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Short communication

Clonazepam improves the symptoms of two siblings with novel variants in the *SYNJ1* gene

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ABSTRACT

Background: Mutations in the *SYNJ1* gene have been associated with early-onset of atypical Parkinson's disease or severe neurodegeneration with intractable seizures. Due to the rarity of the disease, there were limitations in the quality of available treatment options for SYNJ1-related diseases.
Methods: Two affected siblings from a non-consanguineous family were evaluated through a set of clinical and laboratory tests. The genetic screening was performed through exome next generation sequencing. *SYNJ1* mutant transcripts were purified and cloned into the vectors for Sanger sequence of single-stranded DNA. Relative level of the *SYNJ1* transcript was measured by quantitative PCR.
Results: The clinical features were characterized by a triad of symptomatic progression including diplopia, dystonia, and Parkinsonism. The dystonic symptoms were outstanding in the siblings, which preceded the Parkinsonism symptoms and became the main symptoms. Clonazepam resolved the clinical symptoms, especially the severe trunk dystonia and dystonic postures of limbs. Compound heterozygous variants (c.2579-2A > G; p.A860Gfs*5 and c.3845C > A; p.P1282L) were identified in the *SYNJ1* gene co-segregating in this family. The proline residue is highly conserved across species and predicted to be damaging by several *in silico* tools. The splice site variant caused a skip of exon 20 and a significant reduction of the *SYNJ1* transcript expression.
Conclusions: Our study expanded the clinical and genetic spectrums of the SYNJ1-related diseases. Although our study was a preliminary observation, it indicated that clonazepam could improve the dystonic symptoms caused by mutations in the *SYNJ1* gene.

1. Introduction

During the past years, several loci for autosomal recessive early-onset Parkinson's disease (PD) have been identified as follows [1]: PARK2 caused by mutations in the *PRKN* gene, PARK6 caused by mutations in the *PINK1* gene, PARK7 caused by mutations in the *DJ1* gene, PARK14 caused by mutations in the *PLA2G6* gene, PARK15 caused by mutations in the *FBXO7* gene, PARK19 caused by mutations in the *DNAJC6* gene, PARK20 caused by mutations in the *SYNJ1* gene, and PARK23 caused by mutations in the *VPS13C* gene. Mutations in the *PRKN*, *PINK1*, and *DJ-1* genes cause more typical Parkinsonism, while mutations in the *ATP13A2*, *PLA2G6*, *FBXO7*, *DNAJC6, SYNJ1*, and *VPS13C* genes are associated with complex Parkinsonism accompanied by dystonia, pyramidal signs, cognitive decline, as well as other neurological and psychiatric symptoms, and may become irresponsive to levodopa treatment [1].

Synaptojanin 1 (SYNJ1) is a highly conserved polyphosphoinositide phosphatase that is concentrated at synapses [2]. Recently, defects of the SYNJ1 have been associated with early-onset complex Parkinsonism syndrome [2–5], late-onset typical Parkinson's disease [6], and severe neurodegeneration with intractable seizures [7,8]. However, most patients with *SYNJ1* mutations exhibited poor prognosis or poor responsiveness to levodopa treatment. Herein, we described two siblings who presented with dystonia-Parkinsonism symptoms caused by compound heterozygous mutations in the *SYNJ1* gene, and showed a good responsiveness to clonazepam.

2. Methods

2.1. Subjects

All available members from a non-consanguineous family (Fig. 1A) were examined by two neurologists after consent was obtained. Multiple laboratory tests were conducted including blood count,

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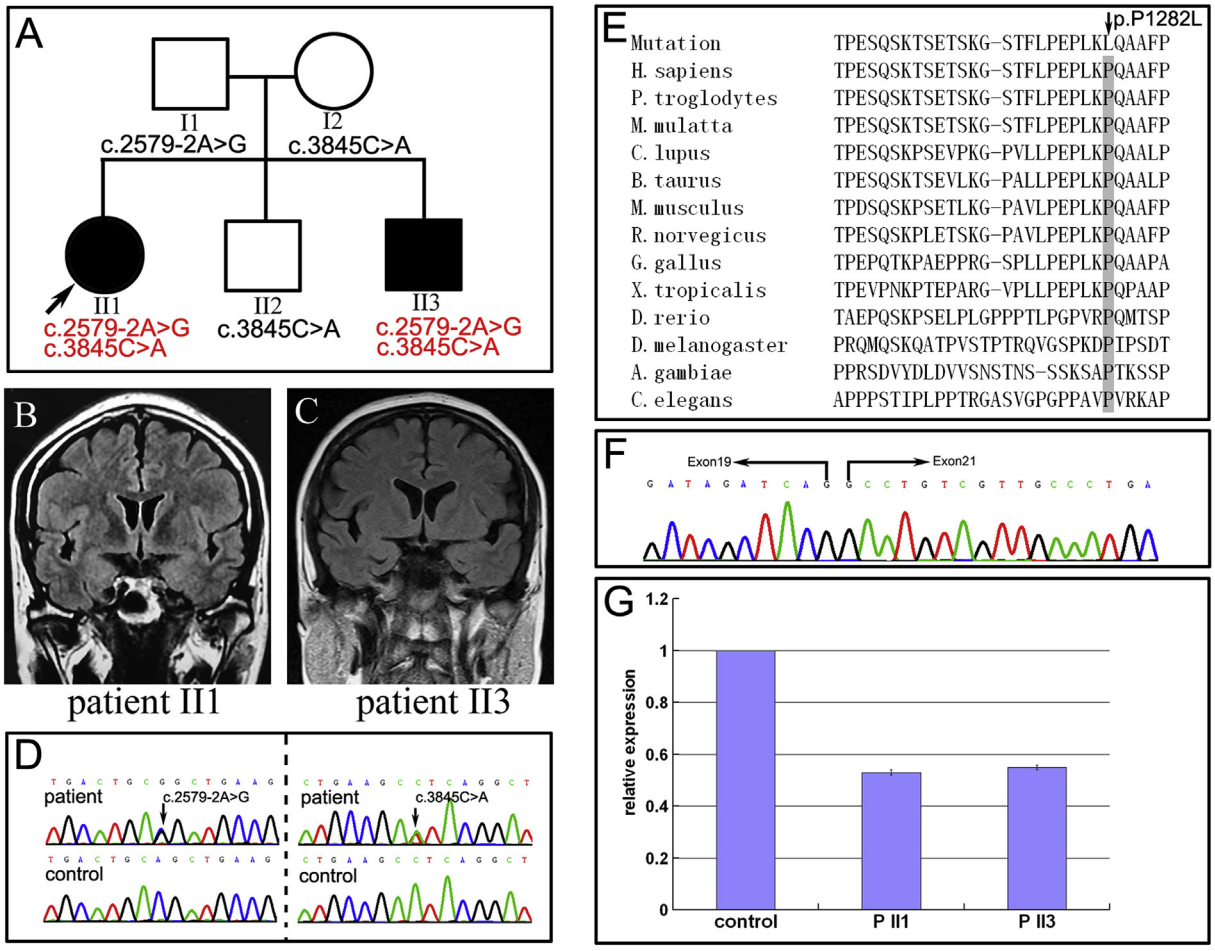

Fig. 1. Pedigree of the family (A). Arrow indicates the index patient. The genotype co-segregated with the phenotype in the family. The index patient (II1) showed mild cerebral atrophy on MRI (B). Patient II3 had a normal cerebral MRI (C). The chromatogram of two novel variants in the *SYNJ1* gene (D); The proline at amino acid residue 1282 is highly evolutionarily conserved in different species (E); cDNA sequence demonstrated that there was a strand of RNA with a skip of exon 20 (F). Quantitative PCR measurements showed a significant reduction of the SYNJ1 transcript expression in both patients' lymphocytes compared to those of control cells (G). Experiments were repeated three times for every test. Data were analyzed by one-way ANOVA and Tukey's post hoc test; Error bars are SEM; *p < 0.05.

biochemical analysis, vitamin B12, thyroid hormones, transferrin, ceruloplasmin, anti-glutamic acid decarboxylase (GAD) and anti-amphiphysin antibodies, and blood acylcarnitine and urine organic acid profiles. Brain and spinal MRI were performed. The study was approved by the ethics committee of Peking University People's Hospital.

2.2. Genetic evaluations

DNA was extracted from the peripheral blood of all available members. Initially, the genetic test was performed commercially in the index patient through exome next generation sequencing (PrecisionMD, Beijing, China). In order to validate the variants, fragments containing the novel *SYNJ1* variants were amplified for Sanger sequencing in the index patient and other family individuals.

B-lymphocytes from the patients were immortalized through EB-virus transformation. RNA was extracted using the RNeasy Kit (Qiagen) according to the manufacturer's instructions. The cDNA was converted using random hexamer primers. To visualize SYNJ1 mutant transcripts, the PCR products of the *SYNJ1* gene were purified and cloned into the cloning vector pBluescript SK + that served to produce single-stranded DNA. Quantitative PCR (qPCR) for relative quantification of SYNJ1 transcript level was conducted using primers specific for the *SYNJ1* and *GAPDH* gene. Measurements were normalized against the *GAPDH* gene. Levels of mutant transcript were relatively quantified to the level of wild-type transcript from a control cell line.

3. Results

3.1. Clinical outcomes

The index patient (II1) was a 35-year-old woman. Her early history was unremarkable with normal development to adulthood. She complained of a left horizontal diplopia at the age of 31. A few months later, she felt a muscle “tight feeling” on her back and neck. The involuntary muscle contractions soon spread to shoulders and waist, thus she had difficulty in stretching arm and bending down, more markedly in cold condition. Meanwhile she felt limb stiffness. Her left arm involuntarily bent inward, but without disturbance of finger posture. At the age of 33, she had difficulty in stretching her legs, especially when she felt nervous. She fell backward several times at such situation. At the age of 35, she consulted our department with complaints of progressive slowness of movements, fatigue, abnormal posture, and gait abnormalities. Non-motor symptoms and autonomic signs were not found in the patient. Physical examination revealed a slow irregular speaking rhythm with mild dysarthria. The facial expression was reduced. The eye movements showed a limitation of left abduction, but a normal movement in the other directions. Her gait was small-stepped without arm swing. She displayed fine postural tremor and bradykinesia. Limb strength was normal, but axial and four-limb rigidity were apparent. Her left arm tended to be flexed at the elbow involuntarily. Deep tendon reflexes were normal; Babinski sign was negative. No signs of cerebellar ataxia

Table 1
The summarization of clinical data for patients with *SYNJ1* gene mutations.

Literature	Case number	Sex/age	Age at onset	Clinical phenotype	MRI change	Mutation	Treatment
Our study	1	F/35	31	Parkinsonism, dystonia, diplopia	CA	p.A860Gfs*5, p.P1282L	clonazepam
	2	M/30	28	Parkinsonism, dystonia, diplopia	normal	p.A860Gfs*5, p.P1282L	clonazepam
Quadri, 2013	3	M/47	22	Parkinsonism, dystonia, CI	CA, HH	p.R258Q (hom)	LID
	4	F/31	28	Parkinsonism, dystonia, CI	CA	p.R258Q (hom)	LID
Krebs, 2013	5	M/29	3	Parkinsonism, seizure	CA, WMH	p.R258Q (hom)	LID, MIB
	6	F/39	neonatal	Parkinsonism, seizure	meningioma	p.R258Q (hom)	LID, DAID, MIB
Olgiati, 2014	7	M/31	28	Parkinsonism, seizure, dystonia, CI	normal	p.R258Q (hom)	IP
	8	F/27	16	Parkinsonism, seizure, dystonia, CI	normal	p.R258Q (hom)	IP, IB
Kirola, 2016	9	F/32	12	Parkinsonism	HSN	p.R459P (hom)	LID
	10	M/22	18	Parkinsonism	normal	p.R459P (hom)	LID
Bouhouche, 2017	11	M/76	74	Parkinsonism	NA	p.S552Ffs*5, p.T1236 M	GRL
Taghavi, 2018	12	M/NA	24	Parkinsonism, seizure	NA	p.R839C (hom)	LID
Romdhan,2018	13	M/23	7	Parkinsonism, seizure	CA, WMH	p.L1406Ffs*42, p.K1321E	GRL
	14	F/24	21	Parkinsonism	normal	p.L1406Ffs*42, p.K1321E	GRL
Dyment, 2015	15	M/6.5*	neonatal	seizure, neurodegeneration	mild CA	p.R136X (hom)	PRAP
Hardies, 2016	16	F/7	neonatal	seizure, neurodegeneration	normal	p.Y888C (hom)	PRAP
	17	M/6	neonatal	seizure, neurodegeneration	normal	p.Y888C (hom)	PRAP
	18	F/5	neonatal	seizure, neurodegeneration	normal	p.W843X (hom)	PRAP
	19	F/2.5	neonatal	seizure, neurodegeneration	normal	p.W843X (hom)	PRAP
	20	M/2.5*	neonatal	seizure, neurodegeneration, dystonia	normal	p.Q647Rfs*6, p.S1122Tfs*3	PRAP
	21	M/8*	neonatal	seizure, neurodegeneration, dystonia	TCC, WMH	p.Q647Rfs*6, p.S1122Tfs*3	PRAP
Al Zaabi, 2018	22	F/2	neonatal	seizure, neurodegeneration	normal	p.Q237X (hom)	PRAP
	23	M/2	NA	seizure, neurodegeneration	mild CA	p.Q237X (hom)	PRAP

The references can be found in the supplementary data.
*the age of death; CA: cortical atrophy; CI: cognitive impairment; DAID: dopamine agonists induced dystonia; F: female; GRL: good responsive to levodopa; HH: hyppocampi hyperintensity; hom: homozygous; HSN: hyperintensity in substantia nigra; LID: Levodopa induced dystonia; IB: improvement for biperiden; IP: improvement for pramipexole; M: male; MIB: moderate improvement for bromocriptine; NA: no available; PRAP: poor response to antiepileptic medications; TCC: thin corpus callosum; WMH; white matter hyperintensity.

were observed. The score of the Unified Parkinson's disease rating scale (UPDRS-III motor scale) was 52/108. The mini-mental state examination (MMSE) scored 28/30. Electroencephalogram (EEG) was normal. Brain MRI showed mild cortical atrophy (Fig. 1B). All laboratory tests were within normal limits.

The younger brother (III3) of the index patient experienced horizontal diplopia at the age of 28. One year later, he had intermittent muscle twitching on the back and neck, and soon the involuntary muscle contractions were persistent along the spine. The contractions became more severe when he stood up after sitting for a prolonged time, or when he was at cold condition, or when he felt nervous. He also complained of slowness of movements, fatigue, abnormal posture, and gait abnormalities. On examination at age 30, he showed a mildly masked face and mild bradykinesia. Hands had fine postural tremor. Gait pattern exhibited small steps without arm swing. His right upper limb was flexed at the elbow and wrist, and legs tended to extend at the knee. Rigidity was observed in his neck, back, and limbs. Deep tendon reflexes were mildly exaggerated. Limb strength was normal. Babinski sign was negative. No signs of cerebellar ataxia were found. The score of the UPDRS-III motor score was 48/108. The MMSE scored 29/30. EEG was normal. Brain MRI was normal (Fig. 1C). All laboratory tests were within normal limits.

The two patients were initially treated with L-dopa/carbidopa (250 mg, tid) for two weeks, no any improvements, but rather, exacerbated dyskinesias, were observed. Next, considering the combination of dystonia and Parkinsonism symptoms, L-dopa/carbidopa (250 mg, tid), pramipexole (0.25 mg, tid), baclofen (20 mg, tid), and eperisone (50 mg, tid) were administered together for two weeks, but only mild improvements of the gait symptoms and bradykinesia were observed, and the UPDRS-III score decreased to 40 in the index patient and to 38 in her brother. At last, we stopped all above medicines and tried to treat the patients with clonazepam (1 mg, qn). To our surprise, their axial and limb rigidity almost completely recovered after two days. The dystonia and gait freezing when they suddenly stood up after sitting for a prolonged time, or when they felt nervous were also significantly improved two weeks later. The residual symptoms of bradykinesia and mild tremor were improved after administration of pramipexole (0.25 mg, tid). Except for the diplopia, clonazepam still had effect on the symptoms after half a year follow-up.

3.2. Genetic mutation

In the exome-sequencing process, two heterozygous variants were found in the *SYNJ1* gene (Fig. 1D). A splice site variant c.2579-2A > G predicted to cause a premature stop (p.A860Gfs*5) and a missense c.3845C > A predicated to cause a substitution (p.P1282L). In the family, the c.2579-2A > G was from the father, while the c.3845C > A was from the mother. The younger brother (II3) also had the compound heterozygous variants, but a healthy brother (II2) only had a c.3845C > A variant. The variants were validated by Sanger sequencing. The variants were not found in 1000 genomes database, ExAC database, and gnomAD database. A homology search in different species demonstrated that the proline at amino acid residue 1282 was highly evolutionarily conserved (Fig. 1E). The missense variant was predicted to be probably damaging with PolyPhen-2 score of 1.00, be deleterious with SIFT score of 0.00, and be disease causing by MutationTaster. The splice site variant changed the splice score from 0.91 to 0 predicted by NetGene2 server. The cDNA sequence demonstrated that there was a strand of RNA with a skip of exon 20 (Fig. 1F). qPCR measurements showed that a significant reduction of the SYNJ1 transcript expression was found in both patients' lymphocytes compared to those of control cells (Fig. 1G).

4. Discussion

SYNJ1-related diseases covered a group of heterogeneous symptoms such as seizures, Parkinsonism, dystonia, cognitive impairment, and other severe neurodegenerative symptoms. Dystonia can be a concomitant symptom, evenly an initial symptom, of PD [9]. When dystonia is one of the presenting features of PD, the PD patients are usually younger than PD patients in general. We summarized the clinical data of patients with *SYNJ1* gene mutations previously reported (Table 1).

Among the 21 cases with *SYNJ1* gene mutations, the symptom of dystonia was observed in 7 cases who presented with trunk dystonia [4], dystonic postures in both hands and feet [2], dystonic posture in the arms [10], jerky involuntary movements in the lips and tongue [2], and opisthotonus [7]. Our siblings showed a triad of symptomatic progression including diplopia, dystonia, and Parkinsonism with the onset age around 30. Similarly, they presented with severe trunk dystonia, dystonic postures of unilateral arm, and feet dystonia. The dystonic symptoms were outstanding in the siblings, preceded the Parkinsonism symptoms, and became the main symptoms. Interestingly, the dystonic symptoms were often exacerbated by cold and sudden new actions, which were similar to those found in paroxysmal dyskinesias, while a baseline dystonia was persistent in our patients, which was different from the features of paroxysmal dyskinesias. Collectively, our patients displayed a symptomatic combination of dystonia and Parkinsonism. Therefore, the expanding list of genes assigned to the "dystonia-Parkinsonism symptom" should also include the *SYNJ1* gene based on our observation [9].

Oculomotor disturbances such as supranuclear vertical gaze limitation or eyelid apraxia were reported in some patients with mutations in the *SYNJ1* gene [3]. Similarly, a limitation of eyeball abduction was observed in our patients. Although no obvious structural abnormalities were identified in the areas important for the control of eye movements in our patients, eyeball movement abnormalities were common in PD due to the underlying failure of neural circuits [11]. Future study is warranted to better define the phenotypic spectrum and pathogenesis of oculomotor disturbances in the SYNJ1-related diseases.

A total of 13 mutations were found in the SYNJ1-related diseases (Table 1). Our patient had a novel compound heterozygous state with a missense variant and a premature stop variant in the *SYNJ1* gene. The variant combination was similar to those in cases reported by Bouhouche and colleagues [6] or Romdhan and colleagues [10]. The missense variant (p.P1282L) is located at the C-terminal domain of SYNJ1 protein containing Asn-Pro-Phe repeats that functions as a protein-protein interactor and binds a variety of SH3 domain-containing proteins such as amphiphysin and endophilin [8], and might preserve a partial enzymatic property of dephosphorylation for phosphatidylinositol 4,5-bisphosphate. The acceptor splice site variant was proved to result in a skip of exon 20, and cause a significant reduction of SYNJ1 transcript expression. Therefore, the variants would be qualified as pathogenic according to the American college medical genetics and genomics criteria.

Due to the rarity of the disease, there were limitations in the quality of available treatment options for SYNJ1-related diseases. Overall, the seizures were intractable to most antiepileptic medications. Most patients with Parkinsonism symptoms were poor responsive to levodopa or dopamine agonists, and dyskinesias were soon induced by low-dose levodopa [2], though very few cases showed a moderate improvement to levodopa, bromocriptine, or biperiden [3,5,10]. Interestingly, we found that clonazepam can effectively resolve the dystonic symptoms in our patients. Clonazepam is a gamma-aminobutyric acid receptor alpha 1 (GABARA1) agonist and can enhance GABA-gated chloride channel function. Clonazepam also can effectively relieve the symptoms of stiff-person syndrome associated with anti-amphiphysin antibody. Experimental studies in patients with stiff-person syndrome showed compromised tonic inhibition of GABAergic presynaptic terminals as an underlying pathomechanism [12]. SYNJ1 plays some key roles in regulating presynaptic processes, and can bind with amphiphysin through the proline-enriched domain. Therefore, clonazepam might recover the dystonic inhibition through improving the GABAergic transmission. However, the detailed pharmacology of clonazepam for SYNJ1-related dystonia still needs to be investigated.

In summary, our data should be considered a preliminary observation, as it is based on a single follow-up assessment of two siblings. Thus the identification of additional families with *SYNJ1* mutations and their follow-up are warranted to better define both the phenotypic spectrum and the treatment responsiveness to clonazepam.

Disclosure statement

The authors declare no conflicts of interest.

Declarations of interest

None.

Financial declarations

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Author contributions

The study was designed by HD, GX and ZJ. HD drafted the manuscript. CL and ZS carried out detailed clinical assessments of both patients. All the genetic experiments, bioinformatics analysis and interpretation of results were carried out by HY. XL gave critical advice, and helped drafting the manuscript. All authors critically reviewed the manuscript for intellectual content and agreed on the final version of manuscript for publication.

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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.2018.11.020.

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