

LETTER TO THE EDITOR

Association analysis of *SYT11*, *FGF20*, *GCH1* rare variants in Parkinson's disease

Dear editors,

Mounting evidence has demonstrated that the genetic factors play an important role in PD (Parkinson's disease). Previous studies have identified several genetic variants that are associated with Parkinson's disease in eastern China.^{1,2} Recently, Uladzislau Rudakou and colleagues identified a rare 3' UTR variant (rs945006601), a rare 5' UTR variant (rs1034608171), the rare nonsynonymous variants that drive the *SYT11*, *FGF20*, *GCH1* associated with PD in three cohorts, respectively.³ In addition, they found that common variants in *MAPT*, *TMEM175*, *BST1*, *SNCA*, and *GPNMB* are linked with PD. Since race is an important factor for the genetic association test, we investigated these rare variants in *SYT11*, *FGF20*, and *GCH1* genes in our eastern Chinese Han population.

In our study, 516 patients with sporadic PD (mean age: 61.35 ± 10.56; male/female: 289/227) and 498 ethnicity-, age-, and sex-matched healthy controls (mean age: 60.14 ± 11.44; male/female: 287/211) were included by two movement disorder specialists according to Movement Disorder Society clinical diagnostic criteria in the second affiliated hospital of Zhejiang University of Medicine (Hangzhou, China).⁴ All participants have provided written informed consent. The ethics of this study was approved by the ethics committee of the second affiliated hospital of Zhejiang University of Medicine.

All blood samples were collected, and then genomic DNA was extracted from peripheral blood leucocytes using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's protocol. We sequenced these rare variants using the MassArray and KASP assays, and the details of the primers were provided in Tables S1 and S2. The results were analyzed using a MassARRAY Analyzer (Agena Bioscience) and an ABI 7900 Analyzer (Applied Biosystems), respectively. We used Pearson's chi-square test to compare allelic frequencies of these 10 rare variants between PD and healthy controls. All statistical analyses were conducted using SPSS20.0 (IBM). We predicted the pathogenicity of the variant (position: 55312562; c.C552T, p.R186C) by CADD, Polyphen2, SIFT, MutationTaster in VarCards website (<http://varcards.biols.ac.cn/>). The threshold values were set for the harmfulness of the variants: CADD>15; Polyphen2 > 0.86; SIFT<0.05.

In our cohort, we did not find any significantly different rare variants between PD patients and healthy controls reported

by Uladzislau Rudakou. One nonsynonymous variant (position: 55312562; c.C552T, p.R186C) in the *GCH1* gene was found in eight PD patients and four healthy controls (Table 1). The p.R186C variant is located in the GTP cyclohydrolase 1 domain and is highly conserved across different species (Figure 1), suggesting that the p.R186C variant might change the function of *GCH1* and contribute to the pathogenesis of PD. Moreover, all pathogenicity predictions in silico (CADD, Polyphen2, SIFT, MutationTaster) indicated that the p.R186C variant was harmful to PD (Figure 1). However, there is no statistically significant difference between PD patients and healthy controls in our cohort.

The *SYT11* protein, synaptotagmin-11, is a synaptotagmin isoform, which regulates membrane trafficking in synaptic transmission.⁵ Genetic studies have reported that the mutations in the *SYT11* gene were linked with PD and schizophrenia.^{6,7} Previous studies have suggested that the variants in the nearby *GBA* gene drive the *SYT11* GWAS association with PD.⁸ However, after exclusion of all *GBA* variant carriers, Uladzislau Rudakou and colleagues found that the rs945006601 in the *SYT11* gene remained statistically significant between PD patients and healthy controls. Therefore, the variant rs945006601 in the *SYT11* gene linked with PD was independent of *GBA* variants. Conversely, in our eastern Chinese Han population, we did not find rs945006601 which likely indicated the important effect of ethnic context in *SYT11* variants.

The protein encoded by *FGF20*, fibroblast growth factor 20, is a neurotrophic factor preferentially expressed in the substantia nigra pars compacta. *FGF20* can significantly improve the survival of cultured dopaminergic neurons via activating the MAPK pathway. *FGF20* protects against the loss of dopaminergic neurons in the rat model of PD.⁹ Mounting evidence has indicated that variants in the *FGF20* gene were risk factors for PD in different ethnic populations.^{10,11} Uladzislau Rudakou et al. found a novel rare variant (rs1034608171) located in the promoter region of the *FGF20* gene which drives the significant association with PD. However, this variant did not exist in our PD patients and healthy controls. The relatively smaller sample size or different ethnic context might have contributed to our failure in detecting this variate.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *CNS Neuroscience & Therapeutics* Published by John Wiley & Sons Ltd.

TABLE 1 Variants of *SYT11*, *FGF20*, and *GCH1* genes identified in our cohort

Gene	Position	dbSNP ID	Ref/Alt	PD	Control	<i>p</i>	OR (95% CI)
<i>SYT11</i>	155852207	rs945006601	G/C	1030/0	998/0		
<i>FGF20</i>	16859615	rs1034608171	G/C	1032/0	998/0		
<i>GCH1</i>	55312498	NA	A/T	1032/0	1000/0		
	55312559	NA	G/C	1032/0	1000/0		
	55312562	NA	G/A	1012/8	986/4	0.269	1.949 (0.585–6.492)
	55332087	NA	C/T	1032/0	1000/0		
	55310826	rs104894434	A/G	1032/0	998/0		
	55312502	rs200891969	C/T	1012/0	986/0		
	55310817	rs41298442	T/C	1020/0	992/0		
	55326454	rs756256944	C/T	1032/0	996/0		

Abbreviations: Alt, alternate allele; OR, odds ratio; PD, Parkinson's disease; Ref, reference allele.

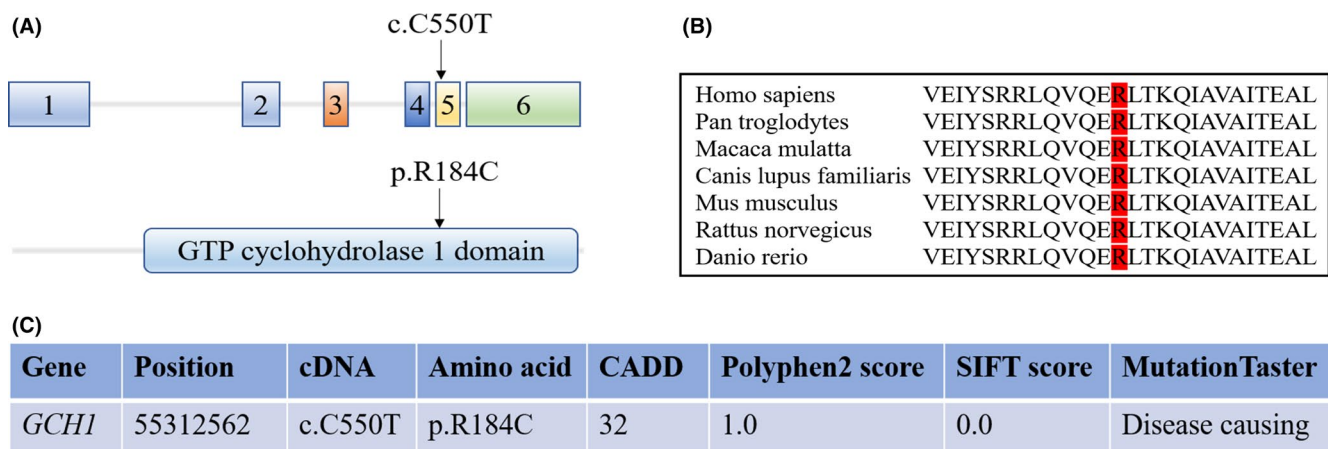


FIGURE 1 Schematic of human *GCH1* and pathogenetic analysis of c.C552T, p.R186C variant. (A) The c.C552T, p.R186C variant locates in *GCH1* gene and *GCH1* protein. (B) The position and the surrounding sequence of *GCH1* p.R186C are highly conserved across different species. (C) Pathogenicity prediction of c.C552T, p.R186C variant in silico. Threshold values for deleteriousness: CADD greater than 15; Polyphen2 greater than 0.86; SIFT less than 0.05

The *GCH1* gene, which encodes GTP cyclohydrolase 1, belongs to the GTP cyclohydrolase family of enzymes known to be involved in the biosynthesis of tetrahydrobiopterin. Tetrahydrobiopterin is a cofactor for tyrosine hydroxylase that is a rate-limiting enzyme for dopamine biosynthesis. Therefore, variants in the *GCH1* gene could cause dopa-responsive dystonia.¹² Moreover, many genetic association studies have also identified *GCH1* variants that increased the risk for PD.^{13,14} In our cohort, we only found one nonsynonymous variant (c.C552T, p.R186C) that exists in eight Parkinson's disease patients and four healthy controls. Moreover, analysis of conservation across different species and prediction of pathogenesis all suggested that the p.R186C variant may contribute to the pathogenesis of PD.

Taken together, our results indicated that the 10 variants in *SYT11*, *FGF20*, *GCH1* genes may not be causative variants for PD in eastern China. However, p.R186C variant likely increases the risk of PD. In consideration of the different results due to the diverse ethnic

contexts, additional genetic association studies with larger samples and different ethnic cohorts are needed to identify the role of the variants in *SYT11*, *FGF20*, *GCH1* gene in PD.

FUNDING INFORMATION

This work was supported by the National Natural Science Foundation of China (grant numbers 81771216) and the Primary Research and Development Plan of Zhejiang Province (grant number 2020C03020).

ACKNOWLEDGEMENTS

We express our gratitude to all the patients and their families who participated in this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

Jia-li Pu 


Zhi-Hao Lin

Ran Zheng

Yi-Qun Yan

Nai-jia Xue

Xin-zhen Yin 

Bao-Rong Zhang 

Department of Neurology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China

Correspondence

Bao-Rong Zhang, Department of Neurology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310009, China.
Email: brzhang@zju.edu.cn

Jia-li Pu and Zhi-Hao Lin contributed equally to this work.

ORCID

Jia-li Pu  <https://orcid.org/0000-0002-6719-4060>

Xin-zhen Yin  <https://orcid.org/0000-0001-9063-4012>

Bao-Rong Zhang  <https://orcid.org/0000-0002-8099-7407>

REFERENCES

1. Shen YT, Wang JW, Wang M, et al. BST1 rs4698412 allelic variant increases the risk of gait or balance deficits in patients with Parkinson's disease. *CNS Neurosci Ther.* 2019;25(4):422-429.
2. Gao T, Wu J, Zheng R, et al. Assessment of three essential tremor genetic loci in sporadic Parkinson's disease in Eastern China. *CNS Neurosci Ther.* 2020;26(4):448-452.

3. Rudakou U, Yu E, Krohn L, et al. Targeted sequencing of Parkinson's disease loci genes s SYT11, FGF20 and other associations. *Brain.* 2020;144(2):462-472.
4. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord.* 2015;30(12):1591-1601.
5. Xie Z, Long J, Liu J, Chai Z, Kang X, Wang C. Molecular mechanisms for the coupling of endocytosis to exocytosis in neurons. *Front Mol Neurosci.* 2017;10:47.
6. Inoue S, Imamura A, Okazaki Y, et al. Synaptotagmin XI as a candidate gene for susceptibility to schizophrenia. *Am J Med Genet B Neuropsychiatr Genet.* 2007;144B(3):332-340.
7. Sesar A, Cacheiro P, Lopez-Lopez M, et al. Synaptotagmin XI in Parkinson's disease: new evidence from an association study in Spain and Mexico. *J Neurol Sci.* 2016;362:321-325.
8. Blauwendraat C, Bras JM, Nalls MA, et al. Coding variation in GBA explains the majority of the SYT11-GBA Parkinson's disease GWAS locus. *Mov Disord.* 2018;33(11):1821-1823.
9. Itoh N, Ohta H. Roles of FGF20 in dopaminergic neurons and Parkinson's disease. *Front Mol Neurosci.* 2013;6:15.
10. Sun XY, Wang L, Cheng L, et al. Genetic analysis of FGF20 in Chinese patients with Parkinson's disease. *Neurol Sci.* 2017;38(5):887-891.
11. Sathukhan D, Das G, Biswas A, et al. Evaluation of FGF 20 variants for susceptibility to Parkinson's disease in Eastern Indians. *Neurosci Lett.* 2018;675:68-73.
12. Cao L, Zheng L, Tang WG, et al. Four novel mutations in the GCH1 gene of Chinese patients with dopa-responsive dystonia. *Mov Disord.* 2010;25(6):755-760.
13. Mencacci NE, Isaias IU, Reich MM, et al. Parkinson's disease in GTP cyclohydrolase 1 mutation carriers. *Brain.* 2014;137(Pt 9):2480-2492.
14. Pan HX, Zhao YW, Mei JP, et al. GCH1 variants contribute to the risk and earlier age-at-onset of Parkinson's disease: a two-cohort case-control study. *Transl Neurodegener.* 2020;9(1):31.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.