Insufficient evidence for pathogenicity of SNCA His50Gln (H50Q) in Parkinson’s disease

Cornelis Blauwendraat 1*, Demis A. Kia 2*, Lasse Pihlstrom 3, Ziv Gan-Or 4,5, Suzanne Lesage 6, J. Raphael Gibbs 7, Jinhui Ding 7, Roy N. Alcalay, 8,9, Sharon Hassin-Baer 10,11 Alan M. Pittman 2, Janet Brooks 7, Connor Edsall 7, Sun Ju Chung 12, Stefano Goldwurm 13,14, Mathias Toft 3,15, Claudia Schulte 16, International Parkinson’s Disease Genomics Consortium (IPDGC), COURAGE-PD Consortium, Dena Hernandez 7, Andrew B. Singleton 7, Mike A. Nalls 7,17, Alexis Brice 6, Sonja W. Scholz 1,18, Nicholas W. Wood 2

1 Neurodegenerative Diseases Research Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA
2 Department of Molecular Neurosciences, Institute of Neurology, University College London, London, United Kingdom
3 Department of Neurology, Oslo University Hospital, Oslo, Norway
4 Department of Human Genetics, McGill University, Montréal, Quebec, Canada
5 Department of Neurology & Neurosurgery, Montreal Neurological Institute, McGill University, Montréal, Quebec, Canada
6 Inserm U1127, CNRS UMR7225, Sorbonne Universités, UPMC Univ Paris 06, UMR_S1127, Institut du Cerveau et de la Moelle épinière, Paris, France
7 Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA
8 Department of Neurology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA

9 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

10 The Movement Disorders Institute, Department of Neurology and Sagol Neuroscience Center, Chaim Sheba Medical Center, Tel-Hashomer, 5262101, Ramat Gan, Israel

11 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

12 Department of Neurology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea

13 Parkinson Institute of Milan, ASST "Gaetano Pini/CTO", Milano, Italy

14 Department of Neuroscience, "Rita Levi Montalcini", University of Turin, Italy

15 Institute of Clinical Medicine, University of Oslo, Oslo, Norway

16 Department for Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany

17 Founder/Consultant with Data Tecnica International, Glen Echo, MD, USA

18 Department of Neurology, Johns Hopkins University Medical Center, Baltimore, MD, USA

* these authors contributed equally

Corresponding authors:

Nicholas W. Wood -> n.wood@ucl.ac.uk

Cornelis Blauwendraat -> cornelis.blauwendraat@nih.gov
Highlights

- Publicly available data show that \textit{SNCA} His50Gln has a frequency of \(~0.01\%\) in Europeans
- \textit{SNCA} His50Gln was reported to cause Parkinson’s disease, but it is too common to do so
- There is no evidence of variant enrichment in Parkinson’s disease cases compared to controls

Abstract

\textit{SNCA} missense mutations are a rare cause of autosomal dominant Parkinson’s disease (PD). To date, six missense mutations in \textit{SNCA} have been nominated as causal. Here, we assess the frequency of these six mutations in public population databases and PD case-control datasets in order to determine their true pathogenicity. We found that one of the six reported \textit{SNCA} mutations, His50Gln, was consistently identified in large population databases and no enrichment was evident in PD cases compared to controls. These results suggest that His50Gln is probably not a pathogenic variant. This information is important to provide counseling for His50Gln carriers and has implications for the interpretation of His50Gln \(\alpha\)-synuclein functional investigations.
Introduction

Parkinson’s disease (PD) disease is one of the most common neurodegenerative disorders. The pathological hallmark of PD are Lewy bodies, which are neuronal cytoplasmic inclusions consisting of misfolded α-synuclein encoded by the \textit{SNCA} gene (Spillantini et al., 1997). To date, six missense mutations in the \textit{SNCA} gene have been reported to cause PD: three well established mutations (Ala30Pro, Glu46Lys and Ala53Thr) and three more recently described mutations (His50Gln, Gly51Asp and Ala53Glu) (Figure 1 and Table 1). Whilst atypical presentations and a later onset have been reported, \textit{SNCA} mutation carriers typically develop autosomal dominant early-onset PD characterized by a severe, rapidly progressive course and cognitive decline that commonly progresses to Lewy body dementia (Papadimitriou et al., 2016; Trinh et al., 2014). A fuller understanding of exactly which mutations are truly causal for PD will help direct research on the pathophysiology of PD driven by \textit{SNCA} mutations, and is of crucial importance for counseling of mutation carriers and their family members. Here, we explore the frequency and spectrum of these different \textit{SNCA} mutations in several large public datasets, and then examine their presence in several large PD case-control datasets.

Results

The only \textit{SNCA} missense mutation identified in the population databases was His50Gln (Table 1). To assess whether the His50Gln mutation is found in PD cases, we accessed several PD case/control datasets, which cumulatively totaled 11,095 PD cases and 12,615 controls. From these data, we identified two controls and one case carrying the \textit{SNCA} His50Gln mutation. Additionally, two PD cases carrying Ala53Thr and a single PD case with Gly51Asp mutation were found (Table 2 and Supplementary data). We next assessed pathogenicity prediction
algorithms for all six \textit{SNCA} mutations and overall \textit{SNCA} His50Gln scored poorly compared to the other five \textit{SNCA} mutations (Figure 1, Table 1 and Supplementary data). For comparison, we used the known pathogenic \textit{LRRK2} Gly2019Ser mutation as it is also present in the general population (Table 2). Unlike for \textit{SNCA} His50Gln, analysis in PD case-control datasets revealed a consistent increase in the frequency of \textit{LRRK2} Gly2019Ser mutation carriers amongst PD cases than controls (Table 2).

**Discussion**

Here, we examined the presence of reported pathogenic \textit{SNCA} missense mutations in large population control databases and identified that His50Gln is relatively frequent in both the European and African population. In contrast, the other five reported pathogenic mutations were not observed in these control databases. Follow-up analysis in large PD case-control cohorts identified two additional control individuals carrying this variant, representing a similar frequency to the public population databases. We identified the His50Gln mutation in a homozygous state in one sporadic early-onset PD case with an age at onset at 32 years and two heterozygous controls with last known ages of 62 and 89. Notably, two other \textit{SNCA} mutations, Ala53Thr and Gly51Asp, were found twice and once respectively in cases, demonstrating the power of our large dataset to detect rare mutations in the \textit{SNCA} gene. The \textit{SNCA} His50Gln case presented with a classic PD phenotype and was free of dementia and cognitive decline after almost 10 years of disease, which is unusual for patients with pathogenic \textit{SNCA} missense mutations (Papadimitriou et al., 2016). Currently, with the lack of other homozygous cases or controls there is insufficient evidence to conclude that the His50Gln mutation is pathogenic in a homozygous state. One possibility is that the His50Gln mutation has reduced penetrance, as has
been reported for other PD mutations, such as \textit{LRRK2} Gly2019Ser (Healy et al., 2008; Latourelle et al., 2008). However, the lack of enrichment of \textit{SNCA} His50Gln in PD cases versus controls argues against this and this contrasts with the enrichment observed for the \textit{LRRK2} Gly2019Ser mutation, Table 2. Assuming a life-time risk of 1.3-2\% (depending on sex) to be diagnosed with PD (Elbaz et al., 2002), one would expect \sim 2,600 individuals from gnomAD to develop PD. If the \textit{SNCA} His50Gln mutation is indeed pathogenic, fully penetrant and inherited in an autosomal dominant fashion, the 22 carriers of this allele would represent around \sim 1\% of all PD cases. Similarly, we would expect to identify over 100 PD patients in our PD case cohorts. This was not observed and argues against pathogenicity of this variant. In general, segregation data, case-control enrichments, absence in population databases and pathogenicity prediction algorithms are considered important criteria for establishing the causality of sequence variants for genetic disease (MacArthur et al., 2014; Richards et al., 2015), however the \textit{SNCA} His50Gln mutation does not fulfill any of these criteria (Table1). In conclusion, while it is tempting to speculate about the pathogenicity of \textit{SNCA} His50Gln, especially given limited \textit{in vitro} evidence indicating an increased propensity to form \(\alpha\)-synuclein fibrils (Rutherford et al., 2014), we conclude that insufficient evidence exists to nominate the His50Gln mutation as a causative mutation or high risk mutation. This finding has important implications for the interpretation of functional investigations of His50Gln mutated \(\alpha\)-synuclein isoforms as well as for future study design. Furthermore, when identifying the \textit{SNCA} His50Gln mutation in either a patient or an asymptomatic individual, caution should be used by clinicians and genetic counselors, as genetic evidence suggests this is a rare benign variant.
Acknowledgements

The authors would like to thank all members of the IPDGC (http://pdgenetics.org/partners) and COURAGE-PD consortia for proving data, support and comments. This work was supported (in part) by the Intramural Research Program of the National Institutes of Health (National Institute of Neurological Disorders and Stroke, National Institute on Aging; projects 1ZIA-NS003154-2 and Z01-AG000949) and supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. Data used in the preparation of this article were obtained from the Parkinson’s Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). For up-to-date information on the study, visit www.ppmi-info.org.

PPMI - a public-private partnership - is funded by the Michael J. Fox Foundation for Parkinson’s Research and funding partners, including Abbvie, Avid, Biogen, Bristol-Myers Squibb, Covance, GE Healthcare, Genentech, GlaxoSmithKline, Lilly, Lundbeck, Merck, Meso Scale Discovery, Pfizer, Piramal, Roche, Servier, Teva, UCB, and Golub Capital. The study on the cohort from McGill University was financially supported by the Michael J. Fox Foundation and by the Canadian Consortium on Neurodegeneration in Aging (CCNA). The study on the cohort from France was performed by the French Parkinson’s disease genetics study group (PDG) and supported by the France Parkinson’s association. Also we thank dr. Thibaud Lebouvier as the neurologist who examined the homozygous His50Gln SNCA patient. Columbia University Spot cohort was funded by the NIH (K02NS080915) and the Parkinson’s Disease Foundation. We thank Guy Rouleau, Jennifer Ruskey, Sandra B Laurent, Pascale Hince, Dan Spiegelman, Alexandre Dionne-Laporte, Helene Catoire, Cynthia Bourassa, Pierre Provencher, Cathy Mirarchi and Vessela Zaharieva for their assistance. We thank the Quebec Parkinson’s Network and its members (http://rpq-qpn.ca/) for their collaboration. COURAGE-PD (COnprehensive
Unbiased Risk factor Assessment for Genetics and Environment in Parkinson's Disease) is a transnational project funded by the EU Joint Programme - Neurodegenerative Disease Research (JPND). transnational project funded by the EU Joint Programme - Neurodegenerative Disease Research (JPND).

References:


Table 1: Prevalence of six reported pathogenic SNCA mutations and LRRK2 Gly2019Ser in large population databases and pathogenicity algorithm scores. Twenty-two individuals in gnomAD (3 Africans and 19 Europeans, n=138,587) and seven individuals in HRC (all Europeans, n=32,488) were heterozygous for SNCA His50Gln (Lek et al., 2016; McCarthy et al., 2016). The LRRK2 Gly2019Ser mutation was found in 136 individuals in gnomAD (3 African, 8 Latino, 118 European [including Ashkenazi Jewish] and 7 other) and 13 individuals in the HRC (all Europeans). gnomAD = Genome Aggregation Database, HRC = Haplotype Reference Consortium, D = damaging, T = tolerant, B = benign.
<table>
<thead>
<tr>
<th>Dataset</th>
<th>Population description</th>
<th>No. controls</th>
<th>No. cases</th>
<th>His50Gln alleles in controls (frequency)</th>
<th>His50Gln alleles in cases (frequency)</th>
<th>Gly2019Ser alleles in controls (frequency)</th>
<th>Gly2019Ser alleles in cases (frequency)</th>
<th>Average sequencing coverage at SNCA locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPDGC whole-exome sequencing</td>
<td>Mainly European ancestry</td>
<td>5,774</td>
<td>2,440</td>
<td>1 (0.0087%)</td>
<td>0</td>
<td>4 (0.0346%)</td>
<td>25 (0.51%)</td>
<td>&gt;35x</td>
</tr>
<tr>
<td>IPDGC resequencing data</td>
<td>Mainly European ancestry</td>
<td>2,391</td>
<td>3,481</td>
<td>1 (0.021%)</td>
<td>0</td>
<td>0</td>
<td>56 (0.80%)</td>
<td>&gt;60x</td>
</tr>
<tr>
<td>McGill resequencing data¹</td>
<td>Mainly European and Jewish ancestry</td>
<td>2,460</td>
<td>2,175</td>
<td>0</td>
<td>0</td>
<td>11 (0.22%)</td>
<td>125 (2.9%)</td>
<td>&gt;300x</td>
</tr>
<tr>
<td>COURAGE-PD resequencing data²</td>
<td>Both Asian and European ancestry</td>
<td>1,490</td>
<td>1,490</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 (0.20%)</td>
<td>&gt;400x</td>
</tr>
<tr>
<td>French PDG group resequencing data</td>
<td>Mainly of French European origin</td>
<td>500</td>
<td>1,509</td>
<td>0</td>
<td>2 (1 case in HMZ state) (0.07%)</td>
<td>0</td>
<td>42 (1 HMZ + 40 HTZ) (1.39%)</td>
<td>&gt;260x</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12,615</td>
<td>11,095</td>
<td>2 (0.0079%)</td>
<td>0 (0.0090%)</td>
<td>15 (0.059%)</td>
<td>254 (1.1%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Frequency of SNCA His50Gln and LRRK2 Gly2019Ser in large PD case/control datasets. HMZ = homozygous and HTZ = Heterozygous. ¹ Samples included cohorts from Quebec, France, Israel and Columbia University NY (Alcalay et al., 2015). ² Allele counts in the COURAGE-PD dataset were called from sequencing of DNA pools representing 10 cases or 10 controls. Average coverage is given per sample pool. For subsets of COURAGE-PD participants with available data on LRRK2 Gly2019Ser status, known mutation carriers have been excluded from the resequencing study.
Figure 1: Overview of the SNCA gene on DNA, mRNA and protein level. The SNCA gene has six exons of which several are non-coding (light-blue). NB the SNCA gene is located on the antisense strand of the human genome. On mRNA level five exons are left totaling 423 nucleotides (NM_000345) which result in a 141-amino acid protein. All six missense mutations are located in exon two and three in relatively close proximity. When comparing pathogenicity algorithm scores (CADD (Quang et al., 2015), GERP (Davydov et al., 2010)) between the six missense variants, His50Gln scores very poor compared to the other five missense mutations.