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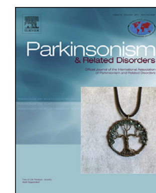

Pipeline to gene discovery - Analysing familial Parkinsonism in the Queensland Parkinson's Project

Steven R. Bentley [a, *], Stephanie Bortnick [b], Ilaria Guella [b], Javed Y. Fowdar [a], Peter A. Silburn [c], Stephen A. Wood [a], Matthew J. Farrer [b], George D. Mellick [a]

[a] Griffith Institute for Drug Discovery, Griffith University, Don Young Road, Nathan, Queensland, Australia
[b] Djavad Mowafhagian Centre for Brain, University of British Columbia, Vancouver, British Columbia, Canada
[c] Centre for Clinical Research, University of Queensland, Brisbane, Queensland, Australia

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ABSTRACT

Introduction: Family based study designs provide an informative resource to identify disease-causing mutations. The Queensland Parkinson's Project (QPP) has been involved in numerous genetic screening studies; however, details of the families enrolled into the register have not been comprehensively reported. This article characterises the families enrolled in the QPP and summarises monogenic forms of hereditary Parkinsonism found in the register.
Method: The presence of pathogenic point mutations and copy number variations (CNVs) were, generally, screened in a sample of over 1000 PD patients from the total of 1725. Whole exome sequencing (WES) was performed on eighteen probands from multiplex families.
Results: The QPP contains seventeen incidences of confirmed monogenic forms of PD, including *LRRK2* p.G2019S, *VPS35* p.D620N, *SNCA* duplications and *PARK2* p.G430D (hom) & exon 4 deletion (hom). Of these seventeen, five belong to multi-incident families, while another eight have a family history of at least one other case of PD. In additional families, WES did not identify known forms of monogenic Parkinsonism; however, three heterozygous mutations in *PARK2*, p.R275W, p.Q34fs, and a 40bp deletion in exon 3 were identified. Of these three mutations, only the 40bp deletion segregated with disease in a dominant inheritance pattern.
Conclusion: Eighteen probands have screened negative for known CNVs and mutations that cause clear monogenic forms of PD. Each family is a candidate for further genetic analysis to identify genetic variants segregating with disease. The families enrolled in the QPP provide a useful resource to aid in identifying novel forms of monogenic PD.

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1. Introduction

The discovery of monogenic forms of Parkinson's disease (PD) aids in our understanding of the underlying molecular pathways that lead to neuronal cell death and the development of PD. Genetic studies have confirmed that mutations in several genes can cause hereditary Parkinsonism in multiplex families; these include *LRRK2*, *SNCA*, *VPS35*, *PARK2* (Parkin), *PINK1*, *PARK7* (DJ-1), *DNAJC6*, *GBA*, *ATP13A2*, *FBXO7*, *PLA2G6*, *SYNJ1*, *RAB39B*, *VPS13C* and *PTRHD1* [1,2]. A number of new candidates are emerging that may harbour disease-causing mutations, including *DNAJC13*, *CHCHD2*, *TMEM230* and *RIC3* [2]. However, it is interesting to note that, the majority of multi-incident families that have been studied do not share a known or highly suspected disease-causing mutation in these genes. This suggests that there are undiscovered genetic factors that can cause seemingly hereditary PD. The Queensland Parkinson's Project (QPP) research registry is a resource that has been used previously to aid in validating disease-causing mutations and genetic risk factors [3–5]. However, a comprehensive report of the registry design, family structures and extent of hereditary PD found within the QPP has not previously been published. In this manuscript, we summarise the structure of the QPP and the known forms of monogenic Parkinsonism identified in the participants.

* Corresponding author.
E-mail addresses: steven.bentley2@griffithuni.edu.au (S.R. Bentley), sbortnick@can.ubc.ca (S. Bortnick), iguella@can.ubc.ca (I. Guella), j.fowdar@griffith.edu.au (J.Y. Fowdar), p.silburn@uq.edu.au (P.A. Silburn), s.wood@griffith.edu.au (S.A. Wood), mfarrer@can.ubc.ca (M.J. Farrer), g.mellick@griffith.edu.au (G.D. Mellick).

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Additionally, we report family structures and preliminary findings WES in eighteen probands from kindreds from the QPP registry.

2. Methods

The study used the patient information and genetic resources collected from the QPP, a register of over 4250 individuals recruited from throughout the State of Queensland, Australia, who have agreed to participate in research into PD and related disorders. The QPP Register contains clinical, demographic and risk-factor information for 1725 patients clinically diagnosed with PD by a movement disorders specialist using established criteria [6]. The majority of PD patients were recruited into the project by referral from Brisbane's two major public tertiary referral movement disorder clinics (at the Princess Alexandra Hospital and the Royal Brisbane and Women's Hospital) and several private movement disorders practices in Brisbane, Queensland. Where possible, we attempted to also recruit unaffected control subjects to match the affected participant. Three groups of controls were recruited: (1) Family controls - blood related family members of the PD cases who screened negative for symptoms of PD using a sensitive screening tool [7] (n = 451); (2) Spouse controls – we invited the spouses of cases to participate (n = 549) and (3) Community dwelling controls (n = 811). The community controls consisted of 548 suburb- & age-matched participants enrolled through a telephone call-out study [8] with the remainder convenience samples made up of volunteers recruited from the general community. Upon enrolment, participants provided a sample of whole blood and completed a structured self-administered questionnaire, which collected information regarding clinical symptoms (if they are a patient), life-style and environmental risk factors, as well as family history of Parkinsonism and other medical conditions. Additionally, family members of patients reporting PD-family histories were invited into the project for kindred studies.

All donated blood and information were collected in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research, with written consent obtained from each participant. Ethical approval for this study was obtained from the Human Research Ethics Committee (HREC) at Griffith University (Protocol No: ESK/04/11/HREC). Genomic DNA from donated blood was extracted using the salting-out procedure previously described, with two key changes: 10 M ammonium acetate was used instead of 6 M NaCl, and genomic DNA was washed with 70% ethanol before storage in TE pH 7.4 buffer.

Patients in the QPP were screened for genetic mutations that are known to be causal for Parkinsonism. Collectively, the PD cases were screened for *LRRK2* p.G2019S (n = 1295), p.I2020T (n = 1304), p.R1441C (n = 1222), p.R1441G (n = 1094), p.R1441H (n = 1075), p.I1122V (n = 1091) and p.Y1699C (n = 1090) variants. Additionally, 1138 patients were screened for the *VPS35* p.D620N variant. CNVs were assessed in 860 patients. Due to Parkinsonism being attributed to polymorphic nucleotide expansions in some Spinocerebellar Ataxia (SCA) genes [9], we have screened 893 cases and 916 controls from QPP for polyglutamine repeats in *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6) and *TBP* (SCA17) [5]. We have also screened 920 cases and controls for the *C9orf72* (G4C2)n repeats [10]. The rationale for this is that a substantial number of FTLD/ALS patients (14–35%) who carry *C9orf72* $(G_4C_2)_{>60}$ expansions present with atypical Parkinsonism in early disease stages and there is an increased incidence of Parkinsonism with or without features of the FTLD/ALS complex in these cases' relatives.

This was done by a variety of methods including: (1) Direct genotyping of specific single nucleotide sequence variants such as: *LRRK2* p.G2019S & p.I2020T and *VPS35* p.D620N was done using TaqMan™ (Thermo Fisher) and MassARRAY® (Sequenom) platforms, (2) Indirect genotyping using high resolution melt (HRM) analysis, (3) Analysis of polymorphic nucleotide expansions in *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6), *TBP* (SCA17) and *C9orf72* using polymerase chain reaction (PCR) followed by capillary gel electrophoresis and (4) Copy number variation (CNV) analysis using multiplex ligation-dependent probe amplification (MLPA) to screen for CNVs in the known PD-related genes *SNCA* (*PARK1*), *PARK2*, *PINK1*, *PARK7* and *ATP13A2*, supplemented with CNV predictions generated by PennCNV [11] using data from SNP arrays. Single nucleotide sequencing variants were confirmed using Sanger sequencing. Additional information for each specific method is available from the authors on request.

Probands from eighteen multi-incident families, which had DNA samples available and had family structures that were conducive for follow-up recruitment of additional affected members, were selected for WES.

WES of selected probands was conducted using one of two next-generation sequencing platforms, the MiSeq (Illumina) or the Ion Torrent™ (Thermo Fisher). The MiSeq platform was used in conjunction with the Nextera Rapid Capture Exome Enrichment chemistry (Illumina) following the manufacturer's protocol. The Ion Torrent™ platform was used in conjunction with the Ion AmpliSeq™ chemistry (Thermo Fisher) following the manufacturer's protocol.

Similarly, two bioinformatic pipelines were used to analyze the data. The recommended bioinformatic pipeline from GenomeAnalysis ToolKit (GATK) (Broad Institute, 2017) was used to generate variants for the MiSeq platform. Briefly, reads were aligned to the human reference genome (hg19) using the BWA v0.7.12. Sequences with a mapping quality score below Q30 were removed and the file was sorted using SAMtools v1.2. Read group information was attached and PCR duplicates were marked with Picard tools v2.7.1. Base quality scores were recalibrated using GATK v3.5. Sites different to the reference genome were called for individual samples using HaplotypeCaller. Individual GVCF files were then merged and a joint genotyping analysis was performed using GATK v3.5. The genotypes in the subsequent file were then assigned confidence scores using the 'variant quality score recalibration'. The Torrent Suite™ (v4.0) of bioinformatic tools (Thermo Fisher) was used to process sequencing data from the Ion Torrent™ platform. This included mapping and alignment using Torrent Mapping Alignment Program v5.0.6 and variant calling using Torrent Variant Caller v5.0.6.

All variants that differed from the consensus sequence were

Table 1
Summary and general information of the 137 multiplex families.

Family Characteristic	Number of multi-incident families
Proband with EOPD (<= 52years)[a]	41/137
Proband with EOPD (<= 40years)	10/137
3 or 4 affected family members	118/137
5 + affected family members	19/137
Affected first degree relatives	93/137
Affected second degree relatives	96/137
Affected third degree relatives	49/137
Affected fourth degree relatives	4/137
Affected fifth degree relatives	1/137
Summary information of 137 multi-incident families	
Mean unaffected siblings to proband:	2.5
Mean age at onset of proband:	55.90 yrs
Mean affected family members:	3.7

EOPD: Early Onset Parkinson's disease.
[a] 52 years represents the first quartile of all onset ages from patients in the QPP.

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