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Short communication

A novel homozygous *SYNJ1* mutation in two siblings with typical Parkinson's disease

Fei Xie[a,b], Si Chen[b,c], Zhi-dong Cen[b,c], You Chen[b,c], De-hao Yang[b,c], Hao-tian Wang[b,c], Bao-rong Zhang[b], Wei Luo[b,*]

[a] Department of Neurology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[b] Department of Neurology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[c] Cancer Institute, Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

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ABSTRACT

Background: Mutations in the *SYNJ1* have been associated with early onset of atypical Parkinson's disease (PARK20). Patients with PARK20 exhibit a wide phenotypic variability. Here, we report the clinical and genetic findings in two affected siblings with a novel homozygous *SYNJ1* mutation.
Methods: A consanguineous family with two affected siblings with Parkinson's disease was recruited. Both siblings underwent detailed neurological examinations. Whole genome sequencing was performed in the proband.
Results: Both affected siblings presented with pure parkinsonism with no other atypical symptoms and a slow disease progression. The proband had an excellent response to levodopa. Performing the levodopa challenge test in the proband's older brother resulted in improvements in the parkinsonism signs. Genetic analysis identified a homozygous missense mutation in *SYNJ1* (c.2495A > G, p.Y832C) in both of siblings. In silico analyses revealed that the mutation was deleterious.
Conclusions: Screening for *SNYJ1* should be considered in patients with typical levodopa-responsive Parkinson's disease.

1. Introduction

To date, 27 monogenic forms of genes have been identified as linked to Parkinson's disease (PD), of which 12 (*PRKN*, *PINK1*, *PARK7*, *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6*, *SYNJ1*, *SPG11*, *VPS13C*, *PODXL*, *PTRHD1*) are associated with autosomal recessive (AR) inheritance [1]. Patients with mutations in *PRKN*, *PINK1* and *PARK7* present with typical levodopa-responsive early-onset PD and a slow disease progression. However, mutations in other AR genes cause juvenile or early onset atypical Parkinsonism, with poor responsiveness to levodopa and other neurological symptoms, such as cognitive decline, dystonia, epilepsy, and pyramidal features.

Bi-allelic mutations in the *SYNJ1* are associated with two different rare neurological diseases, early-onset PD (PARK20) [2,3] and severe neurodegenerative disorder with intractable seizure and tauopathies [4–6]. Patients with mutations in *SYNJ1* exhibit wide phenotypic variability. Here, we report the clinical and genetic findings in two siblings with a levodopa-responsive PD associated with a homozygous *SYNJ1*mutation.

2. Patients and methods

2.1. Patients

We studied a consanguineous Chinese family that included two siblings with PD. Patients underwent detailed neurological examinations by a specialized neurologist. This study was approved by the Research Ethics Committees of the Second Affiliated Hospital, School of Medicine, Zhejiang University (Hangzhou, China). Written informed consent was obtained from all participants. All persons visible in the video consented to the publication, including online publication and dissemination, of the video material.

2.2. Genetic analysis

Blood samples of the proband and her older brother were collected. Genomic DNA was extracted from peripheral blood leukocytes using phenol and chloroform extraction. Whole-Genome Sequencing (WGS) was performed on DNA from the proband. Paired-end DNA libraries of

* Corresponding author. Department of Neurology, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, 310009, China.
E-mail address: luoweirock@zju.edu.cn (W. Luo).

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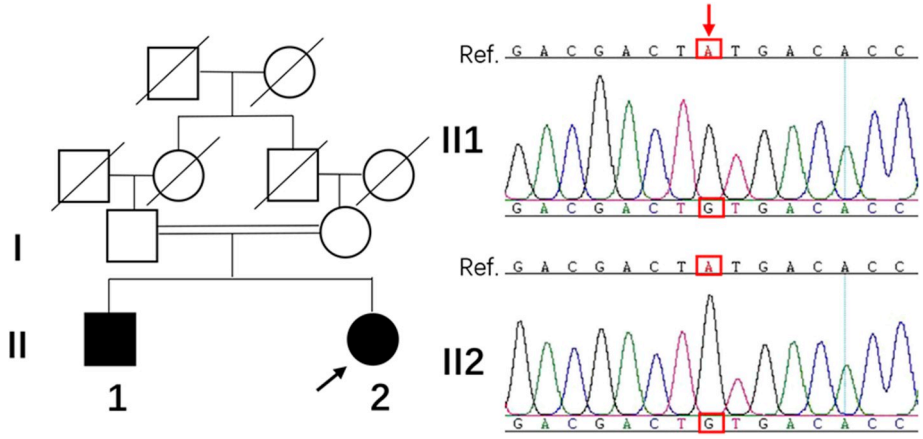

Fig. 1. Pedigree of the family and electropherogram of the Sanger sequencing of *SYNJ1* c.2495A > G mutation. Cycle indicates woman. Square indicates man. The black symbols represent the affected individuals, and the blank symbols represent the unaffected individuals. Double lines indicate consanguineous parents.

patients for WGS analyses were prepared using Illumina Truseq Library Construction and sequenced on Illumina Hiseq X. The average sequencing depth was 31.35 × and 90.1% of the whole genome was covered at least 20 × . The identified missense variant was analyzed using VarCards [7] to assess the pathological character of single amino acid mutations.

3. Result

3.1. Clinical findings

The proband was a 52-year-old woman who exhibited normal development until adulthood. She complained of right hand resting tremor at age of 40 years. One year later, the resting tremor had spread to the right leg. After disease onset, she was treated with piribedil and benzhexol hydrochloride with an excellent response resulting in near complete relief of motor symptoms. Subsequently, the disease progressed slowly. When she presented at our clinic at age 52 years, neurologic examination revealed a masked facies, mild bilateral limb rigidity, mild bradykinesia in the right limbs, and apparent resting tremor in the right hand and both legs. Reduced arm swings were noticed in both hands. She responded well to treatment with levodopa/carbidopa (250 mg/day), selegiline hydrochloride (5 mg/day) and benzhexol hydrochloride (6 mg/day), and her Unified Parkinson's Disease Rating Scale subset III (UPDRS-III) score improved from 24 without medication to 7 in with this regimen. No other atypical symptoms, such as seizures, cognitive decline, abnormal eye movements or dystonia, were observed. The mini-mental state examination scored 30/30. Electroencephalogram results were normal.

Her older brother was a 54-year-old man who had suffered a resting tremor in the right hand at age 52 years. Subsequently, he developed a slight bradykinesia of the right hand. Upon neurological examination at age 54 years, he showed a mild masked facies, right hand resting tremor, and mild bradykinesia in the right limbs. The right arm swing was absent during walking. He was treated with selegiline hydrochloride, which partially improved his motor symptoms. The patient had exhibited parkinsonism for no more than 2 years. Therefore, daily levodopa administration had not been prescribed. Nonetheless, the levodopa challenge test with 200/50 mg of levodopa/benserazide resulted in an improvement in the UPDRS-III score from 13 in the “off” state to 9 in the “on” state (1 hour after the administration). He refused an additional brain MRI or dopamine transporter PET imaging for further evaluation. Videos of the parkinsonism signs in the “off” state and “on” state exhibited by both siblings are available in the supporting material.

3.2. Genetic findings

WGS analysis identified a homozygous missense mutation in *SYNJ1* (c.2495A > G, p.Y832C). The mutation was verified by Sanger sequencing (Fig. 1). No other rare homozygous or heterozygous deleterious variations were identified in genes associated with PD. The mutation c.2495A > G has been reported in 3 European cases at the heterozygous state in the Genome Aggregation Database (total allele frequency: 0.00001061) and not found in the dbSNP (build 152), the 1000 Genomes, the NHLBI Exome Sequencing Project, the CONVERGE, the GME Variome and the Iranome databases. In silico analyses revealed that *SYNJ1* p.Y832C was highly conserved among vertebrates and predicted to be deleterious in all of silico pathogenicity prediction tools in VarCards (23 of 23). The proband's brother harbored the same homozygous mutation. DNA from their parents were not available.

4. Discussion

To date, 3 homozygous and 6 compound heterozygous mutations of *SYNJ1* have been identified in 15 patients with PARK20 (Table 1) [2,3,8–13]. Patients with PARK20 are characterized by juvenile or early onset of parkinsonism. They exhibited with various atypical clinical symptoms, including seizures, cognitive decline, abnormal eye movements, dystonia, dysarthria and dysphagia without cerebellar signs. Here, we report a new *SYNJ1* mutation (p.Y832C) in two siblings with typical levodopa-responsive parkinsonism with no atypical symptoms. This led to a milder phenotype and relatively slow disease progression compared with previous cases. The age at onset of parkinsonism in our patients was considerably older (40 and 52 years) than previous cases (an average of 21.5 years; range: 12–31 years). The efficacy of levodopa treatment in 15 previous cases with PARK20 was variable. Most cases (10/15) showed a poor response to levodopa or did not tolerate levodopa because of severe and disabling dyskinesias, dystonias, or postural hypotension. In this report, the proband exhibited an excellent response to levodopa even after 12 years of disease evolution. A levodopa challenge test of the brother resulted in improvement in parkinsonism signs. The clinical course in our cases appeared to be relatively slow and no atypical signs were observed after 12 and 2 years, respectively, which is not distinct from patients with PD caused by *PRKN*, *PINK1*, and *PARK7* mutations. Though in silico analyses revealed that the mutation was deleterious, we cannot be absolutely certain the mutation is the cause of the patients' disorder. More case reports with similar phenotype are warranted to confirm the phenotype-genotype correlation.

The SYNJ1 protein has two major isoforms: 170-kD and 145-kD isoform [14]. Both isoforms contain an *N*-terminal Sac1-like inositol domain (Sac-1), an inositol-5-phosphatase domain (5′PP), and a *C*-

Table 1
The clinical features of patients with PARK20.

Cases	Patient no.	Mutation	Gender/age	AAO of parkinsonism	Developmental milestones	Clinical phenotype (age)	Brain MRI	DaT-SCAN imaging	FDG-PET imaging	Response to levodopa
Our study	1	p.Y832C (hom.)	F/52	40	Normal	Parkinsonism	NA	NA	NA	GRL
	2	p.Y832C (hom.)	M/54	52	Normal	Parkinsonism	NA	NA	NA	NPL
Krebs, 2013 [2]	3	p.R258Q (hom.)	M/29	20	NA	Parkinsonism, ALO, seizures (3), OD.	Mild CA, WMH	NA	NA	NTL due to LID
	4	p.R258Q (hom.)	F/39	Early 20's	Normal	Parkinsonism, ALO, seizures (infancy).	Meningioma	NA	NA	NTL due to LID
Quadri, 2013 [3]	5	p.R258Q (hom.)	M/47	22	Normal	Parkinsonism, dystonia, ALO, OD, CI.	CA, HH, TMQP.	bilateral NDD	CCH	NTL due to LID & LIPH
	6	p.R258Q (hom.)	F/31	28	Normal	Parkinsonism, dystonia, OD, CI.	CA, HH, TMQP.	NA	CCH	NTL due to LID & LIPH
Olgiati, 2014 [8]	7	p.R258Q (hom.)	M/31	28	Delayed	Parkinsonism, dystonia, OD, CI.	Normal	bilateral NDD	Mild CCH	NPL
	8	p.R258Q (hom.)	F/27	26	Delayed	Parkinsonism, seizure (16), dystonia, CI.	Normal	bilateral NDD	Mild CCH	NPL
Kirola, 2016 [9]	9	p.R459P (hom.)	F/32	12	NA	Parkinsonism	HSN;	NA	NA	NTL due to LIDD
	10	p.R459P (hom.)	M/22	18	NA	Parkinsonism	NA	NA	NA	NTL due to LIDD
Rauschendorf 2017 [10]	11	p.W171*/R258Q	M/21	15	Normal	Parkinsonism, Generalized dystonia,	NA	bilateral NDD	CCH	NTL due to LID
	12	p.W171*/Ar R258Q	M/32	13	Delayed	Parkinsonism, Generalized dystonia,	NA	bilateral NDD	CCH	GRL
Taghavi, 2018 [11]	13	p.R839C (hom.)	M/NA	24	NA	Parkinsonism, seizure (24).	NA	NA	NA	PRL
Romdhan, 2018 [12]	14	p.L1406Ffs*42, p.K1321E	M/23	16	NA	Parkinsonism, seizure (7), Dystonic, moderate CI.	normal	NA	NA	GRL
	15	p.L1406Ffs*42, p.K1321E	F/24	21	normal	Parkinsonism, OD, moderate CI.	normal	NA	NA	GRL
Hong 2018 [13]	16	p.A860Gfs*5, p.P1282L	F/35	31	normal	Parkinsonism, dystonia, OD.	CA	NA	NA	PRL
	17	p.A860Gfs*5, p.P1282L	M/30	28	NA	Parkinsonism, dystonia, OD.	normal	NA	NA	PRL

No.: number; hom.: homozygous; AAO: age at onset; F: female; M: male; NA: Not available; ALO: apraxia of eyelid opening; CI: cognitive impairment; OD: oculomotor disturbances; CA: cortical atrophy; HH: hyperintensity of hippocampi; TMQP: thinning of the midbrain quadrigeminal plate; WMH: white matter hyperintensities HSN: hyperintensity in substantia nigra; NDD: nigrostriatal dopaminergic deficit; CCH: Cortical and caudate hypometabolism; NTL: Not tolerated to levodopa; GRL: good responsive to levodopa; PRL: poor responsive to levodopa; LID; levodopa induced dyskinesia; LIDD; levodopa induced dyskinesia and dystonia; LIPH: levodopa induced postural hypotension; NPL: not prescribed to levodopa.

terminal proline-rich domain (PRD) [14]. While, the 170-kD isoform has an additional *C*-terminal tail that contains binding sites for clathrin (AP2). It has been postulated that phenotype of *SYNJ1* variants correlated with residual SYNJ1 phosphatase activity [5]. Missense mutations limited to the Sac1 domain, which reduced the dephosphorylation activity limited to the Sac1 domain, lead to a milder phenotype of PARK20 [2,3,8,9]. In contrast, missense mutations localized in the inositol-5-phosphatase domain and nonsense, frameshift mutations, which cause a critical reduction of the phosphatase activity of both the 5′PP and the Sac1 domains, lead to a severe phenotype of early onset refractory seizures and progressive neurological decline [4–6]. However, a recent report has revealed a homozygous mutation lied within the 5′PP domain of *SYNJ1* (p.R839C) leaded to PARK20 [11]. Similarly, the mutation reported in our cases is located in 5′PP domain. Although the residual SYNJ1 phosphatase activity of both 2 mutations remains unknown, these data suggest that mutations found in the 5′PP domain can lead to PARK20.

In conclusion, this is the first report of a phenotype compatible with typical levodopa-responsive parkinsonism in two siblings with bi-allelic mutations in *SYNJ1*. Our results suggest that screening for the *SNYJ1* gene should be considered in patients with typical levodopa-responsive PD.

Financial disclosure

None.

Ethical statement

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. Written informed consent was obtained from all participants.

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Authors' roles

F.X, collected the clinical data, designed the study, performed genetic analysis and wrote the first draft of the manuscript. ZD.C, Y.C, S.C, DH.Y, HT.W, collected the clinical data, performed the genetic analysis and revised the manuscript. BR.Z offered suggestions for the study and revised the manuscript. W.L obtained funding, organized the study, revised the manuscript and recruited patients. All coauthors reviewed and accepted the final manuscript.

Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.11.001.

References

[1] A. Lunati, S. Lesage, A. Brice, The genetic landscape of Parkinson's disease, Rev. Neurol. (Paris) 174 (9) (2018) 628–643.
[2] C.E. Krebs, S. Karkheiran, J.C. Powell, M. Cao, V. Makarov, H. Darvish, et al., The Sac1 domain of SYNJ1 identified mutated in a family with early-onset progressive Parkinsonism with generalized seizures, Hum. Mutat. 34 (9) (2013) 1200–1207.
[3] M. Quadri, M. Fang, M. Picillo, S. Olgiati, G.J. Breedveld, J. Graafland, et al., Mutation in the SYNJ1 gene associated with autosomal recessive, early-onset Parkinsonism, Hum. Mutat. 34 (9) (2013) 1208–1215.
[4] D.A. Dyment, A.C. Smith, P. Humphreys, J. Schwartzentruber, C.L. Beaulieu, F.C. Consortium, et al., Homozygous nonsense mutation in SYNJ1 associated with intractable epilepsy and tau pathology, Neurobiol. Aging 36 (2) (2015) 1222 e1–5.
[5] K. Hardies, Y. Cai, C. Jardel, A.C. Jansen, M. Cao, P. May, et al., Loss of SYNJ1 dual phosphatase activity leads to early onset refractory seizures and progressive neurological decline, Brain 139 (Pt 9) (2016) 2420–2430.
[6] N. Al Zaabi, N. Al Menhali, F. Al-Jasmi, SYNJ1 gene associated with neonatal onset of neurodegenerative disorder and intractable seizure, Mol. Genet. Genom. Med. 6 (1) (2018) 109–113.
[7] J. Li, L. Shi, K. Zhang, Y. Zhang, S. Hu, T. Zhao, et al., VarCards: an integrated genetic and clinical database for coding variants in the human genome, Nucleic Acids Res. 46 (D1) (2018) D1039–D1048.
[8] S. Olgiati, A. De Rosa, M. Quadri, C. Criscuolo, G.J. Breedveld, M. Picillo, et al., PARK20 caused by SYNJ1 homozygous Arg258Gln mutation in a new Italian family, Neurogenetics 15 (3) (2014) 183–188.
[9] L. Kirola, M. Behari, C. Shishir, B.K. Thelma, Identification of a novel homozygous mutation Arg459Pro in SYNJ1 gene of an Indian family with autosomal recessive juvenile Parkinsonism, Park. Relat. Disord. 31 (2016) 124–128.
[10] M.A. Rauschendorf, M. Jost, F. Stock, A. Zimmer, B. Rosler, M. Rijntjes, et al., Novel compound heterozygous synaptojanin-1 mutation causes l-dopa-responsive dystonia-parkinsonism syndrome, Mov. Disord. 32 (3) (2017) 478–480.
[11] S. Taghavi, R. Chaouni, A. Tafakhori, L.J. Azcona, S.G. Firouzabadi, M.D. Omrani, et al., A clinical and molecular genetic study of 50 families with autosomal recessive parkinsonism revealed known and novel gene mutations, Mol. Neurobiol. 55 (4) (2018) 3477–3489.
[12] S. Ben Romdhan, S. Sakka, N. Farhat, S. Triki, M. Dammak, C. Mhiri, A novel SYNJ1 mutation in a Tunisian family with juvenile Parkinson's disease associated with epilepsy, J. Mol. Neurosci. 66 (2) (2018) 273–278.
[13] D. Hong, L. Cong, S. Zhong, Y. He, L. Xin, X. Gao, et al., Clonazepam Improves the Symptoms of Two Siblings with Novel Variants in the SYNJ1 Gene, Parkinsonism Relat Disord, 2018.
[14] R.M. Perera, R. Zoncu, L. Lucast, P. De Camilli, D. Toomre, Two synaptojanin 1 isoforms are recruited to clathrin-coated pits at different stages, Proc. Natl. Acad. Sci. U. S. A. 103 (51) (2006) 19332–19337.