Truncating variants in the *SHANK1* gene are associated with a spectrum of neurodevelopmental disorders

May, Halie J; Jeong, Jaehoon; Revah-Politi, Anya; Cohen, Julie S; Chassevent, Anna; Baptista, Julia; Baugh, Evan H; Bier, Louise; Bottani, Armand; Carminho Amaro Rodrigues, Maria Teresa; Conlon, Charles; Fluss, Joel Victor; Guipponi, Michel; Kim, Chong Ae [**and 13 more**]

Case Reports

Case Report: Individual 1

Individual 1 is an 11-year-old male born to Caucasian parents of Italian background. Clinical synopsis includes learning disability, expressive language deficits, persistent low tone, attention deficit and hyperactivity disorder (ADHD) and anxiety disorder. Family history is significant for a maternal first cousin with a learning disability and a paternal uncle with bipolar disorder. The pregnancy was complicated by non-reassuring fetal tracing (variable deceleration), but was otherwise normal. Labor was induced at 38 weeks gestation and he was delivered with vacuum-assisted vaginal delivery. Labor was complicated by meconium stained amniotic fluid and nuchal cord. Birth weight was 3.34-kg (34^{th} centile, -0.42 SD), birth length was 50.5 cm (55^{th} centile, +0.13 SD), and head circumference at birth was 33.5 cm (15^{th} centile, -1.05 SD). He was noted to be floppy and pale, and was intubated for tracheal suctioning. Apgar scores were 2, 6, and 7 at 1, 5, and 10 minutes, respectively.

His mother reports that he never crawled, and sat unassisted at a late age. He began walking at 16 months of age. He has persistent low tone, functional weakness and decreased endurance. There is also difficulty with balance, motor planning, and overall mild gross motor delay. His first words were at 2 years of age, and there were still concerns with speech by age 3, including significant expressive language deficits and articulation disorder. He received physical therapy (PT), occupational therapy (OT), and speech therapy (ST) from 18 months until age 3. He was diagnosed with ADHD and auditory processing disorder at age 5 by a developmental pediatrician, and behavioral therapy was recommended. He now has an Individualized Education Program (IEP) with OT/PT 2 times per week. He has difficulty with executive functioning, verbal reasoning and problem solving. He is able to study independently and do his homework, but mathematic concepts are difficult. There is a history of generalized anxiety and difficulty with transitions, and he is constantly worried. He experiences highs and lows and changes in mood, and is followed by a psychiatrist and a therapist monthly. He is presently taking guanfacine 2mg nightly at bedtime. No brain MRI has been performed.

Surgical history is significant for tonsillectomy. Physical examination during a genetic evaluation at 11 years and 9 months was unremarkable with no dysmorphic features appreciated. His weight was 36 kg (51st centile, +0.01 SD) and his height was 143 cm (47th centile, -0.07 SD). Head circumference was not obtained at that time, but was noted to be 50.5 cm (36th centile, -0.36 SD) at a previous evaluation at 5 years and 10 months.

FMR1 analysis revealed he is hemizygous for a premutation with 78 CGG repeats. Chromosomal microarray analysis (CMA) was normal. Initial trio research exome sequencing (ES) identified a *de novo* frameshift variant (NM_016148.5, c.3488dupC, p.Ser1164fs) in *SHANK1*. No other variants were identified or reported.

Case Report: Individual 2

Individual 2 is a 6-year-old female born to Egyptian parents. Clinical synopsis includes autism, global developmental delay, intellectual disability, mixed receptive expressive language disorder (MRELD) and behavioral problems. Family history is unremarkable for similarly affected individuals. She has an unaffected older sister in whom targeted testing did not detect the variant, and she also has an unaffected younger brother. The pregnancy was uncomplicated. She was delivered by C-section at 39 weeks gestation with no complications. Birth weight was 3.08 kg (22th centile, -0.77 SD). Birth length and head circumference are unavailable.

She began walking between 18 and 19 months of age. She spoke her first words at 2 years of age and eventually acquired approximately ten words which she used inconsistently; however, she experienced gradual language regression at 5 years-old and is now non-verbal. She has intellectual disability and a history of generalized behavioral issues including hyperactivity and hair pulling when upset. These behavioral issues as well as poor sleep improved with risperidone. After presenting with poor social interaction, repetitive behaviors and restricted interests, she was formally diagnosed with autism by a developmental pediatrician at 3 years and 9 months of age. She was diagnosed with MRELD at that time,

and was noted to have mild hypotonia upon physical exam. She was toilet trained at 3 years old, and at 4 years old, she could feed herself with her hands and drink from a cup, although she still has feeding difficulties related to sensory issues. She receives OT and ST, and is in a special education class.

She has no surgical history. MRI of her brain at the age of 2 years was normal. Sleep EEG performed as part of routine investigations at 3 years showed bilateral occipital spike-wave epileptogenic activity. Staring spells prompted a follow-up EEG at 4 years, which showed sharp waves in the occipital head regions bilaterally and independently on the left and right. This was interpreted to be consistent with a seizure disorder of multifocal origin, but she has not experienced a clinical seizure and has not been treated with any antiepileptic medications. Physical examination at 4 years and 8 months of age was significant for a prominent forehead and midface hypoplasia. Her weight was noted to be 18.1 kg (78^{th} centile, +0.76 SD), height was noted to be 109 cm (95^{th} centile, +1.60 SD), and head circumference was noted to be 50 cm (37^{th} centile, -0.32 SD).

Previous normal genetic testing included *FMR1* analysis, CMA, and metabolic studies, which included plasma amino acids, urine organic acids, blood acylcarnitine profile and carnitine. Initial trio exome sequencing (ES) identified a *de novo* nonsense variant (NM_016148.5, c.3355G>T, p.Glu1119*) in *SHANK1*. Her unaffected sister underwent targeted analysis of the identified *SHANK1* variant, and was found not to have this variant. No other variants were reported.

Case Report: Individual 3

Individual 3 is a 7-year-old male born to parents of Caucasian, Mexican, and Pakistani background. Clinical synopsis includes cognitive delay and MRELD. Family history is unremarkable for similarly affected individuals. The pregnancy was uncomplicated. He was delivered vaginally at 41 weeks gestation with no complications. Birth weight was 4.31 kg (94^{th} centile, +1.52 SD). Birth length and head circumference are unavailable.

He began walking at 16 months of age. He spoke his first words at 1 year of age. Although his first words were on time, his subsequent speech development was slow. At 28 months of age, he infrequently said phrases and family members' names, but had no other words. MRELD was diagnosed at 5 years of age. At 7 years of age, his speech is still behind and he has difficulty articulating. He has persistent cognitive delays at 7 years of age. MRIs of his brain at 4, 5, and 7 years of age were all normal. He had two separate incidents of seizures at 7 years old. The first was a generalized tonic/clonic seizure with a subsequent abnormal EEG. The second looked like 3-4 minutes of generalized convulsions and vomiting and consisted of approximately 10 minutes of very agitated post-ictal state with coughing, then a more placid post-ictal time. He was started on levetiraceteam, but because of behavioral problems, he was switched to topiramate which has been tolerated. There is a history of constipation. He receives PT, OT, and ST.

He has no surgical history. Physical examination during a genetic evaluation at 5 years of age was significant for mild hypotonia, exotropia, a left epicanthal fold, and clinodactyly. The patient wears glasses for his exotropia and astigmatism. His weight was noted to be 21.5 kg (84^{th} centile, +0.99 SD), height was noted to be 115 cm (91^{st} centile, +1.34 SD), and head circumference was noted to be 50.5 cm (36^{th} centile, -0.36 SD).

Previous genetic testing included CMA and *FMR1* analysis, both of which were normal. Trio exome sequencing (ES) identified a *de novo* frameshift variant (NM_016148.5, c.3314delG, p.Gly1105fs) in *SHANK1*. No other variants were reported.

Case Report: Individual 4

Individual 4 is a 9-year-old male born to Caucasian parents of Swiss background. Clinical synopsis includes developmental delay and macrocephaly (>90th centile) associated with a mild autism spectrum disorder. Family history is significant for an 11-year-old brother diagnosed with Asperger syndrome, in whom targeted testing did not detect the variant. The pregnancy was uncomplicated. He was delivered

vaginally at 37 6/7 weeks gestation with no complications. Birth weight was 2.64 kg (7th centile, -1.47 SD), birth length was 48 cm (21st centile, -0.81 SD), and head circumference at birth was 33 cm (10th centile, -1.27 SD). The newborn was noted to have left clubfoot. Apgar scores were 9, 10, and 10 at 1, 5, and 10 minutes, respectively.

He began walking at 2 years of age and had mild gross and fine motor delays. A speech delay was also observed. On successive neurodevelopmental assessments, a global development delay characterized by reduced intelligibility, low receptive skills, oromotor dysfunction, mild autistic traits as well as generalized hypotonia and motor incoordination was observed. Repetitive behaviors, hand-flapping, and discursive speech were noticed at around 5 years of age. A formal diagnosis of autistic spectrum disorder with the ADOS-2 was established at age 10. Concurrent cognitive assessment using the Psychoeducational Profile-third edition (PEP-3) demonstrates competencies ranging from 12 to 40 months. He has severe learning difficulties, although has a good memory for names, events and people. He is toilet trained and can manage his personal hygiene independently or with some assistance. He is in a special needs school where he receives psychomotor therapy, speech therapy and ergotherapy. He has a history of generalized anxiety.

Surgical history is significant for unilateral tenectomy at 5 months old to repair his unilateral talipes equinovarus. He has not had a brain MRI. Physical examination during a genetic evaluation at 9 years of age was significant for macrocephaly, dolichocephaly, high implantation of the hair with bilateral frontal upsweep, posteriorly rotated ears, short philtrum, thick lower lip, diastema, tapering fingers, and long toes. His weight was 28.7 kg (51^{st} centile, +0.02 SD), his height was 134.5 cm (57^{th} centile, +0.16 SD), and his head circumference was 55.5 cm (>99^{th} centile, +2.17 SD).

Previous genetic testing includes CMA and *FMR1* analysis, both of which were normal. Trio exome sequencing (ES) identified a *de novo* nonsense variant (NM_016148.5 c.1198C>T, p.(Arg400*)) in *SHANK1*. His affected brother underwent targeted analysis of the identified *SHANK1* variant, and was found not to have this variant. No other variants were reported.

Case Report: Individual 5

Individual 5 is a 10-year-old male born to healthy unrelated parents of Caucasian background. Clinical synopsis includes autism, severe intellectual disability, joint laxity and macrocephaly (>98th centile). Family history is unremarkable for similarly affected individuals. The pregnancy was complicated by maternal hypothyroidism and maternal hypertension treated with labetalol. He was delivered via C-section due to the maternal health factors at 40 weeks gestation with no complications. Birth weight was 4.26 kg (92nd centile, +1.42 SD), birth length was 55 cm (97th centile, +1.84 SD), and head circumference at birth was 36.5 cm (64th centile, +0.37 SD). Apgar scores were 9, 9, at 1 and 5 minutes, respectively. Neonatal period was unremarkable with no feeding issues reported.

Parents noticed that their son showed little interest in his surroundings as a baby. He walked at 20 months of age. He started babbling at 10 months of age, but his words were unintelligible until 5 years of age. He was diagnosed with autism at 2 years and 9 months of age due to repetitive behaviors and poor language development. At age 10 years, his language is extremely limited. He understands simple commands. To date he is not yet toilet trained. Behavioral problems including aggressive outbursts towards his parents, self-biting, anxiety, and tics such as head jerks have been noted. There is persistent mild hypotonia. Gastroesophageal reflux was diagnosed at 8 years of age. He currently receives OT.

Brain MRI has not been performed. There have been some absence-type episodes but these have not been confirmed to be epileptic in nature. EEG was not done. Physical examination during a genetic evaluation at 10 years and 9 months of age was significant for full lips with a prominent cupid's bow, pes planus and generalized joint laxity with soft, doughy skin. His height was 144.5 cm (81st centile, +0.88 SD) and head circumference was 58.5 cm (>99th centile, +3.97 SD). His weight was not able to be recorded during this visit, but was noted to be 53.7kg (99th centile, +2.39 SD) at 10 years and 1 month of age.

Previous genetic testing included *FMR1* analysis and CMA, both of which were normal. Thyroid function tests showed mildly raised TSH and normal T4. Trio research exome sequencing (ES) performed through the United Kingdom (UK) Deciphering Developmental Disorders (DDD) study identified a *de novo*

frameshift variant (NM_016148.5, c.4496_4499del, p.(Gln1499fs)) in *SHANK1*. No other likely causative variants were reported.

Case Report: Individual 6

Individual 6 is an 11-year-old female born to Brazilian parents. Clinical synopsis includes severe cognitive delay and seizures. Family history is unremarkable for similarly affected individuals. The pregnancy was uncomplicated. She was delivered vaginally at 38 weeks gestation with no complications. Birth weight was 2.87 kg (15^{th} centile, -1.06 SD), birth length was 47 cm (14^{th} centile, -1.10 SD), and head circumference at birth was 32 cm (3^{rd} centile, -1.90 SD). Apgar scores were reported to be normal.

She sat at 48 months of age, and is currently unable to walk. She is nonverbal and only makes guttural sounds as a mode of expression. She has severe cognitive delay and is completely dependent for activities of daily life. Behavioral problems include placing her hands inside her mouth, but not biting hard. She receives OT, ST, and PT, and is in a special education class.

She presented with myoclonic seizures at 11 months, and developed focal to bilateral tonic-clonic seizures with motor onset at 14 months. She is taking sodium valproate and lamotrigine, as well as clobazam (benzodiazepine ansiolitic) to control her seizures. Cerebral CT performed at 11 months old was significant for ventricular enlargement and corpus callosum agenesis. There were no ischemic lesions identified. Brain MRI at 3 years and 10 months was significant for absent cerebellar vermis, and possible dysgenesis with partial fusion of the cerebral hemispheres and the thalami, suggestive of a tubulinopathy. Her most recent EEG at 11 years 5 months showed diffuse disorganization of basic activity characterized by lack of sleep elements and a higher content of irregular slow waves in the theta and delta bands, predominantly in the left hemisphere. It also revealed very frequent epileptiform paroxysms of spikes and acute waves of multifocal projection, predominately in the left anterior temporal region, during which the patient presented seven crises of eyelid clonus predominantly on the right as well as a slight cephalic version

on the left. Renal and abdominal ultrasound, and echocardiogram were normal. She has been hospitalized multiples for recurrent urinary tract infections.

Physical examination during a genetic evaluation at 11 years of age was significant for microcephaly, strabismus, nystagmus, myopia, hypertelorism, macrotia, broad nose, thick lips, short philtrum, widely spaced teeth, hypertrichosis, camptodactyly, pes planus, hypotonia, and spasticity. Weight was 24 kg (1st centile, -2.36 SD), height was 136 cm (<1st centile, -19.98 SD), and head circumference was 49.8 cm (1st centile, -2.29 SD).

Previous genetic testing included a normal CMA. Proband-only research exome sequencing (ES) identified a nonsense variant (NM_016148.5, c.650delT, p.(Leu217*)) in *SHANK1*. Sanger sequencing was performed to confirm absence of variant in parent samples and validate *de novo* inheritance[1]. No other likely causative variants were identified or reported.

Additional Case Information

We have also identified an individual with a maternally inherited in-frame indel c.2265_2267dup (p.Val756dup). This individual was not included in our cohort as effect of the in-frame indel is unknown. His phenotype includes developmental delay, intellectual disability, ASD, ADHD, and neuropsychological diagnoses including oppositional defiant disorder, bipolar disorder, behavioral contact disorder, and schizophrenia. Maternal history included bipolar disorder, anxiety, post-traumatic stress disorder, depression, and a history of seizures (type unknown) at 17 years of age with abnormal EEG. She does not require anti-epileptic drugs (AEDs) to control her seizures. Proband also has a maternally inherited 560.8 kb duplication of uncertain significance in chromosomal region 3q25.32, spanning the entire *SHOX2*, *RSRC1*, and *MLF1* genes, as well as a VOUS in the *NPEPPS* gene (NM_006310.4:c.1466delC (p.Pro489fs)) that was not maternally inherited. It is possible that this individual's 3q25.32 duplication and/or variant in *NPEPPS* contributed to aspects of his phenotype. Of note, this proband has a brother with ASD who does not share this variant in *SHANK1*.

Supplementary Methods

Exome sequencing

Exome sequencing for patient 1 was performed in the Institute for Genomic Medicine (IGM) at Columbia University Irving Medical Center. For this analysis sequencing libraries were prepared with the KAPA library preparation kit; targeted capture of the exome was performed with the Roche SeqCap EZ Exome v3.0 exome enrichment kit. Paired-end sequencing reads were generated on an Illumina HiSeq 2500 or NovaSeq 6000. Sequencing reads were aligned to the reference genome (GRCh37/hg19) according to standard IGM protocols. Variants were annotated with SnpEff 4.2. After annotation, qualifying variants were prioritized for analysis. Trio sequence data were analyzed with an updated version of our established trio sequencing framework[2], which identifies ‘‘qualifying’’ genotypes not observed in the parents, 1,395 control individuals from the Institute for Genomic Medicine, or two external databases of 6,503 and 60,706 control individuals provided by the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP6500SI [March 2013 release]) and the Exome Aggregation Consortium (ExAC Browser v.0.3 [January 2015 release]), respectively. The presence of the variant was confirmed via Sanger sequencing.

Clinical exome sequencing for patients 2 and 3 were performed at GeneDx. Using genomic DNA from the proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the IDT xGen Exome Research Panel v1.0. Massively parallel (NextGen) sequencing was done on an Illumina system with 100bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol has been previously described[3]. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Exome sequencing for patient 4 was captured using the Agilent SureSelect QXT Human All Exon V5 kit and sequenced on a NextSeq500 instrument (Illumina). Reads mapping and variant calling were performed using BWA 0.7.13, Picard 2.9.0, GATK HaplotypeCaller 3.7 and annotated with ANNOVAR

2017-07-17 and UCSC RefSeq (refGene) downloaded on 2018-08-10. The variants were searched in various databases including dbSNP151, gnomAD 2.1, ClinVar 2018 and HGMD 2016. Pathogenicity prediction scores were obtained for missense variants using SIFT[4], PolyPhen[5], MutationTaster[6], CADD[7]. Splicing effect alterations were assessed using dbscSNV [8].

Clinical exome sequencing for patient 5 (Decipher# 299762) was performed as part of the DDD project, Wellcome Sanger Centre, Cambridge, United Kingdom [9]. Material and methods description outlined in Wright et al[10].

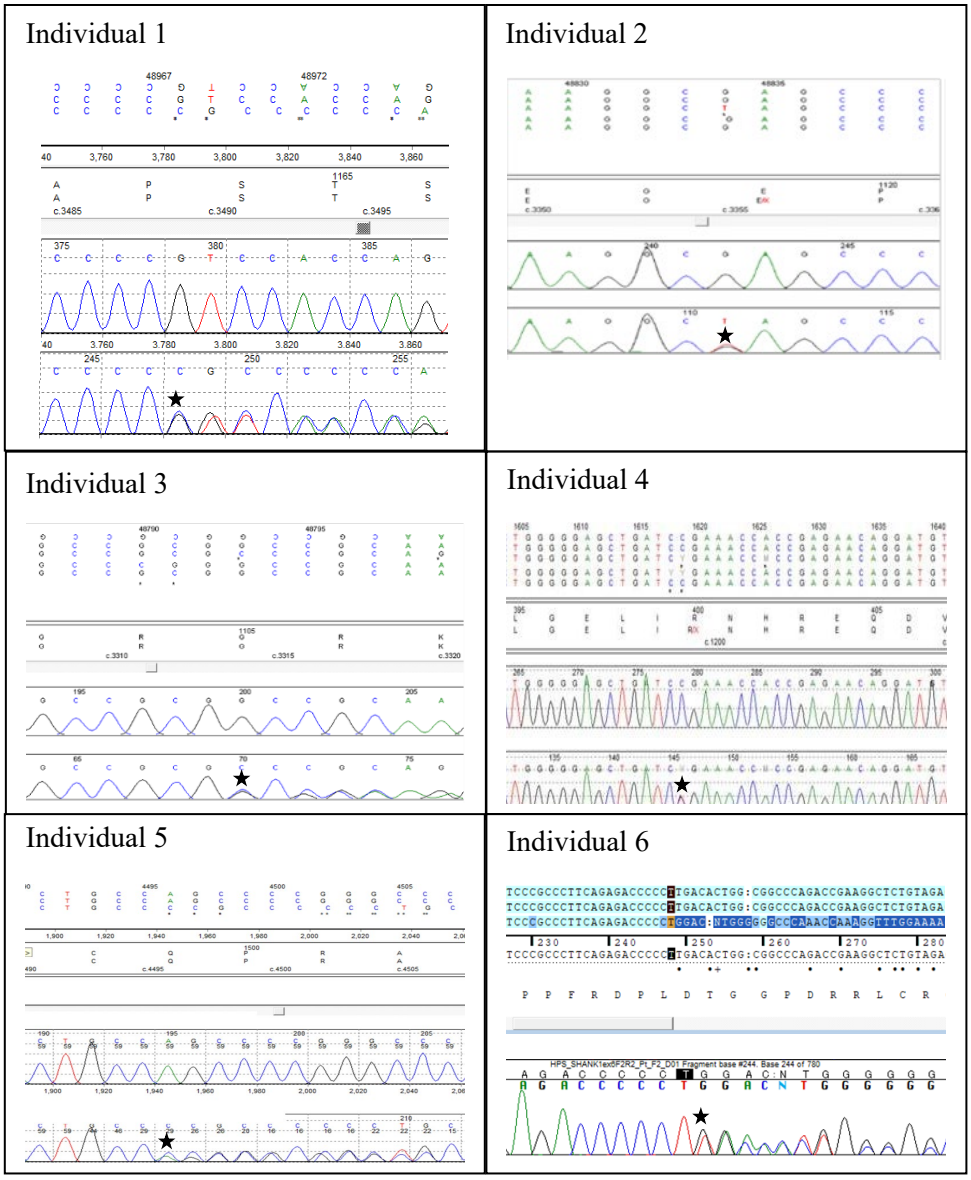
Research exome sequencing for patient 6 was performed on a NovaSeq platform and captured by SureSelect Human All Exon v6. Briefly, reads were aligned to the human genome reference sequence GRCh37 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/) using NovoAlign (v3.02.13) (http://www.novocraft.com/products/novoalign/) and PCR duplicates were excluded using Picard (https://broadinstitute.github.io/picard/). Indel realignment and recalibration of base-quality scores were performed using the Genome Analysis Tool Kit (GATK) (3.7-0) (https://software.broadinstitute.org/gatk/). The single nucleotide variant analysis was performed as described previously[1]. The presence of the variant was confirmed via Sanger sequencing.

Bioinformatics analysis

Human SHANK1 amino acid sequence and predicted amino acid sequences of SHANK1 variants (Gly1105Afs or Glu1119* variants) were analyzed for amino acid composition, secondary structure, and solvent accessibility using the PredictProtein server (www.predictprotein.org).

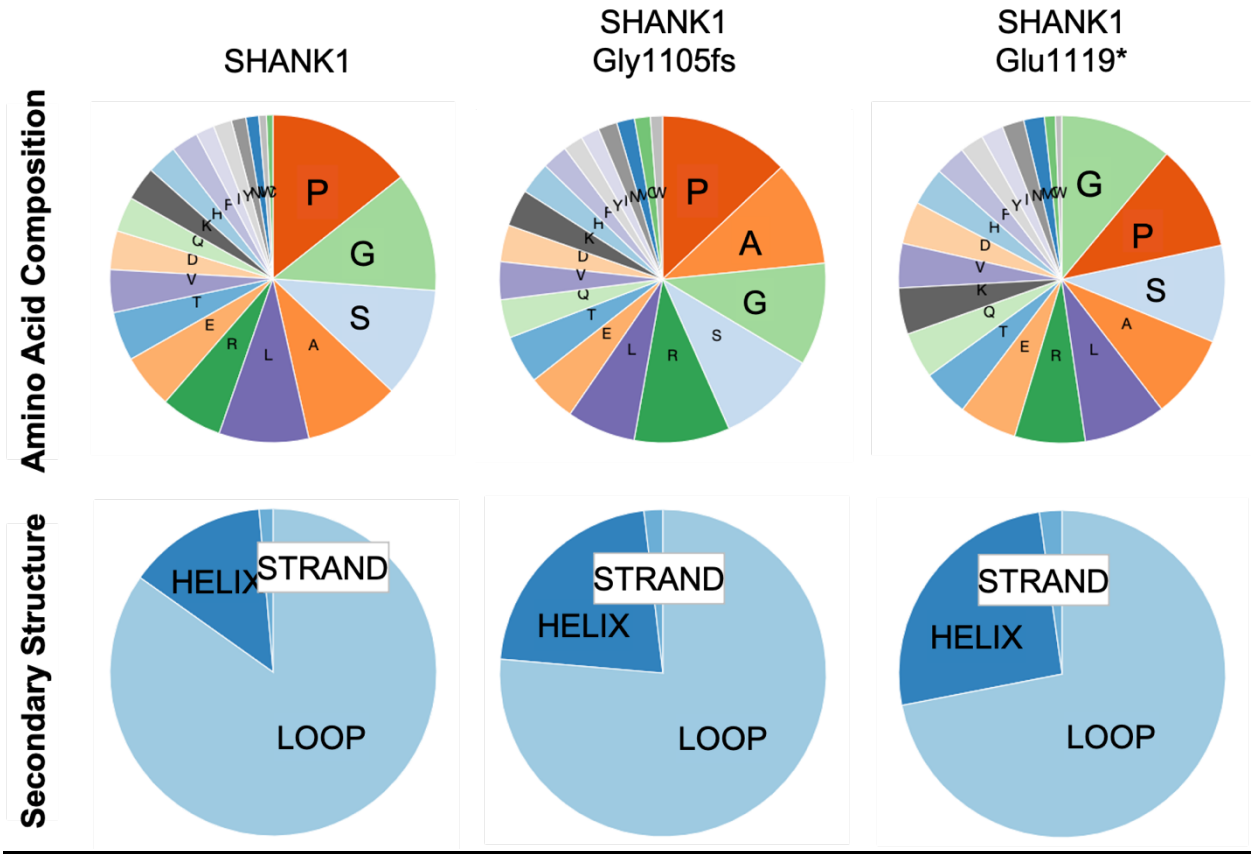
Figures and Legends

Figure S1. Sanger sequencing of *SHANK1* variants identified in patients.



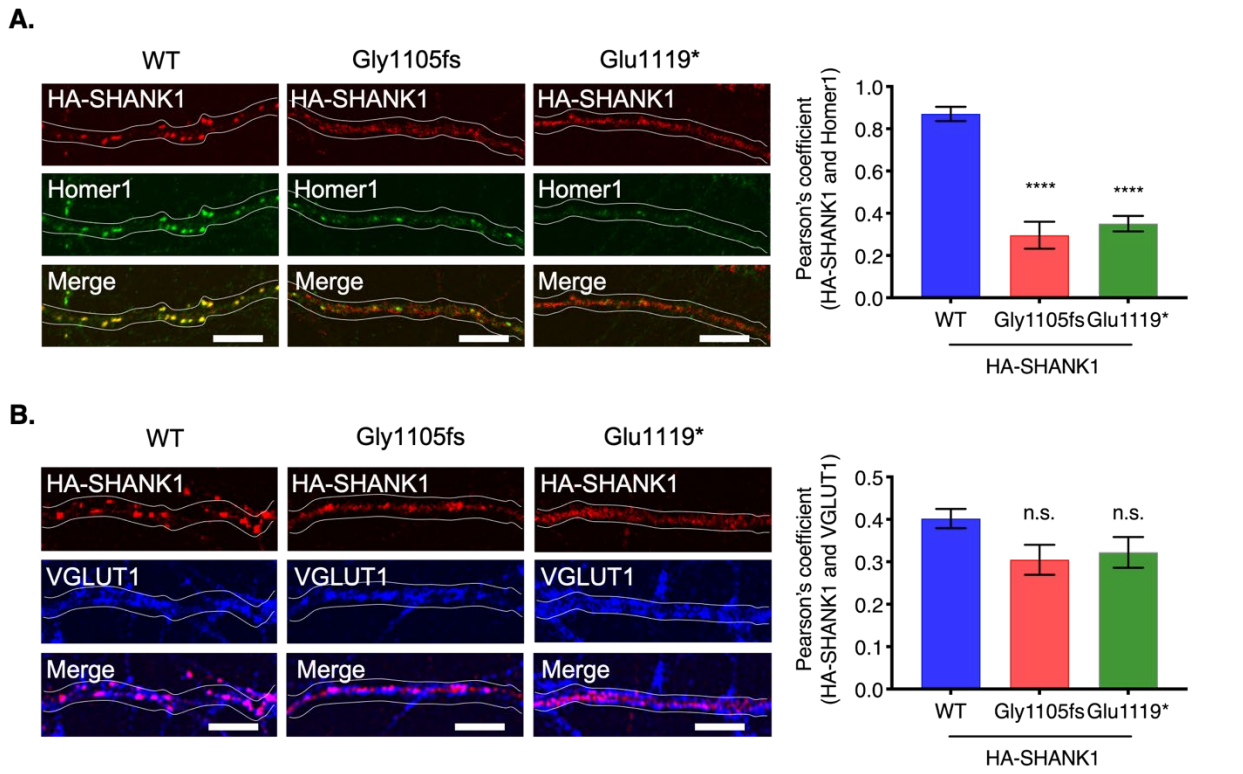
Sanger chromatograms of the identified *SHANK1* variants. *SHANK1* variants are marked with stars. From left to right: Patient 1: c.3488dup; Patient 2: c.3355G>T; Patient 3: c.3314del; Patient 4: c.1198C>T; Patient 5: c.4496_4499del; Patient 6: c.650del

Figure S2. Bioinformatic analysis of the effect of Gly1105fs and Glu1119* variants on the protein features.



Protein sequences of SHANK1 Gly1105fs and Glu1119* proteins were compared to SHANK1 WT protein sequence. Amino acid composition and secondary structure were analyzed by bioinformatics tools (www. predicprotein.org).

Figure S3. The effect of Gly1105fs and Glu1119* variants on SHANK1 colocalization with synaptic proteins.



HA-*SHANK1* (WT, Gly1105fs, or Glu1119*) was expressed in cultured rat hippocampal neurons. HA-SHANK1 was labeled with anti-HA and Alexa 555-conjugated secondary antibody (red). Endogenous Homer1 was labeled with anti-Homer and Alexa 488-conjugated secondary antibody (green). Endogenous VGLUT1 was labeled with anti-VGLUT1 and Alexa 647-conjugated secondary antibody (blue). White lines indicate dendritic outlines. Regions from three dendrites per each neuron were analyzed for Pearson's coefficient. Graph indicates mean ± SEM (n = 7-12). ****P < 0.0001 using one-way ANOVA with Dunnett's multiple comparison test (Scale bar, 5 μm).

Supplementary Table

Table S1. Comparison of clinical features across all reported patients with LOF variants in *SHANK1*

Symptoms	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	New patients	Sato et al. 2012[11]	Wang et al. 2016[12]	Fromer et al. 2016[13]	Total (when compared with assessed individuals)
Motor development/ delays	Yes	No	No	Yes	No	Yes	3/6	1/7	1/1	1/1	6/15
Speech & language development/ delays *Said first words on time, however has a dx of MRELD[a] or other speech delays	Yes	Yes	*Yes	Yes	Yes	Yes	6/6	3/7	1/1	N/A[b]	10/14
Cognitive delays/ID learning disabilities	Yes	Yes	Yes	Yes	Yes	Yes	6/6	0/7	1/1	N/A[b]	7/14
Autism spectrum disorder	No	Yes	No	Yes	Yes	No	3/6	5/7	1/1	N/A[b]	9/14
Other neuropsychiatric and behavioral issues	Yes	Yes	No	Yes	Yes	Yes	5/6	3/7	0/1	1/1	9/15
Seizures	No	No	Yes	No	No	Yes	2/6	N/A[b]	0/1	N/A[b]	2/7
Hypotonia	Yes	Yes (mild)	Yes (mild)	No	Yes (mild)	No	4/6	N/A[b]	N/A[b]	N/A[b]	4/6
Headaches/migraines	Yes	No	No	No	No	No	1/6	N/A[b]	N/A[b]	N/A[b]	1/6
Macrocephaly (≥ 2SD)	No	No	No	Yes (+2.17 SD)	Yes (+3.97 SD)	No	2/6	N/A[b]	0/1	N/A[b]	2/7
Microcephaly (≤2SD)	No	No	No	No	No	Yes (-2.29 SD)	1/6	N/A[b]	0/1	N/A[b]	1/7
Brain imaging abnormalities	N/A[d]	No	No	N/A[b]	N/A[b]	Yes	1/3	1/1 (6/7 N/A[b])	0/1	N/A[b]	2/5
Dysmorphic features	No	Yes	Yes	Yes	Yes	No	4/6	N/A[b]	0/1	N/A[b]	4/7

MRELD[a]: mixed receptive language disorder, N/A[b]: not assessed

Web resources

Consensus Coding Sequence (CCDS), https://www.ncbi.nlm.nih.gov/CCDS/

dbSNP: https:// https://www.ncbi.nlm.nih.gov/snp/

ExAC Browser: http://exac.broadinstitute.org/

Ensembl genome assembly GRCh37: http://grch37.ensembl.org/Homo_sapiens/Info/Index

Ensembl Variant Effect Predictor (VEP): http://grch37.ensembl.org/Homo_sapiens/Tools/VEP

gnomAD browser: https://gnomad.broadinstitute.org/

NHLBI Exome Sequencing Project (ESP) Exome Variant Server: http://evs.gs.washington.edu/EVS/

OMIM: http://www.omim.org/

Pubmed: https://www.ncbi.nlm.nih.gov/pubmed

The Human Gene Mutation Database (HGMD): http://www.hgmd.cf.ac.uk/ac/index.php

CADD: https://cadd.gs.washington.edu/

MutationTaster: http://www.mutationtaster.org/ChrPos.html

SIFT: https://sift.bii.a-star.edu.sg/

PolyPhen: http://genetics.bwh.harvard.edu/pph2/index.shtml

dbSNP151:

http://bioconductor.riken.jp/packages/3.8/data/annotation/html/SNPlocs.Hsapiens.dbSNP151.GRCh38.html

Supplementary References

1. Uchiyama Y, Yamaguchi D, Iwama K, et al. Efficient detection of copy-number variations using exome data: Batch- and sex-based analyses. Hum Mutat. 2021;42(1):50-65.
2. Zhu X, Petrovski S, Xie P, et al. Whole-exome sequencing in undiagnosed genetic diseases: interpreting 119 trios. Genet Med. 2015;17(10):774-781.
3. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med. 2016;18(7):696-704.
4. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31(13):3812-3814.
5. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248-249.
6. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):361-362.
7. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46(3):310-315.
8. Jian X, Boerwinkle E, Liu X. In silico prediction of splice-altering single nucleotide variants in the human genome. Nucleic Acids Res. 2014;42(22):13534-13544.
9. Firth HV, Richards SM, Bevan AP, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 2009;84(4):524-533.
10. Wright CF, Fitzgerald TW, Jones WD, et al. Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet. 2015;385(9975):1305-1314.
11. Sato D, Lionel AC, Leblond CS, et al. SHANK1 Deletions in Males with Autism Spectrum Disorder. Am J Hum Genet. 2012;90(5):879-887.
12. Wang T, Guo H, Xiong B, et al. De novo genic mutations among a Chinese autism spectrum disorder cohort. Nat Commun. 2016;7:13316.
13. Fromer M, Pocklington AJ, Kavanagh DH, et al. De novo mutations in schizophrenia implicate synaptic networks. Nature. 2014;506(7487):179-184.