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Co-occurring *SYNJ1* and *SHANK3* variants in a girl with intellectual disability, early-onset parkinsonism and catatonic episodes

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Parkinsonism in childhood and adolescence is a clinically and genetically heterogeneous condition, often occurring in the setting of complex neurodevelopmental or neurodegenerative disorders. While the association between parkinsonism and psychiatric features has been well characterized in adulthood, it still represents a poorly investigated field in childhood. Moreover, the association of parkinsonism and catatonic features is anecdotal.

This 19 year-old girl was born after a normal a pregnancy and delivery from nonconsanguineous healthy parents. Neonatal period was uneventful and developmental milestones normal until the age of 18 months when developmental stagnation with prominent language involvement became evident, leading to global developmental delay at the age of 3 years and mild intellectual disability (ID) at the age of 6.

The clinical condition remained stable until the age of 13 years, when she presented with subacute onset of parkinsonism, pseudobulbar signs, facial grimacing, visual hallucinations, and severe apathy (video 1). This status rapidly responded to levodopa (video 2), and for two years the patient had no psychiatric exacerbations and showed good control of motor symptoms. Cognitive re-evaluation at the age of 13 years disclosed a moderate ID. At the age of 16, under levodopa treatment, she presented with phases of perseveration and behavioral dyscontrol, alternated with akinetic mutism, bizarre postures, and mannerisms. Psychotic features and rapid mood changes were also observed. Levodopa withdrawal resulted in a worsening of motor symptoms without any amelioration of psychiatric status. DaTSCAN imaging and brain MRI were normal. Epilepsy was not part of her clinical presentation.

High dose alprazolam (6 mg/day) and lithium (750 mg/day) resulted in a stabilization of psychiatric symptoms and prevented new exacerbations. Dopaminergic therapy (L-DOPA/carbidopa, 200/50 mg/day) was restarted with clear motor improvement, but the neurological status worsened over the next two years with cognitive regression, axial dystonia, and nonselective gaze palsy. At the last follow-up at the age of 19 years, after two years of a combination therapy with high dose alprazolam, lithium, and levodopa her cognitive and psychiatric status had much improved with no further deterioration.

Trio-based WES analysis identified co-occurrence of *SHANK3* and *SYNJ1* variants. The c.2223+1G > A intronic change in *SHANK3* (Table S1), affecting the donor splice site of exon 19, was shown to be *de novo* (Fig. 1A), did not occur in the healthy brother, was predicted to be disruptive by *in silico* tools (CADD = 26.4), and was not found in public databases. This change was classified as pathogenic according to ACMG criteria (PVS1, PS2, PS3, PM2, and PP3). A minigene assay was performed to validate the disruptive consequences of this variant on mRNA processing. COS1 cells were transiently transfected with pSPL3 constructs containing either the wild-type or mutant *SHANK3* genomic region encompassing exon 19. Sequencing of the processed mRNA products revealed aberrant splicing of the mutant allele (Fig. 1B). *De novo*, loss-of-function mutations in *SHANK3* cause a wide spectrum of neurodevelopmental and psychiatric disorders, including autism, isolated ID, schizophrenia, and catatonic episodes, and are associated with Phelan McDermid syndrome [1]. While *SHANK3* haploinsufficiency is likely to underlie the ID and catatonic episodes occurring in the patient, movement disorders have not previously been reported as part of the phenotypic spectrum linked to *SHANK3* mutations.

Besides the inactivating *SHANK3* mutation, three variants in *SYNJ1* (Table S1), a known gene associated with autosomal recessive (AR) early-onset Parkinson disease (PARK20) [2–4], were identified. A rare maternally inherited change [c.548A > C, p.(Asn183Thr), MAF = 3.2×10^{-5}] was predicted to be "deleterious" by *in silico* tools (CADD = 18), and affected a highly conserved residue among *SYNJ1* orthologues, which is located in the SAC1-like phosphatase domain, a mutational hotspot for pathogenic *SYNJ1* variants. This change was classified as "likely pathogenic" according to ACMG criteria (PM1, PM2, PP2, and PP3). Molecular dynamics (MD) simulations showed that the p.Asn183Thr substitution promotes a local structural rearrangement of the hydrogen bond network, with long range consequences on protein structure and flexibility, likely impacting catalysis (Fig. S1).

Two relatively common variants [c.3596-29T > G, MAF = 0.011; c.4358G > A, p.(Gly1453Glu), MAF = 0.012] were inherited from the father and were considered as benign by most predictive tools. While a minigene assay did not provide any evidence supporting a deleterious effect of the intronic change on mRNA processing (Fig. 1B), at least under the used experimental conditions, Gly^{1453} is located adjacent to

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the EPS15 binding motif and close to the clathrin and clathrin adaptor protein complex 2 (AP2) binding sites, and a disruptive effect of the glycine-to-glutamic acid substitution on binding to SINJ1 interactors cannot be ruled out. While we failed in identifying a second *bona fide* variant in *SYNJ1*, the neurologic features observed in the patient are highly suggestive of the involvement of biallelic inactivating variants in this gene.

SHANK3 encodes a critical scaffolding protein in excitatory synapses involved in the maturation of dendritic spines and in neurotransmission mediated by NMDA and AMPA receptors [5]. Defective SHANK3 function is one of the most important genetic determinants implicated in the pathogenesis of catatonia, due to dysfunction of the postsynaptic NMDA receptor-SHANK3 unit. Beside its role in postsynaptic density, SHANK3 can directly bind to hyperpolarization-activated cyclic nucleotide-gated channel proteins (HCN) mediating I(h) currents at the postsynaptic sites and controlling rhythmic activity of spontaneously firing neurons. Loss of SHANK3 severely impairs I(h) currents during neuronal development. External lithium has a positive effect on HCN gating, which can explain the positive outcome of lithium administration on catatonia-like deterioration in patients with dominant *SHANK3* mutations [6], as well as in our patient. *SYNJ1* encodes the synaptojanin-1, a phosphoinositide phosphatase involved in synaptic vesicle dynamics. Of note, parkinsonism caused by impaired SYNJ1 activity can be, as in our patient, levodopa-responsive and associated with dystonia, oculomotor disturbances, and cognitive deterioration [2–4].

In conclusion, our findings identify a novel pathogenic mutation in *SHANK3* and further document the power and limits of exome sequencing.

1. Methods

1.1. Methods are reported as supplementary information

1.2. Video legend

Video 1. Onset of parkinsonism at the age of 13 years, manifesting with hypomimia, dysarthric and hypophonic speech, bradykinesia, and abolished arm swings during gait. Facial grimacing, upper limb dystonic posturing, and slight axial dystonia were also observed.

Video 2. Patient at the age of 14 after 6 months under levodopa

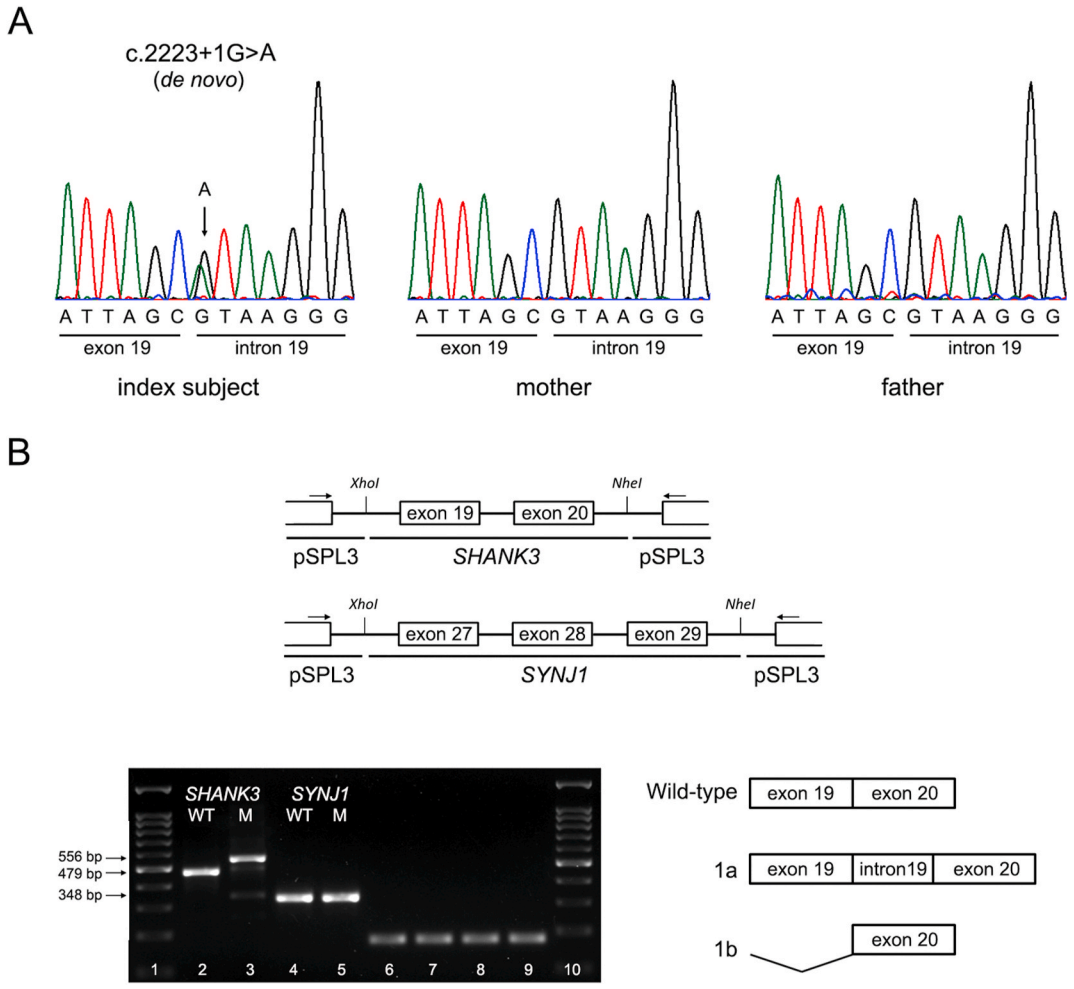

Fig. 1. (A) Electropherograms showing the *de novo* origin of the *SHANK3* splice site variant. (B) Minigene assay performed to evaluate the impact of the c.2223+1G > A (*SHANK3*) and c.3596-29T > G (*SYNJ1*) intronic changes on mRNA processing. Four genomic fragments encompassing exons 19 and 20 of *SHANK3* and exons 27–29 of *SYNJ1* (wild-type and mutant forms) were amplified and cloned into the pSPL3 vector (upper panel). COS1 cells were transiently transfected; 24 h after transfection, cells were harvested. cDNAs obtained from retrotranscription of total RNA were amplified using a pSPL3-specific primer pair (arrows). PCR products were separated by 2% agarose gel electrophoresis (lower panel, left). Lanes 1 and 10, 100 bp molecular weight marker; lane 2, *SHANK3* WT allele; lane 3, *SHANK3* M allele; lane 4, *SYNJ1* WT allele; lane 5, *SYNJ1* M allele; lanes 6–9, *GAPDH* amplification from each cDNA indicating absence of genomic DNA contamination. A schematic representation of the consequences of the *SHANK3* splice-site mutation is shown (lower panel, right). Sanger sequencing identified two major aberrant products: 1a (556 bp; retention of 77 bp of intron 19) and 1b (348 bp; skipping of exon 19).

treatment leading to a dramatic improvement of hypomimia and speech as well as walking with recovery of arm swing and increased speed and stride length.

Financial disclosure/conflicts of interest

The authors report no conflicts of interest relevant to the manuscript.

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Declaration of competing interest

None.

Final disclosures

Vincenzo Leuzzi has served on scientific advisory boards for Nutricia, BioMarin, and PTC Therapeutics.

Ethical approval and informed consent

All authors declare that the manuscript is in accordance with the statement of ethical standards for manuscripts submitted to *Parkinsonism and related disorders*. Parents have consented for video publication and provided a signed release form authorizing the offline and/or online distribution of this video material.

Data availability

Any anonymized data not published within the article will be shared by request from any qualified investigator.

Authorship

SG: conception and design of the study, analysis and interpretation of data, drafting the article, revising the manuscript critically for important intellectual content.

SM: conception and design of the study, analysis and interpretation of data, drafting the article, revising the manuscript critically for important intellectual content.

LP: functional analysis and interpretation of data.

AT: analysis and interpretation of data, drafting the article, revising the manuscript critically for important intellectual content.

MV: analysis and interpretation of data.

SP: analysis and interpretation of data.

AC: analysis and interpretation of data.

GC: structural analysis.

FG: acquisition of data.

SC: acquisition of data.

MT: analysis and interpretation of data, drafting the article, revising the manuscript critically for important intellectual content.

VL: analysis and interpretation of data, drafting the article, revising the manuscript critically for important intellectual content.

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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.2020.12.022.

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