deep brain stimulation; LEDD, levodopa equivalent daily dose (mg/day); LT, long-term (~5 years); ST, short-term (~1 year); STN, subthalamic nucleus.

Table 4
Results of the association study between polygenic risk score and the four outcome clusters, including results adjusted for sex, age at surgery, and disease duration at surgery

				Adjusted values		
Outcome	Beta	SE	*P*	Beta	SE	*P*
ST LEDD	0.04	0.19	0.82	–0.02	0.20	0.93
ST Axial	–0.24	0.20	0.22	–0.31	0.21	0.13
LT LEDD	–0.07	0.19	0.72	–0.09	0.19	0.63
LT Axial	0.14	0.24	0.56	0.14	0.25	0.59

Axial, axial subscore of the Unified Parkinson's Disease Rating Scale; LEDD, levodopa equivalent daily dose (mg/day); ST, short-term (~1 year); LT, long-term (~5 years); SE, standard error.

more widespread pathology at baseline might predict a faster disease progression [31]. Reliable indicators of surgical outcome would dramatically improve the application of this increasingly popular treatment for PD.

Based on the limited data seen in monogenetic forms of dystonia and PD [32], it has been suggested that genetic factors might be taken into account to select DBS candidates. So far, only a single study has attempted to establish the link between STN-DBS outcome and a few variants in two PD genes. It reported that in a cohort of 85 patients a more favorable motor outcome two years post-surgery was associated with two SNPs tagging a PD-related haplotype at the 3' untranslated region of *SNCA,* strongly supporting the evaluation of genetic biomarkers in surgical cohorts [15]. Importantly, in our cohort, we found that both SNPs were nominally associated with an improved ST Axial subscore ($p = 0.018$ for rs356219 and $p = 0.029$ for rs356220).

Our study aimed to correlate NeuroX variants with detailed post-operative clinical data in our deeply phenotyped cohort, to identify loci associated with STN-DBS outcome in the hope that eventually such knowledge could be applied clinically to target patient selection for STN-DBS, leading to improved surgical outcome. Importantly, patients included in this study are representative of the typical population undergoing STN-DBS and likewise, the effect of surgery on motor signs and LEDD reduction is in keeping with the extensive literature published to date [33, 34]. Our analysis suggested three nominally significant candidate genes (*CRHR1*, *IP6K2*, and *PRSS3*) that may influence the development of axial symptoms post STN-DBS and one gene (*SH3GL2*) linked to a reduction of dopamine replacement therapy post STN-DBS.

CRHR1 was associated with axial symptoms one year post DBS. It encodes a corticotropin-releasing hormone receptor that has previously been associated with PD in two large GWASs [26, 35]. *CRHR1* is widely expressed in the brain and its stimulation in the pituitary gland leads to the release of glucocorticoids [36]. There is growing appreciation for the role of glucocorticoids and their receptors in PD, with increased signaling worsening symptoms and enhancing neurodegeneration in experimental models of PD [37].

Our analysis also suggests an association between axial symptoms one year post DBS and *IP6K2*, which was previously linked to PD in two GWASs [26, 38]. *IP6K2* encodes Inositol hexakisphosphate kinase 2 that has been shown to mediate apoptosis [39]. *IP6K2* is abundantly expressed in the brain and has been implicated in the neurodegenerative

process of several diseases. In amyotrophic lateral sclerosis, *IP6K2* has been shown to promote cell death associated with TDP-43 aggregation [40]. Similarly, activation of *IP6K2* has been associated with pathogenicity in Huntington's disease [39]. As both *CRHR1* and *IP6K2* are implicated in neurodegenerative processes, it is possible that they influence the development of axial symptoms post DBS by an effect on disease progression.

Our data suggested that axial symptoms 5 years post DBS were linked to *PRSS3*, encoding the trypsinogen protease serine 3, which is highly expressed in both the pancreas and the brain. *PRSS3* was identified by GWAS through quantitative trait locus mapping (nominating the gene under the disease linked LD-block based on the functional data) [26]. Also, four *PRSS3* variants were noted in a South African PD population using exome sequencing [41]. Little is known about trypsinogens and PD; however, trypsin-2 mRNA has been found in PD substantia nigra where it has been associated with levodopa-induced dyskinesia and psychosis [42]. Thus, it is plausible that trypsinogen signaling might impact PD-related symptoms.

A tempting but speculative view concerns the role of these three genes in inflammatory and apoptotic responses of the brain, an area receiving increasing attention in PD [43]. Interestingly, recent animal data indicate that DBS may act as a regulator of the inflammatory response in PD states, attenuating classical activation of astrocytes and cytokine induction [44], thus confirming the hypothesis of a preliminary study in PD patients undergoing DBS [45]. On the other hand, autopsy data have shown inflammatory responses around the electrodes of DBS patients [46] and systemic inflammation has been reported to impair brain's ability to tolerate a DBS procedure [47]. Taken together, these notions might indicate that inflammatory response may be associated with the post-surgical decline of some PD patients undergoing STN-DBS, possibly indicating that *CRHR1*, *IP6K2,* and *PRSS3* are involved in such a response.

Finally, our study points to an association between LEDD post STN-DBS and common SNPs in *SH3GL2* encoding endophilin A1, a known risk factor for PD. The observed association with endophilin A1 could stem from its contribution to synaptic vesicle exocytosis influencing vulnerability of dopaminergic neurons in PD [38, 48]. Alternatively, since LEDD reduction is a proxy of overall DBS outcome, *SH3GL2* might have a more general role in surgical efficacy.

Baseline clinical characteristics for patients with and without the observed rare variants in *IP6K2*, *PRSS3*, and *CRHR1* and between patients with AA vs. GG genotype of rs10810812 in *SH3GL2*, were not significantly different, other than one nominally significant result that was observed for older age of onset in *IP6K2*-carriers (Supplementary Table 3). Thus, based on the available clinical data, these identified variants do not appear to have different baseline clinical characteristics that could help with patient selection. Indeed, this finding emphasizes the potential for genetic testing to detect possible markers to inform patient selection.

We appreciate that the conclusions drawn from our study are subject to limitations. First, although one of the largest DBS cohort studies to date, we recognize for a genetic cohort the sample size is relatively small. Second, the use of arbitrary cut-offs to categorize patients has obvious limitations that we tried to minimize with their *a priori* definition. In spite of these limitations, our study of 150 STN-DBS patients evaluated at a single center with a standardized battery up to 5 years after surgery replicated the findings of two *SNCA* SNPs (rs356219 and rs356220) previously associated with neurostimulation outcome [15]. In addition, high effect sizes were observed for several nominally significant variants. For example, rs35507033 in *LRRK2* for ST LEDD outcome, rs75638861 for ST Axial subscore, and several variants for LT Axial subscore outcome (e.g., rs184013125, rs142022985, rs117922937, rs146051626). Although our findings should be considered preliminary in nature, we hope our work encourages future studies to validate our findings in independent cohorts. Such future experiments might also address epigenetic modifications that could dictate surgical outcomes and investigate the effects of DBS itself on DNA methylation and the brain transcriptome. Finally, NeuroX is a commonly used array in genetic investigation of neurodegenerative diseases; however, it was designed prior to the recent GWASs that identified novel PD loci. Therefore, future studies could have more power using the more updated platform designed with a focus on neurodegenerative disorders (e.g., Illumina's Neuro Booster).

CONCLUSIONS

Our study has enhanced understanding of the mechanisms responsible for the successes of DBS

and gene-phenotype correlations in PD and adds to growing calls to evaluate genetic biomarkers in surgical cohorts. We emphasize the potential for genetic testing to detect markers to inform patient selection. To this end, standardized assessment of our future surgical population will represent an invaluable tool. Although outside the scope of the present study, it is critical that these findings are validated in independent populations to pave the way for the implementation of new recommendations with respect to patient selection and/or target selection (e.g., STN vs. globus pallidus interna) for DBS in PD.

ACKNOWLEDGMENTS

The authors would like to acknowledge the patients who participated in this research, as well as the McLaughlin and Blidner Family Foundations and funder of the Chair in Neuromodulation and Multi-Disciplinary Care at the University of Toronto and University Health Network for their generous support. This study was funded by the McLaughlin, Blidner Family Foundations, and the Canadian Consortium on Neurodegeneration in Aging.

CONFLICT OF INTEREST

NPV, AF, and ER received a grant from the McLaughlin Foundation to support this work. AF received research support and/or speaker honoraria from Abbott, Brainlab, Boston Scientific, and Medtronic; he is a consultant for Abbott, Boston Scientific, Cere Gate, INBRAIN Neuroelectronics, and Medtronic.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JPD-212730.

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