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Identification of a novel in-frame *de novo* mutation in *SPTAN1* in intellectual disability and pontocerebellar atrophy

Supplementary Information

Gene screening and bioinformatics

PCR primers targeting all coding exons and intronic splice junctions of *SPTAN1* (longest isform NM_001130438.2) were designed using Exon-Primer from the UCSC Genome Browser (Table S1). PCR was done in 384 well plates using 5 ng of genomic DNA, according to standard procedures. The PCR products were sequenced at the McGill University and Genome Quebec Innovation Centre (Montreal, Canada) (www.genomequebecplatforms.com/mcgill/) on a 3730XL DNA Analyzer. In each case, rare mutations (identified only once in the disease cohort) were confirmed by re-amplifying the fragment and re-sequencing of the proband and both parents using reverse and forward primers. PolyPHRED (v.5.04), PolySCAN (v.3.0) and Mutation Surveyor (v.3.10 Soft Genetics Inc.) were used for mutation detection.

Cell culture, transfection, and immunofluorescence

Mouse neuroblastoma 2A (N2A) cells were grown as previously described.[1] N2A cells on 200 μg/ml poly-D-lysine (Millipore)/20 μg/ml laminin (Invitrogen)-coated glass coverslips (in 24 well plates) were transfected with 200 ng of plasmid DNA using FuGene6 reagent (Roche diagnostics, Tokyo, Japan). After 4 hrs, culture medium was changed to low serum medium (5% FBS) with 20 μM all trans-retinoic acid (Sigma) in order to induce neural differentiation, and cells were subsequently cultured for 24 hours. Primary neuronal culture and transfection were also performed as previously described.[2]

N2A cells and primary neurons were fixed with 2% paraformaldehyde in PBS for 20 min, and permeabilized with 0.25% and 0.1% Triton X-100 for 5 min, respectively. Cells were then blocked with 10% normal goat serum for 30 min. The following primary antibodies were used: mouse anti-Flag M2 (1:1000 dilution; Sigma), rabbit anti-β-II spectrin (1:400 dilution; Abcam), rabbit anti-β-III spectrin (1:400 dilution; Abcam). Alexa Fluor 488- and 546-conjugated secondary antibodies to rat and mouse primary antibodies (Invitrogen) were used. Photographs were taken as previously described [2]. For evaluation of spectrin aggregation in N2A cells and primary neurons at 7 days in vitro with anti-Flag antibody, more than 50 and 100 isolated transfected neurons were analyzed in each experiment,

respectively, and representative cells were photographed. The results were confirmed in three independent experiments. Non-repeated measures using ANOVA followed by a Bonferroni post-test was applied for examination of statistical significance ($P<0.01$).

Clinical description of patients with *de novo* mutations in *SPTAN1*

Patient-1. Patient 1 is a 9 year-old boy born to non-consanguineous French Canadian parents. Family history is positive since the patient also has a sister with mild ID. Perinatal history is unremarkable. Cognitive and adaptive assessments performed at 6 years and 6 months of age with the Wechsler Intelligence Scale for Children-III and the Vineland Adaptive Behavioural Scale were consistent with mild intellectual disability. The patient also shows attention deficit with impulsivity. At 9 years of age, height is 130.1 cm (75th centile), weight is 26.5 kg (75-90th centile) and head circumference is 55.5 cm (97th centile). On physical examination, no specific dysmorphic features were noticed. Neurological examination was unremarkable. Karyotyping (at a resolution of 750 bands), subtelomeric FISH studies and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Brain CT scan performed at 5 years and 6 months of age was normal.

Patient-2. Patient-2 is a 11 year-old boy born to non-consanguineous French Canadian parents. Family history is negative. He was delivered at term after an unremarkable pregnancy. APGAR score was 9^{1}, 10^{5} and 10^{10}. At birth, his weight was 3.3 kg (50^{th} percentile). Hypotonia was noted over the first few months of life. At 16 months of age, he showed a few febrile seizures suggestive of flexion spasms over a few days. EEG was normal. At 2 years of age, he showed non-febrile generalized tonic-clonic and absence seizures. EEG was again normal. Valproic acid administration was then initiated. He had a few seizures over the next 3 years and none since 5 years of age. Valproic acid administration was stopped at 7 years of age.

Development was characterized by global delay. At 11 years of age, the patient can stay sit and can drive a wheel chair but cannot walk. He does not say words but he understands a few commands and communicate using a few signs. He can eat with a fork and has acquired the pincer grasp. He can operate a TV and a DVD player. Height is 155 cm (90-97th centile), weight is 52 kg (90-97th centile) and head circumference is 56 cm (90-97th centile). On physical examination, no specific dysmorphic features were noticed. Neurological examination revealed severe axial and mild appendicular hypotonia. Karyotyping (at a resolution of 540 bands), subtelomeric FISH studies and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any

abnormality. Blood lactate and ammonia levels were 1.66 mmol/L (reference values: 0,50 -2,20 mmol/L) and 37 μmol/L (reference values: 0-55 μmol/L), respectively. Plasma amino-acid and urine organic acid chromatography were unremarkable. Nerve conduction velocity and electromyogram studies were normal. Brain MRI studies were performed at 17 months and at 3 years and 5 months (Figure 3) of age. Both studies showed severe atrophy of the cerebellum and brainstem without any other abnormality.

References

1. Saitsu H, Kato M, Mizuguchi T *et al*: De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 2008; 40: 782-788.

2. Saitsu H, Tohyama J, Kumada T *et al*: Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am J Hum Genet* 2010; 86: 881-891.

Table S1. Paternity/maternity testing for patients 1 and 2 done using 6 informative polymorphic unlinked microsatellite markers.

Family-1	**D3S1754**		**D4S3351**		**D8S1179**		**D15S659**		**D14S587**		**D19S215**	
Patient-1	7	8	4	6	6	7	7	8	2	2	8	6
Father	7	8	6	6	6	8	6	7	2	7	5	8
Mother	8	8	4	5	6	7	7	8	2	6	6	7
Affected Sister	8	8	4	6	6	8	6	8	2	7	5	7
Family -2												
Patient-2	7	7	5	9	6	8	6	6	3	5	2	6
Father	7	8	5	9	6	8	2	6	2	3	2	6
Mother	7	7	5	6	5	6	2	6	10	5	6	12

Shown here are the segregation profiles of microsatellite markers tested in both families.

Table S2. Primers used to amplify *SPTAN1* (NM_001130438.2) coding exons and intronic junctions.

primer	forward_seq	reverse_seq	amplicon size (bp)	exons	Chr	start (hg18)	Stop (hg18)
G265_1	TCAATTCATTTGTCTCCTGGG	GGTCATAATTAAGTAACTTTCCTCGTC	451	2	9	130368719	130369169
G265_2	TAAGAGAATGGGCAAGGTGC	GCTGTCATATTACAAAGGCAGG	439	3	9	130370729	130371167
G265_3	TTGTCTAAACTCTATGGAAGAGCC	TGTGCTAACACTGGGTCTCAC	311	4	9	130376697	130377007
G265_4	TGTTTGATGTTTCTGGAAGCC	AGAAATTAGGGGACCAATCATC	317	5	9	130377225	130377541
G265_5	GATGCTTCAAGGAACCAACC	TTGACCTAGAAATGAACTCCGTC	620	6-7	9	130378837	130379456
G265_6	AGGTGGGTTTTACAAGCAGC	CAGAACAGACCCTTAACTCTGC	319	8	9	130379372	130379690
G265_7	GCTTTAGGGAGGCCATTTTC	GCCTCACCCAACACTGATAAAC	284	9	9	130380139	130380422
G265_8	TGCTTATGTAAAATTCAGCACC	AAGGATGAATAATAGCTTTCAGAGAC	260	10	9	130381656	130381915
G265_9	TAGCTGTTCTGTCTGCCTCG	AAAACAAAACAAGCACACTACCC	294	11	9	130382950	130383243
G265_10	GCCAAAATACCCTTTGCTTC	CTGTTTGGAATCCTTCCAGG	272	12	9	130383801	130384072
G265_11	GCAGTTACTTTTCTCCAGAGGC	TCCCTTATTTCGTCCTTTAGC	539	13-14	9	130384504	130385042
G265_12	AAGTATGAAGGTAGTGCAAGGG	TGTCCCTAAAATACTTAATAGAAAGGG	358	15	9	130385102	130385459
G265_13	ACTGTTCATGGGCAGTGTTG	TTACTGGCTTTTCCTCCCTC	323	16	9	130385817	130386139
G265_14	CTTTCTAGGAGCCCCAGATG	ATTTAAATTCGACCCTGCCC	483	17	9	130386242	130386724
G265_15	CTGGAACCATGGTGGTAGC	ATGCCCTCGGCAAGAAG	280	18	9	130386732	130387011
G265_16	AGTGCCGTGAACACACAGAG	ACTGTAATGCACACCTCCCG	416	19	9	130387756	130388171
G265_17	GAGAAAGGCTGTTGAGGCAG	GGAACACCTGCTGACAGTATC	263	20	9	130389608	130389870
G265_18	TCTTGGATCAGATTTTATTGGTAGC	AACCCACAGAAACCCAACTG	368	21	9	130390752	130391119
G265_19	TTAAAACAACGCTCTCGTGTG	CTTCGGCCCATGTGGAAC	318	22	9	130393478	130393795
G265_20	GAGCTGCTGCCTTTCATCC	TGGACAAAGGCCTTCAAGAG	224	23	9	130395009	130395232
G265_21	AGCCTGGAATCCACATCTTG	CCAGCTTGGATGACAGTGAG	412	24	9	130396169	130396580
G265_22	GCCATAGTTTGTGACTATGTCTCC	GAAACACTGCTTGTTGGGC	275	25	9	130400422	130400696
G265_23	TCCTGTGTTTCCAGGTTTGG	TGGCTTGATATTCTTCCCAAG	185	26	9	130400973	130401157
G265_24	AGAGTCATTCGCTGGAAAGG	AGAAAGGCACACCAGAAAAG	195	27	9	130402100	130402294
G265_25	TCAATGACACTTGCAGCTCAG	TGCTGTAGACAGGGAAGGAAG	196	28	9	130405566	130405761
G265_26	TTTTCCCAAAGTCTGACTGG	TCTAGGATCTCAGTCAGCAAGC	253	29	9	130406354	130406606
G265_27	TGTCCTGTAATCAAGGCAGTTG	TTTTCACAATAAAAATGACCCC	577	30-31	9	130407073	130407649
G265_28	CCCAAGATAACTTTCTGAGGC	AGCTAAAGAGGGCAAGAGGC	637	32-33	9	130409595	130410231
G265_29	TGCTCCTGGTTTCTGACC	GGTATTCCTTGGAGATATAACAGTG	317	34	9	130410157	130410473
G265_30	CCTGCCACCAGCTAGTTCTC	CATGGGTGCAAGGAGATTG	627	35-36	9	130410858	130411484
G265_31	GTTCCCAAATGCTGAGCTTC	GAAGAGACACCAGCAAACCG	242	37	9	130411634	130411875
G265_32	GTTGGCATTTACCCCAGTTC	CATCTGTAATTCAGCCTAAAGTCTTC	385	38	9	130413693	130414077
G265_33	TCAAATTGAGCTTTAGGAGAGG	ACTGATGGACCCACTGTGC	280	39	9	130414137	130414416
G265_34	CTGGGCAACCTGAATTTTCC	CACTCTCTTTTCTCAAACACGC	273	40	9	130415405	130415677
G265_35	CAACACTCCACATCTCAGTAAGG	AGTCAGCCTGTGGTTCCTG	379	41	9	130417633	130418011
G265_36	AGTTGACCTGATGTCCCCAC	CCACAGCTCAGAGTCTGCC	616	42-43	9	130419666	130420281
G265_37	ATGCCCAGCTTTTGTGACTG	ACAGGGAAATGATGACCAGC	301	44	9	130420873	130421173
G265_38	TTGTCACTCTCTGTCCCCG	CCCAGGAAGTGAACTTTGGG	269	45	9	130423178	130423446
G265_39	TTAGAGCCTTTCCAGGGAGG	AACCAATGACACGGAAGGAG	312	46	9	130426347	130426658
G265_40	GTTAGGAAGATTGGGATTTATCTG	AGTGAAAGACGCCACCAGTC	270	47	9	130427084	130427353
G265_41	TGCCATCTGAGCCTAGGAAG	CAGAGCTGGGCAGACAGAAG	362	48	9	130427794	130428155
G265_42	CACCCCACCTCCTGCAC	GTGACTCAGTTAAGTGTGGGC	456	49	9	130428418	130428873
G265_43	TGAGCCCATCTGTGAAGGAG	CACAAAGCACCACCACCTC	283	50	9	130429411	130429693
G265_44	TGTGTCTTCTCTCTGTCCCC	GGATCCTCCACAAGCCAG	186	51	9	130429942	130430127
G265_45	TGAGAGAAGGTTCATTCTGAGC	AGGGAAGGGGTACCCTGG	198	52	9	130432348	130432545
G265_46	CAGAGGGCAGTAAGTGGCTC	TGTCTGCAGCACAAAGCC	528	53-54	9	130434147	130434674
G265_47	GAGTTCAGCCTTACTCGCCC	CCTGTTACCCCAGACTCAGC	554	55-56	9	130434587	130435140
G265_48	AGCATCCTGAGACCTGGGAG	ACAGAGGAGCGGACATGC	274	57	9	130435230	130435503

Table S3. Variants identified in *SPTAN1* from screening 95 ID patients

c.DNA change	Amino acid change	dbSNP131 rs#	Occurrence/ n=95	Inheritance	SIFT	PolyPhen
c.1679A>G	p.E560G	NA	1	Mother[1]	0.20	0.84
c.1697G>C	**p.R566P**	**NA**	**1**	***de novo***	**0.02**	**2.36**
c.2070T>C	p.I690I	NA	1	Mother[1]	NA	NA
c.5908G>A	p.A781A	34084388	2	ND	NA	NA
c.2610A>G	p.Q870Q	NA	1	Mother[1]	NA	NA
c.3300G>A	p.A1100A	2227865	2	ND	NA	NA
c.3456T>C	p.D1182D	945831	2	ND	NA	NA
c.4410C>T	p.T1470T	2228951	1	ND	NA	NA
c.5437C>A	p.R1813R	3750333	4	ND	NA	NA
c.5523C>T	p.I1841I	79569204	1	ND	NA	NA
c.5925G>A	p.A1975A	11543345	1	ND	NA	NA
c.6549C>A	p.T2178T	NA	1	Father[1]	NA	NA
c.6605_6607del	**p.Q2202del**	**NA**	**1**	***de novo***	NA	NA
c.7389C>T	p.T2463T	2228952	1	ND	NA	NA

[1]Unaffected (healthy) parent. cDNA and amino acid positions are based on *SPTAN1* reference sequence NM_001130438.2. NA, not available. ND, not determined. Missense predicted protein damaging if SIFT (http://sift.jcvi.org) < 0.05 and/or PolyPhen (http://genetics.bwh.harvard.edu/pph/) > 1.8.

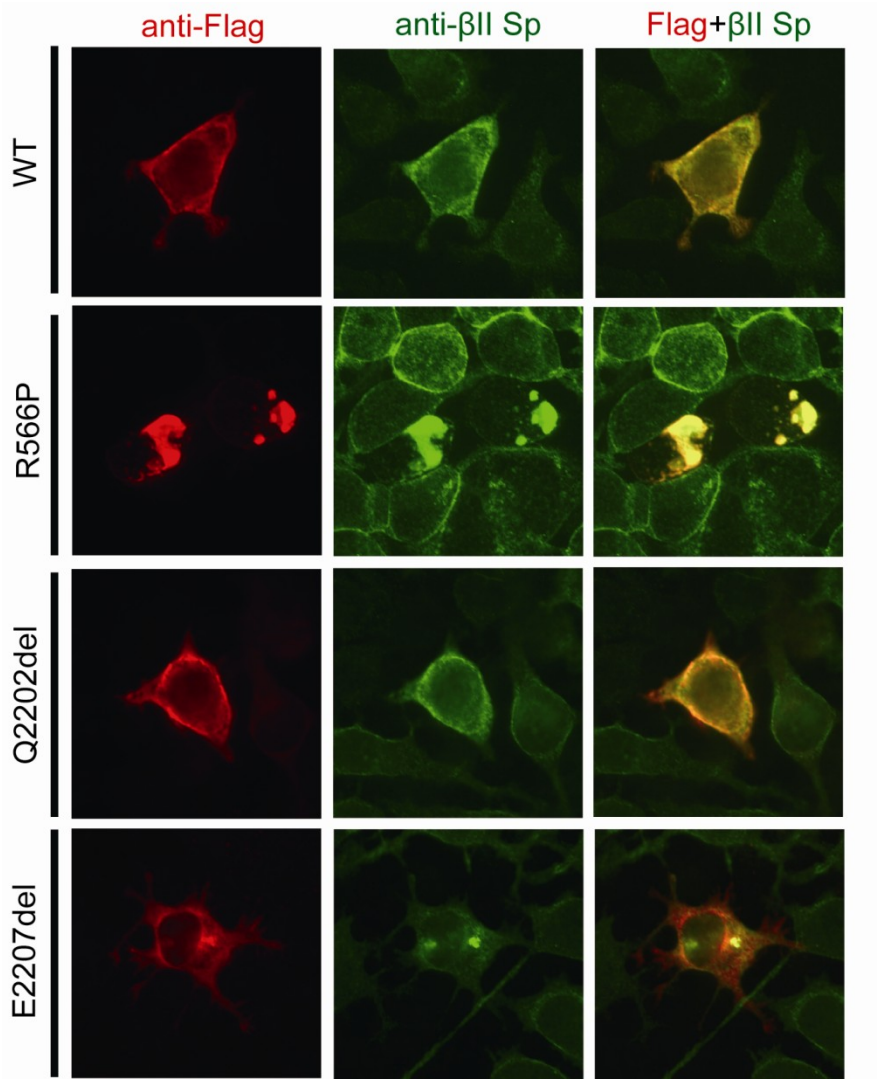

Figure S1. Double immunostaining in N2A cells showing overlapping expression between SPTAN1 mutant constructs and endogenous β-II spectrin subunit. β-III spectrin was not detected (not shown).