Screening PARK Genes for Mutations in Early Onset Parkinson's Disease Patients from Queensland, Australia

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Abstract

A family history of Parkinson's disease (PD) is the most commonly reported risk factor after age, suggesting a genetic component to the disease in a sub-group of patients. Mutations in at least six genes have been identified that can lead to monogenic forms of PD. We screened a sample of 74 early onset PD cases out of a cohort of 950 patients (onset <50 years) for genetic abnormalities in known familial Parkinsonism genes. A self-reported family history of PD existed for 30 patients (40.5%).

The entire coding region of the *parkin* (MIM 602544), *DJ-1* (MIM 602533) and *PINK1* (MIM 698309) genes, and exon 41 of the *LRRK2* gene (MIM 609007) were screened by direct sequencing. All exons of *parkin* were examined for gene dosage abnormalities.

Screening identified six patients with putative genetic disease: three patients carried PARKIN mutations (p.G12R heterozygous, p.G430D homozygous and a heterozygous deletion of exons 2-4), one patient carried a p.G411S heterozygous amino acid change in the *PINK1* gene and two individuals were heterozygous for the common p.G2019S mutation in *LRRK2*. No alpha-synuclein or *DJ-1* variants were observed.

Our data suggest that approximately 7% of early onset PD cases seen in Queensland movement disorders clinics have mutations involving known PARK genes.

Key words: Parkinson's disease, PARK genes, mutations

Introduction

Parkinson's disease (PD) is a neurodegenerative condition with a typical onset of the in the seventh decade, however about 4% of PD patients present with early onset before the age of 50 [1]. It is a complex, multifactorial disorder, comprising genetic and environmental components. The majority of cases appear to be sporadic or idiopathic, however in the recent past a number of mutations in at least six genes (PARK1, 2, 5, 6, 7, and 8) have been identified as being causative in the familial form of the condition, accounting for a small number of all PD cases. Mutations in these genes may lead to the disease phenotype and are often characterized by an earlier onset (under the age of 50 years) with or without Lewy body pathology. It is to be expected that more mutations causative for the disease in 'idiopathic' PD will be identified in the future, adding to the number of distinct genetic forms of PD. The aetiology of the sporadic form of PD is still unclear but identification of molecular mechanisms and gene products underlying the disease in its monogenic form have shed some light on possible pathways involved in the non-hereditary form of the condition.

As an early disease onset is frequent in familial PD, we undertook in this study to estimate the prevalence of known genetic forms of Parkinsonism in a typical Australian population (Queensland) by screening a subset of early onset cases, derived from a large movement disorders clinic in Brisbane, Australia.

Methods

Sampling Frame

Patients were derived from a case series of 950 patients with a diagnosis of PD seen in one specialist movement disorders practice in Brisbane, between 2000 and 2005. Informed consent was obtained from all participating patients.

Patient Selection

Patients were included in the study if they: (1) received a diagnosis of probable PD according to stringent clinical and neurological criteria; (2) exhibited onset of symptoms ≤ 50 years; and (3) had been seen at clinic between 2001 and 2005.

Patient Ethnicity

Patients in this sample were in the majority (95%) of European extraction. Two patients reported Australian Aboriginal ancestry, one case was of New Zealand Maori extraction and one patient reported Asian ancestry.

Screening Methodology

DNA was extracted from peripheral blood according to standard methods for use in gene-dosage studies. A whole genome amplification of the original DNA was performed prior to sequencing studies. The entire coding region of the SNCA (MIM 163890), Parkin (MIM 602544), DJ-1 (MIM 602533) and PINK1 (MIM 698309) genes, and exon 41 of the LRRK2 gene (MIM 609007) were screened by direct sequencing using standard methods. All exons of Parkin were examined for gene dosage abnormalities using TaqMan based methods. Details have been given elsewhere [2, 3]. Primers and TaqMan probes used in the quantitative PCR amplification analysis are listed in table I. UCHL1 (PARK5) was excluded from the study for reason of its extreme rareness; the importance of the gene in PD is still unclear.

Results

Seventy-four patients met the inclusion criteria. Demographic data are shown in Table II. In this sample, males were slightly over-represented ($n = 44$, 59.5%). A self-reported family history of PD existed for 30 patients (40.5%). The average age of onset was 42.4 ± 5.7 years.

Screening identified mutations in five patients with putative genetic disease (Table III and Fig. 1). Two patients carried *parkin* mutations (c.34G>C and c.1289G>A, the first leading to a heterozygous p.G12R amino acid change in exon 2, and the second to a homozygous p.G430D amino acid change in exon 12).

One patient possessed a heterozygous p.G411S phenotype resulting from a c.1231G>A mutation in exon 6 of the PINK1 gene.

Two individuals were found to be heterozygous for the common p.G2019S mutation in LRRK2.

No alpha-synuclein or DJ-1 variants were observed. The results are summarized in Table IV.

The previously reported *parkin* (S167N) and PINK1 (Q115L) polymorphisms were also identified (data not shown).

Discussion

The importance of genetic factors for the aetiology of PD has been debated controversially for a long time. Longitudinal twin studies argued for a genetic element that contributed to the condition whereas other cross-sectional studies could find no evidence for inheritance [4, 5]. In the mean-time, at least five genes have been identified that are implicated in the development of a monogenic form of PD (PARK1, 2, 6, 7, and 8). These monogenic forms of the condition may mimic clinically idiopathic PD but generally (though not exclusively) appear at an earlier age of onset. In this study we therefore screened a cohort of 74 PD patients with age of onset earlier than 50 years, taken from a case series of 950 patients, for monogenic disease.

The first gene implicated in the development of PD was SNCA, coding for the alpha-synuclein protein (PARK1). Three point mutations and gene multiplications leading to familial Parkinsonism have been reported, although these mutations are considered rare and are estimated to contribute < 1% of monogenic cases of PD. In our cohort no mutations in this gene were detected, in agreement with statistical expectations with respect to the sample size.

Mutations in the *parkin* gene (PARK2) were first found to be causative for autosomal recessive juvenile parkinsonism in Japanese families [6]. The frequency of PARK2 mutations has been estimated to be as high as 40-50% in early onset disease [7-9] and 10-20% in idiopathic cases [7, 9-11]. In our study two patients (2.7% of all subjects screened) possessed *parkin* mutations (Table III). Notably, no confirmed exon-dosage abnormalities were observed. This number is lower than that reported in several comparable studies, which report *parkin* mutations to be present in between 10.4 and 18.0% of early onset cases [9, 10, 12, 13]. Our data is comparable to the reported 3.8% frequency of *parkin* mutations in patients screened in a cohort of 313 North American PD cases [14]. The ability to detect *parkin* mutations depends on factors such as sample size, ethnic extraction, inclusion criteria

for cases and the methods used for mutation detection. Of the two identified *parkin* mutations reported in the current study, one has been reported previously (p.G430D) [12]. To the best of our knowledge, there have been no previous reports of the p.G12R variant. The functional significance of these sequence variants remains to be established. *Parkin* mutations are generally presumed to be recessive, so the possibility that additional non-coding region sequence variants or additional factors contributing to the disease outcome in these individuals cannot be ruled out. There is a growing body of evidence that heterozygous *parkin* mutations can be pathogenic and may be causative for disease [7, 9, 15]. In a recent report from Denmark, 10 out of 87 patients screened possessed putative disease-causing *parkin* mutations; eight of these mutations were heterozygous in nature [13]. It has also been proposed that a heterozygous genotype may lead to a comparatively later age of onset. Our data are not necessarily consistent with this argument. Our mutation carriers developed symptoms well before age 45 at 40 and 30 years of age, respectively (Table IV).

PARK6 (PINK1) mutations have been reported in 3-15% of early onset recessive parkinsonism cases while 5% of sporadic cases reportedly carry a single heterozygous PINK1 mutation [16-18]. An estimated contribution of < 1% for PINK1 mutations to familial PD is probably more realistic [19, 20]. Statistically, the one PINK1 mutation carrier identified in our study accounts for 1.4% of the cohort. This mutation leading to a G411S amino acid substitution has been previously described in at least five PD patients [21-23]. Whether this particular mutation in its heterozygous form is causative for the disease phenotype is a matter of speculation. However, given that the amino acid change occurs within the kinase domain, in a sequence highly conserved in vertebrates, and that it has not been observed in normal subjects despite considerable investigation [21, 22] it seems reasonable to assume that the mutation at least contributes significantly to the development of parkinsonism.

No PARK7 (DJ-1) mutations were detected in our study, consistent with previous studies that suggest that <2% of early onset cases of PD carry coding region mutations in PARK7 [24].

Two of our patients carried the common LRRK2 p.G2019S mutation in exon 41 (2.7%, Table III). Funayama and colleagues linked the PARK8 locus to chromosome 12 in 2002 [25]. It has subsequently been found that LRRK2 mutations constitute the most frequent form of monogenic PD. The p.G2019S mutation occurs in more than 2% of North American and English patients [26, 27], and is found in >10% of North African, Ashkenazi Jewish and Portuguese populations [28-30]. The mutation falls within an activation segment of the MAPKKK domain, changing a highly conserved glycine at the start of the activation loop. Alternatively, it has been proposed that a reduction in kinase enzyme activity may be caused through changes in the magnesium-binding loop or by introduction of new phosphorylation sites. A recent Australian study, that did not include any of subjects investigated in the current report, identified eight of 830 PD patients (1%) with this mutation.

Conclusions: Our data suggest that approximately 7% of early onset PD cases seen in Queensland movement disorders clinics have mutations involving known PARK genes. However, whether these mutations were disease-causing in all patients must remain open. The number of mutations found will increase as additional causal genes are identified from current gene-hunting strategies.

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Figure 1

Table I: Primers and TaqMan probes used in the PARK2 analysis

PRIMER NAME	FORWARD (5' - 3')	REVERSE (5' - 3')	PROBE
PARKIN Ex1(MGB)	CGCAGCCGCCACCTA	GGCGCAGAGAGGCTGTAC	FAM-CCCAGTGACCATGATAGGTA-MGB
PARKIN Ex2	CACAGTCCAGTCATTCCTCAGC	GTTCAACTCCAGCCATGGTTTC	FAM-CCTTCCCTGCGAAAATCACACCGCAT -TAMRA
PARKIN Ex3	GAGGACTGAGCTGCTGAGG	AGAGCATTGTTACATTGTGC	FAM-TCGTCGCCTCCAGTTGCATTCAT -TAMRA
PARKIN Ex4	TCTTCTCCAGCAGGTAGATCAATC	TGCTGACACTGCATTTCTTAC	FAM-ATGTGTATTGCAAAGGCCCTGTCAAT -TAMRA
PARKIN Ex5(MGB)	CCCAAAGGGTCCATCTTGCT	ACTAGTCCCAGGGCAGTGT	FAM-ACCACTCATCCGGTTTGG -MBG
PARKIN Ex6	TAGAGGAAAAATGAGCAGCCG	CGTAATGCAAGTGATGTTCCGA	FAM-AGCACACCCCACCTCTGACAAGGAAAT -TAMRA
PARKIN Ex7	TGCCGATCATTGAGTCTTGTC	CCAGTTGCCTTTCCACACTGA	FAM-AGTGGAACAGTCTAAGCAAATCACGTGGCT -TAMRA
PARKIN Ex8	ATTCTTCTTTCCAACAGCTGGC	ATGACAGTCTGATGCAGCCTTT	FAM- CCCAACTCCTTGATTAAAGAGCTCCATCACT-TAMRA
PARKIN Ex9	TTTTGCAGTACAACCGGTACCA	AGCAAACAAGGACAGGAACACA	FAM-AGTATGGTGCAGAGGAGTGTGTCCTGCAT -TAMRA
PARKIN Ex10	CCAAATGCAACCTAATGTCCC	TGGAGGAATGAGTAGGGCATT	FAM-AGTGCAGTGCCGTATTTGAAGCCTCAT -TAMRA
PARKIN Ex11	AGCTGAGATTAAACGCCTTTCC	TTTTTCCACTGGTACATGGCAG	FAM- CTTTGTTCCTCCAGGCCTACAGAGTCGGAT-TAMRA
PARKIN Ex12	GTTTTCCAGGTAAGTGTGCG	AAGGTAGACACTGGGTATGCTCC	FAM-ACCACACCTTTGTTTTCTGCCCCCT-TAMRA

Table II. Patient demographic data

Total number of patients n (%)	74 (100.0)
Male n, (%)	44 (59.5)
Female n, (%)	30 (40.5)
Age at onset (years)	
All*	42.4 ± 5.7 (26 – 50)
Male*	42.4 ± 6.3 (26 – 50)
Female*	42.5 ± 4.9 (35 – 49)
Age at examination (years)	
All*	58.0 ± 8.1 (40 - 78)
Male*	58.9 ± 8.6 (40 – 77)
Female*	56.5 ± 7.2 (43 – 78)

*data given as mean ± SD (range)

Table III. Detected mutations

Gene and ID	Mutations	Family history of PD	Age at onset
<i>PARK1 (alpha-synuclein)</i>	None detected.		
<i>PARK2 (parkin)</i>			
10782	p.G12R (c.34G>C: exon 2) heterozygous.	negative	40
12238	p.G430D (c.1289G>A: exon 12) homozygous.	negative	30
<i>PARK6 (PINK)</i>			
11280	p.G411S (c.344A>T: exon 6) heterozygous.	Positive: Uncle, grandmother, cousin	26
<i>PARK7 (DJ1)</i>	None detected.		
<i>PARK8 (LRRK2)</i>			
10002	p.G2019S (c. 6055G > A: exon 41) heterozygous.	Positive: Aunt	49
12248	p.G2019S (c. 6055G > A: exon 41) heterozygous.	Positive: Father, Mother	46

Table IV. Clinical characteristics of mutation carriers

10782

Female, aged 48 years at consultation. Initial symptoms: rigidity, loss of dexterity and dystonia of right foot. Good response to levodopa. No family history of PD

12238

Female, aged 35 years at consultation. Initial symptoms: right hand tremor and dystonia of right foot. No family history of PD. Good response to 100 mg levodopa b.i.d. suffers from depression, requiring treatment.

11280

Male, aged 39 years at consultation. Initial symptoms were speech problems and loss of dexterity at age 26 years. Family history of Parkinson's disease: paternal grandmother, uncle and cousin.

10002

Female, aged 58 years at consultation. Initial symptoms: unilateral tremor and gait disturbance at age 49 years. Family history: aunt. Currently well responding to treatment with 100 mg levodopa t.d.s.

12248

Male, aged 58 years at consultation. Initial symptoms: cramping, "turning in" of right leg and gait disturbances. Family history: father and mother (father subsequently found to carry the LRRK2 G2019S mutation). Currently well responding to treatment with 200/50 levodopa and 5 mg benzhexol hydrochloride t.d.s.