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Common and rare *GCH1* variants are associated with Parkinson's disease

Uladzislau Rudakou [a,b], Bouchra Ouled Amar Bencheikh [b,c], Jennifer A. Ruskey [b,d], Lynne Krohn [a,b], Sandra B. Laurent [b,d], Dan Spiegelman [b,d], Christopher Liong [e], Stanley Fahn [e], Cheryl Waters [e], Oury Monchi [f,g], Edward A. Fon [b,h], Yves Dauvilliers [i], Roy N. Alcalay [e,j], Nicolas Dupré [k,l], Ziv Gan-Or [a,b,d,*]

[a] *Department of Human Genetics, McGill University, Montréal, Quebec, Canada*
[b] *Montreal Neurological Institute, McGill University, Montréal, Quebec, Canada*
[c] *Centre de Recherche, Centre Hospitalier de l'Universite de Montreal, Montreal, Quebec, Canada*
[d] *Department of Neurology and Neurosurgery, McGill University, Montréal, Quebec, Canada*
[e] *Department of Neurology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA*
[f] *Department of Clinical Neurosciences and Department of Radiology, University of Calgary, Calgary, Alberta, Canada*
[g] *Cumming School of Medicine, Hotchkiss Brain Institute, Calgary, Alberta, Canada*
[h] *McGill Parkinson Program and Neurodegenerative Diseases Group, Montreal Neurological Institute, McGill University, Montréal, Quebec, Canada*
[i] *National Reference Center for Narcolepsy, Sleep Unit, Department of Neurology, Gui-de-Chauliac Hospital, CHU Montpellier, University of Montpellier, Inserm U1061, Montpellier, France*
[j] *Taub Institute for Research on Alzheimer's Disease and the Aging Brain, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA*
[k] *Division of Neurosciences, CHU de Québec, Université Laval, Québec City, Quebec, Canada*
[l] *Department of Medicine, Faculty of Medicine, Université Laval, Québec City, Quebec, Canada*

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ABSTRACT

GCH1 encodes the enzyme guanosine triphospahte (GTP) cyclohydrolase 1, essential for dopamine synthesis in nigrostriatal cells, and rare mutations in *GCH1* may lead to Dopa-responsive dystonia (DRD). While *GCH1* is implicated in genomewide association studies in Parkinson's disease (PD), only a few studies examined the role of rare *GCH1* variants in PD, with conflicting results. In the present study, *GCH1* and its 5′ and 3′ untranslated regions were sequenced in 1113 patients with PD and 1111 controls. To examine the association of rare *GCH1* variants with PD, burden analysis was performed. Three rare *GCH1* variants, which were previously reported to be pathogenic in DRD, were found in five patients with PD and not in controls (sequence Kernel association test, $p = 0.024$). A common haplotype, tagged by rs841, was associated with a reduced risk for PD (OR = 0.71, 95% CI = 0.61–0.83, $p = 1.24 \times 10^{-4}$), and with increased *GCH1* expression in brain regions relevant for PD (www.gtexportal.org). Our results support a role for rare, DRD-related variants, and common *GCH1* variants in the pathogenesis of PD.

1. Introduction

The genetics of Parkinson's disease (PD) is a rapidly evolving field, which may help identifying patients with specific variants that will be eligible for future, specific precision medicine. Genetic studies from recent years reported conflicting results on the involvement of rare and common guanosine triphosphate cyclohydrolase I (*GCH1*) variants in PD. Guanosine triphosphate (GTP) cyclohydrolase 1, encoded by *GCH1*, controls the first, rate-limiting step of the biosynthesis of tetrahydrobiopterin (BH_4), which is an essential cofactor for synthesis of dopamine in nigrostriatal cells (Kurian et al., 2011). Loss-of-function mutations in *GCH1* have been shown to cause two rare disorders: autosomal dominant DOPA-responsive dystonia (DRD) and autosomal recessive GCH-deficient hyperphenylalaninemia (Furukawa et al., 1998). Co-occurrence of DRD and Parkinsonism has been reported in families with *GCH1* mutations (Lewthwaite et al., 2015; Rengmark et al., 2016), and a study on patients with sporadic PD demonstrated an increased frequency of pathogenic *GCH1* mutations that were previously reported to cause DRD (Mencacci et al., 2014). Three subsequent studies also supported an association between rare *GCH1* variants

* Corresponding author at: Montreal Neurological Institute, McGill University, 1033 Pine Avenue, West Ludmer Pavilion, Room 312, Montreal, Quebec, H3A 1A1, Canada. Tel.: +1-514-398-5845; fax: +1-514-398-8248.
E-mail address: ziv.gan-or@mcgill.ca (Z. Gan-Or).

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and PD in different populations (Guella et al., 2015; Lewthwaite et al., 2015; Xu et al., 2017). However, other studies did not provide convincing evidence for association of rare *GCH1* DRD-causing mutations and PD (Bandres-Ciga et al., 2016; Rengmark et al., 2016; Yan et al., 2018).

Some conflicting results were also reported on common variants in the *GCH1* locus. Large genomewide association studies (GWASs) identified common variants near *GCH1*, associated with risk for PD (Chang et al., 2017; Nalls et al., 2014). These were replicated in several studies (Chen et al., 2016; Safaralizadeh et al., 2016), but not in others (Newman et al., 2014; Yang et al., 2017; Zou et al., 2018), possibly due to the different sizes and ethnicities of the population studied. Understanding whether rare and common *GCH1* variants have a role in PD is of major importance, as GTP cyclohydrolase 1 can become a target for PD drug development in the upcoming era of precision medicine.

To further examine the potential role of rare and common *GCH1* variants in PD, we sequenced its entire coding regions, as well as the 5′ and 3′ untranslated regions and the intronic regions around the exon-intron boundaries in two cohorts of patients with PD and controls.

2. Subjects and methods

2.1. Study population

Two cohorts with a total of 1113 patients with PD and 1111 controls were included. A cohort composed of French and French-Canadian unrelated patients with PD (n = 538) and controls (n = 831), recruited in Quebec (Canada) and in France. Average patient age was 65.7 ± 10.0 years, with 62.9% men. The control population of this cohort included 2 groups, elderly controls (n = 201, average age at enrollment of 62.7 ± 8.2 years) and young controls (n = 619, average age at enrollment of 35.4 ± 6.5 years, data on age were not available for 11 controls). There was no significant difference in *GCH1* variant frequencies between the 2 groups, which allowed us to combine them for the analysis (average age of 41.9 ± 13.6 years with 51.7% men). The second cohort was recruited in New York (Columbia University) and included 575 patients with PD (average age 66.3 ± 10.55 years, 64% men) and 280 unrelated controls (average age 65.0 ± 9.7 years, 35.4% men). As detailed in the following, owing to the differences in age and sex, statistical analysis was adjusted. All patients with PD were diagnosed by movement disorder specialists according to the UK brain bank criteria (Hughes et al., 1992), without excluding patients with a family history of PD. All patients signed informed consent forms before entering the study, and the institutional review boards approved the study protocols.

2.2. DNA extraction and GCH1 sequencing

DNA was extracted using a standard salting out protocol. The coding sequence and regulatory regions of *GCH1* were targeted using molecular inversion probes (MIPs), designed as previously described (O'Roak et al., 2012). MIPs were selected based on their predicted coverage quality and overlap. All MIPs used to sequence *GCH1* in the present study are detailed in Supplementary Table 1. Targeted DNA capture and amplification was carried out as previously described (Ross et al., 2016), and the full protocol is available on request. The library was sequenced using Illumina HiSeq 2500 platform at the McGill University and Genome Quebec Innovation Centre. Sequence processing was performed by Burrows-Wheeler Aligner for alignment (Li and Durbin, 2009), the Genome Analysis Toolkit (GATK, v3.8) for postalignment cleanup and variant calling (McKenna et al., 2010), and ANNOVAR for annotation (Wang et al., 2010). Data on the frequency of each *GCH1* variant were extracted from the public database Exome Aggregation Consortium. Only variants with high coverage (>30×) and read quality were included in the analysis. Pathogenicity of variants was examined in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and through specific searches in PubMed. Because exon 1 of *GCH1* was not covered well by the targeted sequencing, Sanger sequencing of exon 1 was also performed in all samples using the following primers: forward 5'-GAGGCAACTCCGGAAACT-3′, reverse 5'-GCTCATTCCGCAATAA GTGG-3'.

2.3. Quality control steps

During quality control (QC) filtration using the PLINK software, we excluded SNPs that deviated from Hardy-Weinberg equilibrium, set at 0.001 threshold, and the SNPs with genotyping rate of less than 90%. Same genotyping rate cutoff was used for individual samples. Threshold for rate of missingness between cases and controls was set at 0.05. After the QC, 1082 patients and 1110 controls were included in the analysis. The final genotype call rate after QC filtration was greater than 99%.

2.4. Statistical analysis

The association between common *GCH1* variants and PD was analyzed using a logistic regression with the status (patient or control) as a dependent variable. Because there were differences in age and sex between patients and controls, and because the different recruitment sites recruited patients with different ethnical background, age, sex, and recruitment site were used as covariates. To analyze all the rare variants (minor allele frequency [MAF] <1%), optimized sequence Kernel association test (SKAT-O, R package) was performed (Lee et al., 2012). Association of presumed pathogenic variants was tested using burden analysis (R package SKAT) (Lee et al., 2012) because the direction of the association was presumed as pathogenic before the test. All other statistical analysis was performed using the SPSS software, version 24 (IBM Inc).

3. Results

Table 1 details the identified nonsynonymous *GCH1* variants, and Fig 1 depicts the location of mutations in *GCH1* reported in patients with PD in the present and previous studies on PD. A total of 11 rare variants (MAF <1%) and one less-frequent variant P23L (MAF 1%–5%) were identified. There were 6 novel variants that

Table 1
Summary of all nonsynonymous variants detected in the present study

dbSNP	Position	Substitution	PD N = 1082	Controls N = 1110	ExAC MAF in Europeans
rs41298432	14:55369314	p.P23L	3	8	1.502E-02
rs1030068813	14:55369276	p.A36S	0	1	-
-	14:55369231	p.D51N	0	1	-
-	14:55369219	p.G55S	0	1	-
rs56127440	14:55369176	p.P69L	2	0	5.934E-04
-	14:55312562	p.R184C[a]	1	0	-
rs200891969	14:55312502	p.V204I	0	3	1.798E-04
-	14:55310569	p.N215K	0	1	-
-	14:55310570	p.N215I	0	1	-
-	14:55310841	p.R216Q	1	0	-
rs104894434	14:55310826	p.M221T[a]	1	0	4.495E-05
rs41298442	14:55310817	p.K224R[a]	3	0	4.195E-04
Total			11 (1.02%)	16 (1.44%)	

Key: ExAC, exome aggregation consortium; MAF, minor allele frequency; PD, Parkinson's disease.
[a] Variants reported to be pathogenic in DRD.

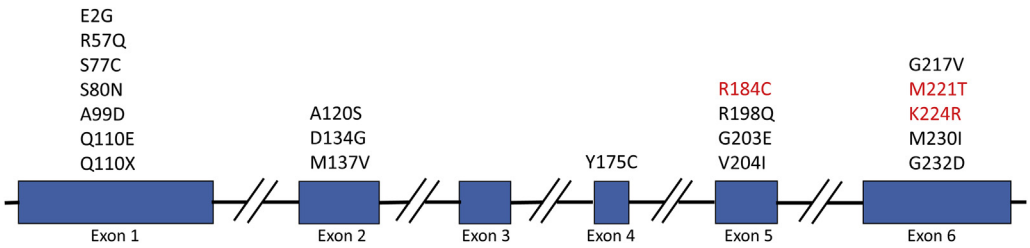

Fig. 1. Mutations in the guanosine triphosphate cyclohydrolase I (*GCH1*) gene detected in patients with Parkinson's disease. The mutations found in this study are indicated as highlighted in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were not found in public databases (2 in patients and 4 in controls). Three variants (p.R184C, p.M221T, and p.K224R), which were previously reported to be pathogenic in DRD (Chenbhanich et al., 2017; Furukawa et al., 1998; Guella et al., 2015; Leuzzi et al., 2002; Mencacci et al., 2014), were found in five (~0.5%) patients with PD and none in controls (SKAT burden test $p = 0.024$). The other variants are of unknown significance. When analyzing all non-synonymous variants, regardless of their contribution to DRD, no association was found (SKAT-O, $p = 0.223$). One variant (p.V204I) that is reported in the ClinVar database to have conflicting interpretation of pathogenicity in DRD was found in 3 controls (~0.3%) and in no patients with PD.

When examining common variants, logistic regression showed a common SNP, rs841, to be associated with reduced PD risk in our data (OR = 0.73, 95% CI = 0.62–0.86, $p = 1.24 \times 10^{-4}$). The rs841 SNP was also associated with reduced risk for PD in the PD GWAS portal (www.pdgene.org) (meta-OR = 0.91, 95%CI = 0.87–0.95, $p = 1.00 \times 10^{-6}$) (Lill, 2016; Nalls et al., 2014). This SNP is in partial linkage disequilibrium with the GWAS tagging SNP rs11158026 (Chang et al., 2017; Nalls et al., 2014) (D' = 0.98, $r^2 = 0.5$, owing to lower frequency of rs841). To examine whether this SNP is associated with *GCH1* expression, we accessed the Genotype-Tissue Expression portal (www.gtexportal.org). Supplementary Fig. 1 demonstrates that this SNP is significantly associated with increased expression of *GCH1* in multiple brain tissues, including the substantia nigra and other basal ganglia. The rs841 SNP was not associated with age at onset in 947 patients for which data were available.

3.1. Clinical presentation of patients with PD with DRD-causing GCH1 mutation

Partial clinical data were available for four of the five patients carrying a DRD-causing *GCH1* mutation. Patient 1 (p.K224R) is a male patient who was diagnosed with idiopathic PD at the age of 58 years, presenting with right hand tremor that later progressed to bilateral hand tremor. The patient also presented with rigidity in both the knees as well as facial freezing and tremors at the rest of lower limbs. The patient had good response to L-DOPA. At the age of 66 years, he developed mild cognitive impairment. Patient 2 (p.K224R) is a male patient with PD with age at onset of 64 years. He is not treated with L-DOPA and information about dystonia is not available. His unified PD rating scale (UPDRS) part III was 15.5 and his Montreal Cognitive Assessment (MoCA) score was 28 in his last examination. Clinical data were not available for the third patient with the p.K224R mutation. Patient 4 (p.R184C) is a male patient with age at onset of 48 year and a family history of PD (father). His initial clinical presentation was mainly left-sided rigidity, yet later tremor became more predominant. He is treated with L-DOPA medication with good response; however, he suffered from dyskinesia and on/off fluctuations. This patient also suffers from anxiety, hallucinations, and probable REM sleep behavior disorder, based on a questionnaire. In 2013, the patient went through a successful deep brain stimulation surgery, which led to improvement of motor symptoms. Patient 5 (p.M221T) is a female patient with age at onset of 61 years. She had an excellent response to L-DOPA. Her UPDRS and MoCA scores are 11 and 27, respectively.

4. Discussion

The present study provides further support to previous reports suggesting that rare DRD-causing *GCH1* variants may also cause PD and that common *GCH1* variants are associated with a small effect on PD risk, perhaps through regulation of *GCH1* expression. The negative results previously reported for common and rare *GCH1* variants in PD (Cobb et al., 2009; Hertz et al., 2006; Newman et al., 2014; Rengmark et al., 2016; Yang et al., 2017) may be due to small sample sizes, differential sequencing techniques, or population-specific effects (Table 2 details results from previous sequencing and genotyping studies on *GCH1* in PD).

A total of 20 rare *GCH1* variants have been reported in PD/Parkinsonism (Fig. 1). However, it is still not clear if all 20 variants indeed have a pathogenic role in PD, as some of them may be rare benign variants that were randomly found in patients with PD. For example, there is conflicting evidence regarding the role of p.V204I; this variant was reported in three patients with PD (Mencacci et al., 2014) but was also found in compound heterozygosity with another pathogenic *GCH1* variant. If the p.V204I was indeed pathogenic as well, this patient should have had the infant-onset severe phenotype (Weissbach and Klein, 2014). In our study, this variant was found in three controls and none in patients, further supporting lack of pathogenicity. Furthermore, in Exome Aggregation Consortium, it is found in about 1:500 individuals of South Asian origin (http://exac.broadinstitute.org/variant/14-55312502-C-T), which is a somewhat high frequency for a disease-causing variant. Similarly, we have identified two patients with PD with the p.P69L variant, which was previously reported in patients with PD with dystonia (Furukawa et al., 2004), but was also reported as a benign variant by others (Mencacci et al., 2014). However, this mutation is slightly less common, found in about 1:1000 Europeans. At this point, the pathogenicity of these variants, or alternatively, their role as risk factors with reduced penetrance (as seen with some *GBA* variants, for example [Hernandez et al., 2016]), cannot be ruled out, and larger genetic studies or functional studies are required to examine their pathogenicity. Other variants such as p.S80N (Cao et al., 2010; Xu et al., 2017; Yan et al., 2018), p.R184C (Chenbhanich et al., 2017; Dobricic et al., 2017), p.M221T (Furukawa et al., 1998), and p.K224R (Guella et al., 2015; Mencacci et al., 2014) are more rare and repeatedly reported in DRD and PD, and thus can be considered as pathogenic for both.

Large GWASs identified a risk locus that includes *GCH1* (Chang et al., 2017; Nalls et al., 2014), suggesting that common, possibly regulatory variants in *GCH1* may affect the susceptibility for PD. The common SNP rs841, which was identified in our study, seems to be associated with reduced risk of PD. In the Genotype-Tissue

Table 2
Role of *GCH1* in PD—summary of previous reports

Article	Role in PD	#Controls	#Cases	Diagnosis of cases	Ethnicity	GCH1 findings
Hertz et al., 2006	No	0	87	EOPD	Danish	No pathogenic GCH1 variants found
Cobb et al., 2009	No	0	53	Familial EOPD, 21 with EOPD + dystonia	North American Caucasian	No coding changes/CNV
Momma et al., 2009	Yes	96	2	EOPD	Chinese	1 rare mutation found in patients
Mencacci et al., 2014	Yes	5935	1318	PD	North American of European descent, Estonians	11 different heterozygous variants at low frequency, 4 of them associated with DRD
Nalls et al., 2014	Yes	95282	13708	PD	European ancestry	GWAS signal
Newman et al., 2014	No	862	1105	PD/dystonia	Australian	No association between PD and the analyzed SNPs
Weissbach et al., 2014	Yes	0	15	PD/Parkinsonism/dystonia	N/A	GCH1 mutation carriers with parkinsonism and idiopathic PD (one had dystonia)
Guella et al., 2015	Yes	290	528	361 PD/167 atypical Parkinsonism + DLB + MSA + PSP	N/A	Rare heterozygous nonsynonymous substitutions found in patients
Lewthwaite et al., 2015	Yes	6	6	2 EOPD, 1 Parkinsonism, 3 DRD	Caucasian	1 novel heterozygous substitution found in a very conserved region
Bandres-Ciga et al., 2016	No	0	134	97 LOPD/28 EOPD/9 FPD	South Spanish	No mutation carriers for GCH1
Chen et al., 2016	Yes	553	528	PD	Taiwanese	rs11158026 increased the risk of developing PD
Rengmark et al., 2016	No	230	509	LOPD	Norwegian/Swedish	No pathogenic GCH1 variants found
Safaralizadeh et al., 2016	Yes	1200	600	PD (excluded EOPD, FPD)	Iranian	Replicated the association of rs11158026 with PD
Chang et al., 2017	Yes	302042	6476	PD	European ancestry	GWAS signal
Xu et al., 2017	Yes	1565	1758	PD	Chinese	7 rare heterozygous nonsynonymous mutations in patients
Yang et al., 2017	No	634	589	sporadic PD (FPD excluded)	Han Chinese	No association of rs11158026 with PD
Yan et al., 2018	Yes	438	421	170 EOPD/251 LOPD (FPD excluded)	Han Chinese	1 LOPD patient (maybe + dystonia) with rare GCH1 mutation(+1 found earlier)
Zou et al., 2018	No	624	579	sporadic PD (FPD excluded)	East Asians	No association of rs11158026 with PD

Key: CNV, copy number variation; DLB, dementia with Lewy bodies; DRD, dopa-responsive dystonia; EOPD, early-onset PD; FPD, familial PD; *GCH1*, guanosine triphosphate cyclohydrolase I; GWAS, genomewide association study; LOPD, late-onset PD; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

Expression portal (www.gtexportal.org), this SNP is associated with increased *GCH1* expression in brain regions important in PD, including the substantia nigra (Supplementary Fig. 1). Of note, because this SNP is in linkage disequilibrium with the GWAS tagging SNP rs11158026, it is possible that the rs841 SNP is responsible for the association in this locus; however, functional studies are required to determine whether this or other SNPs in this locus drive the association. Considering that rare, probably loss-of-function mutations in *GCH1* cause DRD or PD, that the biologic function of GTP cyclohydrolase 1 is in the synthesis of dopamine, together with the protective effect of common variants that are likely to increase expression of *GCH1*, may suggest that increasing the expression and/or the activity of *GCH1*/GTP cyclohydrolase 1 could be an attractive target for drug development for sporadic PD as well.

Clinically, late-onset DRD may present as Parkinsonism (Lill, 2016), which may suggest that patients diagnosed with PD who carry a presumed pathogenic *GCH1* mutation actually have a rare phenotype of DRD, which presents similar to PD (Lewthwaite et al., 2015; Mencacci et al., 2014; Rengmark et al., 2016). Alternatively, it is possible that *GCH1* mutations may predispose to both, and whether a carrier will develop DRD or PD is dependent on other genetic or environmental factors. For example, in a family with a novel pathogenic *GCH1* variant, p.E2G, it seemed to cause both PD and DRD phenotypes in different members of the family studied (Lewthwaite et al., 2015). Further supporting this notion, neuropathological studies of patients with DRD with *GCH1* mutations demonstrated that in most patients with DRD, there was an absence of Lewy bodies pathology, whereas a subset of patients with late-onset Parkinsonism was positive for Lewy bodies pathology (Schneider and Alcalay, 2017). Chart reviews of two of our patients from NY and two patients from Canada confirmed the clinical diagnosis of PD.

Our study has several limitations. First, in the French-Canadian and French controls, some of the controls are significantly younger than the patients. It is important to note that this is a bias toward the null hypothesis, which only means that our results could be more significant had we used an age-matched control group. Furthermore, we accounted for the age and sex differences by demonstrating that there was no difference in frequencies of rare or common variants between the young and elderly controls, and by adjusting the regression model with age and sex as covariates. Finally, despite having a relatively large cohort, because many of the *GCH1* variants are rare, a larger study, or rather a meta-analysis of multiple studies, will be required to determine the role of some of the variants which are still questionable.

Overall, our results support a role for rare *GCH1* variants in PD and for common variants as modifiers of risk for PD. Although larger genetic studies, as well as functional studies are still warranted, *GCH1* should already be considered as a target for drug development.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.008.

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