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Supplemental information

Germline variants in tumor suppressor *FBXW7* lead to impaired ubiquitination and a neurodevelopmental syndrome

Sarah E.M. Stephenson, Gregory Costain, Laura E.R. Blok, Michael A. Silk, Thanh Binh Nguyen, Xiaomin Dong, Dana E. Alhuzaimi, James J. Dowling, Susan Walker, Kimberly Amburgey, Robin Z. Hayeems, Lance H. Rodan, Marc A. Schwartz, Jonathan Picker, Sally A. Lynch, Aditi Gupta, Kristen J. Rasmussen, Lisa A. Schimmenti, Eric W. Klee, Zhiyv Niu, Katherine E. Agre, Ilana Chilton, Wendy K. Chung, Anya Revah-Politi, P.Y. Billie Au, Christopher Griffith, Melissa Racobaldo, Annick Raas-Rothschild, Bruria Ben Zeev, Ortal Barel, Sebastien Moutton, Fanny Morice-Picard, Virginie Carmignac, Jenny Cornaton, Nathalie Marle, Orrin Devinsky, Chandler Stimach, Stephanie Burns Wechsler, Bryan E. Hainline, Katie Sapp, Marjolaine Willems, Ange-line Bruel, Kerith-Rae Dias, Carey-Anne Evans, Tony Roscioli, Rani Sachdev, Suzanna E.L. Temple, Ying Zhu, Joshua J. Baker, Ingrid E. Scheffer, Fiona J. Gardiner, Amy L. Schneider, Alison M. Muir, Heather C. Mefford, Amy Crunk, Elizabeth M. Heise, Francisca Millan, Kristin G. Monaghan, Richard Person, Lindsay Rhodes, Sarah Richards, Ingrid M. Wentzensen, Benjamin Cogné, Bertrand Isidor, Mathilde Nizon, Marie Vincent, Thomas Besnard, Amelie Piton, Carlo Marcelis, Kohji Kato, Norihisa Koyama, Tomoo Ogi, Elaine Suk-Ying Goh, Christopher Richmond, David J. Amor, Jessica O. Boyce, Angela T. Morgan, Michael S. Hildebrand, Antony Kaspi, Melanie Bahlo, Rún Friðriksdóttir, Hildigunnur Katrínardóttir, Patrick Sulem, Kári Stefánsson, Hans Tómas Björnsson, Simone Mandelstam, Manuela Morleo, Milena Mariani, TUDP Study Group, Marcello Scala, Andrea Accogli, Annalaura Torella, Valeria Capra, Mathew Wallis, Sandra Jansen, Quinten Waisfisz, Hugoline de Haan, Simon Sadedin, Broad Center for Mendelian Genomics, Sze Chern Lim, Susan M. White, David B. Ascher, Annette Schenck, Paul J. Lockhart, John Christodoulou, and Tiong Yang Tan

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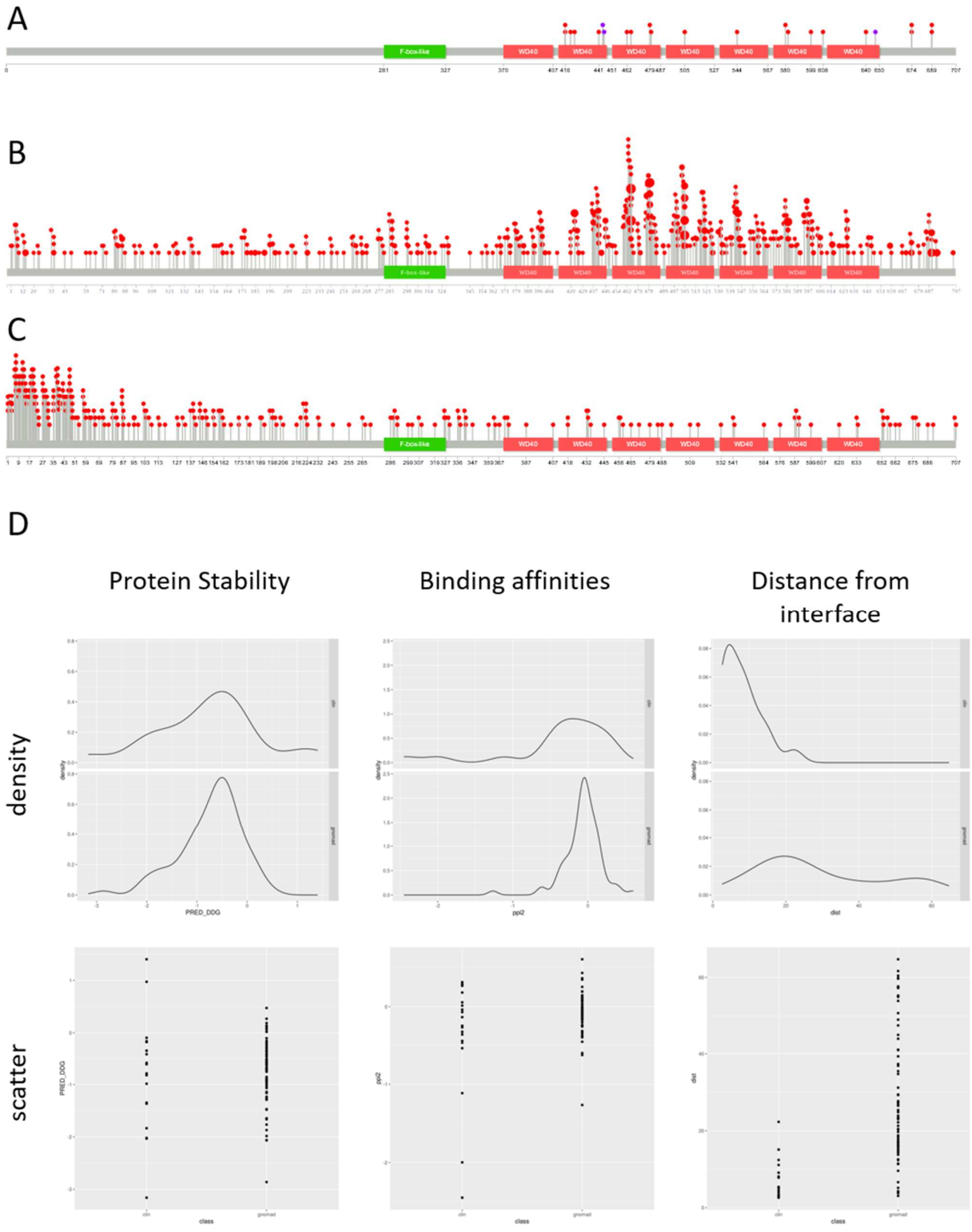

Figure S1: Distribution of neurodevelopment variants within FBXW7 relative to known COSMIC and gnomAD variants. (**A**) Location of patient-ascertained missense variants (red) and stop-gained and frameshift variants (purple). (**B**) Distribution of 1481 (440 unique) Catalogue Of Somatic Mutations In Cancer (COSMIC) somatic mutations (red) in FBXW7, where bubble size corresponds to the number of observations.

(**C**) Distribution of 280 missense variants (277 unique) in FBXW7 gnomAD v2.1 (140k exomes and genomes) variants with bubble size corresponding to the number of observations. (**D**) Comparison of structural predictors of neurodevelopmental disease variants to gnomAD variants. The gnomAD dataset was filtered to only those within the FBXW7 experimental structure, which includes residues 263 - 706, giving 78 variants only. Of these, the majority are very rare in the population (Allele Count: No. observations – 1:55, 2:14, 3:5, 6:2, 7:1, 12:1). Protein stability, determined using mutation Cutoff Scanning Matrix (mCSM), predicted the majority of gnomAD variants to also have a destabilizing effect, and to be similarly distributed to the patient cohort variants. Binding affinity, determined using mCSM-Protein–protein interactions (PPI) 1&2 (ΔΔG), suggests that gnomAD variants have a much smaller effect on binding affinity compared to the patient variants. Additionally, the gnomAD variants are dispersed throughout the structure and are, on average, further from the predicted interface with CYCLIN E1. See Table S3 for individual variant data.

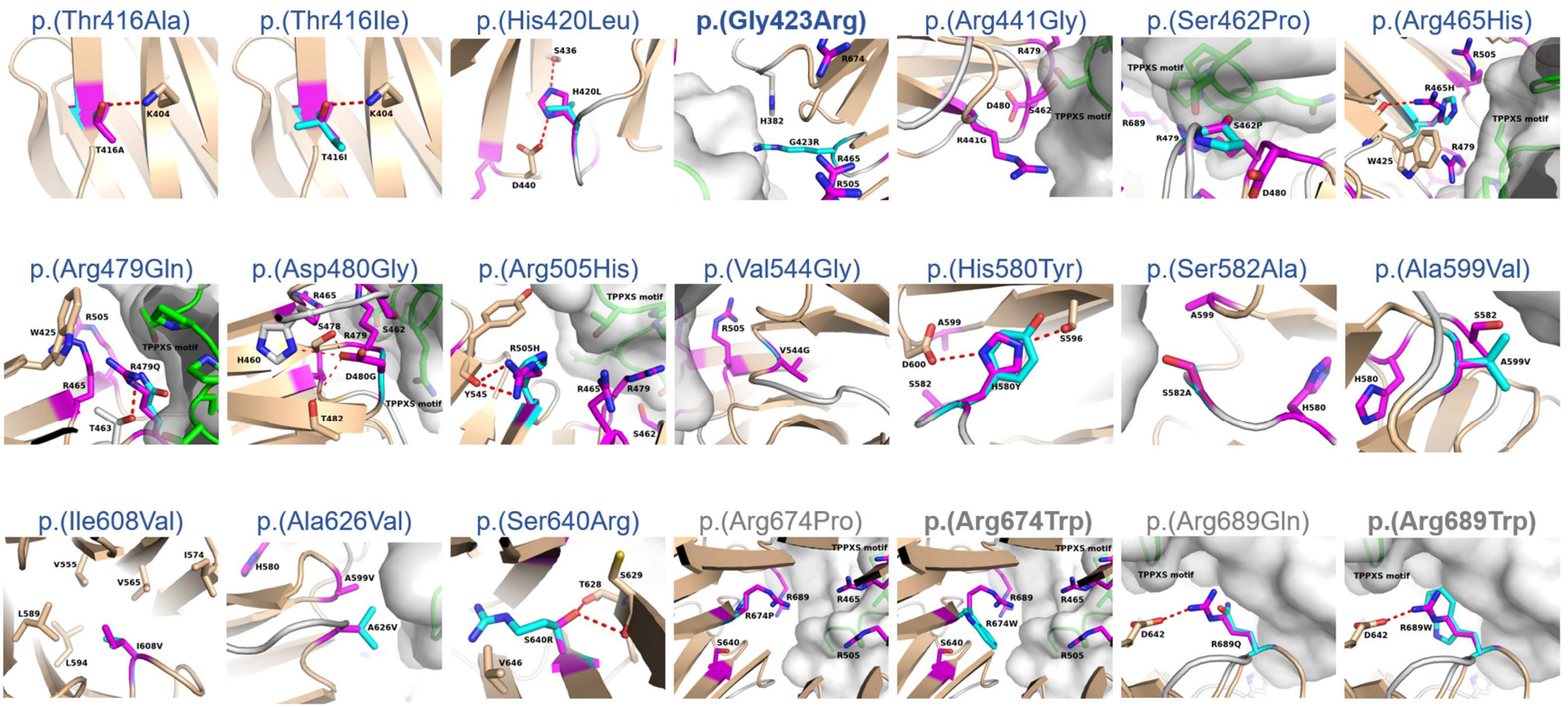

Figure S2: Change in interaction with CYCLIN E1 predicted by each variant

Zoom-in of the interaction of wild-type/variant residues of FBXW7 and its surrounding residues. The variant residues are overlaid on wild-type residues to identify the changes in interaction when variant occurs. FBXW7 is shown in brown ribbon, while CYCLIN E1 is shown in a light gray surface. All wild-type, and variant residues are shown in magenta and cyan sticks, respectively, while surrounding residues of FBXW7 and TPPXS motif of CYCLIN E1 are shown in brown and green sticks. The Oxygen and Nitrogen atoms are in red, and blue, respectively. Hydrogen bond interactions are shown in the red dash lines. Variants that reoccur are indicated by the bold title.

Figure S3: Amino acid alignment of human FBXW7 and Drosophila Ago sequences

Uniprot (www.uniprot.org) sequence alignment of Homo sapiens (ENSP00000474725/1-707) and Drosophila melanogaster (FBpp0073101/1-1326) FBXW7 (ago) proteins. Highlighted in yellow the F-Box, in gray the seven D40 repeats of the WD40 domain.

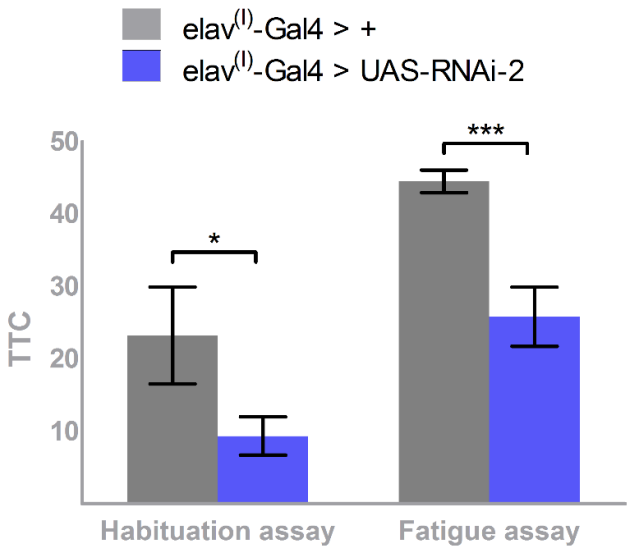

Figure S4: Knockdown of ago in Drosophila neurons induced by the elav$^{(l)}$-Gal4 driver causes faster decline of jump response due to fatigue

The knockdown of ago leads to a lower mTTC compared to the controls. Increasing the inter-trial interval in the fatigue assay, preventing habituation from being formed, demonstrates that this lower TTC is not due to improved habituation but due to motor fatigue. Precise genotypes tested in the fatigue assay: elav$^{(l)}$ C155-Gal4, GMR-wIR /Y; +/+; +/+ of genetic background control in gray and elav$^{(l)}$ C155-Gal4, GMR-wIR /Y; +/+; UAS-RNAi-2/+ of RNAi-2 knockdown in blue. $N_{control}$ = 42, N_{RNAi-2} = 48, $mTTC_{control}$ = 44.53, $mTTC_{RNAi-2}$= 25.85, p = 1.35E-5 . Statistical significance was assessed by a linear model regression analysis on the log transformed mTTC values, * P < 0.05, ** P < 0.01, *** P < 0.001.

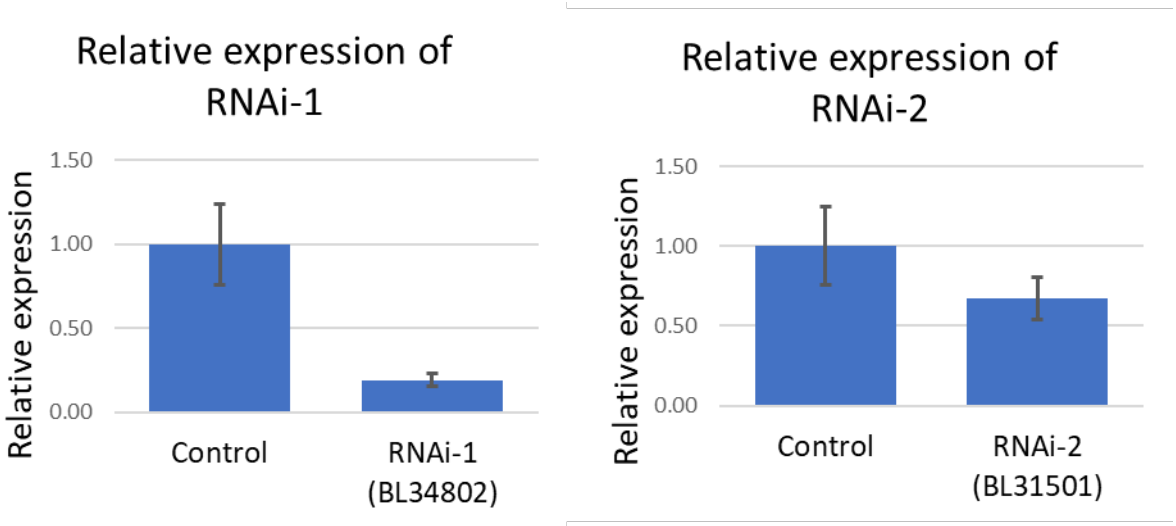

Figure S5: Relative expression of ago in Drosophila knockdown lines

Quantitative RT-PCR was performed on wandering L3 larva from RNAi-1 and RNAi-2 lines crossed to the ubiquitous Act-Gal4/TM3 Sb Tb driver to determine the level of *ago* expression using exon spanning primers to *ago* and *β'COP*. Error bars represent standard deviation.

Table S1: Analysis of FBXW7 neurodevelopmental syndrome variants

Individual	Inheritance	gDNA (Chr4; GRCh37)	Exon	cDNA	Protein	Occurs within WD40 domain	gnomAD - germline variants		COSMIC - somatic variants		CADD		FATHMM			GERP++ RS		MPC		MTR		MutationAssessor			PROVEAN			Polyphen2			SIFT			VEST4	
							Same variant	Alternative change at same amino acid residue	Same variant	Alternative change at same amino acid residue	score	rank score	score	rank score	predicted conseq-uence	score	rank score	score	rank score	score	rank score	score	rank score	predicted conseq-uence	score	rank score	predicted conseq-uence	score	rank score	predicted conseq-uence	score	rank score	predicted conseq-uence	score	rank score
1	de novo	g.153249446_153249447del	11	c.1331_1332del	p.(Lys444Serfs*27)	NMD predicted	Absent	3 LoF variants upstream	Absent	p.(Lys444Glyfs*55), p.(Lys444Argfs*32), p.(Lys444fs*2), more than 10 NMD predicted variants	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	de novo	g.153249446dup	11	c.1332dup	p.(Val445Serfs*27)	NMD predicted	Absent	3 LoF variants upstream	Absent	p.(Val445Cysfs*53), p.(Val445Aspfs*27), more than 10 NMD predicted variants	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	familial	g.153245477_153245478del	13	c.1713_1714del	p.(Asn572Leufs*32)	Truncation predicted	Absent	3 LoF variants upstream	Absent	More than 10 NMD predicted variants	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4																																				
5																																				
6																																				
7	de novo	g.153244218T>A	14	c.1939A>T	p.(Lys647*)	Truncation predicted	Absent	3 LoF variants upstream	Absent	p.(Lys647Asnfs*5) in stomach carcinoma, more than 10 NMD predicted variants	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	de novo	g.153250822A>T	intron 10	c.1236+2T>A	p.?	N/A	Absent	Alternative change at same nucleotide: Absent	Present (stomach carcinoma)	Alternative change at same nucleotide: c.1236+2T>C (large intestine adenocarcinoma)																										
9	de novo	arr[GRCh37] 4q31.3(152720434_153661857)x1 dn	N/A	N/A	Entire gene deleted	N/A	N/A	N/A	N/A	N/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	de novo	arr[GRCH37] 4q31.3q32.1(152854578_156285170)x1 dn,4q32.1q34.1(161464002_175617314)x3 dn,4q34.1q34.3(175858796_179802170)x3 dn	N/A	N/A	Entire gene deleted	N/A	N/A	N/A	N/A	N/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	de novo	g.153249531G>A	11	c.1247C>T	p.(Thr416Ile)	Yes	Absent	Absent	Absent	Absent	4.22	0.86	0.13	0.61	T	5.90	0.95	2.78	0.99	0.56	0.97	1.37	0.34	L	-5.66	0.87	D	1.00	0.92	D	0.05	0.56	D	0.78	0.80
12	familial	g.153249532T>C	11	c.1246A>G	p.(Thr416Ala)	Yes	Absent	Absent	Absent	Absent	4.08	0.81	0.04	0.62	T	5.90	0.95	2.40	0.97	0.56	0.97	1.71	0.44	L	-4.80	0.81	D	0.98	0.81	D	0.02	0.59	D	0.80	0.81
13	de novo	g.153249519T>A	11	c.1259A>T	p.(His420Leu)	Yes	Absent	Absent	Absent	Multiple	4.13	0.83	-1.50	0.81	D	5.90	0.95	3.08	0.99	0.87	0.59	4.72	1.00	H	-10.92	0.99	D	1.00	0.97	D	0.00	0.91	D	0.94	0.95
14	de novo	g.153249511C>T	11	c.1267G>A	p.(Gly423Arg)	Yes	Absent	Absent	Present (multiple tissue types)	Multiple	4.35	0.89	0.16	0.61	T	5.90	0.95	3.01	0.99	0.67	0.92	1.21	0.30	L	-7.94	0.96	D	1.00	0.97	D	0.00	0.91	D	0.94	0.96
15																																				
16	de novo	g.153249457G>C	11	c.1321C>G	p.(Arg441Gly)	Yes	Absent	Absent	Present (lung carcinoma, rhabdomyosarcoma)	Multiple	3.72	0.70	2.02	0.21	T	4.08	0.47	3.14	0.99	0.55	0.97	0.42	0.13	N	-6.83	0.93	D	0.99	0.80	D	0.03	0.91	D	0.84	0.86
17	de novo	g.153249394A>G	11	c.1384T>C	p.(Ser462Pro)	Yes	Absent	Absent	Absent	p.(Ser462Tyr), p.(Ser462Phe)	4.33	0.89	0.86	0.74	T	6.05	0.98	2.78	0.99	0.54	0.97	2.65	0.77	M	-4.96	0.82	D	0.93	0.92	D	0.00	0.78	D	0.87	0.89
18	de novo	g.153249384C>T	11	c.1394G>A	p.(Arg465His)	Yes	Absent	p.(Arg465Cys) (allele balance 45% or lower)	Present (more than 5 tissue types)	Multiple	4.17	0.84	0.97	0.43	T	6.05	0.98	3.08	0.99	0.61	0.95	1.26	0.32	L	-4.96	0.82	D	1.00	0.97	D	0.02	0.91	D	0.78	0.79
19	de novo (mosaic 14%)	g.153247366C>T	12	c.1436G>A	p.(Arg479Gln)	Yes	Absent	p.(Arg479Gly) (allele balance 25-30%)	Present (more than 5 tissue types)	Multiple	4.17	0.84	1.02	0.41	T	5.72	0.89	2.93	0.99	0.80	0.75	0.84	0.21	L	-3.97	0.74	D	1.00	0.97	D	0.02	0.51	D	0.64	0.65
20	de novo	g.153247363T>C	12	c.1439A>G	p.(Asp480Gly)	Yes	Absent	Absent	Present (colon adenocarcinoma)	Multiple	4.46	0.91	-2.49	0.89	D	5.72	0.89	3.19	0.99	0.86	0.60	3.93	0.96	H	-6.95	0.93	D	1.00	0.97	D	0.00	0.91	D	0.99	0.99
21	de novo	g.153247288C>T	12	c.1514G>A	p.(Arg505His)	Yes	Absent	Absent	Present (more than 5 tissue types)	Multiple	4.17	0.85	0.05	0.62	T	4.87	0.63	3.08	0.99	0.55	0.97	1.50	0.38	L	-4.96	0.82	D	1.00	0.97	D	0.01	0.78	D	0.75	0.75
22	de novo	g.153247171A>C	12	c.1631T>G	p.(Val544Gly)	Yes	Absent	Absent	Present (large intestine adenocarcinoma)	p.(Val544Asp) (mouth squamous cell carcinoma)	4.29	0.88	-0.79	0.74	T	5.72	0.89	3.18	0.99	0.55	0.97	4.32	0.98	H	-6.95	0.93	D	1.00	0.92	D	0.00	0.91	D	0.64	0.66
23	de novo	g.153245453G>A	13	c.1738C>T	p.(His580Tyr)	Yes	Absent	Absent	Present (large intestine adenocarcinoma)	Multiple	4.09	0.82	-1.49	0.81	T	5.70	0.89	2.95	0.99	0.75	0.84	4.04	0.97	H	-5.96	0.89	D	1.00	0.97	D	0.01	0.56	D	0.89	0.89
24	de novo	g.153245447A>C	13	c.1744T>G	p.(Ser582Ala)	Yes	Absent	Absent	Absent	Multiple	4.02	0.79	1.00	0.41	T	5.70	0.89	0.97	0.97	0.76	0.83	1.46	0.37	L	-2.70	0.61	D	0.98	0.75	D	0.10	0.59	T	0.66	0.67
25	de novo (mosaic 23%)	g.153245395G>A	13	c.1796C>T	p.(Ala599Val)	Yes	Absent	p.(Ala599Gly) (allele balance 35-40%)	Absent	Absent	4.29	0.88	0.94	0.44	T	5.45	0.80	2.69	0.99	0.86	0.62	0.86	0.21	L	-3.96	0.74	D	1.00	0.84	D	0.02	0.78	D	0.63	0.64
26	de novo	g.153244280G>A	14	c.1877C>T	p.(Ala626Val)	Yes	Absent	Absent	Present (large intestine adenocarcinoma)	p.(Ala626Thr), p.(Ala626Pro), p.(Ala626Asp)	4.13	0.83	0.96	0.43	T	5.67	0.88	2.73	0.99	0.63	0.94	1.71	0.44	L	-3.91	0.74	D	1.00	0.91	D	0.00	0.78	D	0.74	0.76
27	de novo	g.153244237G>T	14	c.1920C>A	p.(Ser640Arg)	Yes	Absent	Absent	Absent	Absent	3.40	0.62	0.97	0.43	T	3.03	0.34	2.87	0.99	0.81	0.72	2.58	0.75	M	-4.91	0.82	D	1.00	0.92	D	0.06	0.60	T	0.87	0.92
28	de novo	g.153245369T>C	13	c.1822A>G	p.(Ile608Val)	No	Absent	Absent	Absent	Absent	2.43	0.40	0.90	0.45	T	5.45	0.80	1.39	0.85	0.37	0.99	0.18	0.09	N	-0.86	0.23	N	0.05	0.30	B	0.22	0.19	T	0.36	0.41
29	de novo	g.153244136C>G	14	c.2021G>C	p.(Arg674Pro)	No	Absent	Absent	Absent	p.(Arg674Trp), p.(Arg674Gln)	4.13	0.83	2.21	0.18	T	4.82	0.62	3.35	1.00	0.52	0.98	2.67	0.78	M	-6.83	0.93	D	1.00	0.89	D	0.00	0.78	D	0.81	0.85
30	de novo	g.153244137G>A	14	c.2020C>T	p.(Arg674Trp)	No	Absent	Absent	Present (glioma, cervix squamous cell carcinoma, atypical meningioma, prostate adenocarcinoma)	p.(Arg674Gln) (colon adenocarcinoma, stomach adenocarcinoma)	3.77	0.71	2.20	0.19	T	1.62	0.23	2.77	0.99	0.52	0.98	2.67	0.78	M	-7.80	0.96	D	1.00	0.92	D	0.00	0.91	D	0.80	0.88
31																																				
32	de novo	g.153244091C>T	14	c.2066G>A	p.(Arg689Gln)	No	Absent	Absent	Present (more than 5 tissue types)	p.(Arg689Glu), p.(Arg689Trp)	4.14	0.83	2.25	0.18	T	4.82	0.62	2.62	0.98	0.40	0.99	2.70	0.79	M	-3.92	0.73	D	1.00	0.89	D	0.03	0.68	D	0.82	0.87
33	de novo	g.153244092G>A	14	c.2065C>T	p.(Arg689Trp)	No	Absent	Absent	Present (more than 5 tissue types)	p.(Arg689Glu), p.(Arg689Gln)	4.22	0.86	2.20	0.19	T	5.67	0.88	2.77	0.99	0.40	0.99	2.70	0.79	M	-7.88	0.96	D	1.00	0.97	D	0.03	0.91	D	0.86	0.91
34																																				
35																																				

Tolerated (T); Deleterious (D); Low (L); Medium (M); High (H); Combined Annotation Dependent Depletion (CADD); Functional Analysis through Hidden Markov Models (FATHMM); Genomic Evolutionary Rate Profiling (GERP) ++ rejected substitutions" (RS) score; missense badness, PolyPhen-2, and constraint (MPC); Missense Tolerance Ratio (MTR); Protein Variation Effect Analyzer (PROVEAN); Polymorphism Phenotyping v2 (PolyPhen-2); Sorting Intolerant From Tolerant (SIFT); Variant Effect Scoring Tool (VEST).

Table S2: Clinical details of Individuals with FBXW7 neurodevelopmental syndrome

INDIVIDUAL	1	2	3	4	5	6	7	8	9	10	11
VARIANT TYPE	Predicted Loss of Function								GENE DELETION		MISSENSE, AFFECTING WD40 DOMAIN
Variant Details											
Mode of discovery	Trio WES (Broad)	Exome sequencing with parental variant segregation	Family-based WGS	Family-based WGS	Family-based WGS	Family-based WGS	Solo WES	Trio WES	Array CGH Agilent 180K	SNP array (Illumina Infinium CoreExome-24 v1.1, hg19) [VCGS]	Gene Dx Trio WES
cDNA (NM_001349798.2)	c.1331_1332del	c.1332dup	c.1713_1714del	c.1713_1714del	c.1713_1714del	c.1713_1714del	c.1939A>T	c.1236+2T>A	N/A	N/A	c.1247C>T
gDNA (Chr4; GRCh37)	g.153249446_153249447del	g.153249446dup	g.153245477_153245478del	g.153245477_153245478del	g.153245477_153245478del	g.153245477_153245478del	g.153244218T>A	g.153250822A>T	arr [GRCh37] 4q31.3(152720434_153661857)x1 dn	arr[GRCH37] 4q31.3q32.1(152854578_156285170)x1 dn,4q32.1q34.1(161464002_175617314)x3 dn,4q34.1q34.3(175858796_179802170)x3 dn	g.153249531G>A
Protein level	p.(Lys444Serfs*27)	p.(Val445Serfs*27)	p.(Asn572Leufs*32)	p.(Asn572Leufs*32)	p.(Asn572Leufs*32)	p.(Asn572Leufs*32)	p.(Lys647*)	p.?	DEL	DEL	p.(Thr416Ile)
Same variant in gnomAD	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	N/A	N/A	Absent
Alternative change at same amino acid residue in gnomAD	3 LoF variants upstream	3 LoF variants upstream	3 LoF variants upstream	3 LoF variants upstream	3 LoF variants upstream	3 LoF variants upstream	3 LoF variants upstream	Alternative change at same nucleotide: Absent	N/A	N/A	Absent
Same variant in COSMIC	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present (stomach carcinoma)	N/A	N/A	Absent
Alternative change at same amino acid residue in COSMIC	p.(Lys444Glyfs*55), p.(Lys444Argfs*32), p.(Lys444fs*2), more than 10 NMD predicted variants	p.(Val445Cysfs*53), p.(Val445Aspfs*27), more than 10 NMD predicted variants	More than 10 NMD predicted variants	More than 10 NMD predicted variants	More than 10 NMD predicted variants	More than 10 NMD predicted variants	p.(Lys647Asnfs*5) in stomach carcinoma, more than 10 NMD predicted variants	Alternative change at same nucleotide: c.1236+2T>C (large intestine adenocarcinoma)	N/A	N/A	Absent
Exon (NM_001349798.2)	11	11	13	13	13	13	14	intron 10	N/A	N/A	11
Occurs within WD40 domain	NMD predicted	NMD predicted	Truncation predicted	Truncation predicted	Truncation predicted	Truncation predicted	Truncation predicted	N/A	N/A	N/A	Yes
Inheritance	De novo	De novo	Paternal	Paternal	Paternal	Unknown	De novo	De novo	De novo	De novo	De novo
Other candidate variants	*PDP2*: de novo LoF variant	No	No	No	No	No	No	No	Other inherited CNV (see below)	No	arr[GRCh37] 22q11.22q11.23(22997609_24961234)x3 pat
Demographic Features											
Sex	Male	Male	Female	Female	Female	Male	Male	Female	Male	Male	Male
Age at last assessment	11 years	3 years 2 months	14 years 9 months	11 years 9 months	6 years 3 months	44 years 6 months	9y	5 years	7 years 2 months	2 years 2 months	18 yo
Other											
Medical History											
Prenatal history	Increased nuchal fold at 12/40; normal chromosomes on amniocentesis	suspicion of preeclampsia	NAD	NAD	NAD	NAD	NAD	Born at 36+5 weeks, birth weight 3390 gr, Apgar scores 7 and 8. Neonatal jaundice treated with phototherapy.	Feet malposition during pregnancy. Mother had positive syphilis serology. Born at 40 weeks: (weight 4,080g; height 51 cm; OFC 35.5 cm). Neonatal jaundice. Negative syphilis serology at age 4 years	40+2/40 Emergency LUSCS for non-reassuring CTG. Nil antenatal concerns. Growth scans normal.	Unremarkable
Neurological problems	Hypotonia, tactile issues	Hypotonia	Hypotonia	NAD	NAD	NAD	NAD	No hypotonia	No neurological signs at testing.	Early hypotonia. No seizures. No regression.	After onset of seizures at age 7 years, his academic and intellectual functions deteriorated. Now in special ed.
Brain imaging	CT brain only - no structural abnormalities reported	N/A	Large cerebellar vermis with low-lying tonsils 3.6 mm below foramen magnum (Arnold-Chiari malformation)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Developmental / cognitive / psychiatric problems	Global developmental delay ; now borderline IQ, attends mainstream school with some assistance; some anxiety	neonatal sucking difficulties delayed motor milestones : crawled on four legs at 18 months, walked independantly at 21 months hypotonia and hyperlaxity. first words at 2,5 years. But at 3y 2 months : can solve puzzles easily, recognizes some letters, can count to five, recognizes colours. Can stay focused on an activity, nice. Speech iompediment but can produce more than 50 significant words	Global developmental delay; learning difficulty, ID, speech/language problems; delayed fine motor skills; sensory processing; ?ASD, no dx - paed says not enough for diagnosis, but all have mild symptoms	Delayed language; late walker; delayed socially; attention, mood; learning disorder; psychiatrist involved; ?ASD, no dx - paed says not enough for diagnosis, but all have mild symptoms	Delayed language; poor gross motor skills; attention/mood. ?ASD, WL for ASD ax through Austin health	Borderline non-verbal IQ	walk at 14m/speech delay/learning difficulties	Walking 18 months, first words 18 months. Speech development disorder. At the age of 4 years sentences of 3-4 words. Problems with pronounciation. Non-verbal development at low average level. Problems with loud noises, lives in her own world. No formal diagnosis of ASD. Mild sleeping problems.	Head control before 4 months, sit alone between 9 and 11 months, walk at age 16 months, mild delayed speech, learning difficulties and global reeducation since age 4 years (speech therapy, psychomotor therapy, psychological therapy). Anxiety, low self-confidence, impulsivity, low treatment speed index (74) and normal indices (between 88 and 97) of WPPSI-IV testing at age 5 years 3 months	Moderate global developmental delay. Early gross motor delays with minimal weight-bearing and lower limb use prior to age 2 years. First independent steps 22 months. No reported fine motor concerns. Severe speech delay: grunts / vocalises. Only "mum" and "dad" at 2 years.	Very mild delays in gross motor skills, mild cognitive delays (eg, color naming) until onset of seizures at age 7 yo, then more learning, attentional and cogntiive delays. Still with impaired coordination
Ophthalmologic problems	No	Astigmatism	No	No	No	No	No	Hypermetropia, amblyopia	Hypermetropia, strabismus (orthoptic therapy)	No	No
Audiology / hearing	Normal	Normal	Acute OME, x2 sets of grommets, no concerns with hearing	Normal	Acute OM as baby, no gommets, no concerns with hearing	Normal	Normal	Normal	Normal	Normal	Normal
Oral / dentition / other ENT problems	Submucous cleft palate	High palate, widely spaced teeth	tonsillectomy; cleft soft palate; Class III malocclusion; orthodontics; on WL for rhinoplasty	Submucous cleft palate; tonsillectomy; orthodontics	tonsillectomy and superior adenoidectomy after speech assessment; orthodontics; speech did not improve after T&As; on waiting list for pharyngoplasty	Class III malocclusion due to midface retrusion	Normal	Normal	no	Ankyloglossia. Poor suck/swallow coordination. Obstructive/noisy upper airway breathing.	No
Cardiac problems	Bicuspid aortic valve, PDA requiring surgical closure	Atrial septal defect	No	No	Heart mumur when born, innocent, no follow up needed, ECG and echo normal	No	No	No	No	No(referred for echocardiogram, pending)	No
Respiratory problems	No	No	Moderate OSA; no asthma	OSA and snoring; hospitalised for pneumonia at 3, no asthma	OSA, now resolved	Mild asthma as child, flares up every once in a while	No	No	No	No	No
Gastrointestinal problems/ feeding difficulties	Poor feeding in newborn period requiring NGT feeds; Chronic constipation	neonatal sucking difficulties. Nocturnal saliva swallowing difficulties responsible for coughing and nocturnal wakings	Cleft-related feeding difficulties; fussy eater; mainly carbs	Hyperphagia; eats good variety of foods and textures; constipation	fussy eater	No	No	No	No	Early difficulties with latching and ongoing poor coordination of suck and swallow. Early difficulty initiating solids.	No
Renal / genitourinary problems	Bilateral cryptorchidism; non-functioning right multicystic dysplastic kidney	initial inguinal testis but now normal. Normal abdominal and kidneys US	No	No	No	No	No	No	No	No	No
Hematologic problems	No	No	No	No	No	No	No	No	No	No	No
Endocrine problems	No	No	No	Seem by Endo at RCH; above % charts for both height and weight; lost 20kg in a year, high BMI; involved in study related to diabetes, does not have diabetes; no longer linked in with Endo at RCH	High end of %ile charts on height and weight, no official measurements yet because of COVID, but Mum said heading in same direction as Marcelle	No	No	No	No	No	No
Musculoskeletal problems	Sprengel deformity scapulae, webbed neck	Joint laxity	No	No	No	No	No	No	No	Small fingers, broad thumbs.	No
Immunological problems	No	No	No	No	No	No	No	No	No	No	No
Skin	Normal	Normal	Acne, on roaccutane	Acne, on roaccutane	Normal	Normal	Normal	Normal	Skin telangiectasia (cheeks, upper part of the back), regression of a congenital blue nevi of the back	Normal	Normal
Malignancy	No	No	No	No	No	No	No	No	No	No	No
Other			Sensory processing difficulties; on medication for anxiety and depression	On medication for anxiety and depression	WL for pharyngoplasty, post pharyngeal wall flap, 'non-cleft VPI'; anxiety, but not medicated	On medication for anxiety and depression			fragile X syndrome		
Family History											
Similarly affected family members	No	no	3	3	3	3 (daughters)	no	No	Difficult to assess, child growing in a foster family, mother has possibly similar difficulties but no evaluation data during her scholing, she did her first year of primary school twice and specialized education at age 10 years; father had mild learning difficulties with attention deficiency, difficulties in writting, and general clumsiness without ataxia or neurological deficit	No	Father had absence epilepsy in childhood, shares the same duplication listed above
Consanguinity	No	No	No	No	No	No	No	No	No	No	
Full siblings	1		2	2	2	NA	NA	0, two half sisters	2	No	Older brother - healthy, 5
Other	Paternal SLE		Father	Father	Father	Daughters		Brother of mother with ADHD and dev delay. Other brother of mother with ADHD only.	3 paternal half brother from 2 women, mother pregnant from another partner	Mother learning difficulties (16p13.11 duplication)	
Physical Examination											
Height	50th centile	105.1cm (+3SD)	Age 17 y: 160 cm (32nd centile)	Age 15 y: 175 cm (98th centile)	Age 10 y: 156 cm (>98th centile)	Age 47 y: 187 cm (92nd centile)	152 cm (+4SD)	At age 3 years 6 months 110.2 cm (2.02 SD)	127 cm (+1.4 SD)	72nd centile (3y)	159.4 cm (1st-3rd centile)
Weight	10th centile	21.5 kg (+5 SD)	Age 17 y: 53 kg (36th centile)	Age 15 y: 82kg (98th centile)	Age 10 y: 64 kg (>98th centile)	Age 47 y: 86 kg (87th centile)	42 kg (+4SD)	20.1 kg (0.97 SD)	27.4 kg (+1.8 SD)	99th centile (3y)	53.1 kg (3rd-10th centile)
Head circumference	50th centile	54.5 cm (+3.2 SD)	Age 13 y: 2nd centile	Age 5y3m: 53 cm (98th centile)	Age 4y9m: 52.5 cm (95th centile)	N/A	54.5 cm (+1SD)	53.5 cm (2.28 SD)	52.4 cm (median)	80th centile (2y)	50.6 cm (<2nd centile)
Facial features	Broad forehead, high nasal root, deeply set eyes, periorbital fullness, malar flattening, short philtrum, thin upper and lower lips vermilion, webbed neck, underdeveloped superior crus of ears with left-sided preauricular pit	"de novo postnatal macrosomia", epicanthus, thick eyebrows, synophris, large lobules of ears, deeply set eyes, periorbital fullness, downturned mouth,	Deeply set eyes, periorbital fullness	Not dysmorphic	Deeply set eyes, periorbital fullness	Midface retrusion	Not dysmorphic	Prominent forehead, no frontal bossing. Upslant, hypertelorism/telecanthus, almond shaped eyes. Nose normal. Thin upper lip. Ears low set and posteriorly rotated, normal shape.	Mild facial asymmetry with low set right ear, thick eyebrows, thin upper lip	Prominent metopic suture. Flat occiput. Broad nasal bridge / hypertelorism. Lateral eversion of eyelids. Small ears with thickened helices. Mild micrognathia. Thin upper lip with flat philtrum.	Mildly dysmorphic
Hands / feet	Tapering fingers, single palmar crease on R, small feet with pes planus and overlapping toes	Single palmar crease, clinodactyly	Normal	Normal	Normal	Normal	Normal	Toes 2-5 clinodactyly.	Metatarsus varus (surgery at age 2.5 years, intermittent physiotherapy and orthopaedic sole)	Small, fleshy hands & fingers with broad thumbs. Small toes. Single palmar creases.	Normal
Other findings	No	unaffected Mother 170 cm (+1,2SD) OFC 54,8 (-0,5SD) ; father 174 cm (-0,2SD) OFC 58 cm (+1,5SD)		Obesity	No	No	other people in the maternal family with over weight/height	No Analyses peformed: FMR1, SNP-array, WES and metabolic screening. With metabolic screening low activity of very long chain acyl-CoA dehydrogenase (VLCAD). She turned out te be a carrier of VLCADD.	arr [GRCh37] 4q31.3(152720434_153661857)x1dn, 6q21(114465667_114548253)x3 mat, 7q36,2(154223820_154390398)x3pat	No	No
		at birth : 38+2WG : 3240g/51cm/OFC35cm							intragenic *DPP6* duplication inherited from the father		
									HS3ST5 exon 3 duplication (UTR) inherited from the mother		

INDIVIDUAL	12	13	14	15	16	17	18	19	20	21	22	23
VARIANT TYPE	MISSENSE, AFFECTING WD40 DOMAIN											
Variant Details												
Mode of discovery	Solo WES	Trio WES (GeneDx)	Trio WES	Trio WES	Trio WES	Trio WGS	Trio WES	Pair WES (Research) Deep WES on skin biopsy; normal depth for patient blood	Trio WES (DDD)	Trio WES (Mayo)	Trio WES	Trio WES (GeneDx)
cDNA (NM_001349798.2)	c.1246A>G	c.1259A>T	c.1267G>A	c.1267G>A	c.1321C>G	c.1384T>C	c.1394G>A	c.1436G>A	c.1439A>G	c.1514G>A	c.1631T>G	c.1738C>T
gDNA (Chr4; GRCh37)	g.153249532T>C	g.153249519T>A	g.153249511C>T	g.153249511C>T	g.153249457G>C	g.153249394A>G	g.153249384C>T	g.153247366C>T	g.153247363T>C	g.153247288C>T	g.153247171A>C	g.153245453G>A
Protein level	p.(Thr416Ala)	p.(His420Leu)	p.(Gly423Arg)	p.(Gly423Arg)	p.(Arg441Gly)	p.(Ser462Pro)	p.(Arg465His)	p.(Arg479Gln)	p.(Asp480Gly)	p.(Arg505His)	p.(Val544Gly)	p.(His580Tyr)
Same variant in gnomAD	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Alternative change at same amino acid residue in gnomAD	Absent	Absent	Absent	Absent	Absent	Absent	p.(Arg465Cys) (allele balance 45% or lower)	p.(Arg479Gly) (allele balance 25-30%)	Absent	Absent	Absent	Absent
Same variant in COSMIC	Absent	Absent	Present (multiple tissue types)	Present (multiple tissue types)	Present (lung carcinoma, rhabdomyosarcoma)	Absent	Present (more than 5 tissue types)	Present (more than 5 tissue types)	Present (colon adenocarcinoma)	Present (more than 5 tissue types)	Present (large intestine adenocarcinoma)	Present (large intestine adenocarcinoma)
Alternative change at same amino acid residue in COSMIC	Absent	Multiple	Multiple	Multiple	Multiple	p.(Ser462Tyr), p.(Ser462Phe)	Multiple	Multiple	Multiple	Multiple	p.(Val544Asp) (mouth squamous cell carcinoma)	Multiple
Exon (NM_001349798.2)	11	11	11	11	11	11	11	12	12	12	12	13
Occurs within WD40 domain	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Inheritance	Inherited from mother with ID	De novo	De novo	De novo	De novo	De novo	De novo	De novo, post-zygotic (mosaic: 14%)	De novo	De novo	De novo	De novo
Other candidate variants	Yes NCOR1:NM_001190440:exon27:c.3734G>A:p.Arg1245Gln (mat) + NCOR1:NM_001190440:exon3:c.244C>A:p.(Pro82Thr) [no known disease association]	No	No	No	No	No	No	Yes CACNA1A:NM_023035.2:c.835C>T p.(Arg279Cys)	No	No	No	No
Demographic Features												
Sex	Female	Male	Male	Male	Male	Male	Female	Male	Male	Male	Female	Male
Age at last assessment	3y	10 years, 2 months	14 yo	15	5 years	Information gathered from clinical record and obtained from parents	9.5 years	(currently 6 years)	16 years	3 years	23 months	3 years
Other				Macedonian					DDD4K.02106 in DDD paper			
Medical History												
Prenatal history	no	non-contributory, born at 38 weeks 1 day	uneventful	Unremarkable	Uncomplicated pregnancy and term delivery	Planned Caesarean after 42 weeks of gestation, because of size and drop in fetal movement, weight at birth: 5230 g (<99 percentile, +3.4 SD), length: 57 cm (<99 percentile, +2.6 SD), head circumference: 40 cm (99 percentile, +2.5 SD)	normal	Born at 40 weeks. Uneventful pregnancy. Weight at birth : 3520g.	Normal (well bar ear infection in mum and she took ear drops)	Delivered at 36 weeks, induced for maternal cholestasis. BW 8lbs. 15oz., hospitalized for 6 days.	Normal	Unremarkable. Term delivery, Cesarean section for breech presentation
Neurological problems	Unsteady gait, Broad-based gait	hypotonia diagnosed shortly after birth	history of seizures; hypotonia	Hypotonia; macrocephaly	Generalized hypotonia	hypotonia	neonatal hypotonia and failure to thrive	Generalized severe hypotonia;	apgars 8 @1 min, 10 at 5 min. home day 4 fine, ducky episode day 12, floppy, generalised hypotonia; on CBZ for his seizures which has stabilised;	Hypotonia	Severe progressive spasticity;	Hypotonia. Broad-based gait but not frankly ataxic. No seizures. Normal EMG/NCS.
Brain imaging	thin corpus callosum	N/A	N/A	agenesis of corpus callosum	Brain MRI (2 years of age) shows dysmorphic corpus callosum, scattered foci of T2 prolongation in periventricular white matter	N/A	N/A	Brain MRI: cerebellar hypoplasia but large folia and big cisterna magna	Crowded posterior fossa with enlarged cerebellum and thick vermis with low-lying tonsils just above foramen magnum; thick brainstem, normal corpus callosum	Brain: abnormal sulcal pattern suggestive of polymicrogyria, thin corpus callosum, delayed myelination	Brain Atrophy per MRI Scattered small subcortical calcifications (Brain CT)	Normal brain MRI/spine with MRS.
Developmental / cognitive / psychiatric problems	Global moderate developmental delay, walking at 20m, firts words at 2y	normal	Developmental delay waled at age 3; intellectual disability. Very few words. Dx of Autism spectrum disorder	Global dev delay all milestones/moderate intellectual disability	Moderate global developmental delay (language limited and has speech apraxia, ambulatory)	motor developmental delay, normal language development, normal fine motor development	Global developmental delay	Global developmental delay with marked generalized hypotonia, only syllabes for speech; (walking independently after 4 years)	Severe global developmental delay (babbling, mama dada but not consistent, non ambulatory); Sleep disturbance	Motor and speech delay	Severe progressive developmental delay with episodes of regression	Global developmental delay: gross motor (walking independently after 2 years) and expressive speech
Ophthalmologic problems	strabismus	No	No	Bilateral esotropia/hypermetropia	Mild hypermetropia, nasolacrimal duct obstruction	No	No	No	Esophoria	No	No	No reported concerns
Audiology / hearing	Normal	bilateral mixed hearing loss requiring hearing aids	Normal	Normal	Chronic OM s/p TM tubes, normal audiometry	Normal	Normal	moderate conductive hearing loss	Normal	Normal	Normal	Normal
Oral / dentition / other ENT problems	hypoplastic uvula	normal	High palate	High palate	Normal dentition, no caries	No	Anterior open-bite malocclusion	No	Carious teeth	Laryngeal cleft	High palate	No reported concerns
Cardiac problems	spontaneously resolving VSD	No	interrupted aortic arch; multiple ASD and VSDs; subaortic stenosis. Severe LV dysfunction.	No	Normal ECHO	delay in ductus arteriosus closure after birth, but normal echocardiogram at 3 weeks of age	Surgical correction for patent ductus arteriosus, now she has minimal shunts for perimembranous interventricular defect and ostium secundum interatrial defect.	No	No	No	Persistent left superior vena cava to coronary sinus	No
Respiratory problems	No	respiratory distress at birth, no current issues	No	No	No	No	Recurrent pneumonia, low ciliar functioning	No	No	No	recurrent pneumonia	No
Gastrointestinal problems/ feeding difficulties	No	feeding dysfunction in infancy requiring Gtube, Nissen fundoplication (GERD), currently eats by mouth with no vomiting, diarrhea or constipation	Gtube fed; Constipation	Severe persistent constipation	Severe feeding difficulties with velopharyngeal insufficiency, GERD, constipation	poor feeding, constipation	Episodic constipation	Constipation	Chronic constipation, gastro-oesophageal reflux	Constipation (negative testing for Hirschprung); G-tube dependence; GERD	failure to gain weight	Severe constipation. Negative rectal biopsy for Hirschprung.
Renal / genitourinary problems	No	No	Cryptorchidism	Nephromegaly/Normal function	Nocturia	normal renal ultrasound at 14 mo	No	No	Cryptorchidism	No	No	No
Hematologic problems	No	No	Neutropenia; Thrombocytopenia	No	Neutropenia (resolved) and intermittent normocytic anemia	iron deficiency (4 µmol/L at 5 mo)	neonatal anemia	No	No	No	No	No
Endocrine problems	testosterone assay and pelvis ultrasound normal	No	No	No	No	No	No	No	Vitamin D deficiency	No	No	No
Musculoskeletal problems	Joint laxity	muscle fatigue	Tall	Normal birth centiles;subsequent overgrowth with all centile being >>> 97th ; review at age 15 growth now normalizing with centiles ; as adolescent more Marfan like habitus with long torsi and US:LS ratio 0.79 & arm span >length by 3.7 cm; small chest with mild pectus excavatum;	Left sided hemihyperplasia	No	Beighton score 6/9 + pectus carinatum + mild scoliosis	discrete limb length asymetry (Left>Right)	Dislocated hip	No	increased muscle tone	No
Immunological problems	No	No	No	No	Hypogammaglobulinemia	No	IgG1 deficit	No	No	Recurrent infections/pneumonia, Possible IgA deficiency	No	No
Skin	hypertrichosis	Normal	Normal	Pigmented naevus on lower right buttock	Normal	white patches on skin and a rash	Hypopigmented skin across Blaschko lines	Pigmentary mosaicism including cafe-au-lait spots in a patchy pattern without midline separation and hyperpigmentation in a blaschkolinear pattern Naevus flammeus	Normal	Normal	Normal	Normal
Malignancy	No	No	No	No	No	No	No	No	No	No	No	No
Other		normal microarray in 2013, mildly low carnitine responding to supplementation	Admission to ICU for hyperammonemia		Severe fatigue, drop attacks (unclear etiology)	narrow shoulders						CMA, MS-MLPA for Angelman, and FMR1 testing negative
Family History												
Similarly affected family members	mother with ID and hypertrichosis, father with ID and microcephaly (-3.5SD),	no	None	Nil	7 year old brother with hemihyperplasia, but developmentally normal. Does not carry FBXW7 variant.	No	no	No	No	NO	none	No
Consanguinity	no	no	No	Nil	No	No	no	No	No	NO	no	No
Full siblings	fetus with ventriculomegaly, hypoplastic thumbs, IUGR, anal imperforation, pelvic kidney, right aortic arch.	yes	Full Sister - 16 yo - Healthy	2- well and developmentally age appropriate	1	1	1 male, 1 female	No	0	5		2
Other					Non-contributory	Parents unaffected		No	One half sibling	NO		
Physical Examination	at 2y 3m											
Height	88 (+0,5SD)	142.3 cm (72nd centile)	167 cm (53rd centile)	All consistently above 97th centile till about age 13 then has started normalizing and at last exam (age 15) head circumference remains above 97th centile but other centiles on 50th.	89th centile	At 10 mo: 75 cm (64th percentile)	141 (75-90°%)	120 cm (+1,5 SD) at 6 years	Age 5 m: 66 cm (52nd centile)	107.0 cm (95th percentile)	142.3 cm	102 cm (65th centile)
Weight	10,5 (-1,5SD)	30.9 kg (44th centile)	58.8 kg (69th centile)	50th centile	92nd centile	At 10 mo: 11 kg (81st percentile)	32,9 (50°%)	23,6 Kg (+1 SD) at 6 years	Age 5 y: 21.7 kg (87th centile)	18.1 kg (90-95th percentile)	30.9 kg	16kg (60th centile)
Head circumference	44 (-3SD)	57 cm (99th centile)	N/A	head circumference remains above 97th centile	>99th %ile, z= +3	At 10 mo: 49 cm (99th percentile, +2.4 SD)	57 (>97°%)	54.2 cm (+2 SD) at 6 years	Age 5 y: 52 cm (66th centile)	Macrocephaly - 54.6 cm (>95th centile)	46 at 2y	52cm (83rd centile)
Facial features	Downturned corner of the mouth, synophrys, highly arched eyebrows	Not dysmorphic	Myopathic facies; Hypoteloric, thin upper and lower lips vermilion, highly arched eyebrows with synophrys; thick eyelashes, left preauricular pit.	Long palpebral fissures which were down slanting with long thick curly eyelashes uptilted nose with a flat tip; low set posterior rotated ears with overfolded helices and prominent inferior and superior crus ; jowl-like cheeks and his mouth was small with down turned corners; high palate	somewhat flat face, short nose, downturned corners of mouth	small chin, deep set eyes	long face, bulbous nose, posteriorly rotated ears, high narrow palate, nasal voice	Mild dysmorphic facial -metopic ridge, overfolded helices, epicanthal folds, bulbous nose, uptented upper lip,	Long palpebral fissures, dimple lower lip, philtral pillars prominent	Mild dysmorphic facial features- Overfolded helices, Bilateral epicanthal folds, down slanted palpebral fissures, deep-set eyes, periorbital fullness, High arched palate, Wide nasal bridge, Scaphocephalic with a prominent occiput, Metopic but not sagittal ridging.	Puffy eyelids and cheeks ,small mouth slightly broad nose	mild frontal bossing, raised philtrum, thin upper lip,
Hands / feet	flat feet, right single transverse palmar fold	Normal	Normal	Although his hands were large consistent with the rest of his habitus, his fingers were actually short (10th centile); broad thumbs. splayed forefeet with 2-3 syndactyly.	hemihyperplasia of left leg	fat pad on top of foot	Normal	finger hyperlaxity and mild proximal rigidity	Tapered digits, hypermobile joints, single palmar crease bilaterally, persistent fetal finger pads, broad small feet	Thickening of palms	Finger swelling	Normal
Other findings	No	No	No	small chest with mild pectus excavatum; small umbilical hernia;	No	long eyelashes	No	No	BW 3.48kg, HC 35.5cm at birth	Thymus bulge with valsalva. T&A removed	No	No
											153247171A>C	

INDIVIDUAL	24	25	26	27	28	29	30	31	32	33	34	35
VARIANT TYPE	MISSENSE, AFFECTING WD40 DOMAIN				MISSENSE, NOT AFFECTING WD40 DOMAIN							
Variant Details												
Mode of discovery	Trio WES	Trio Exome	Trio WES	Trio WGS (research)	Trio WES	Trio WES (GeneDx)	Trio WES	Trio WES	Trio WES	Solo WES	trio WES	Trio WES
cDNA (NM_001349798.2)	c.1744T>G	c.1796C>T	c.1877C>T	c.1920C>A	c.1822A>G	c.2021G>C	c.2020C>T	c.2020C>T	c.2066G>A	c.2065C>T	c.2065C>T	c.2065C>T
gDNA (Chr4; GRCh37)	g.153245447A>C	g.153245395G>A	g.153244280G>A	g.153244237G>T	g.153245369T>C	g.153244136C>G	g.153244137G>A	g.153244137G>A	g.153244091C>T	g.153244092G>A	g.153244092G>A	g.153244092G>A
Protein level	p.(Ser582Ala)	p.(Ala599Val)	p.(Ala626Val)	p.(Ser640Arg)	p.(Ile608Val)	p.(Arg674Pro)	p.(Arg674Trp)	p.(Arg674Trp)	p.(Arg689Gln)	p.(Arg689Trp)	p.(Arg689Trp)	p.(Arg689Trp)
Same variant in gnomAD	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Alternative change at same amino acid residue in gnomAD	Absent	p.(Ala599Gly) (allele balance 35-40%)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Same variant in COSMIC	Absent	Absent	Present (large intestine adenocarcinoma)	Absent	Absent	Absent	Present (glioma, cervix squamous cell carcinoma, atypical meningioma, prostate adenocarcinoma)	Present (glioma, cervix squamous cell carcinoma, atypical meningioma, prostate adenocarcinoma)	Present (more than 5 tissue types)	Present (more than 5 tissue types)	Present (more than 5 tissue types)	Present (more than 5 tissue types)
Alternative change at same amino acid residue in COSMIC	Multiple	Absent	p.(Ala626Thr), p.(Ala626Pro), p.(Ala626Asp)	Yes (one in NCI's GDC)	Absent	p.(Arg674Trp), p.(Arg674Gln)	p.(Arg674Gln) (colon adenocarcinoma, stomach adenocarcinoma)	p.(Arg674Gln) (colon adenocarcinoma, stomach adenocarcinoma)	p.(Arg689Glu), p.(Arg689Trp)	p.(Arg689Glu), p.(Arg689Gln)	p.(Arg689Glu), p.(Arg689Gln)	p.(Arg689Glu), p.(Arg689Gln)
Exon (NM_001349798.2)	13	13	14	14	13	14	14	14	14	14	14	14
Occurs within WD40 domain	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Inheritance	De novo	De novo (mosaic: 23.3% of 103 reads)	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Other candidate variants	No	No	No	No	No	No	No	No	KMT2D: de novo NM_003482.4:c.16012T>C p.(Cys5338Arg)	No	No	No
Demographic Features												
Sex	Female	Male	Male	Female	Male	Male	Male	Male	Male	Male	Male	Male
Age at last assessment	6 years	22 months	3 yrs	7 years	11 years	2 years (currently 4 years)	15 years	14 years	2 years	10 years	12 years	7 y
Other			Caucasian		Caucasian							
Medical History												
Prenatal history	Normal	1565g, 33 weeks premature, SGA, tracked small on US but no dx of IUGR. Vaginal. Mother G1P0, 30years old. NICU for 19 days for jaundice, hypoglycemia and feeding difficulties. Abnormal newborn hearing screen.	Born at 41+4 wks. Uncomplicated pregnancy and delivery. BW 3872g.	Low maternal serum PAPP-A; Velamentous cord insertion; Term birth and Apgars 9/9; Birth weight, length, and head circumference within normal range	Normal	Born at 42wks to a G9P6 41yo mother, BW = 7lbs, Length = 48.5cm, HC = 34.5cm. NICU for 2 days due to mild respiratory distress and poor feeding. Parents report a history of increased nuchal fold and extra skin fold in the back of the neck at birth, which resolved. Mother took Prilosec for GERD during pregnancy. Newborn screen was normal. NHS was completed 3 times.	Unremarkable pregnancy. Born at term. BW 3450g. Apgars 8,9. Mom recalls concerns re being "blue" at delivery but no resuscitation needed.	Normal	Normal	Normal	Normal	Hyperechogenic kidneys; hypotonia and respiratory distress at birth (Apgar 4-7-9)
Neurological problems	no hypotonia, no seizures	motor developmental delay, mild hypotonia. Focal seizures (left anterior temporal area) confirmed by EEG and is now on antiepileptic medication	Hypotonia. Tonic upgaze eye movements in infancy. Myoclonic and myoclonic-tonic seizures from 4-5 months of age. EEG findings suggestive of developmental epileptic encephalopathy with multifocal and generalised discharges.	Generalized hypotonia; Treatment-refractory seizures;	Early Onset Absence Epilepsy (EOAE); rare isolated myoclonic seizures	Generalized hypotonia	Normal tone on exam (from 8 years). Achilles tendon contracture (tight heel cords). Abnormality of coordination. Possible ataxia. Has history of migraines with photophobia.	epilepsy started at 4y6m; hypotonia; ataxia; upper hand dyskinetic involuntary movements and stereotypies	Normal	hypotonia, language trouble	hypotonia	Global DD, hypotonia, drooling
Brain imaging	Delayed myelination; generally thin corpus callosum	Brain MRI 3/2018: prominent extraaxial fluid but otherwise normal for age.	MRI brain abnormal sulcal pattern suggestive of polymicrogyria.	Brain MRI x3 - Large cerebellum with crowded posterior fossa at 9 months, the vermis continues to grow from 42mm to 46mm at 3.5 years, thereafter it mildly shrinks to 41mm at 9 years – this could be related to antiepilepsy medications. The dysmorphic corpus callosum is not thickened at 9 months but is thick at 3.5 and 9 years. On the last scan there is generalised mild ventriculomegaly and increased size of the extra-axial CSF spaces	N/A	N/A	MRI brain shows big cerebellum 46mm with crowded posterior fossa and tonsils low lying at foramen magnum; thick brainstem, scattered bilateral fl/T2 hyperintensities in subcortical white matter,frontal, parietal and temporal similar to Individual 25. Volume and myelination ok; thin corpus callosum	normal brain MRI	N/A	Brain MRI (2 yo) : non specific white matter hypersignals, widening of Virchow–Robin spaces, vermis upper limits 41mm, low right tonsil, mega cisterna magna; thick brainstem; normal myelination and corpus callosum	N/A	Brain MRI: normal
Developmental / cognitive / psychiatric problems	Moderate global developmental delay (4 months head control 9 months sitting 31 months walking)	Developmental concerns at 2 months when he was not lifting his head or tracking. Rolled over at 9 months, sat on own at 10 months, pulled to stand at 14 months, crawled at 14 months, cruising at 16 months, walking at 22 months is very wobbly and still needs support. Speech - at 22 months has 2-3 spontaneous words, but usually only repeats or echos. Developmental delay, global, mild and making progress. Cruising age 16 mos; not walking yet 22 mos. Few words, mostly repeats words at 22 mos. Social and affectionate.	Motor and speech delay. Walked at 19/20 months of age. Minimal speech at 3yrs of age.	Global developmental delay (non-verbal, non-ambulatory)	Learning difficulties/Borderline IQ FSIQ=70	Mild-moderate global developmental delay (Sitting at 8mo, talking at 13mo and walking at 24mo). EI evaluation showed average cognition with delayed expression language and motor skills. At 22mo his pediatrician reported that he was able to point and vocalize to show his wants, as well as use two word combinations, but he could not run without falling. By 26mo months he could use pronouns and verbalize immediate experiences, but he could not walk up and down stairs one step at a time.	Moderate ID. GDD - walked at 29 months. Speech delay with first words at 4 years and sentence at 6 years, still not fully understandable at age 9. In grade 9 was operating at grad 4-5 level for academics, and is in life skills program at school. Has compulsive behaviours, paricularly trichotillomania. NO autism.	severe delay; no words; understands simple orders; walked at 32 m;	Speech delay	moderate global developmental delay, walking at 23 mo, global motor delay, global and facial hypotonia, fine motor trouble, 1st words at 4yo, can say words, language trouble, good non-verbal communication, good level of understanding of simple orders, not toilet-trained	moderate developmental delay	Global moderate psychomotor delay, delayed and limited speech, dyslalia
Ophthalmologic problems	No	Intermittent strabismus/esotropia. Hypermetropia but no rx for glasses. Amblyopia and treated with patching.	No	Visual impairment; Bilateral ptosis; Cortical visual impairment	Myopia	No	Hypermetropia	No	No	No	No	No
Audiology / hearing	Normal	5db bilateral SNHL. Hx 2 ear infections. Tubes placed at 17 months. Mixed hearing loss with mild sensorineural hearing loss, conductive hearing loss with chronic middle ear effusion s/p ear tubes. *Note: pt is also compound heterozygote for 2 different pathogenic variants in GJB2*	Normal newborn hearing assessment. Normal assessment at 1 year of age.	Unclear	Normal	Failed NHS, but eventually had a normal ABR in both ears	Normal	Normal	Normal	Normal	Normal	Normal
Oral / dentition / other ENT problems	No	delayed teeth eruption and in unusual order	Tongue tie release at 2 months of age.	Dental caries	No	Delayed dental eruption - first tooth at 12mo	No	Normal	Laryngomalacia; Lip and tongue tie at birth - clipped	Narrow palate	No	Dysmorphic ears, small mouth, high palate, bifid uvula, nasal voice, nasal septum deviation
Cardiac problems	Atrial Septal Defect	No	Small PFO noted at birth. Normal ECHO at 2yrs of age.	Ventricular septal defect (spontaneously closed); Mesocardia	No	No	No	No	Bicuspid aortic valve	No	No	Patent foramen ovale, interatrial defect
Respiratory problems	No	No	Recurrent upper respiratory tract infections	Recurrent aspiration pneumonias; Reactive airway disease; Sinuses essentially unremarkable on brain MRI	No	No	No	No	CT scan performed secondary to frequent sinus infections showed "underdeveloped maxillary sinus".	No	No	No
Gastrointestinal problems/ feeding difficulties	No	Hx feeding difficulty. Still struggles with hard foods due to delayed teeth eruption. Prefers soft foods and struggles a little to chew and swallow hard foods; uses a sippy cup and can drink from a straw	Diarrhoea	Constipation; Oro-motor feeding dysfunction; GERD; G-tube fed	No	Hx poor sucking and feeding difficulties as infant leading to FTT by 3mo. Formulas were tried with no success. Barium swallow showed GERD - tx Prevacid and Zantac with minimal improvement. Normal upper GI and small bowel series; endoscopy showed some mild inflammation, but was otherwise wnl. Hx chronic constipation.	intermittent diarrhea, of unclear etiology	No	FTT and poor suck/swallow coordination. GERD and blood in stool prompting several formula changes. He was diagnosed with milk protein allergy as infant, but skin test negative and now able to tolerate milk without symptoms / blood in stools.	Constipation	severe constipation	Constipation
Renal / genitourinary problems	No	No	No	Bilateral inguinal hernias	Incontinence, resolved at 8 yr with seizure control	No	Small penis and testicles (history of delayed puberty). Normal renal ultrasound	No	Bilateral cryptorchidism	incomplete foreskin	No	Increased corticomedullary differentiation
Hematologic problems	No	No	No	No	No	No	No	No	No	No	No	No
Endocrine problems	No	No	No	Neonatal hypoglycemia (resolved)	No	Hypothyroidism (IGF-1 41 ng/mL, TSH 15.2 uU/mL, free T4 1.43 ng/dL) tx w/ synthroid .05mg, Nl thyroid ultrasound	Delayed puberty	No	No	No	No	No
Musculoskeletal problems	No	No joint problems or muscle concerns. Hypotonia has improved with time	Mild kyphosis. Hypotonia has improved with time.	Right calcaneovalgus deformity at birth (resolved)	No	No	mild valgus deformity of knees. Decreased coverage of R femoral head on pelvic xray. Short stature at 3rd centile	No	No	Joint laxity	early hypotonia	Joint laxity
Immunological problems	No	No	No	Recurrent infections	No	Episodes of fevers	Bacterial meningitis at age 8y, no history of recurrent infections	No	No	few otitis, bronchitis	No	No
Skin	Normal	Normal	Eczema	Capillary hemangiomas; Nevus	Normal	Normal	sensitive skin to temperature change	Normal	May have immunological abnormalities (per parents report, we didn't have anything to review).	Normal	Normal	Normal
Malignancy	No	No	No	No	No	No	No	No	No	No	No	No
Other		Failure to thrive. Weight percentile <1%, length ~1%, weight for length ~32%, head circumference 2%, All growth parametes are increasing along his own curve indicating normal growth velocity.	Narrow/sloping shoulders		Nil		array CGH (60K) normal, frax normal	No	No	No	No	Dolicocephaly, macrocephaly (OFC = 54 cm at 3 y),
Family History												
Similarly affected family members	No, She is an only child, from healthy unrelated Japanese parents.	No. First child to both parents	No	No	No	No	no	no	No	no	no	No
Consanguinity	No	None	No	No	No	No	no	no	No	no	no	No
Full siblings		None	1	2	2	7	3				healthy sister	No
Other		Ashkenazi Jewish ancestry.	Nil	Maternal uncle with congenital heart disease	Nil	Mother had two spontaneous first trimester abortions; AJ ancestry	none contributory					-
Physical Examination		*at 22 months*						5y7m				
Height	103 cm, -1.9 SD	76.20cm (Z=-2.85)	91 cm (10th centile)	125 cm (62nd centile) at 7y5m	138.2 at 11 yrs (22nd percentile)	<<1% (Normal at birth, by 3 months length was 39%, and by 12mo it was <3%)	154 .2 cm (3rd centile) at 15y (was previously at 25th centile)	114.5 cm (+1 SD)	92nd centile	147 cm (+1.5 SD)	166 cm (+1.5 SD)	116.5 cm (50th centile) at 6 y and 3 m
Weight	16.4kg, -1.1SD	9.62kg (Z= -2.40)	12.3 kg (7th centile)	26 kg (74th centile) at 7y5m	32.2 at 11 yrs (26th precentile)	<<1% (Normal at birth, by 3 months weight fell below 3%)	52.8 kg (25-50th centile) at 15y	20 kg (+1 SD)	99th centile	33.3 Kg (+0.5 SD)	50.2kg (0 SD)	22 kg (59th centile) at 6 y and 3 m
Head circumference	48.5cm, -1.5SD	45.60 cm (Z= -2.06)	50 cm (58th centile)	51.0 cm (34th centile) at 6y10m	52 cm at 5 yrs (85% percentile)	25% (consistent since birth)	55.8cm (75th centile) at 15y	53 cm (+1 SD)	90-98th centile	55.5 (+1.5 SD)	60cm (+3 SD)	54.5 cm (+2.5 SD) at 6 y and 3 m
Facial features	Deep-set eyes, periorbital fullness, anteverted ears, depressed nasal bridge and broad nasal tip.	mild flattening of the occiput but otherwise normal head shape with anterior and posterior hairlines normal. Curly, dark brown hair. Level palpebral fissures with normal eye spaces. Pupils equally round and reactive to light with extraocular movements in tandem. Irises are brown. Ears normal formed and placed.There are no preauricular pits or tages. Prominent, somewhat high nasal bridge with normal nose and philtrum. Thin lips. Palate is intact and not high with single midline uvula. Teeth are primary teeth with normal enamel.	Epicanthic folds, depressed & broad nasal bridge, blue sclerae.	Low anterior hairline; Bilateral ptosis; Thin ear helix; Synophrys	Normal	Depressed nasal bridge, high arched palate, tented upper lip	Deep set eyes, mildly narrow pfs. flared eyebrows, long philtrum, prominent lips, large appearing frontal incisors	no	Ears that are mildly prominent and posteriorly rotated but otherwise normally formed and set, normal nasal bridge, good dentition, forehead is tall with a somewhat receded anterior hairline. Horizontal with mildly long palpebral fissures, periorbital fullness noted and gives impression of eyes being somewhat deep set, bilateral epicanthal folds.	small mouth	frontal bossing, downslant, small ears	Dysmorphic features: high forehead, dolichocephaly, wide and protrudin ears with uplifted earlobes, flat nasal philtrum, small mouth, malocclusion, ogival palate, bifid uvula, chin hypoplasia
Hands / feet	Normal	Mild fifth finger brachydactyly. Normal otherwise.	Normal	Bilateral single transverse palmar crease; Tapered fingers; Interphalangeal joint contracture of finger; Short phalanges of fingers; 2-3 toe syndactyly; Prominent calcaneus	Normal	Persistent fetal pads, single palmer crease	2-3 toe syndactyly	Normal	2nd toes bilaterally have a more dorsal insertion point and the 2nd toe overlaps toes 1 and 3, right > left, lymphedema of the dorsum of each hand that was not noted in the feet.	Normal	Normal	Pes planus, bilateral
Other findings	No	No	No	No	No	No	Supernumerary nipple	No	Mild distal pectus excavatum	No	No	Somatosensory evoked potentials and EMG: normal
				Was reported in cohort paper PMID 32960281								

Table S3: HPO terms associated with FBXW7 Neurodevelopmental syndrome

Prenatal history	Thickened nuchal skin fold HP:0000474
	Jaundice HP:0000952
	Breech presentation HP:0001623
	Neonatal hypoglycemia HP:0001998
	Hyperechogenic kidneys HP:0004719
	Neonatal respiratory distress HP:0002643

Neurological problems	Hypotonia HP:0001252
	Seizure HP:0001250
	Progressive spasticity HP:0002191
	Unsteady gait HP:0002317
	Broad-based gait HP:0002136
	Paroxysmal tonic upgaze HP:0033980
	Early onset absence seizures HP:0011152
	Achilles tendon contracture HP:0001771
	Abnormality of coordination HP:0011443
	Migraine HP:0002076
	Photophobia HP:0000613
	Ataxia HP:0001251
	Dyskinesia HP:0100660
	Stereotypy HP:0000733
	Drooling HP:0002307
	Atonic seizure HP:0010819

Brain Imaging	Enlarged cerebellum HP:0012081
	Arnold-Chiari type I malformation HP:0007099
	Thin corpus callosum HP:0033725
	Agenesis of corpus callosum HP:0001274
	Abnormal corpus callosum morphology HP:0001273
	Punctate periventricular T2 hyperintense foci HP:0030081
	Aplasia/Hypoplasia of the cerebellum HP:0007360
	Enlarged cisterna magna HP:0002280
	Abnormal brainstem morphology HP:0002363
	Brain atrophy HP:0012444
	Subcortical white matter calcifications HP:0007346
	Delayed myelination HP:0012448
	Extra-axial cerebrospinal fluid accumulation HP:0012510
	Polymicrogyria HP:0002126
	Ventriculomegaly HP:0002119
	Dilation of Virchow-Robin spaces HP:0012520

Developmental / cognitive / psychiatric problems	Global developmental delay HP:0001263
	Intellectual disability, borderline HP:0006889
	Anxiety HP:0000739
	Poor suck HP:0002033
	Motor delay HP:0001270
	Specific learning disability HP:0001328
	Delayed speech and language development HP:0000750
	Speech articulation difficulties HP:0009088
	Sleep disturbance HP:0002360
	Impulsivity HP:0100710
	Short attention span HP:0000736
	Autistic behavior HP:0000729
	Speech apraxia HP:0011098
	Developmental regression HP:0002376
	Hair-pulling HP:0012167
	Depression HP:0000716

Ophthalmologic problems	Astigmatism HP:0000483
	Hypermetropia HP:0000540
	Amblyopia HP:0000646
	Strabismus HP:0000486
	Nasolacrimal duct obstruction HP:0000579
	Esophoria HP:0025312
	Bilateral ptosis HP:0001488
	Cerebral visual impairment HP:0100704
	Myopia HP:0000545

Audiology / hearing	Mixed hearing impairment HP:0000410
	Conductive hearing impairment HP:0000405
	Sensorineural hearing impairment HP:0000407

Oral / dentition / other ENT problems	Otitis media with effusion HP:0031353
	Submucous cleft of soft and hard palate HP:0410031
	High palate HP:0000218
	Widely spaced teeth HP:0000687
	Cleft soft palate HP:0000185
	Dental malocclusion HP:0000689
	Midface retrusion HP:0011800
	Ankyloglossia HP:0010296
	Poor suck HP:0002033
	Impaired oropharyngeal swallow response HP:0031162
	Episodic upper airway obstruction HP:0012271
	Short uvula HP:0010812
	Anterior open-bite malocclusion HP:0009102
	Carious teeth HP:0000670
	Laryngeal cleft HP:0008751
	Delayed eruption of teeth HP:0000684
	Laryngomalacia HP:0001601
	Narrow palate HP:0000189
	Abnormal ear morphology HP:0031703
	Bifid uvula HP:0000193
	Nasal speech HP:0001611
	Deviated nasal septum HP:0004411

Cardiac problems	Bicuspid aortic valve HP:0001647
	Patent ductus arteriosus HP:0001643
	Atrial septal defect HP:0001631
	Ventricular septal defect HP:0001629
	Interrupted aortic arch HP:0011611
	Subvalvular aortic stenosis HP:0001682
	Abnormal left ventricular function HP:0005162
	Secundum atrial septal defect HP:0001684
	Persistent left superior vena cava HP:0005301
	Patent foramen ovale HP:0001655
	Mesocardia HP:0011599

Respiratory problems	Obstructive sleep apnea HP:0002870
	Snoring HP:0025267
	Asthma HP:0002099
	Recurrent pneumonia HP:0006532
	Recurrent sinusitis HP:0011108
	Abnormality of the maxillary sinus HP:0430023
	Recurrent upper respiratory tract infections HP:0002788
	Bronchitis HP:0012387

Gastrointestinal problems/ feeding difficulties	Feeding difficulties HP:0011968
	Nasogastric tube feeding in infancy HP:0011470
	Chronic constipation HP:0012450
	Polyphagia HP:0002591
	Gastroesophageal reflux HP:0002020
	Velopharyngeal insufficiency HP:0000220
	Constipation HP:0002019
	Failure to thrive HP:0001508
	Diarrhea HP:0002014

Renal / genitourinary problems	Bilateral cryptorchidism HP:0008689
	Multicystic kidney dysplasia HP:0000003
	Enlarged kidney HP:0000105
	Nocturia HP:0000017
	Inguinal hernia HP:0000023
	Urinary incontinence HP:0000020
	Micropenis HP:0000054
	Decreased testicular size HP:0008734
	Ventral shortening of foreskin HP:0012435
	Abnormal renal corticomedullary differentiation HP:0005932

Hematologic problems	Neutropenia HP:0001875
	Thrombocytopenia HP:0001873
	Normocytic anemia HP:0001897
	Iron deficiency anemia HP:0001891
	Anemia HP:0001903

Endocrine problems	Low levels of vitamin D HP:0100512
	Neonatal hypoglycemia HP:0001998
	Hypothyroidism HP:0000821

Musculoskeletal problems	Sprengel anomaly HP:0000912
	Webbed neck HP:0000465
	Joint laxity HP:0001388
	Short finger HP:0009381
	Broad thumb HP:0011304
	Increased muscle fatiguability HP:0003750
	Pectus excavatum HP:0000767
	Pectus carinatum HP:0000768
	Hemihypertrophy HP:0001528
	Scoliosis HP:0002650
	Hip dislocation HP:0002827
	Hypertonia HP:0001276
	Kyphosis HP:0002808
	Calcaneovalgus deformity HP:0001848
	Genu valgum HP:0002857

Immunological problems	Decreased circulating antibody level HP:0004313
	Recurrent fever HP:0001954

Skin problems	Acne HP:0001061
	Telangiectasia HP:0001009
	Blue nevus HP:0100814
	Hypertrichosis HP:0000998
	Melanocytic nevus HP:0000995
	Poliosis HP:0002290
	Hypopigmentation of the skin HP:0001010
	Distributed along Blaschko lines HP:0025293
	Cafe-au-lait spot HP:0000957
	Hyperpigmentation of the skin HP:0000953
	Nevus flammeus HP:0001052
	Capillary hemangioma HP:0005306

Growth	Short stature HP:0004322
	Tall stature HP:0000098
	Obesity HP:0001513
	Macrocephaly HP:0000256
	Microcephaly HP:0000252

Facial features	Broad forehead HP:0000337
	Abnormal nasal bridge morphology HP:0000422
	Periorbital fullness HP:0000629
	Malar flattening HP:0000272
	Deeply set eye HP:0000490
	Thin vermilion border HP:0000233
	Underdeveloped superior crus of antihelix HP:0011246
	Preauricular pit HP:0004467
	Epicanthus HP:0000286
	Thick eyebrow HP:0000574
	Synophrys HP:0000664
	Large earlobe HP:0009748
	Downturned corners of mouth HP:0002714
	Prominent forehead HP:0011220
	Hypertelorism HP:0000316
	Telecanthus HP:0000506
	Almond-shaped palpebral fissure HP:0007874
	Low-set, posteriorly rotated ears HP:0000368
	Prominent metopic ridge HP:0005487
	Dolichocephaly HP:0000268
	Flat occiput HP:0005469
	Wide nasal bridge HP:0000431
	Eversion of lateral third of lower eyelids HP:0007655
	Thickened helices HP:0000391
	Microtia HP:0008551
	Micrognathia HP:0000347
	Smooth philtrum HP:0000319
	Highly arched eyebrow HP:0002553
	Myopathic facies HP:0002058
	Hypotelorism HP:0000601
	Prominent eyelashes HP:0011231
	Long palpebral fissure HP:0000637
	Downslanted palpebral fissures HP:0000494
	Anteverted nares HP:0000463
	Overfolded helix HP:0000396
	Prominent inferior crus of antihelix HP:0011238
	Narrow mouth HP:0000160
	Short nose HP:0003196
	Prominent nasal tip HP:0005274
	Tented upper lip vermilion HP:0010804
	Deep philtrum HP:0002002
	Scaphocephaly HP:0030799
	Prominent occiput HP:0000269
	Anteverted ears HP:0040080
	Depressed nasal bridge HP:0005280
	Low anterior hairline HP:0000294
	Thick vermilion border HP:0012471
	Incisor macrodontia HP:0011081

Hands/feet	Tapered finger HP:0001182
	Single transverse palmar crease HP:0000954
	Short foot HP:0001773
	Pes planus HP:0001763
	Overlapping toe HP:0001845
	Clinodactyly HP:0030084
	Metatarsus adductus HP:0001840
	2-3 toe syndactyly HP:0004691
	Prominent fingertip pads HP:0001212
	Finger swelling HP:0025131
	Short 5th finger HP:0009237
	Interphalangeal joint contracture of finger HP:0001220
	Prominent calcaneus HP:0012428
	Abnormality of the 2nd toe HP:0010319

Other	Supernumerary nipple HP:0002558
	Narrow chest HP:0000774
	Umbilical hernia HP:0001537

Table S4: Impact of neurodevelopmental syndrome variants on protein stability and interaction with CYCLIN E1.

	mCSM-Stability (ΔΔG) - previous structure	mCSM-Stability (ΔΔG)	Change in protein stability	Distance to interface (Å)	mCSM-PPI 1&2 (ΔΔG)	Change in PPI binding affinity	Δ Charge	Δ Volume	Δ Residue nature
p.(Thr416Ile)	-0.134	-0.179	Decrease	15.1	-0.07	Decrease	0	50.6	Polar -> Hydrophobic
p.(Thr416Ala)		-0.788	Decrease	15.1	-0.134	Decrease	0	-27.5	Polar -> Hydrophobic
p.(His420Leu)		-0.823	Decrease	12.4	-0.469	Decrease	Partial	13.5	Partial charge -> Neutral
p.(Gly423Arg)	-1.031	-0.985	Decrease	7.8	-0.264	Decrease	1	113.3	Neutral -> Basic
p.(Arg441Gly)	-1.258	-1.367	Decrease	2.8	0.179	Increase	-1	-113.3	Basic -> Neutral
p.(Ser462Pro)		0.973	Increase	2.6	-2.449	Decrease	0	23.7	Polar -> Hydrophobic
p.(Arg465His)		-1.834	Decrease	5.1	0.286	Increase	Partial	-20.2	Basic -> Partial charge
p.(Arg479Gln)	-0.625	-1.346	Decrease	3.1	0.054	Increase	-1	-29.6	Basic -> Unchanged
p.(Asp480Gly)	0.327	1.401	Increase	4.5	-1.998	Decrease	1	-51	Acidic -> Neutral
p.(Arg505His)	-2.019	-2.032	Decrease	5.4	-0.038	Neutral	Partial	-20.2	Basic -> Partial charge
p.(Val544Gly)	-3.203	-3.16	Decrease	9.1	-1.114	Decrease	0	-79.9	Hydrophobic -> Hydrophobic
p.(His580Tyr)	-0.414	-0.422	Decrease	9.1	0.311	Increase	Partial	40.4	Partial charge -> Aromatic
p.(Ser582Ala)		0.033	Increase	3.54	-0.422	Decrease	0	-0.4	Polar -> Hydrophobic
p.(Ala599Val)	-0.451	-0.093	Neutral	4	0.265	Increase	0	51.4	Hydrophobic -> Hydrophobic
p.(Ala626Val)		-0.106	Decrease	4.4	0.291	Increase	0	51.4	Hydrophobic -> Hydrophobic
p.(Ser640Arg)	-0.639	-0.586	Decrease	11.1	-0.355	Decrease	1	84.4	Polar -> Basic
p.(Ile608Val)		-2.019	Decrease	22.3	-0.244	Decrease	0	-33.4	Hydrophobic -> Hydrophobic
p.(Arg674Pro)	-0.648	-0.614	Decrease	8.1	-0.321	Decrease	-1	-60.7	Basic -> Neutral
p.(Arg674Trp)	-0.283	-0.157	Decrease	8.1	0.013	Neutral	-1	54.4	Basic -> Aromatic
p.(Arg689Gln)	-0.079	-0.353	Decrease	3.4	-0.54	Decrease	-1	-29.6	Basic -> Neutral
p.(Arg689Trp)		-0.357	Decrease	3.4	-0.437	Decrease	-1	54.4	Basic -> Aromatic

mutation Cutoff Scanning Matrix (mCSM); Protein–protein interactions (PPI)

Table S5: Comparison of the impact of neurodevelopmental syndrome variants and gnomAD variants on protein stability and substrate binding

Variant	Amino acid reference	Amino acid position	amino acid change	mCSM-Stability (ΔΔG)	Distance to interface (Å)	mCSM-PPI 1&2 (ΔΔG)	Allele Count	Class	Chromosome	Position	rsID	Reference	Alternate	Source	Protein Consequence	Transcript Consequence
T416A	T	416	A	-0.788	15.1	-0.134	1	clin	4	153247315	-	G	A	This study	p.(Thr416Ile)	c.1247C>T
T416I	T	416	I	-0.179	15.1	-0.07	1	clin	4	153249532	-	T	C	This study	p.(Thr416Ala)	c.1246A>G
H420L	H	420	L	-0.823	12.4	-0.469	1	clin	4	153249519	-	T	A	This study	p.(His420Leu)	c.1259 A>T
G423R	G	423	R	-0.985	7.8	-0.264	2	clin	4	153249511	-	C	T	This study	p.(Gly423Arg)	c.1267G>A
R441G	R	441	G	-1.367	2.8	0.179	1	clin	4	153249457	-	G	C	This study	p.(Arg441Gly)	c.1321C>G
S462P	S	462	P	0.973	2.6	-2.449	1	clin	4	153249394	-	A	G	This study	p.(Ser462Pro)	c.1384T>C
R465H	R	465*	H	-1.834	5.1	0.286	1	clin	4	153249384	-	C	T	This study	p.(Arg465His)	c.1394G>A
R479Q	R	479*	Q	-1.346	3.1	0.054	1	clin	4	153247366	-	C	T	This study	p.(Arg479Gln)	c.1436G>A
D480G	D	480	G	1.401	4.5	-1.998	1	clin	4	153247363	-	T	C	This study	p.(Asp480Gly)	c.1439A>G
R505H	R	505	H	-2.032	5.4	-0.038	1	clin	4	153247288	-	C	T	This study	p.(Arg505His)	c.1514G>A
V544G	V	544	G	-3.16	9.1	-1.114	1	clin	4	153247171	-	A	C	This study	p.(Val544Gly)	c.1631T>G
H580Y	H	580	Y	-0.422	9.1	0.311	1	clin	4	153245453	-	G	A	This study	p.(His580Tyr)	c.1738C>T
A599V	A	599*	V	-0.093	4	0.265	1	clin	4	153245395	-	G	A	This study	p.(Ala599Val)	c.1796C>T
I608V	I	608	V	-2.019	22.3	-0.244	1	clin	4	153245369	-	T	C	This study	p.(Ile608Val)	c.1822A>G
S640R	S	640	R	-0.586	11.1	-0.355	1	clin	4	153244237	-	G	T	This study	p.(Ser640Arg)	c.1920C>A
R674P	R	674	P	-0.614	8.1	-0.321	1	clin	4	153244136	-	C	G	This study	p.(Arg674Pro)	c.2021G>C
R674W	R	674	W	-0.157	8.1	0.013	1	clin	4	153244137	-	G	A	This study	p.(Arg674Trp)	c.2020C>T
R689Q	R	689	Q	-0.353	3.4	-0.54	1	clin	4	153244091	-	C	T	This study	p.(Arg689Gln)	c.2066G>A
R689W	R	689	W	-0.357	3.4	-0.437	2	clin	4	153244092	-	G	A	This study	p.(Arg689Trp)	c.2065C>T
V265I	V	265	I	-1.029	57.368	0.119	1	gnomad	4	153259022	rs1393933844	C	T	gnomAD Genomes	p.Val265Ile	c.793G>A
H267R	H	267	R	-0.581	61.633	-0.111	1	gnomad	4	153259015	rs1172754641	T	C	gnomAD Exomes	p.His267Arg	c.800A>G
V271M	V	271	M	-0.254	64.686	-0.311	1	gnomad	4	153259004	rs764074483	C	T	gnomAD Exomes	p.Val271Met	c.811G>A
K286E	K	286	E	-0.462	60.444	-0.046	1	gnomad	4	153258959	rs1490325931	T	C	gnomAD Exomes	p.Lys286Glu	c.856A>G
L288F	L	288	F	-0.908	59.843	0.132	6	gnomad	4	153253869	-	C	A	gnomAD Exomes	p.Leu288Phe	c.864G>T
A289S	A	289	S	-1.765	57.647	0.121	6	gnomad	4	153253868	rs1444335835	C	A	gnomAD Exomes	p.Ala289Ser	c.865G>T
Y291C	Y	291	C	-0.467	55.112	-0.254	2	gnomad	4	153253861	rs948405432	T	C	gnomAD Exomes	p.Tyr291Cys	c.872A>G
Y291N	Y	291	N	-0.601	55.112	-0.017	2	gnomad	4	153253862	rs369187069	A	T	gnomAD Exomes	p.Tyr291Asn	c.871T>A
K299E	K	299	E	0.02	40.936	0.004	1	gnomad	4	153253838	rs750051282	T	C	gnomAD Exomes	p.Lys299Glu	c.895A>G
L302I	L	302	I	-0.922	44.101	-0.084	1	gnomad	4	153253829	rs150506693	G	T	gnomAD Exomes	p.Leu302Ile	c.904C>A
T307I	T	307	I	-0.413	54.949	-0.006	1	gnomad	4	153253813	rs764174613	G	A	gnomAD Exomes	p.Thr307Ile	c.920C>T
R309H	R	309	H	-0.992	59.667	0.003	1	gnomad	4	153253807	rs760675122	C	T	gnomAD Exomes	p.Arg309His	c.926G>A
L319I	L	319	I	-0.533	49.037	-0.066	1	gnomad	4	153253778	rs1369661240	G	T	gnomAD Genomes	p.Leu319Ile	c.955C>A
K326R	K	326	R	-0.425	39.317	0.12	1	gnomad	4	153253756	rs773325030	T	C	gnomAD Exomes	p.Lys326Arg	c.977A>G
K326T	K	326	T	-0.52	39.317	-0.056	1	gnomad	4	153253756	rs773325030	T	G	gnomAD Exomes	p.Lys326Thr	c.977A>C
E327D	E	327	D	-0.513	37.333	0.1	1	gnomad	4	153253752	rs148769501	T	G	gnomAD Exomes	p.Glu327Asp	c.981A>C
G329E	G	329	E	-1.067	34.737	0.145	1	gnomad	4	153252020	rs1358178925	C	T	gnomAD Genomes	p.Gly329Glu	c.986G>A
I330V	I	330	V	-1.265	36.875	-0.056	1	gnomad	4	153252018	rs767438108	T	C	gnomAD Exomes	p.Ile330Val	c.988A>G
I336M	I	336	M	-0.569	45.102	0.19	1	gnomad	4	153251998	rs1046708929	G	C	gnomAD Exomes	p.Ile336Met	c.1008C>G
K337Q	K	337	Q	-0.295	47.538	0.194	1	gnomad	4	153251997	rs750480880	T	G	gnomAD Exomes	p.Lys337Gln	c.1009A>C
R338K	R	338	K	-0.216	50.737	0.251	3	gnomad	4	153251993	rs1185005670	C	T	gnomAD Exomes	p.Arg338Lys	c.1013G>A
V341A	V	341	A	-0.617	55.273	-0.144	1	gnomad	4	153251984	rs1485389861	A	G	gnomAD Exomes	p.Val341Ala	c.1022T>C
I342T	I	342	T	-0.67	57.24	-0.602	2	gnomad	4	153251981	rs765495879	A	G	gnomAD Exomes	p.Ile342Thr	c.1025T>C
I342V	I	342	V	-0.424	57.24	0.344	1	gnomad	4	153251982	rs1247097813	T	C	gnomAD Exomes	p.Ile342Val	c.1024A>G
I347V	I	347	V	-0.956	53.902	-0.016	1	gnomad	4	153251967	rs762013076	T	C	gnomAD Exomes	p.Ile347Val	c.1039A>G
H359R	H	359	R	-0.889	35.47	0.035	1	gnomad	4	153251930	rs1014611334	T	C	gnomAD Genomes	p.His359Arg	c.1076A>G
T363N	T	363	N	-1.29	31.186	-0.004	3	gnomad	4	153251918	rs1381320045	G	T	gnomAD Exomes	p.Thr363Asn	c.1088C>A
N364S	N	364	S	-1.14	29.412	-0.243	2	gnomad	4	153251915	rs775885576	T	C	gnomAD Exomes	p.Asn364Ser	c.1091A>G
R367Q	R	367	Q	-0.102	29.26	-0.019	7	gnomad	4	153251906	rs745418631	C	T	gnomAD Exomes,gnomAD Genomes	p.Arg367Gln	c.1100G>A
K371R	K	371	R	-0.371	27.686	0.115	1	gnomad	4	153251894	rs761747465	T	C	gnomAD Exomes	p.Lys371Arg	c.1112A>G
P373R	P	373	R	-0.297	23.003	-0.033	1	gnomad	4	153251888	rs748952220	G	C	gnomAD Exomes	p.Pro373Arg	c.1118C>G
K374E	K	374	E	0.473	21.263	-0.168	1	gnomad	4	153251886	rs937391131	T	C	gnomAD Exomes	p.Lys374Glu	c.1120A>G
L387V	L	387	V	-1.645	13.766	0.063	1	gnomad	4	153250901	rs1338105130	A	C	gnomAD Exomes	p.Leu387Val	c.1159T>G
S407L	S	407	L	-0.153	23.243	-0.1	1	gnomad	4	153250840	-	G	A	gnomAD Genomes	p.Ser407Leu	c.1220C>T
V418L	V	418	L	-0.209	15.903	-0.155	2	gnomad	4	153249526	rs755422880	C	A	gnomAD Exomes	p.Val418Leu	c.1252G>T
V418M	V	418	M	-0.517	15.903	-0.098	2	gnomad	4	153249526	rs755422880	C	T	gnomAD Exomes	p.Val418Met	c.1252G>A
N432I	N	432	I	0.078	26.504	-0.075	2	gnomad	4	153249483	rs772668762	T	A	gnomAD Exomes	p.Asn432Ile	c.1295A>T
I433F	I	433	F	-1.662	23.411	0.361	3	gnomad	4	153249481	rs761173677	T	A	gnomAD Exomes	p.Ile433Phe	c.1297A>T
I433V	I	433	V	-1.665	23.411	-0.172	2	gnomad	4	153249481	rs761173677	T	C	gnomAD Exomes	p.Ile433Val	c.1297A>G
I435V	I	435	V	-1.87	16.772	-0.377	1	gnomad	4	153249475	rs1190126709	T	C	gnomAD Exomes	p.Ile435Val	c.1303A>G
V445L	V	445	L	-0.729	18.426	-0.093	1	gnomad	4	153249445	rs776371212	C	A	gnomAD Exomes	p.Val445Leu	c.1333G>T
T456N	T	456	N	-0.895	14.647	-0.131	1	gnomad	4	153249411	rs775244232	G	T	gnomAD Exomes	p.Thr456Asn	c.1367C>A
L457F	L	457	F	-1.16	12.355	0.603	1	gnomad	4	153249407	rs1433184454	T	A	gnomAD Exomes	p.Leu457Phe	c.1371A>T
G459V	G	459	V	-0.347	9.584	-0.311	1	gnomad	4	153249402	rs772056210	C	A	gnomAD Exomes	p.Gly459Val	c.1376G>T
R465C	R	465*	C	-1.48	5.148	0.035	1	gnomad	4	153249385	rs867384286	G	A	gnomAD Exomes	p.Arg465Cys	c.1393C>T
E471G	E	471	G	-0.856	25.356	-0.243	12	gnomad	4	153249366	rs756238684	T	C	gnomAD Exomes	p.Glu471Gly	c.1412A>G
R479G	R	479*	G	-0.614	3.072	-1.265	1	gnomad	4	153247367	rs747241612	G	C	gnomAD Exomes	p.Arg479Gly	c.1435C>G
V485I	V	485	I	-1.163	17.029	-0.053	2	gnomad	4	153247349	rs1325363774	C	T	gnomAD Exomes	p.Val485Ile	c.1453G>A
I488T	I	488	T	-2.861	20.45	-0.025	1	gnomad	4	153247339	rs1222797439	A	G	gnomAD Exomes	p.Ile488Thr	c.1463T>C
H495R	H	495	R	-1.47	17.376	0.073	3	gnomad	4	153247318	rs750717620	T	C	gnomAD Exomes	p.His495Arg	c.1484A>G
Y509C	Y	509	C	-2.06	19.683	-0.627	2	gnomad	4	153247276	rs1334352027	T	C	gnomAD Exomes	p.Tyr509Cys	c.1526A>G
V515I	V	515	I	-0.697	16.152	-0.323	1	gnomad	4	153247259	rs757683191	C	T	gnomAD Exomes	p.Val515Ile	c.1543G>A
T532I	T	532	I	-0.371	20.341	-0.005	1	gnomad	4	153247207	rs1179476070	G	A	gnomAD Exomes	p.Thr532Ile	c.1595C>T
T541S	T	541	S	-0.708	6.634	0.083	1	gnomad	4	153247181	rs1184403966	T	A	gnomAD Exomes	p.Thr541Ser	c.1621A>T
N542S	N	542	S	-1.271	3.775	-0.02	1	gnomad	4	153247177	rs1462861861	T	C	gnomAD Exomes	p.Asn542Ser	c.1625A>G
R564C	R	564	C	-1.983	15.026	-0.448	1	gnomad	4	153245501	rs1024060344	G	A	gnomAD Genomes	p.Arg564Cys	c.1690C>T
T576M	T	576	M	0.072	16.356	-0.047	2	gnomad	4	153245464	rs1429385222	G	A	gnomAD Exomes	p.Thr576Met	c.1727C>T
M587I	M	587	I	-0.303	12.55	-0.345	1	gnomad	4	153245430	rs1269436440	C	T	gnomAD Exomes	p.Met587Ile	c.1761G>A
E588D	E	588	D	-1.234	15.447	0.029	2	gnomad	4	153245427	rs751435265	T	A	gnomAD Exomes	p.Glu588Asp	c.1764A>T
K590E	K	590	E	0.269	22.181	-0.234	1	gnomad	4	153245423	-	T	C	gnomAD Exomes	p.Lys590Glu	c.1768A>G
K590R	K	590	R	-0.309	22.181	-0.109	1	gnomad	4	153245422	rs1290448722	T	C	gnomAD Exomes	p.Lys590Arg	c.1769A>G
A599G	A	599*	G	-0.412	3.998	-0.372	1	gnomad	4	153245395	rs766088325	G	C	gnomAD Exomes	p.Ala599Gly	c.1796C>G
P620S	P	620	S	-0.557	16.899	-0.062	1	gnomad	4	153244299	rs1375643251	G	A	gnomAD Exomes	p.Pro620Ser	c.1858C>T
N633S	N	633	S	-0.757	23.914	-0.073	3	gnomad	4	153244259	rs758163453	T	C	gnomAD Exomes	p.Asn633Ser	c.1898A>G
K634R	K	634	R	0.026	27.347	-0.05	1	gnomad	4	153244256	rs1199177582	T	C	gnomAD Genomes	p.Lys634Arg	c.1901A>G
K652Q	K	652	Q	0.136	26.65	0.13	1	gnomad	4	153244203	rs1182453513	T	G	gnomAD Exomes	p.Lys652Gln	c.1954A>C
T653K	T	653	K	-0.363	24.831	-0.116	1	gnomad	4	153244199	rs775781675	G	T	gnomAD Exomes	p.Thr653Lys	c.1958C>A
T653M	T	653	M	0.115	24.831	0.066	1	gnomad	4	153244199	rs775781675	G	A	gnomAD Genomes	p.Thr653Met	c.1958C>T
F656Y	F	656	Y	-0.584	18.778	-0.201	1	gnomad	4	153244190	rs1176237755	A	T	gnomAD Exomes	p.Phe656Tyr	c.1967T>A
R658Q	R	658	Q	-0.906	20.288	0.191	2	gnomad	4	153244184	rs759610249	C	T	gnomAD Exomes	p.Arg658Gln	c.1973G>A
T662A	T	662	A	-0.612	17.859	-0.022	1	gnomad	4	153244173	rs974902950	T	C	gnomAD Exomes	p.Thr662Ala	c.1984A>G
S665I	S	665	I	-0.629	11.47	-0.132	1	gnomad	4	153244163	rs957874517	C	A	gnomAD Exomes	p.Ser665Ile	c.1994G>T
I675V	I	675	V	-2.065	14.286	0.097	1	gnomad	4	153244134	rs1446303596	T	C	gnomAD Exomes	p.Ile675Val	c.2023A>G
A677T	A	677	T	-0.939	20.339	0.086	2	gnomad	4	153244128	rs781679859	C	T	gnomAD Exomes	p.Ala677Thr	c.2029G>A
N679D	N	679	D	0.186	27.125	0.429	1	gnomad	4	153244122	rs746489993	T	C	gnomAD Exomes	p.Asn679Asp	c.2035A>G
V686I	V	686	I	-0.703	11.334	-0.086	1	gnomad	4	153244101	rs1307998016	C	T	gnomAD Genomes	p.Val686Ile	c.2056G>A
N690S	N	690	S	-0.255	4.292	-0.391	1	gnomad	4	153244088	rs1237327125	T	C	gnomAD Genomes	p.Asn690Ser	c.2069A>G
M706T	M	706	T	-0.185	41.053	-0.248	1	gnomad	4	153244040	rs764051432	A	G	gnomAD Exomes	p.Met706Thr	c.2117T>C

*residue affected in both clincal cohort and gnomAD. Mutation Cutoff Scanning Matrix (mCSM); Protein–protein interactions (PPI).