Title: Pipeline to Gene Discovery - Analysing Familial Parkinsonism in the Queensland Parkinson's Project

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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 1,000 PD patients from the total of 1,725. 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.

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

Methods

The study used the patient information and genetic resources collected from the QPP, a register of over 4,250 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 1,725 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: 10M ammonium acetate was used instead of 6M 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=1,295), p.I2020T (n=1,304), p.R1441C (n=1,222), p.R1441G (n=1,094), p.R1441H (n=1,075), p.I1122V (n=1,091) and p.Y1699C (n=1,090) variants. Additionally, 1,138 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 annotated using the ANNOVAR package [12]. The frequency of the variants in the population were also annotated using the Genome Aggregation Database (gnomAD) exome dataset [13]. Mutations were isolated if they met the following criteria: resided in suspected PD genes outlined in supplementary table 1; had a minor allele frequency (MAF) of <=0.003 from the gnomAD exome dataset; resided in an exonic or splicing region of the gene; and were missense.

Results

The QPP register contains 1,725 PD patients, which includes 262 independent kindreds comprising of 322 patients and 407 controls; an average of 0.23 affected family members and 1.55 unaffected family members have been recruited for each individual proband enrolled in the study. From all PD patients, 463 (26.8%) of whom reported at least one other member of their extended family diagnosed with PD. Additionally, 271 (15.7%) PD patients reported a first-degree relative diagnosed with PD. A sample of 1,716 participants from the QPP with SNP array data available clustered mostly with European ancestry (98%). The register identified 137 families with at least three PD-affected members. The majority (96/137) of the probands had a late onset of symptoms, with an average onset age of 55.90 years (Table 1). Most probands described a first degree or second degree relative with PD, while

35.8% of probands also described an affected third degree relative. The average number of affected members across the 137 families was 3.7 cases.

From the 137 multi-incident families, three probands from 115 samples genotyped (2.6%) carried the *LRRK2* p.G2019S mutation, two probands from 95 samples genotyped (2.1%) carried the *VPS35* p.D620N mutation, and zero from 113 samples genotyped (0%) carried *SNCA* multiplications, or *PARK2*, *PARK7* or *PINK1* exon deletions. Furthermore, no compound heterozygous mutations in *PARK2*, *PARK7* or *PINK1* were identified in these multi-incident families at the time of this publication (See Supplementary Table 3 for detailed family and genotype information for each multi-incident family). Unequal genotyped samples reflects sample availability and quality during times of the genotyping assays.

Previous screening of other patients from the QPP identified a further eight *LRRK2* p.G2019S cases, two *SNCA* duplications cases, one homozygous *PARK2* exon 4 deletion case, and one homozygous *PARK2* p.G430D case (see Table 2 for a summary). No *LRRK2* p.I2020T, p.R1441C, p.R1441G, p.R1441H, p.I1122V or p.Y1699C carriers were identified. Furthermore, no samples possessed pathogenic nucleotide expansions in *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6), *TBP* (SCA17) or *C9orf72*. Details of some of these findings have been published [3, 4, 14, 15]

<**TABLE1**>

<TABLE2>

After screening probands from eighteen multi-incident families negative for the *LRRK2* p.G2019S and *VPS35* p.D620N mutations, and CNVs in *SNCA*, *PARK2*, *PARK7* and *PINK1*, probands underwent WES to identify rare sequence variants in suspected genes that could cause Parkinsonism. Three probands carried heterozygous mutations in *PARK2* including a 40bp deletion in exon 3, p.Q34fs and p.R275W (Table 3). From these *PARK2* mutations, only the heterozygous 40bp deletion in exon 3 segregated with disease in the respective family (Figure 1). The *PARK2* p.Q34fs mutation did not segregate with disease (Figure 1), while additional patients from family #489 could not be recruited to test for mutation-disease segregation.

The proband from family #019 was heterozygous for a *PINK1* p.G411S mutation, previously identified in our laboratory [15]. Sanger sequencing confirmed the heterozygosity of this variant (Data not shown). Further investigation into these *PARK2* and *PINK1* families did not reveal any evidence of compound heterozygous mutations. Additionally, the *PINK1* p.G411S mutation in the proband of family #019 is inherited from the maternal side, and not the paternal side that has multiple people with Parkinsonism (Figure 1).

The proband of family #468 was heterozygous for a *LRRK2* p.H2236R mutation with a very low MAF (Table 3), this variant did not segregate with disease in the family (Figure 1). Other sequence variants were also detected in suspect Parkinsonism genes across the eighteen families, however their MAFs were either higher than expected to cause monogenic Parkinsonism, or the variant's zygosity did not fit the family inheritance of disease (See Supplementary Table 2).

<TABLE3>

<FIGURE1>

Discussion

In this study, we have reported on the genetic causes of Parkinson's disease found in the QPP, as well as describing the initial findings from next generation sequencing analysis of selected probands.

Through routine screening of patients and families of the QPP, we have genotyped a number of known disease-causing mutations (Table 2). As expected, the *LRRK2* p.G2019S mutation, which accounts for disease in 2.6% of the multiplex families genotyped in the QPP is the most frequent genetic cause of PD in our register. The frequency of the p.G2019S mutation in familial PD is heterogeneous across different populations [16], however the estimated frequency in the United Kingdom is 2% (CI: 0.9-3.1%) and the Caucasian population in the USA is 3.5% (CI: 1.9-5.1%) [16]. Our observed *LRRK2* p.G2019S frequency is comparable to these. The eight additional *LRRK2* p.G2019S cases with one or zero affected members (See Table 2) highlights the variable penetrance associated with this substitution. The mutation is reported to be ~70% penetrant over the age of 80 years in a Norwegian population [17]. Our data suggests tremor is a frequent symptom amongst p.G2019S carriers, while falls and depression are not common symptoms. Comparatively, the collation of symptomology from the MDSGene database indicates that patients with any *LRRK2* mutations predominately have rigidity and bradykinesia, while depression rates are even amongst carriers [18]. Discrepancies are likely due to sample size and inclusion of other *LRRK2* mutations in the MDSgene dataset.

Our analysis revealed that two multiplex families in the QPP carry the *VPS35* p.D620N mutation. In line with other reports, this suggests that the p.D620N substitution is also a rare form of PD [3].

We did not identify *SNCA* multiplications or homozygous *PARK2* variants in any multiplex families in this study group. Duplications of the *SNCA* gene are well reported to cause PD [19], and are expected to occur in 1.7% of familial PD cases with a European ancestry [20]. Although, we do not see multiplications of *SNCA* in the QPP multiplex families, we did identify duplications in two smaller QPP families with a lesser number of affected members (Table 2). Similarly, we observed pathogenic *PARK2* mutations in two participants that did not report a family history.

It is noteworthy, that due to long-term storage and repeated use of DNA samples, some samples were not of suitable quality or sufficient quantity for genotyping assays, such as MLPA. Samples that failed quality control assessments or could not be genotyped were omitted from the mutation frequency calculations. Similarly, genotyping assays were performed at different times since establishing the QPP register, and as such, a number of samples were only genotyped for some, but not all, markers due to their availability.

Notably, from the 137 multi-incident families, only five families have been genotyped with the *LRRK2* p.G2019S or the *VPS35* p.D620N substitutions, and none have genotyped positive for pathogenic CNVs. Families remain a highly informative tool to identify disease-causing variants, through observing the segregation of genotype and phenotype. Previous disease-causing mutations have been identified through this method, including the *VPS35* p.D620N mutation [21], the *VPS13C* mutations [22] and the tentative *DNAJC13* p.A855S mutation [23]. Other strategies to identify disease-causing mutations without family structures may reveal interesting candidate genes [24], however without observing the mutation co-segregating with disease in a family, gathering additional evidence to argue causality is problematic. Our aim is to utilise the families from the QPP that have not previously genotyped positive for known genetic lesions, to identify novel Parkinson's disease-causing variants.

The initial stages of our study, which are reported here, include pre-screening and WES of eighteen probands to identify known disease-causing mutations. WES of the probands did not clearly reveal any known monogenic forms of PD. Three probands however possessed heterozygous *PARK2* mutations: a 40 base pair deletion, p.Q34fs and p.R275W (Table 3). In all three probands, no additional mutations or CNVs in *PARK2* were considered pathogenic, ruling out compound heterozygous cases. Individually, these mutations typically cause disease in homozygous or compound heterozygous states [25-27], but alone in heterozygous states, these variants are not considered causes of monogenic Parkinsonism. However, heterozygous *PARK2* variants may increase the risk of developing PD. The mechanism by which the p.R275W mutation increases risk is unclear, but it has been suggested that it may act through dominant mechanisms by causing misfolding of the

parkin protein leading to aggresome formation [28]. Interestingly, the 40bp deletion in *PARK2* has been seen to segregate with disease in a dominant inheritance pattern, albeit with reduced penetrance, in a family with atypical Parkinsonism [29].

Aside from the previously identified p.G411S mutation in *PINK1* in the proband of family #019 [15], we did not identify any additional rare mutations in *PINK1* in the proband through WES. Recently, this variant was shown to increase the risk of PD by 2.9 times through a dominant-negative mechanism [30]; however, the variant itself does not segregate with disease in this family. We propose that additional genetic components contribute to disease in this family and remain to be discovered.

Finally, the *LRRK2* p.H2236R mutation has not been reported previously to cause PD. Although the sequence variation changes a highly conserved histidine residue in the WD40 domain, it does not segregate with disease in the family. Thus, we do not have evidence to suggest a pathogenic role for this sequence variant. All families involved in this study are candidates for discovery of rare segregating sequence variants that can cause PD.

Conclusion

In the current study, we summarise the previous genetic findings from the QPP in regards to pathogenic mutations, as well as provide demographics of families enrolled in the register. From 137 multi-incident families, a causal genetic component has not been identified for 132 families. Preliminary WES sequencing of eighteen candidate families revealed three heterozygous *PARK2* mutations, one heterozygous *PINK1* mutation, and one *LRRK2* mutation. Segregation of the mutation with disease was observed only in family #451. Currently each of the eighteen families is a candidate for further genetic analysis. We aim to use families enrolled in the QPP as a resource to identify new monogenic forms of PD, further increasing our understanding of hereditary Parkinsonism.

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Table 1. Summary and general information of the 137 multiplex families. [a] 52 years represents the first quartile of all onset ages from patients in the QPP. EOPD: Early Onset Parkinson's disease.

Table 2. Patients in the QPP with a known monogenic causes of Parkinsonism. DBS: Deep Brain Stimulation. [a]Sharma et al. 2012, [b]Huang et al. 2007, [c]Mellick et al. 2009, [d]Ross et al. 2011. [e] Information from self-reported survey.

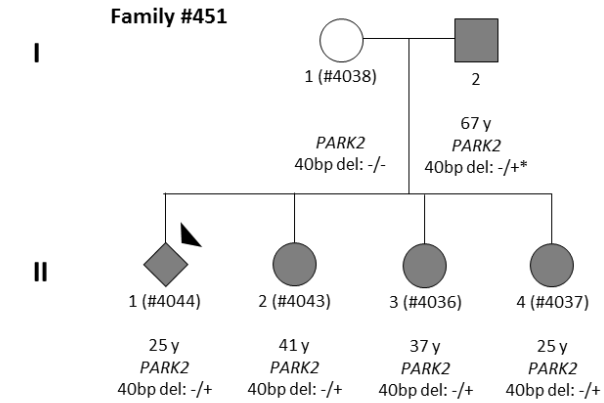
Table 3. Displays details of the probands that have undergone WES. [f]Amino Acid changing variants with minor allele frequency <=0.0001 gnomAD exome (All). [g] Sequenced using Nextera chemistry & MiSeq platform. [h] Patient has been described previously as 'patient 11280' in Mellick et al. 2009 and Puschmann et al. 2017. [I] This member was inaccurately reported by the proband and is not a blood relative. MAF: minor allele frequency from gnomAD exome dataset [13]. NA: Not Available. Family histories are validated by cross-comparison with other family member's questionnaire answers when possible.

Figure 1. Segregation of variants implicated in PD within multi-incident families. Black arrow indicates index patient. Shaded shape indicates PD diagnosis, half shaded shape indicates reports of possible PD symptoms. Lower right quarter shaded shapes indicate atypical PD. Upper right quarter shade indicate tremor disorder. Upper left shade indicates Multiple Systems Atrophy. Middle square shade indicates Dystonia. Subtext describes: ID (#QPP ID), Current age or age at death, Age at Onset, Mutation status. *indicates inferred genotype. Information has been omitted to protect the privacy of the 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

Family ID#	Patient ID#	Age at onset	Unaffected Siblings	Description of Affected Family Members	PD Mutations	First symptom[e]	Tremor[e]	Falls[e]	Postural hypotension[e]	Anxiety disorder[e]	Depression[e]	Memory Loss[e]	Hallucinations[e]	Sleep disturbances[e]	Ever taken L-dopa?[e]	Reason taken off L-dopa[e]
Part of multi-incident family																
261	# 0083	46	0	Proband, Father, Sister, Aunt, Paternal Male Cousin, Paternal Grandmother	VPS35 p.D620N (Het)[a]	N/A	No	No	N/A	No	Yes	No	Yes	No	Yes	N/A
476	# 3725	54	3	Proband, Mother, Paternal Uncle	LRRK2 p.G2019S (Het)	Bradykinesia	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Not Stated
073	# 0069	46	1	Proband, Father, Mother	LRRK2 p.G2019S (Het)[b]	Gait Disturbance	Yes	No	Yes	Yes	No	No	No	Yes	Yes	N/A
N/A	# 1170	53	4	Proband, 2x Paternal Aunts	LRRK2 p.G2019S (Het)[b]	Fatigue, Inability To Write	No	No	No	No	No	No	No	Yes	No	N/A
445	# 2610	59	1	Proband, Mother, Maternal Grandfather	VPS35p.D620N (Het)[a]	Muscular Skeletal Dysfunction	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	N/A
Not part of multi-incident family																
125	# 0135	51	4	Proband, Brother	SNCA duplication (Het)	Speech Defect, Muscular Skeletal Dysfunction	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	N/A
N/A	# 0138	49	N/A	Proband, Aunt	LRRK2 p.G2019S (Het)[c]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
N/A	# 0249	61	N/A	Proband, Maternal Aunt	LRRK2 p.G2019S (Het)[b]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
148	# 0555	53	3	Proband, Brother	LRRK2 p.G2019S (Het)[d]	Tremor	Yes	No	Yes	Yes	No	No	No	Yes	Yes	N/A
436	# 2148	65	5	Proband, Brother	LRRK2 p.G2019S (Het)[d]	Tremor	Yes	No	No	Yes	No	No	Yes	No	No	N/A
N/A	# 2572	56	1	Proband, Maternal Uncle	LRRK2 p.G2019S (Het)[d]	Toe Pain And Anxiety	Yes	Yes	Yes	Yes	No	No	No	No	Yes	DBS
435	# 2802	58	1	Proband, Brother	LRRK2 p.G2019S (Het)[d]	Muscular Skeletal Dysfunction	Yes	No	No	Yes	No	No	No	Yes	Yes	N/A
N/A	# 3027	39	3	Proband, Father	SNCA duplication (Het)	Tremor	Yes	No	No	Yes	No	No	No	Yes	Yes	DBS
Without family history																
434	# 0763	69	3	Proband	LRRK2 p.G2019S (Het)[d]	Tremor And Gait Disturbance	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	N/A
N/A	# 2024	29	1	Proband	PARK2 p.G430D (Hom)[c]	Tremor And Muscular Skeletal Dysfunction	Yes	No	No	Yes	Yes	No	Yes	No	N/A	N/A
N/A	# 2641	63	13	Proband	LRRK2 p.G2019S (Het)[d]	Gait Disturbance	No	No	No	No	No	No	No	No	Yes	N/A
N/A	# 2682	46	4	Proband	PARK2 ex4 del (Hom)	Gait Disturbance	No	No	Yes	No	No	No	No	Yes	Yes	N/A

Family ID#	Patient ID#	Age at onset	Unaffected Siblings	Description of Affected Family Members	Missense & Rare variants[f]	Variants implicated in PD	Rare variants in PD genes with unknown pathogenicity	Mutation segregates with disease in family	Sample extracted date	Exome sequencing date
002	# 1690	66	7	Proband, 2x Sister, Daughter, Mother, 2x Maternal Males Cousins, Maternal Grandmother, Possible Daughter	500[g]				Oct, 2008	May, 2015
006	# 0078	47	5	Proband, Mother, Brother, Maternal Grandfather	629				Mar, 2005	May, 2015
019	# 1743[h]	26	7	Proband, Paternal Uncle, Paternal Grandmother, Paternal Cousin, Maternal Great Uncle	591	PINK1 p.G411S (Het)		No	Nov, 2011	Oct, 2015
052	# 3888	54	0	Proband, Father, Brother, Paternal Male Cousin[i]	587				Apr, 2011	Oct, 2015
080	# 0120	55	7	Proband, Brother, Father, Paternal Female Cousin, Paternal Grandmother, Maternal Aunt, Maternal Uncle, Maternal Cousin	380[g]				Feb, 2005	May, 2015
332	# 1804	41	5	Proband, Mother, Father, Paternal Aunt, Paternal Male Cousin, Maternal Male Cousin	638				Sep, 2007	Oct, 2015
431	# 3953	66	1	Proband, 2x Paternal Male Cousins, Paternal Uncle, Paternal Aunt, Paternal Grandfather	886				Nov, 2011	Dec, 2014
433	# 3749	60	5	Proband, Sister, Maternal Female Cousin	809				Apr, 2010	Dec, 2014
447	# 4012	67	1	Proband, Sister, Paternal Aunt, Paternal Male Cousin, Paternal Grandfather	656				Aug, 2014	Dec, 2014
451	# 4044	25	0	Proband, Father, 3x Sisters	630	PARK2 40bp del ex3 (Het)		Yes	Aug, 2014	Dec, 2014
456	# 4078	55	0	Proband, 2x Maternal Uncle, Maternal Grandfather, Maternal Great Uncle, Possible Sister, Maternal Cousin diagnosed with Dystonia, Mother diagnosed with Multiple Systems Atrophy	417[g]	PARK2 p.Q34fs (Het)		No	Feb, 2013	Nov, 2016
460	# 4024	70	5	Proband, Paternal Male Cousin, Paternal Female Cousin, Maternal Uncle, 2x Maternal Aunt, Brother presents with Undiagnosed Tremor	1041				Sep, 2012	Aug, 2014
468	# 1451	31	2	Proband, Paternal Uncle, Paternal Aunt, Paternal Great Uncle, Paternal Second Cousin, Paternal Cousin (once removed), Possible Paternal Aunt, Possible Paternal Grandfather	372[g]		LRRK2 p.H2236R (Het); MAF=4.06E-06	No	Aug, 2014	May, 2015
479	# 4175	49	3	Proband, Father, Paternal Uncle, Paternal Grandmother, Possible Sister, Sister diagnosed with Corticobasal Syndrome, Mother diagnosed with Dystonia	577				Mar, 2014	Oct, 2015
484	# 4220	48	3	Proband, 2x Paternal Uncles, Paternal Female Cousin	448[g]				Jul, 2014	Nov, 2016
488	# 0533	49	3	Proband, Paternal Uncle, 2x Paternal Aunts	594				Sep, 2014	Oct, 2015
489	# 4095	45	6	Proband, 2x Brothers, Maternal: 2x Uncles, 3x Aunts, 3x Female Cousins, 2x Male Cousins	644	PARK2 p.R275W (Het)		NA	Apr, 2013	Oct, 2015
490	# 3714	60	0	Proband, Brother, Sister, Paternal Male Cousin, Maternal Aunt	599				Sep, 2014	Oct, 2015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2
PARK2
40bp del: -/-
67 y
PARK2
40bp del: -/+*
II
1 (#4044)
2 (#4043)
3 (#4036)
4 (#4037)
25 y
PARK2
40bp del: -/+
41 y
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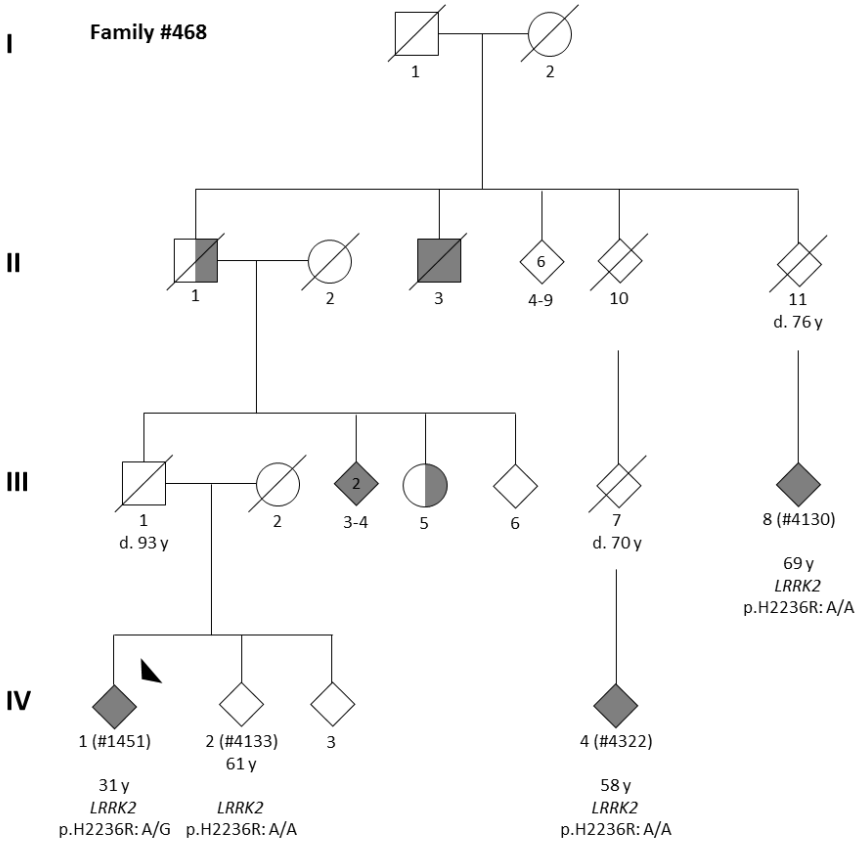
25 y
PARK2
40bp del: -/+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1
2
II
1
2
3
6
4-9
10
11
d. 76 y
III
1
d. 93 y
2
2
3-4
5
6
7
d. 70 y
8 (#4130)
69 y
LRRK2
p.H2236R: A/A
IV
1 (#1451)
31 y
LRRK2
p.H2236R: A/G
2 (#4133)
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4 (#4322)
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LRRK2
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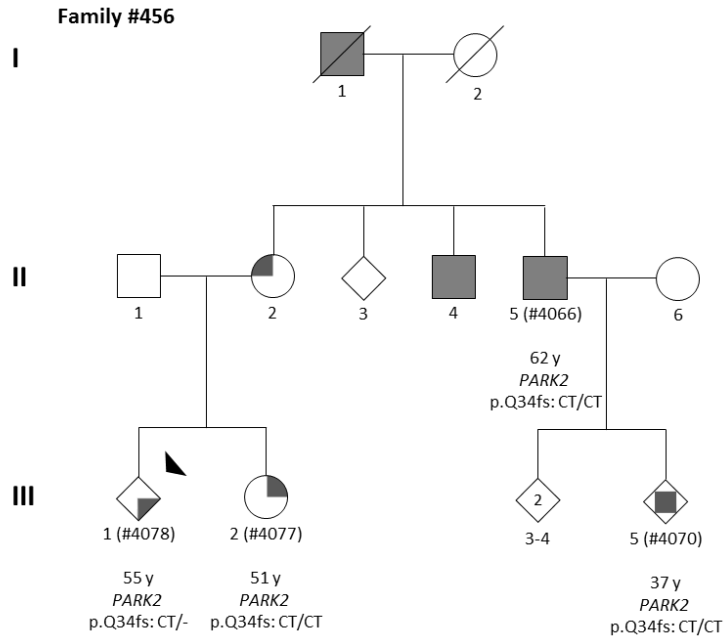
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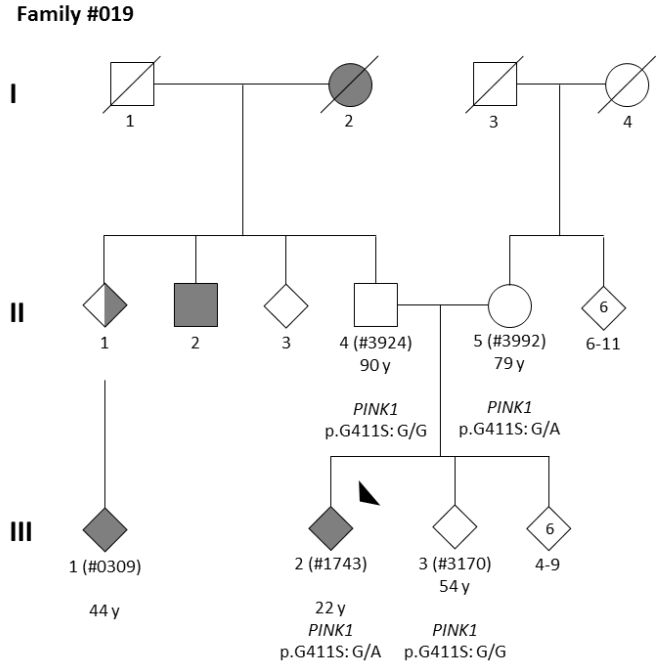
2
II
1
2
3
4
5 (#4066)
6
62 y
PARK2
p.Q34fs: CT/CT
III
1 (#4078)
55 y
PARK2
p.Q34fs: CT/-
2 (#4077)
51 y
PARK2
p.Q34fs: CT/CT
2
3-4
5 (#4070)
37 y
PARK2
p.Q34fs: CT/CT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2
3
4
II
1
2
3
4 (#3924)
90 y
PINK1
p.G411S: G/G
5 (#3992)
79 y
PINK1
p.G411S: G/A
6
6-11
III
1 (#0309)
44 y
2 (#1743)
22 y
PINK1
p.G411S: G/A
3 (#3170)
54 y
PINK1
p.G411S: G/G
6
4-9

- Reporting the demographics of multiplex families found in the QPP
- Summarising previous findings for monogenic parkinsonism found in the QPP
- Report preliminary findings of whole exome sequencing of 18 probands from multiplex families