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Research paper

Exonic rearrangements in the known Parkinson's disease-causing genes are a rare cause of the disease in South African patients

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HIGHLIGHTS

- The frequency of exonic rearrangements in 210 South African PD patients was assessed.
- 20.9% of the cohort had early onset PD (≤50 years), and 26.2% had a positive family history.
- A single heterozygous exon 4 deletion in *PARK2* was found in one individual with an age at onset of 51 years.
- Combined with findings from previous PD studies in the South African population, CNV has been found in only 1.8% (8/439).
- This suggests that in this population CNV in undiscovered PD genes are plausible.

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the loss of dopaminergic neurons in the substantia nigra of the midbrain. To date, a number of PD-causing genes have been found, including *SNCA, LRRK2, VPS35, PARK2, PINK1, DJ-1, ATP13A2*, and most recently *CHCHD2*. Mutations in these genes range from point mutations to larger exonic rearrangements including deletions and duplications. This study aimed to detect possible copy number variation (CNV) in the known PD-causing genes in a cohort of South African patients with PD. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed on a total of 210 South African PD patients, and possible CNVs were verified using quantitative real time PCR. No homozygous or compound heterozygous exon rearrangements in the genes analysed were found in the patient group. A heterozygous *PARK2* exon 4 deletion was found in a sporadic patient with an age at onset of 51 years. Sanger sequencing did not reveal any additional mutations in *PARK2* in this patient. Combining our results with that of previous studies in a South African cohort, the frequency of exonic rearrangements in the known PD-causing genes is only 1.8% (8/439 patients). In conclusion, CNV in the known PD-causing genes are a rare cause of PD in a South African cohort, and there may be as yet unknown genetic causes of PD that are specific to patients of African ethnicity.

1. Introduction

Parkinson's disease (PD) is a relatively common neurodegenerative disorder, second in prevalence only to Alzheimer's disease. PD presents with both motor and non-motor symptoms that are the result of degradation of the dopamine-producing neurons in the substantia nigra pars compacta. The age at onset (AAO) in PD patients varies over a wide range, and can be defined as either early onset (EOPD, ≤50 years) or late onset (LOPD > 50 years). The past eighteen years have been successful in proving the role of genetic factors in the cause of this condition and the following genes have been implicated in PD pathogenesis to date: *SNCA*, *LRRK2*, *PARK2*, *PINK1*, *DJ-1*, *VPS35*, *ATP13A2* and more recently *CHCHD2* [1,2].

In addition to missense mutations and indels, copy number variation (CNV), or exonic rearrangements, has been implicated in PD pathogenesis. CNV described any structural variation, including smaller events less than 1 kb in size, such as insertions, deletions, multiplications (duplications/triplications), inversions and translocations [3]. The discovery and genotyping of structural vari-

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Table 1
Clinical and demographic characteristics of 210 South African Parkinson's disease patients recruited for this study.

	Total N = 210
AAO, mean ± SD, (range)	59.4 ± 12.3 years (20–82 years)
AAO ≤ 50 years	44 (20.9%)
Family history of PD	55 (26.2%)
Ethnicity	
White	131 (62.4%)
Mixed Ancestry	57 (27.1%)
Black	21 (10.0%)
Indian	1 (0.5%)

AAO, age at onset of the disorder.

ation has been central to understanding disease associations, and altered expression levels of CNV genes may be responsible for observed phenotypic variability, disease susceptibility and complex behavioural traits [4]. Previous studies in African PD patients have shown that exonic rearrangements in the known PD-causing genes are rare [5–7]. In a South African context, one family to date has presented with a *SNCA* whole gene triplication, and a total of 7 out of 229 patients screened have been found to have either compound heterozygous or homozygous *PARK2* exonic rearrangements [6]. The present study used Multiplex Ligation-dependent Probe Amplification (MLPA) and real time PCR techniques to further assess the frequency of exonic rearrangements in an additional 210 South African PD patients.

2. Materials & methods

2.1. Patient selection

The present study was approved by the Health Research Ethics Committee, Stellenbosch University. A total of 210 South African PD patients were recruited from the Parkinson's Association of South Africa and the Movement Disorders clinic at Tygerberg Hospital. The patients had been examined by a movement disorder specialist and met the UK Parkinson's Disease Society Brain Bank Research criteria for diagnosis of PD [8]. Patients were from different ethnic backgrounds including White (62.4%), Mixed Ancestry (27.1%), Black (10.0%) and Indian (0.5%). Furthermore, 20.9% of the cohort had early onset PD, and 26.2% had a positive family history (Table 1). For each study participant, a blood sample was obtained and genomic DNA (gDNA) isolated using established methods.

2.2. MLPA assay and verification using quantitative real time PCR

The MLPA assay is a well-established technique to determine exonic rearrangements in various genes responsible for disease. For this study, two commercially available kits for PD were used—namely P051-C2 and P052-C1 (MRC Holland, The Netherlands). Each kit contains probes for exons of the genes of interest, including all exons of *SNCA*, *PINK1* and *PARK2* and selected exons of *LRRK2* (exons 1, 2, 8, 10, 15, 27, 49) and *DJ-1* (exons 1, 3, 5, 7), as well as two point mutations (A30 P in *SNCA* and G2019S in *LRRK2*). The exons for *PARK2* were included in both kits, allowing for verification of any rearrangements found. MLPA was performed on all 210 samples, based on a previously described protocol [5]. The raw data was then analysed using the Coffalyser. Net software, version. 131211 (http://coffalyser.software.informer.com/download/). A ratio comparing the relative peak height of each exon was generated by Coffalyser. Ratios between 0.4 and 0.6 indicated a deletion, between 0.7 and 1.3 was considered normal without exon rearrangements, and larger than 1.4 was a duplication.

Rearrangements detected from the MLPA analysis were verified by quantitative real time PCR (qRTPCR) on the Lightcycler 96 (Roche Diagnostics, Germany) with the SYBR Green Mastermix (Roche Diagnostics, Germany). Each sample was run in triplicate on the plate, and Cq values differing by more than 1 cycle were disregarded. The reaction comprised 60 ng gDNA, 10 pmol of forward and reverse primers, and 1x Mastermix in a total volume of 20 μl. The following cycling conditions were used: (i) Preincubation at 95 °C for 600 s, (ii) Three step amplification for 45 cycles including 95 °C for 10 s, 60 °C for 10s followed by a touchdown to 55 °C after the second cycle, and 72 °C for 10 s, (iii) Melting period of 95 °C for 10 s, 65 °C for 60 s, 97 °C for 1 s and (iv) Cooling period of 37 °C for 30 s. Results were then analysed on the Lightcycler 96 software version 1.1 (www.roche.com), using relative quantification. Selected samples were subjected to Sanger sequencing using the BigDye Terminator Sequence Ready Reaction kit version 3.1 (Applied Biosystems, USA). This was performed at the Central Analytical Facility at Stellenbosch University.

3. Results

No homozygous or compound heterozygous exon rearrangements in the genes analysed were found in the patient group. A heterozygous *PARK2* exon 4 deletion was found in one male patient, and this was verified in both MLPA kits and using qRTPCR with positive controls for homozygous and heterozygous *PARK2* exon 4 deletions (Fig. 1). Sanger sequencing of all 12 exons of the *PARK2* gene revealed no other pathogenic mutations in this patient. The AAO in this patient was 51 years with no family history of PD. He presented with atypical PD with a slow progression. He has minimal non-motor symptoms excepting for sleep disturbance, and had no psychosis or dementia. His current medication is Ropinerole 4 mg tds and Carbilev 25/250 five times a day.

The M192L polymorphism in *PARK2* exon 5 was found in numerous (6.2%; 13/210) individuals. This polymorphism is present at the annealing site of the MLPA P051 probe for *PARK2* exon 5 (providing a false positive result of a deletion of *PARK2* exon 5), and was confirmed by Sanger sequencing. Interestingly, this variant was only observed in black and mixed ancestry patients, and has been previously reported in other studies on African patients [5,7].

4. Discussion

This study has shown that CNV mutations in the known PD-causing genes are a rare cause of PD in South African patients. Combining our results with that of previous studies in a South African cohort, the frequency of exonic rearrangements in the known PD-causing genes in a total of 439 (229 + 210) PD patients is only 1.8% (8/439 patients) [5,6]. The overall frequency of heterozygous CNV is also 1.8% (8 patients; seven from previous studies and one from present study). In specifically EOPD cases, the CNV mutation rate is 4.8% (7/146 patients). Heterozygous mutations in *PARK2* have played a controversial role in recessive genes, and it is still unknown whether they are pathogenic in themselves [9]. Kay and colleagues screened over 2000 patients with PD, as well as age-matched controls and observed that a total of 0.95% of control subjects carried a heterozygous CNV in *PARK2*, compared to 0.86% of patients [10]. This indicates that there is no compelling evidence for association of heterozygous *PARK2* mutations. Conversely, it has been reported in several studies that individuals who are heterozygous carriers of mutations in *PARK2* can also present with PD [11–13]. It is thought that heterozygous mutations are not sufficient to cause PD, but are rather associated with a higher risk of developing the disease and may play a role in a threshold effect for parkinsonism [14].

Mutations in *PARK2* are responsible for 10–20% of EOPD cases worldwide [9], and have been found to be far more frequent in other