

## Short communication

## Novel GCH1 variant in Dopa-responsive dystonia and Parkinson's disease



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## ABSTRACT

**Background:** GTP cyclohydrolase I (*GCH1*) mutations are the commonest cause of Dopa-responsive dystonia (DRD). Clinical phenotypes can be broad, even within a single family.

**Methods:** We present clinical, genetic and functional imaging data on a British kindred in which affected subjects display phenotypes ranging from DRD to Parkinson's disease (PD). Twelve family members were studied. Clinical examination, dopamine transporter (DAT) imaging, and molecular genetic analysis of *GCH1* and the commonest known familial PD-related genes were performed.

**Results:** We have identified a novel missense variant, c.5A > G, p.(Glu2Gly), within the *GCH1* gene in affected family members displaying a range of phenotypes.

Two affected subjects carrying this variant had abnormal DAT imaging. These two with abnormal DAT imaging had a PD phenotype, while the remaining three subjects with the novel *GCH1* variant had normal DAT imaging and a DRD phenotype.

**Conclusions:** We propose that this *GCH1* variant is pathogenic in this family and these findings suggest that similar mechanisms involving abnormal GTP cyclohydrolase I may underlie both PD and DRD. *GCH1* genetic testing should be considered in patients with PD and a family history of DRD.

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## 1. Introduction

Dopa-responsive dystonia (DRD) is an autosomal dominant dystonia, now classified as DYT5, with an estimated incidence of between 0.5 and 1 per million [1]. DRD typically manifests as lower limb dystonia in childhood, although the spectrum of symptoms can be broad, even within the same family. Patients typically have an excellent and sustained response to low dose levodopa. Women are more commonly affected with a lower penetrance of mutations in men [2,3].

Mutations in the GTP cyclohydrolase I gene (*GCH1*) are the most common cause of DRD. *GCH1* is located on chromosome 14 (14q22.1-q22.2) and encodes the 32-kDa guanosine 5'-triphosphate cyclohydrolase 1 (GTPCH1) protein. *GCH1* contains six exons, and more than 200 different mutations have been identified [1,2].

Single positron emission computerized tomography (SPECT) Dopamine Transporter (DAT) imaging is a demonstration of *in vivo* striatal dopamine activity. The DAT ligands for SPECT, including [<sup>123</sup>I]FP-CIT (DaTSCAN) have all shown significantly reduced striatal uptake in PD [4], whilst uptake has usually been normal in DRD [5].

We have studied a family with an inherited movement disorder, with phenotypes ranging from DRD to slowly progressive PD. We report results from clinical, genetic and imaging studies of this kindred.

## 2. Methods

Twelve members of the kindred were studied. A diagnosis of PD was made according to United Kingdom Parkinson's Disease Society (UKPDS) Brain Bank clinical

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diagnostic criteria. The Hoehn and Yahr PD rating scale and a Folstein MMSE (Mini Mental State Examination) were performed on each subject. Olfactory function was assessed by use of the University of Pennsylvania Smell Identification Test (UPSIT-40) (Sensonics, Haddon Heights, NJ) and data compared to normative values. Seven subjects (II:1-II:5, III:1 and III:11) underwent dopamine transporter SPECT scanning (DaTSCAN, Amersham Health) and images reviewed by ER, who was blinded to demographic and clinical data.

Molecular genetic screening of the six affected family members was performed by polymerase chain reaction (PCR) and sequencing of the entire coding sequence, and intron-exon boundaries, of the genes *SNCA*, *Parkin*, *DJ-1*, *PINK1*, *LRRK2* and *GCH1*, using primers and PCR conditions available on request. Multiple ligation-dependent probe amplification (MLPA) was used to detect the presence or absence of copy number variation, using MLPA kits P051 and P052 (MRC Holland), details available on request. Subsequently, six unaffected family members (II:2 and III:4,5,7,9,13) and 150 UK control DNA samples from the PD GEN DNA databank were screened for the novel *GCH1* variant.

All subjects gave informed written consent to take part in the study. The study had appropriate ethical approval from South Birmingham LREC and Sandwell and West Birmingham LREC.

### 3. Results

Twelve individuals in the pedigree (Fig. 1, those annotated with an age) were examined in detail. We noted features consistent with slowly progressive PD in individuals II:1 and II:4 (Supplementary video), with additional levodopa-induced dyskinesias and dementia in subject II:1. Disease onset was at 58 and 50 years in II:1 and II:4 respectively. Subject II:3 had an isolated rest tremor (asymmetrical, right upper limb), with an older age of onset of 75 years, had not progressed over six years and did not meet diagnostic criteria for PD. Subjects II:5, III:1 and III:11 had features consistent with DRD, with median age of disease onset 17 years and median disease duration of 22 years. The remaining members of the family were unaffected. All the studied subjects are Caucasian and clinical details of affected individuals are summarized in Table 1 (more clinical details in Supplementary data).

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.parkreldis.2015.01.004>.

The findings from DaTSCAN images on six affected subjects (II:1, II:3-II:5, III:1 and III:11) and one unaffected subject (II:2) are summarized in Table 1 (Supplementary data). Subjects II:3 and II:4

had a second DaTSCAN performed 18 months after the first scan due to atypical disease progression (ie only very slowly progressing which would be unexpected in idiopathic PD).

No novel or previously documented mutations were identified in the coding sequences of *SNCA*, *Parkin*, *PINK1*, *DJ-1* and *LRRK2*. However, a novel heterozygous substitution was identified in the first exon of *GCH1* affecting the fifth nucleotide of the open reading frame (c.5A > G) (Supplementary data). This change is predicted to replace a glutamic acid residue with glycine (p.(Glu2Gly)). This mutation (GenBank accession NM\_000161.2, NP\_000152.1.) is not listed as a known mutation or polymorphism in standard databases (dbSNP138 (<http://www.ncbi.nlm.nih.gov/SNP/>); 1000 genomes (release October 2013, [http://browser.1000genomes.org.](http://browser.1000genomes.org/)); Exome Variant Server (HLBI-EPS6500, <http://evs.gs.washington.edu/EVS/database> accessed Sept, 2014). The novel *GCH1* variant was identified in five of the affected members of the family, but not in subject II:3 or unaffected subjects II:2 and III:4,5,7,9,13, nor in 300 UK control chromosomes. MLPA did not reveal any copy number variation in *SNCA*, *Parkin*, *PINK1*, *DJ-1* and *LRRK2* or *GCH1*.

### 4. Discussion

We have identified a novel heterozygous missense variant within *GCH1* (c.5A > G) in five family members affected by PD or DRD. Several arguments support the contention that this variant is pathogenic. The variant cosegregates with disease state in the family; it is absent from 300 UK control chromosomes tested here, and from all the large public databases; furthermore, it is almost completely conserved among species (Supplementary Fig. 2), and it is predicted to replace one of the larger hydrophilic amino acids with a small hydrophobic glycine within the N-terminal region of the protein. It is well known that in-silico prediction tools possess limited accuracy [6]. This mutation is predicted as pathogenic by SIFT and SNP&GO, but not by PolyPhen-2 and Mutation Taster. The clinical symptoms and signs observed in subjects II:1 and II:4 met diagnostic criteria for PD. In both cases the disease was more slowly progressive than is usual in idiopathic PD. Subject II:3 had an isolated rest tremor and subjects II:5, III:1 and III:11 were diagnosed

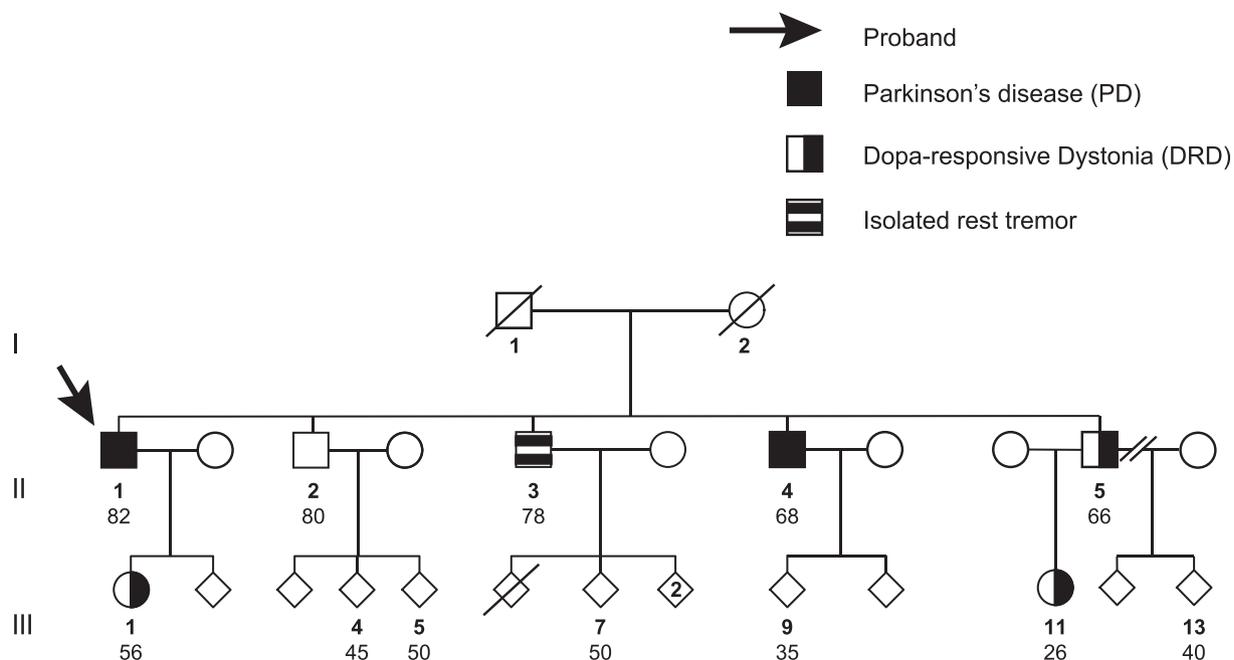


Fig. 1. Pedigree of study family. Numbers refer to the age (in years) at clinical evaluation and blood sampling of the twelve family members studied.

**Table 1**  
Summary of the clinical, functional neuro-imaging and molecular genetic data on subjects from the study family. PD denotes Parkinson's disease; DRD Dopa responsive dystonia; N/A not done or not appropriate; MMSE mini mental state examination score; UPSIT University of Pennsylvania Smell Identification Test; DaTSCAN dopamine transporter imaging; Mod. moderate. Age and sex corrected percentile values for UPSIT scores are presented in brackets.

Subject & gender	Age at eval.	Age at sympt. onset	Clinical diagnosis	L-dopa response	H&Y stage	MMSE	UPSIT score (percentile)	DaT scan	<i>GCH1</i> mut. p.E2G
II:1 M	82	58	PD	Y	3	27	21 (40th)	A	Y
II:2 M	80	N/A	Unaffected	N/A	N/A	27	Severe microsmia 31 (71st)	N	N
II:3 M	78	75	Isolated rest tremor	No Rx	1	30	Mild microsmia 21 (23rd)	1 A 2 N	N
II:4 M	68	50	PD	Y	2	29	28 (31st)	1 A 2 A	Y
II:5 M	66	44	DRD	Y	N/A	28	26 (24th) Mod. microsmia	N	Y
III:1 F	56	17	DRD	Y	N/A	30	35 (38th) Normosomia	N	Y
III:11 F	26	6	DRD	Y	N/A	29	37 (40th) Normosomia	N	Y

with DRD. The symptoms in subjects III:1 and III:11 started at a young age and in the case of subject III:11 were more severe than those seen in subject II:5, illustrating the extreme phenotypic heterogeneity in this kindred and possibly reflecting the increased penetrance of *GCH1* mutations in females.

DaTSCAN data supported the clinical diagnoses in most cases. The clinical phenotypes of DRD and PD and correspondingly normal and abnormal scans were found in subjects carrying the same *GCH1* mutation. DAT imaging is typically normal in DRD. Indeed DAT imaging has been proposed as a diagnostic tool to help differentiate between DRD and early onset PD [5]. There have been case reports of individuals with adult onset dystonia-parkinsonism [7] or PD without any dystonia [8] carrying a *GCH1* mutation and an abnormal DaTSCAN.

Recently, Mencacci and colleagues reported 4 unrelated individuals with adult onset parkinsonism, presumed pathogenic *GCH1* mutations and abnormal DaTSCANS [9].

These cases, and the two in our family (subjects III:1 and III:4), raise the question as to whether PD, in patients with presumed pathogenic *GCH1* mutations, is in fact a rare phenotype of DRD. Certainly the phenotype of DRD is suggested to be broad, as illustrated by a recently reported family with classical DRD, adult onset PD and an MSA-like phenotype associated with a *GCH1* two exon deletion [3]. As recently speculated by Mencacci and colleagues [9], chronic dopamine deficiency resulting from *GCH1* deficiency could directly predispose to nigral cell death (rather than classical lewy body associated neurodegeneration as typically seen in PD). Although earlier studies screening for a *GCH1* mutations in PD cohorts did not identify a significant role for genetic variation in *GCH1*, recent studies have implicated *GCH1* as a risk locus for PD [9,10].

An alternative, but less plausible, explanation in our family is the coincident occurrence of two separate movement disorders: a *GCH1* mutation causing DRD in some individuals an additional mutation (ie an unidentified mutation in an as yet unidentified PD gene) causing PD coincidentally in others. In those with a *GCH1* mutation and PD but no dystonia the lack of DRD phenotype could then be explained by incomplete penetrance of the *GCH1* mutation.

Olfactory dysfunction is found in 70–100% of PD patients. As DRD is a result of dopamine deficiency without neuronal cell death, we would predict that UPSIT-40 data in DRD should be normal. In our study, the mean UPSIT-40 score for affected subjects with the *GCH1* mutation was 29.4, compared to a previous study of olfaction in PD, in which the mean score in 18 subjects with idiopathic PD was 17.1, with a score of 27.6 in 27 age-matched controls [11]. The small sample sizes which result from separating our individuals

with the *GCH1* mutation limit any conclusions but the 2 individuals with PD had a mean score of 24.5 versus the 3 with DRD who had a mean score 32.7.

The DaTSCAN data for subject III:3, who had an isolated rest tremor, is difficult to explain. His first scan was abnormal, but the second scan performed 18 months later was normal. One explanation is that he suffered a vascular embolic event occurring around the time of the first scan. An alternative explanation is that there was a technical problem with one of the scans. There is also a category of subjects with parkinsonism who have normal DaTSCANS, so called SWEDDs (Scans Without Evidence of Dopaminergic Deficit), with 11–15% of subjects with PD being found to have normal nigrostriatal uptake of presynaptic ligands [12]. The novel *GCH1* mutation is not carried by subject III:3 and the most likely explanation for his symptoms is that he has isolated rest tremor due to an unrelated mechanism.

In further studies we will screen for *GCH1* variants in subjects with familial PD, and in subjects with parkinsonism who have normal DAT scans, as well as pursuing further studies into the functional effects of the novel variant described here. We anticipate that future post-mortem histological data on affected individuals, particularly those with the PD phenotype will shed more light on disease mechanisms in this interesting family.

#### Author roles

The study was designed by AJL, with support and advice by DJN, VB, and KEM. AJL and TDL carried out the clinical assessments and the LRRK2 sequencing work. SO, MQ, EJS, and VB carried out the further genetic analyses (sequencing and MLPA). EBR assessed the DaT Scans. AJL and TDL drafted the manuscript. All the co-Authors contributed to revising the manuscript for intellectual content and approved the final version for publication.

#### Conflict of interest

None of the authors has any conflict of interest to disclose.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.parkreldis.2015.01.004>.

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