Partial Loss of USP9X Function Leads to a Male Neurodevelopmental and Behavioral Disorder Converging on Transforming Growth Factor β Signaling

SUPPLEMENTAL INFORMATION

This file contains 17 Figures (Figure S1-S17), 7 Tables (Table S1-S7) and 1 Clinical Data Description

SUPPLEMENTAL FIGURES S1-S17

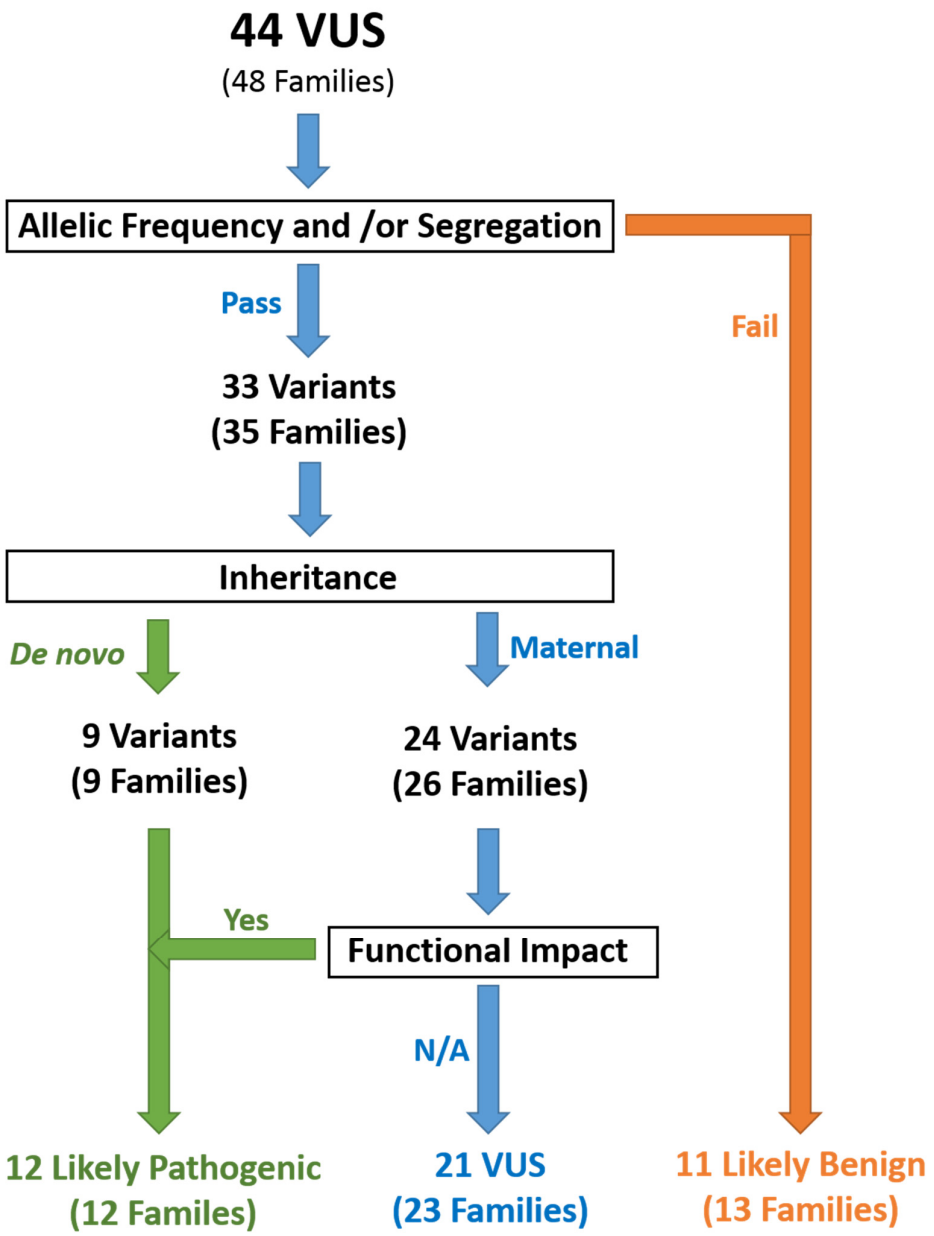


Figure S1. Overview of classification of *USP9X* male variants. Fail at 'Allelic Frequency and/or Segregation' based on either being found in > 1: 10000 alleles, or having >4 alleles found in hemizygous state in gnomAD (genome and exome) data base, or being discovered in a healthy male relative.

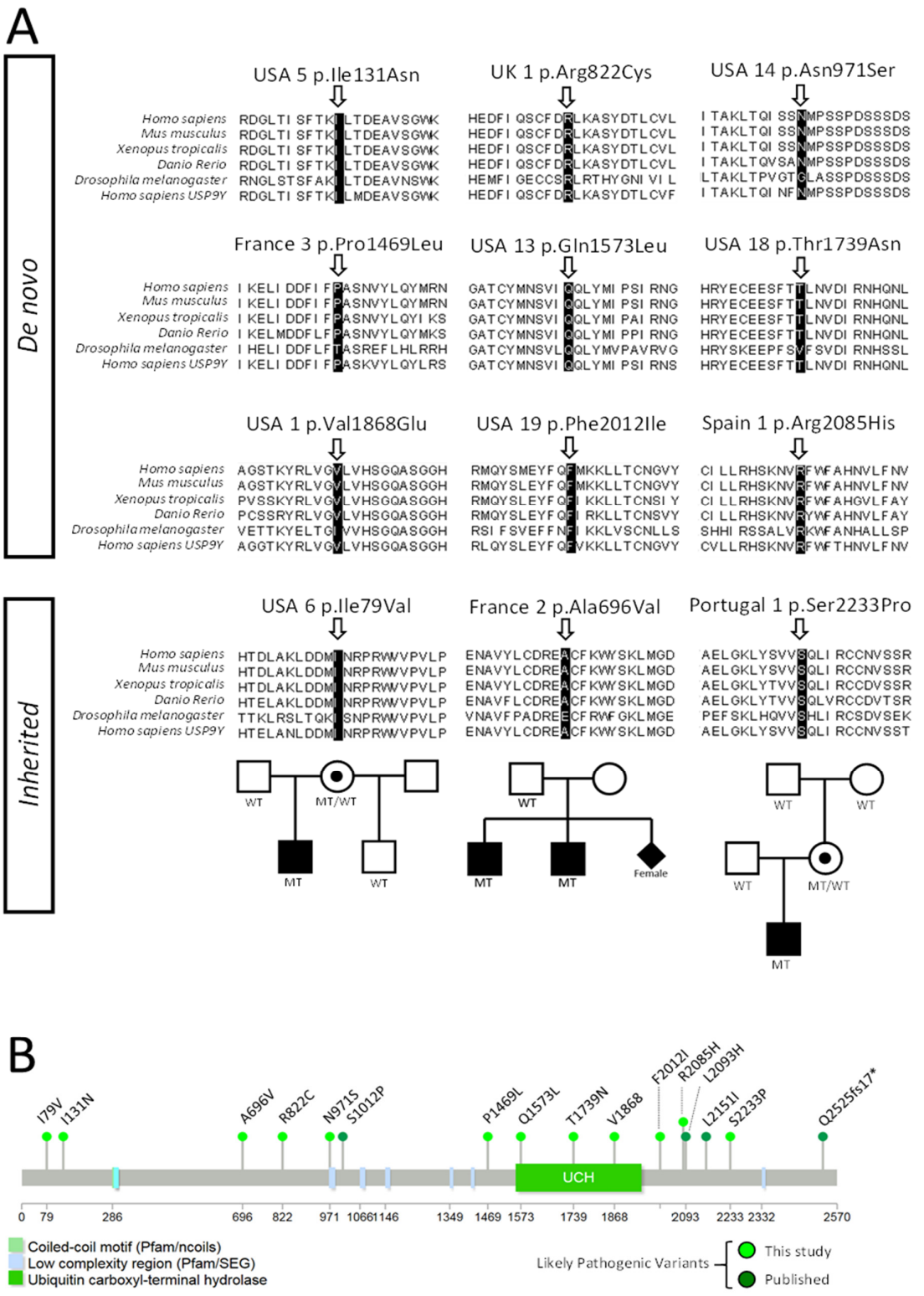


Figure S2. Conservation and protein location likely pathogenic USP9X variants. A. Cross species protein alignment of USP9X showing conservation of altered amino acid residues. Pedigrees are shown for inherited cases. UK1 has Decipher ID: 260068. B. Location of likely pathogenic variants on the USP9X protein structure.

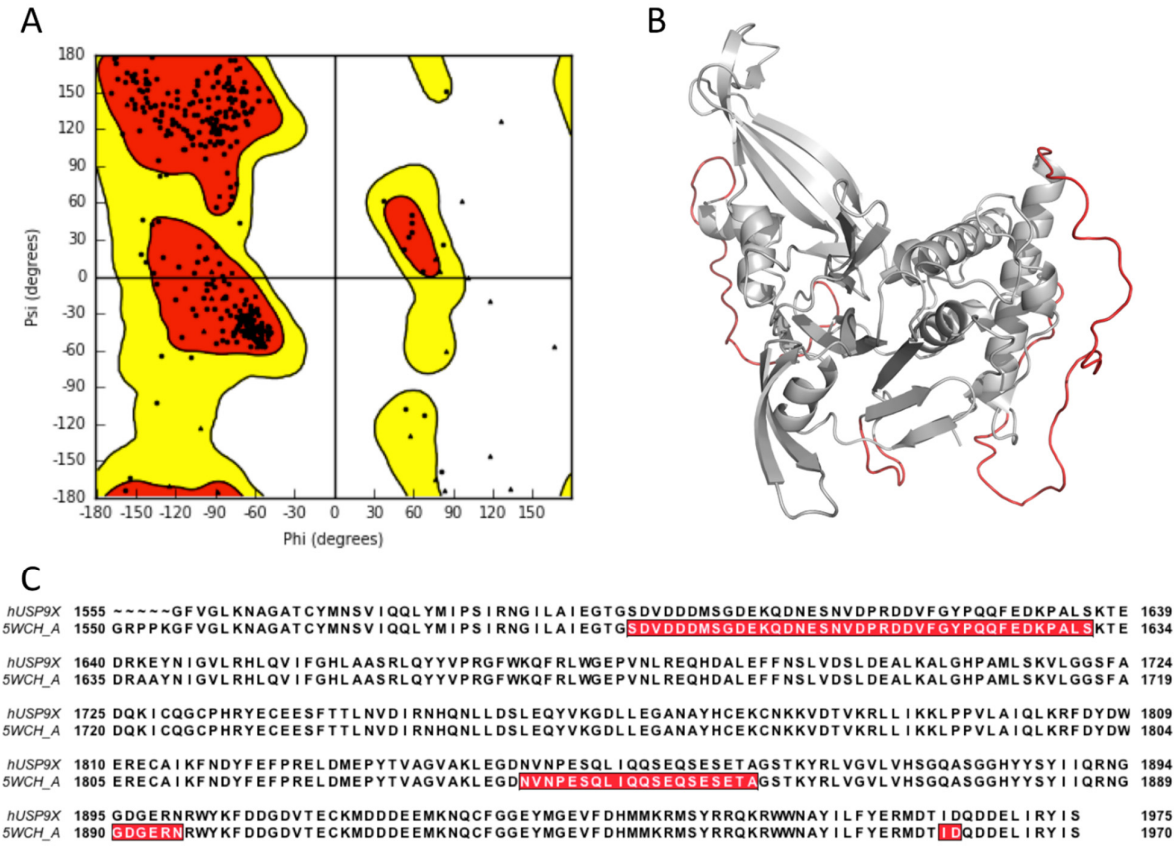


Figure S3. Ramachandran plot of USP9X catalytic domain homology model. Human USP9X/1555-1975 was aligned to crystal structure 5WCH using Maestro multiple sequence viewer (Schrödinger). Homology model was generated using Maestro Bioluminate (Schrödinger) using energy minimisation to model flexible loops absent from 5WCH. A. Ramachandran plot of USP9X homology model. Images were prepared using PyMol (Schrödinger). >97% amino acids are within accepted torsion angles. B. Homology model showing regions present within the crystal structure 5WCH (Zhang et al (1), grey), with absent and likely flexible loops modeled by energy minimization (red). C. Alignment showing the primary sequence positions of flexible loops (red) and sequence alignment used for homology modeling.

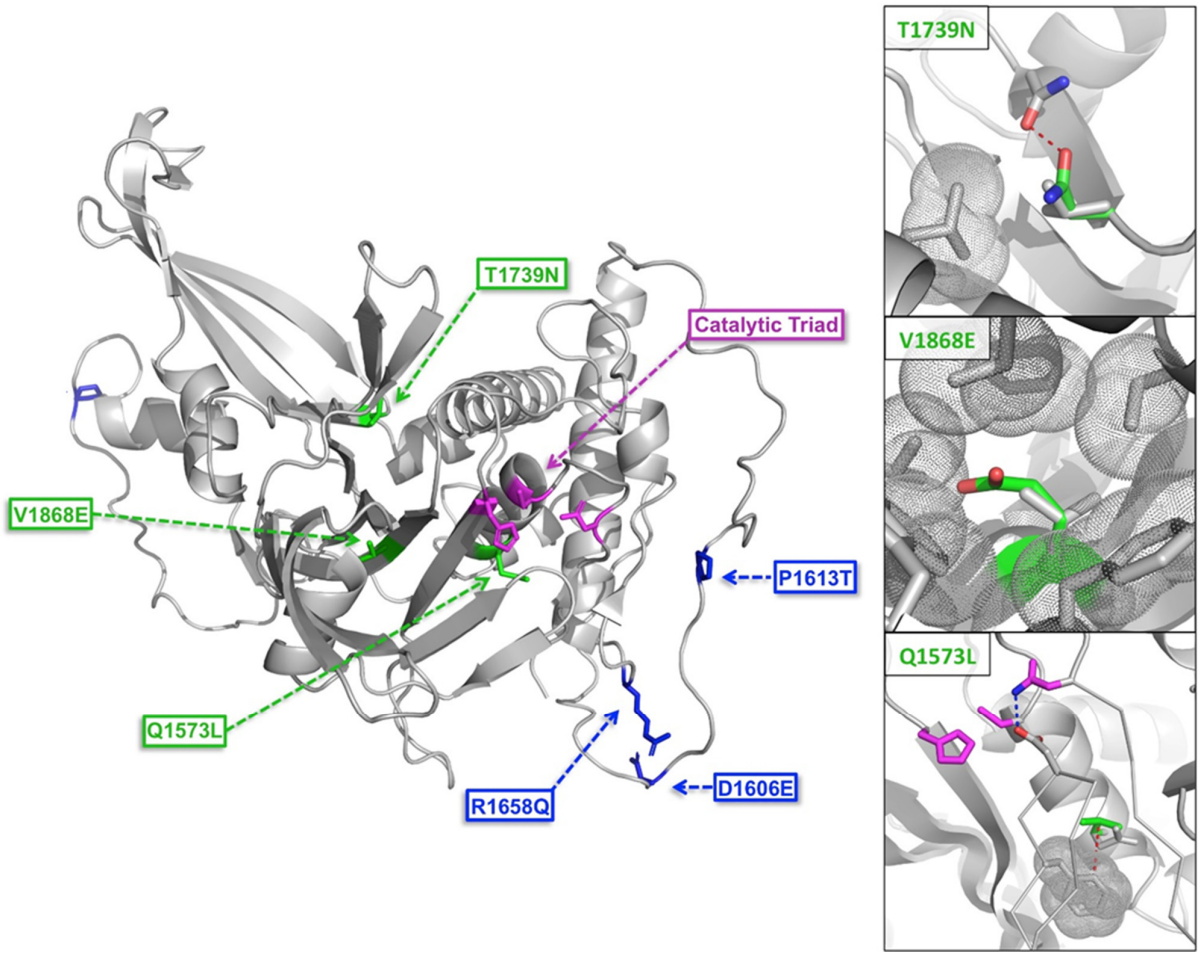


Figure S4. Structural characterization of USP9X variants located in the catalytic domain. Homology model of USP9X (grey) with catalytic site (magenta), likely pathogenic variants (green) and variants of unknown significance (blue) indicated. Likely pathogenic variants are positioned in regions of well-ordered secondary structure which are peripheral to the core catalytic site. Variants of unknown significance occur within two loops of predicted (absent from crystal structure) disordered regions, distal to the catalytic site. Variants within this region are less likely to drastically alter protein stability or catalytic activity, but may produce more nuanced alterations in intra/intermolecular interactions. Insets indicate local structural effects of indicated likely pathogenic variants. All native amino acid side chains are represented as grey sticks, with the position of variants / catalytic residues indicated by colored sticks. Charged atoms are indicated in blue (positive) and red (negative). Hydrophobic van der vaals radii are indicated by dots. T1739N may result in charge-charge repulsion with N1741, indicated by a red dashed line, and steric clash with L1687 altering local secondary structure. V1868 lies within a tight hydrophobic core (composted of L1865, W1897, M1916, Y1883, F1818 and C1920). Change of Valine to the bulky, highly polar glutamate is likely to disrupt hydrophobic packing and produce steric clash with adjacent residues, altering local secondary. Q1573 lies upon an alpha helix adjacent to the catalytic site. Glutamate to Leucine substitution may alter hydrophobic packing (with F1900), inducing conformational change within this loop, and potentially altering the position of D1902. This residue forms charge-charge interactions with the catalytic N1561, indicated by a blue dashed line. Alterations in the positioning of D1902 are likely to affect the availability or efficiency of N1561 during proteolytic activity.

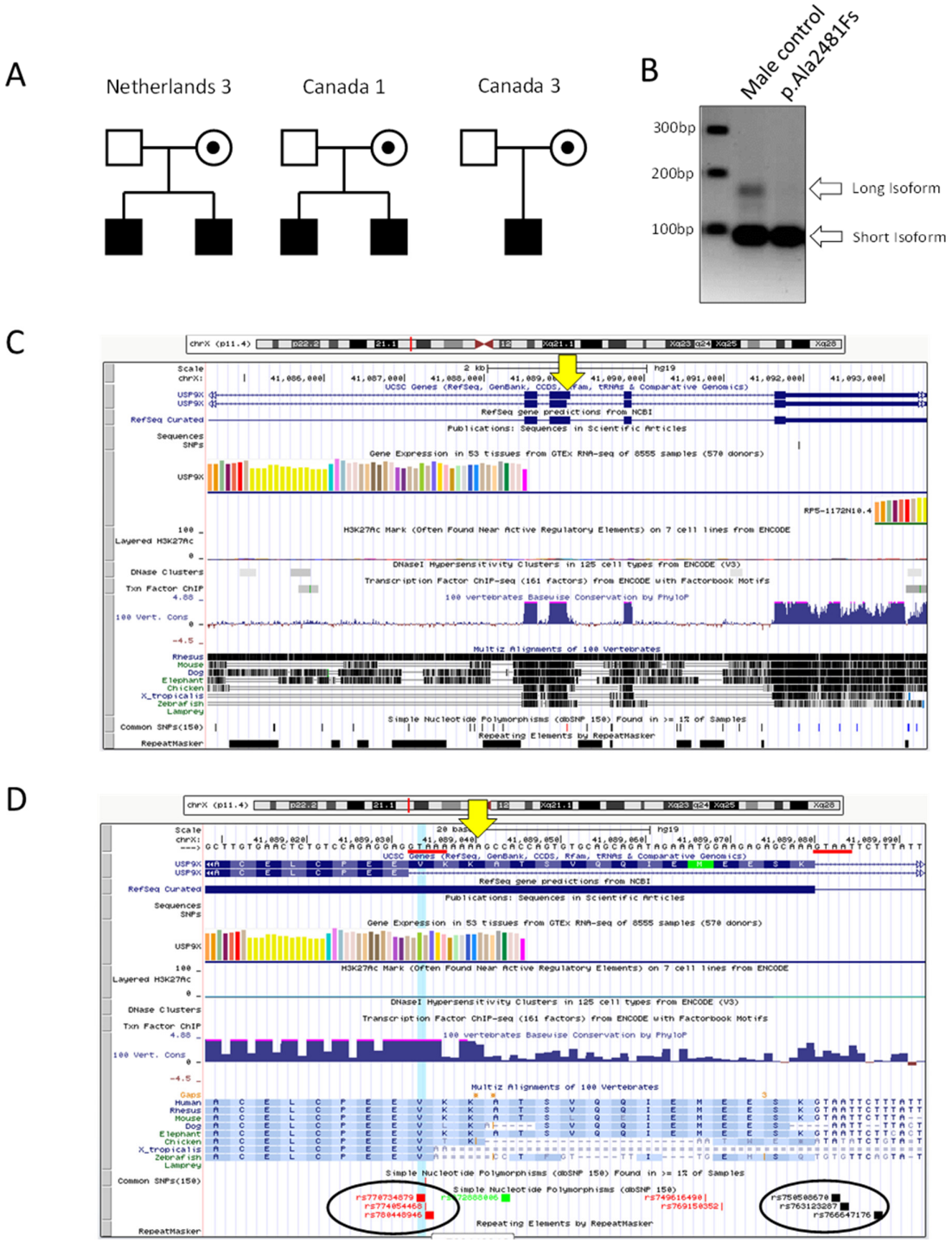


Figure S5. A recurrent variant with deleterious or benign impact depending on USP9X isoform usage. p.Ala2481Fs*17 variant impacts the long *USP9X* isoform (NM_001039590.2:c. c.7440dupA, NP_001034679.2:p.ALa2481Serfs*17) but is intronic in the short isoform (NM_001039591.2;c.[0]. NP_001034680.2 p.[0]). A. Pedigrees of the three independent families with this variant. B. Analysis of mRNA species in fibroblast cell lines from control individual and individual with p.Ala2481fs*17 variant (from family Netherlands 3). Note that long isoform is poorly expressed in control, but absent in the variant cell line (suggestive of non-sense mediated mRNA decay). C-D. UCSC Genome Browser views of the relevant genomic region. Yellow arrows represent site of variant (dup A). Red underlines in D represent splice sites. Black circles highlight SNPs in splice sites.

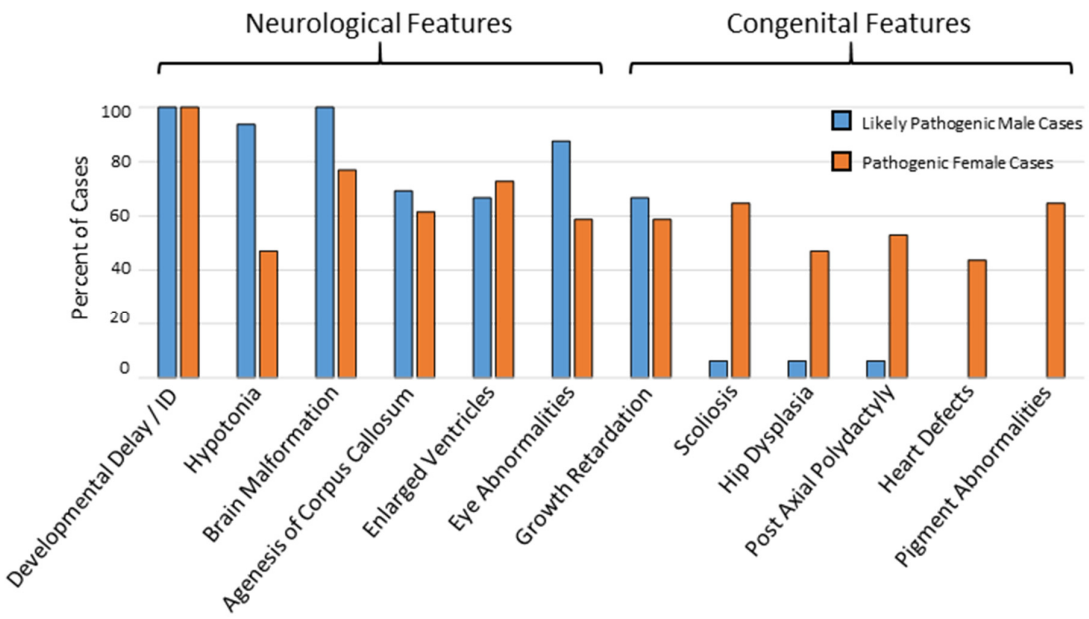


Figure S6. Comparison of the major neurological and congenital features observed in female subjects with male subjects. Most frequent features of female subjects with pathogenic heterozygous loss of function mutations as reported in Reijnders *et al* (2). Assessed against the cohort of males with likely pathogenic missense variants. Note the major neurological findings in females are frequently observed in males, but the congenital features are not.

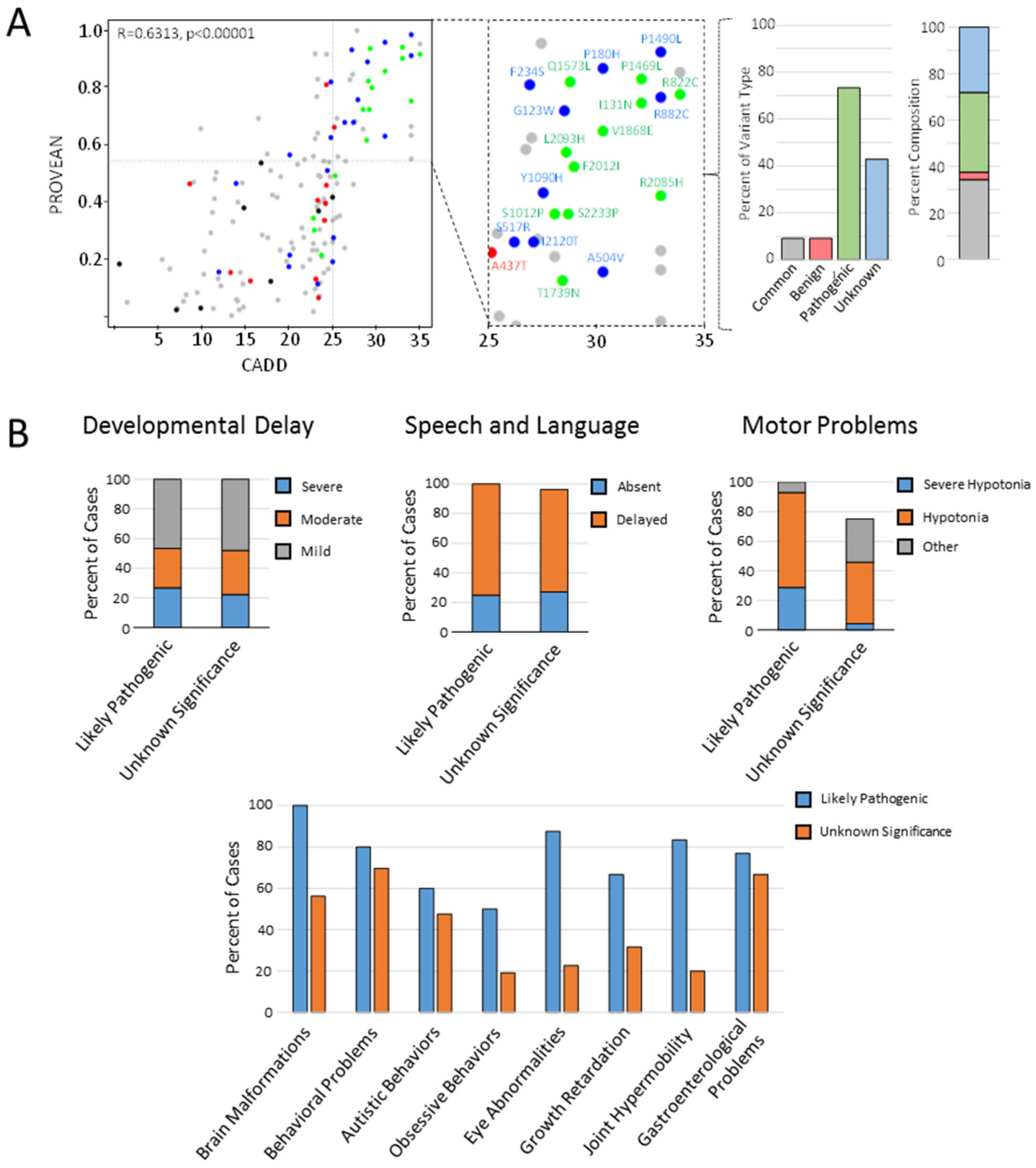


Figure S7. Comparison variants of unknown significance with likely pathogenic variants. A. CADD and PROVEAN scores reveal clustering of subsets of variants of unknown significance with likely pathogenic variants in upper-right quadrant consistent with pathogenicity. Scores are significantly correlated (Pearson's correlation given). Inset identifies variants in the 'pathogenic quadrant'. Graphs show percent of each type of variant, and the overall composition of variant types within the pathogenic quadrant. B Distinctive clinical features of individuals with USP9X likely pathogenic variants are observed also in many individuals with USP9X variants of unknown significance.

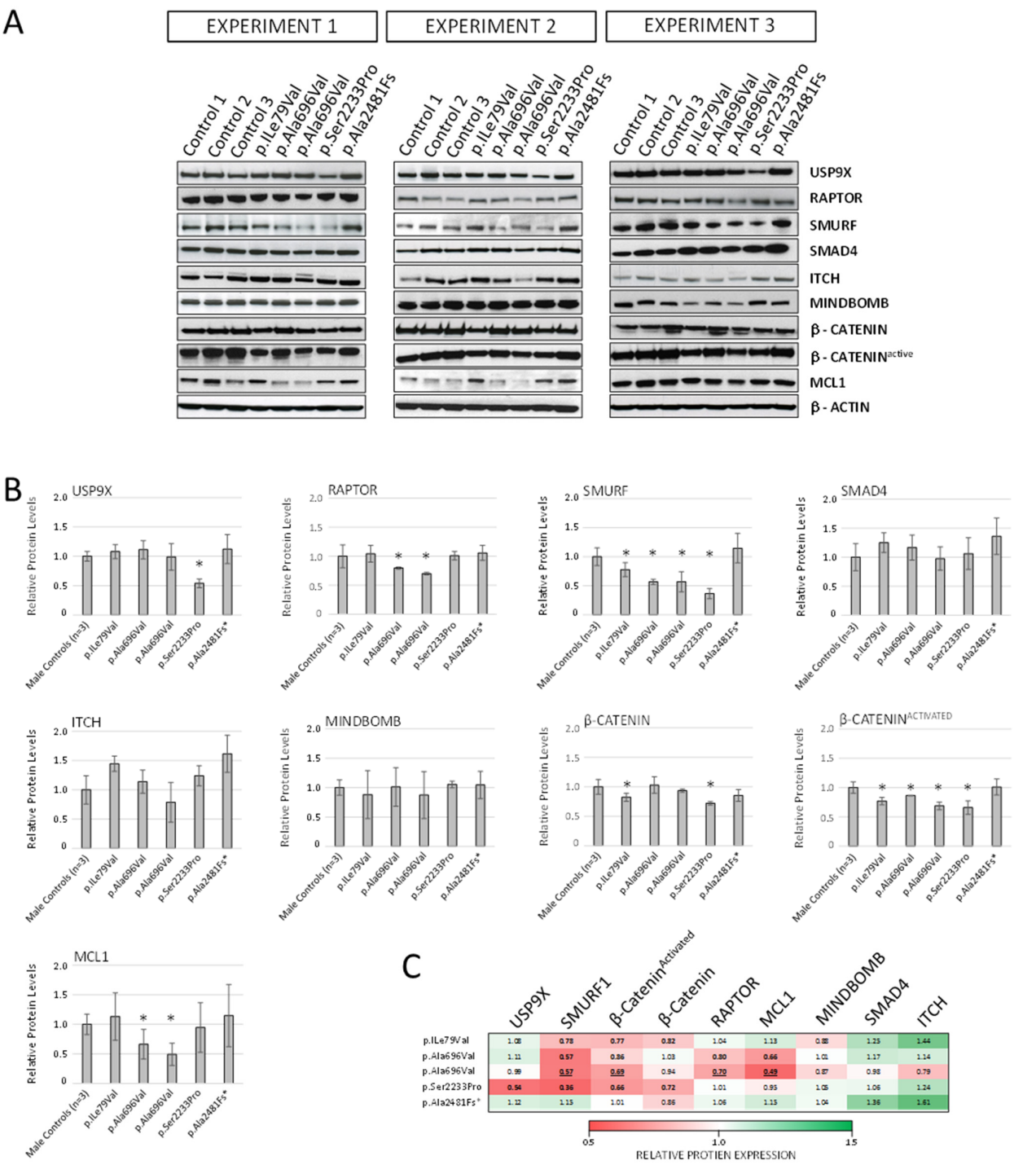


Figure S8. Protein expression of USP9X and its substrates in fibroblast cell lines derived from individuals with USP9X variants. A. Western blot analysis of n=3 independent experiments. B-C. Quantitation of western blots in A. *significantly different to controls, p<0.05 by Student's t-test. In C, significant values are in bold and underlined.

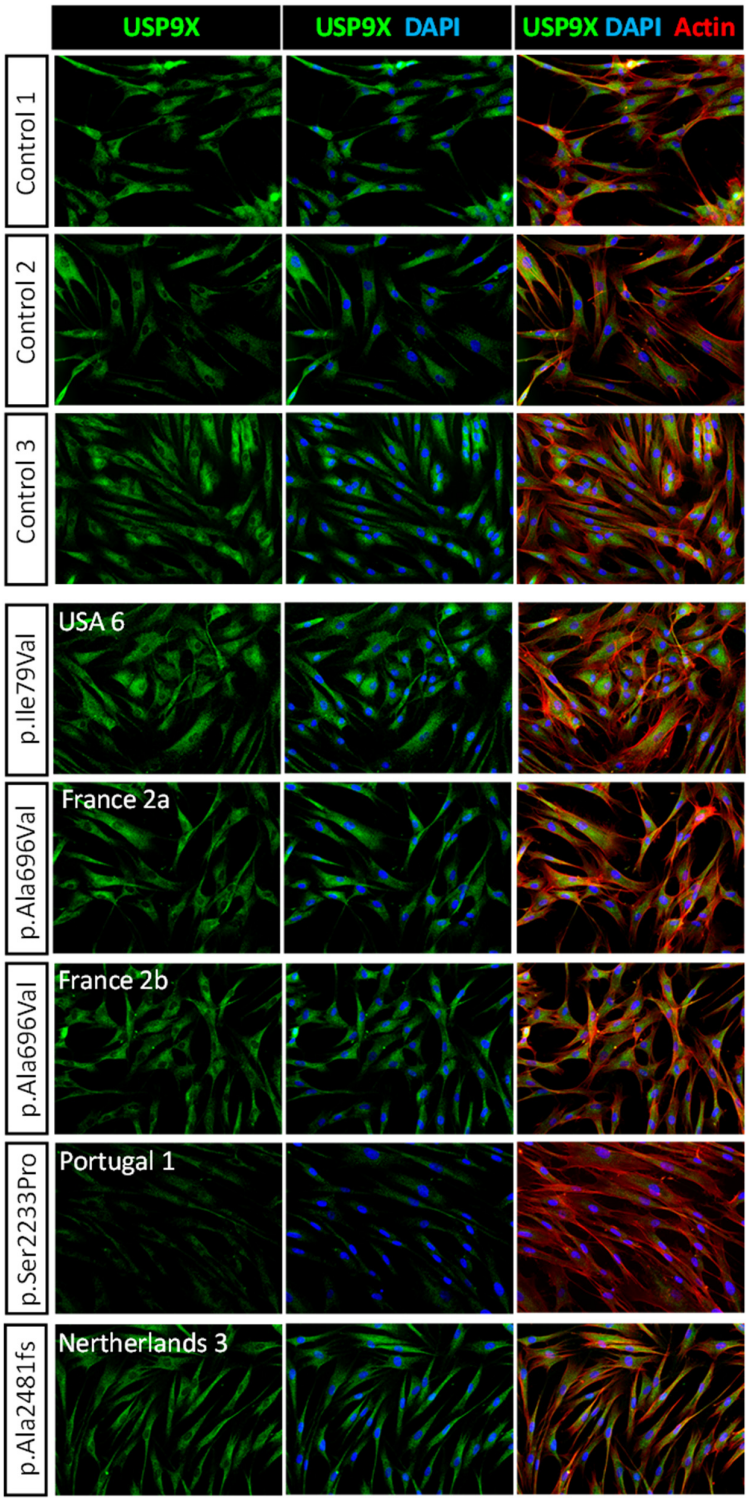


Figure S9. Representative immunofluorescent images of fibroblast cell lines derived from control individuals, and individuals with USP9X variants. Cells strained with antibodies against USP9X (Green), and counterstained with DAPI (Nuclei, Blue) and Phalloidin (Filamentous actin cytoskeleton, Red).

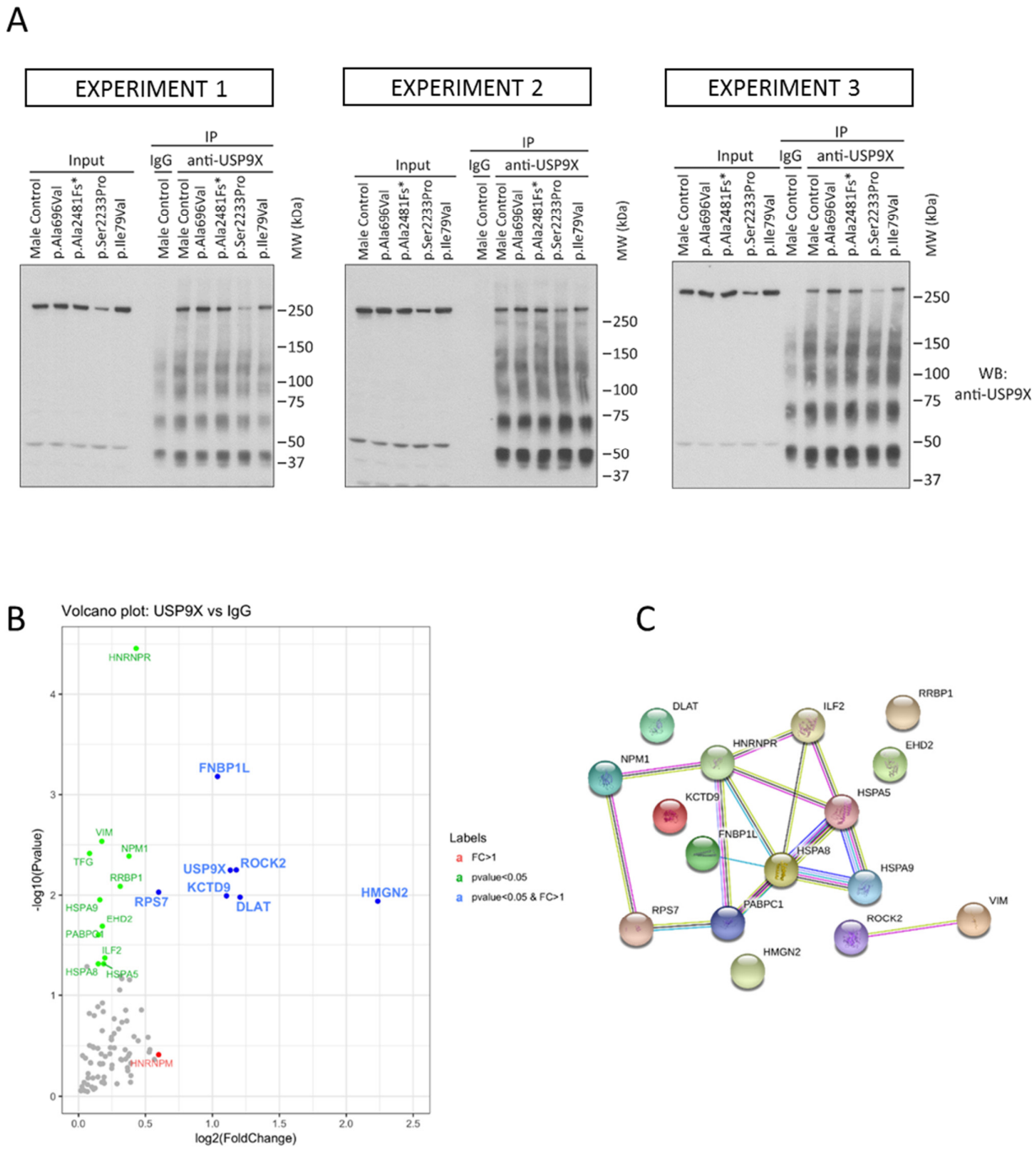


Figure S10. Identification of USP9X interactors in fibroblast cell lines derived from control individuals and individuals with USP9X variants. A. Western blot analysis of USP9X immunoprecipitation experiments. IgG immunoprecipitations served as negative control samples in subsequent proteomics. USP9X and IgG control immunoprecipitated samples from each experiment subsequently analysed using TMT-EIS-MS/MS identification and quantification. B. Volcano plot of proteins immunoprecipitated with USP9X from control fibroblasts. Results derived from 3 independent immunoprecipitation experiments in A. Fold change and statistical values represents comparison to proteins immunoprecipitated with control IgG. p values derived from adjusted Students paired t-test. C. STRING analysis of significantly enriched proteins in B. Statistical analysis reveals a well-connected network (Protein-Protein Interaction enrichment p-value: 0.000589).

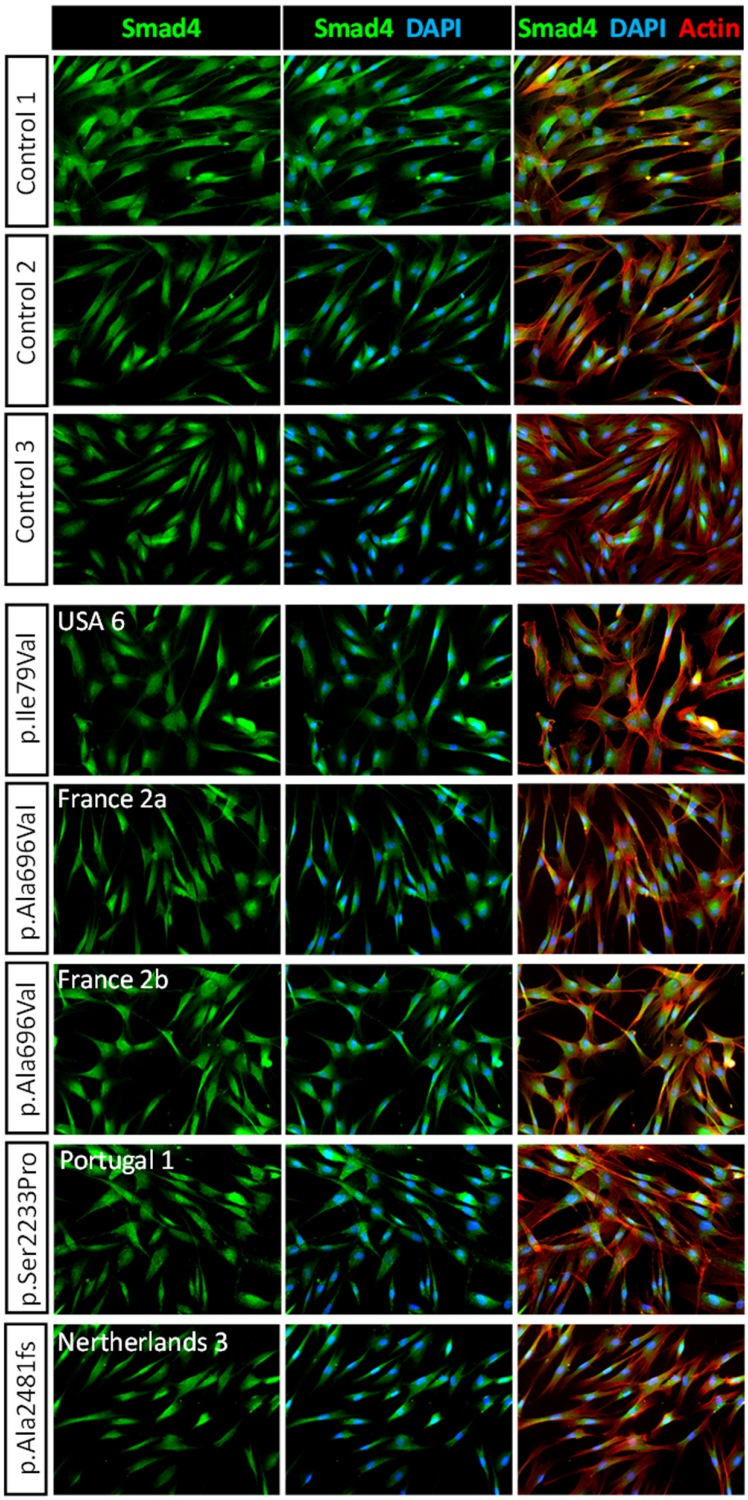


Figure S11. Expression and localisation of SMAD4 in fibroblast cell lines derived from control individuals, and individuals with USP9X variants. Representative immunofluorescent images. Cells strained with antibodies against SMAD4 (Green), and counterstained with DAPI (Nuclei, Blue) and Phalloidin (Filamentous actin cytoskeleton, Red).

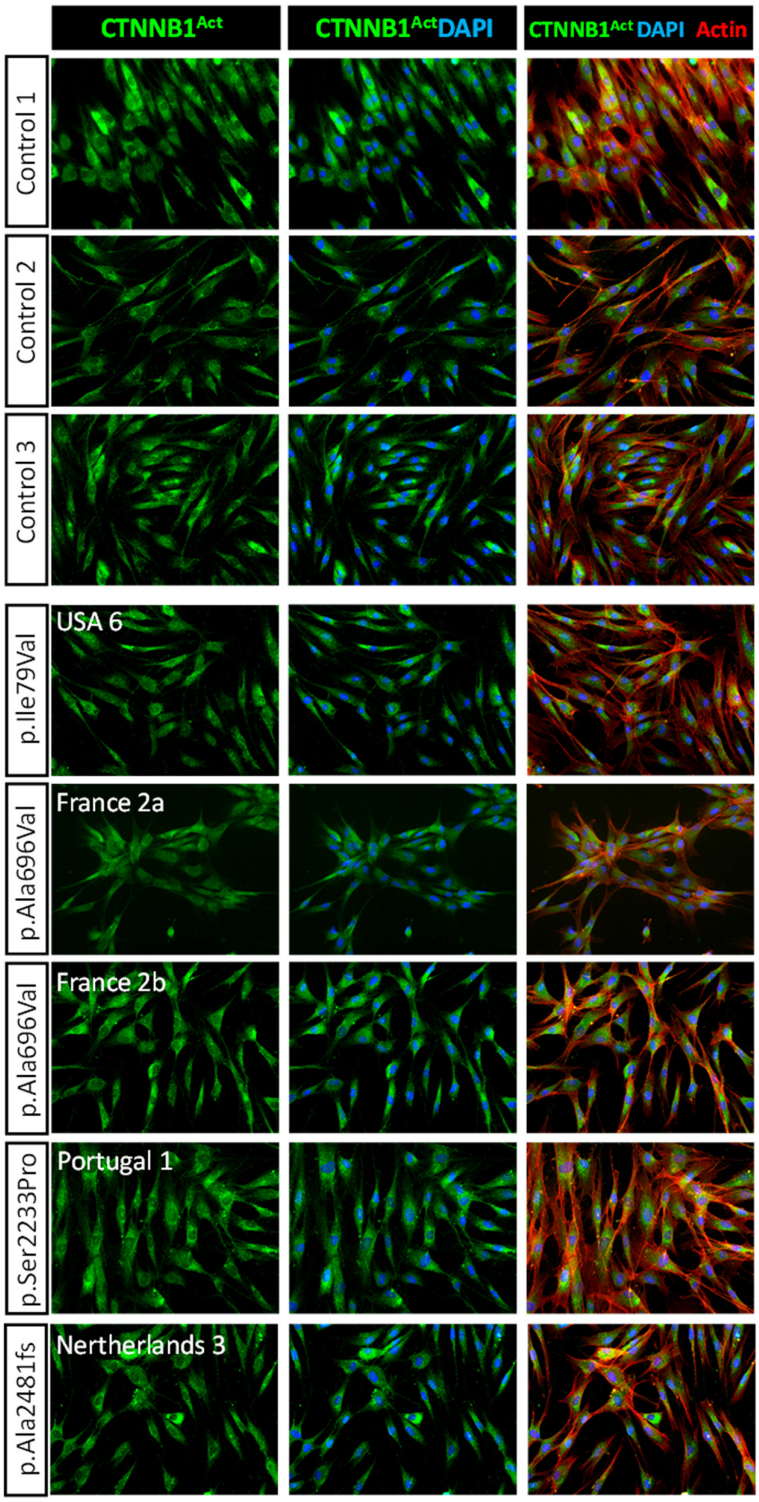


Figure S12. Expression and localisation of CTNNB1 in fibroblast cell lines derived from control individuals, and individuals with USP9X variants. Representative immunofluorescent images. Cells stained with antibodies against activated CTNNB1 (aka β-catenin, Green), and counterstained with DAPI (Nuclei, Blue) and Phalloidin (Filamentous actin cytoskeleton, Red).

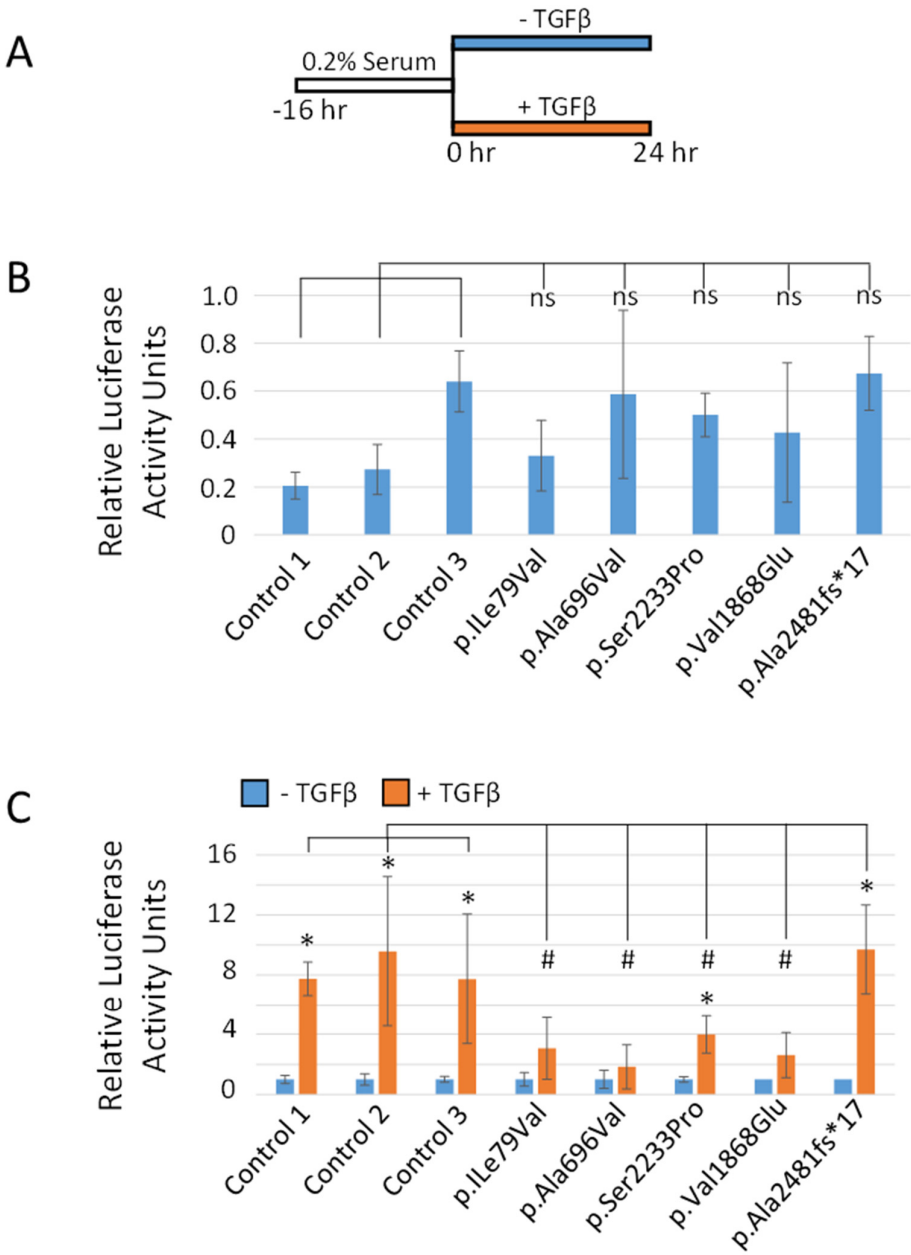


Figure S13. USP9X variants disrupt TGFβ signalling in fibroblast cell line derived from individuals with USP9X variants. A. Pictorial view of the experimental approach. Cells were first serum starved (0.2% serum) for 16 hrs prior to addition of TGFβ and assayed 24 hours later. A. In the absence of added TGFβ, cells display similar basal levels of signalling as assessed by TGFβ luciferase reporter assay. B. Relative increase of TGFβ signalling following addition of ligand as assessed by TGFβ luciferase reporter assays. Experiment done in quadruplicate. * statistical difference between +/- TGFβ. # statistical difference between controls and USP9X variant cell lines. *#p<0.05 Student's t-test. N.B. p.Ala2481fs*17 variant effects only the long USP9X isoform which is barely expressed in fibroblasts.

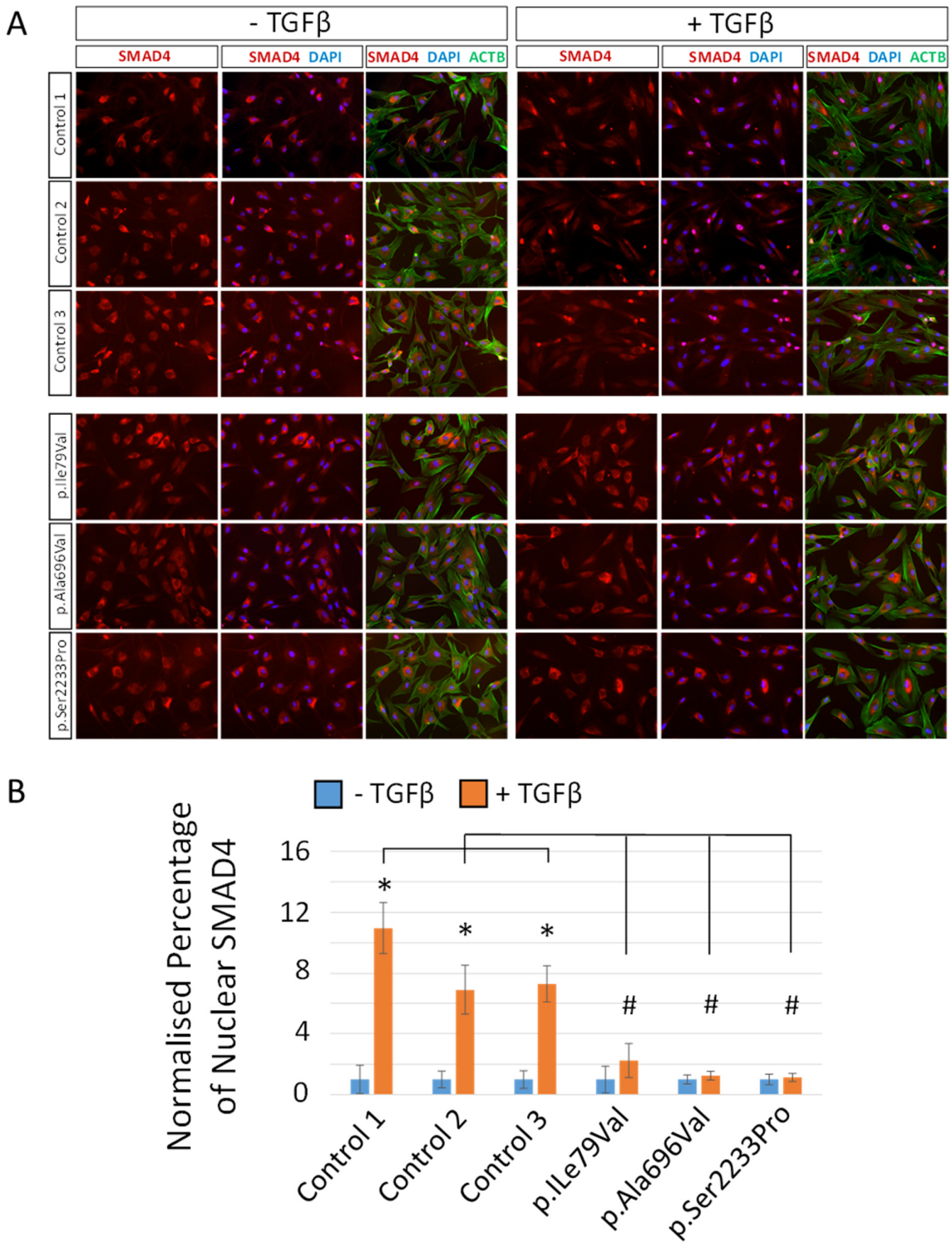


Figure S14. SMAD4 nuclear enrichment is deficient in fibroblast cell lines derived from individuals with USP9X variants in response to TGFβ stimulation. A. Representative immunofluorescent images of SMAD4 localisation before (time = 0 hr) and after (time = 24 hours) addition of TGFβ. Cells stained with antibodies against SMAD4 (Red), and counterstained with DAPI (Nuclei, Blue) and Phalloidin (Filamentous actin cytoskeleton, Green). B. Quantitation of SMAD4 enriched nuclei following addition of TGFβ. * statistical difference between +/- TGFβ. # statistical difference between controls and USP9X variant cell lines. $p<0.05$ Student's t-test; n=5 replicates.

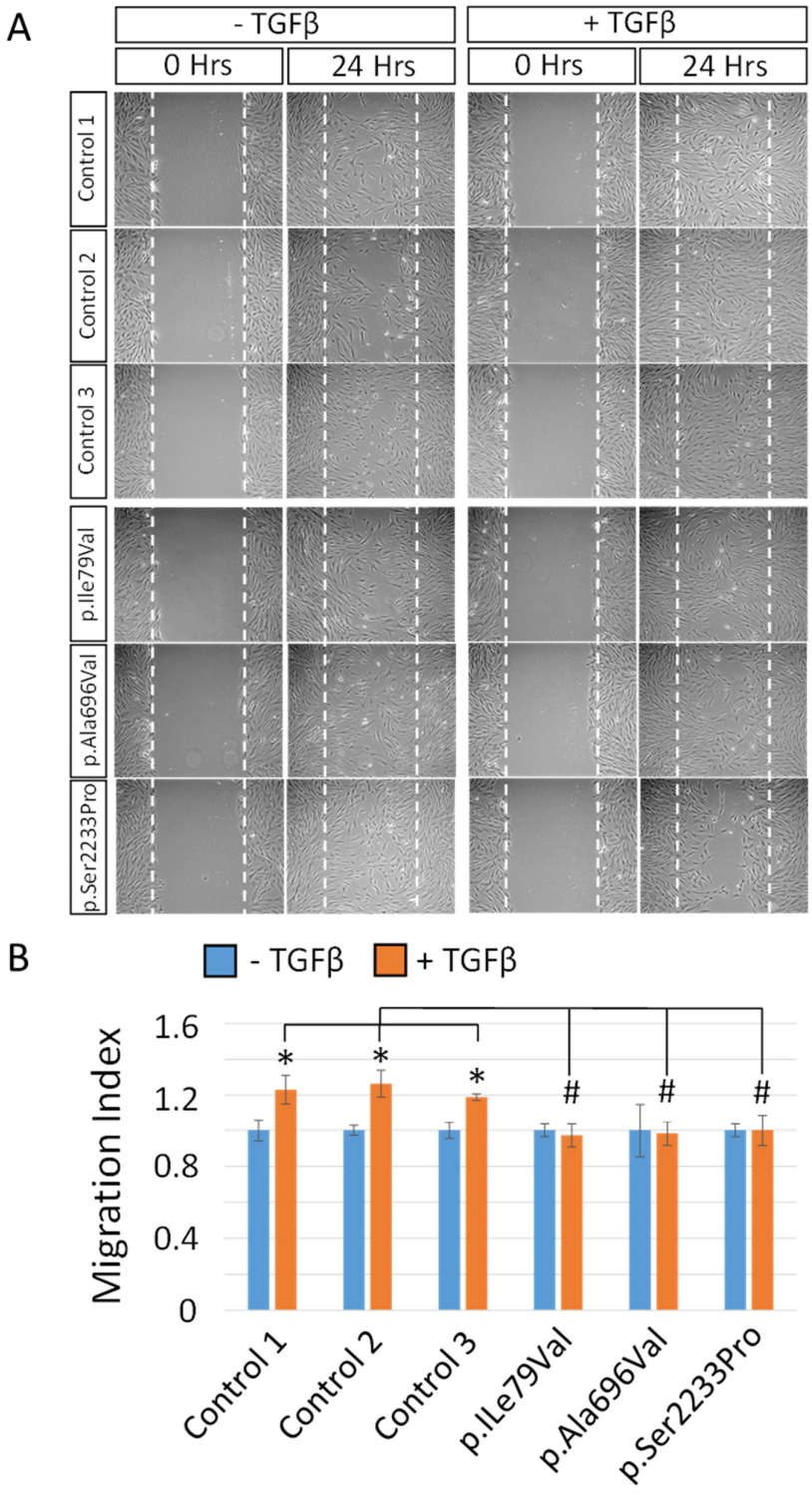


Figure S15. TGFβ-stimulated cell migration is defective in fibroblast cell lines derived from individuals with USP9X variants. A. Representative phase-contrast images of scratch migration assays. B. Quantitation of TGFβ-stimulated migration of cells into the scratch area. n=9 replicates (3 biological x 3 technical (scratches) analysed). * statistical difference between +/- TGFβ. # statistical difference between controls and USP9X variant cell lines. #* $p<0.05$ Student's t-test.

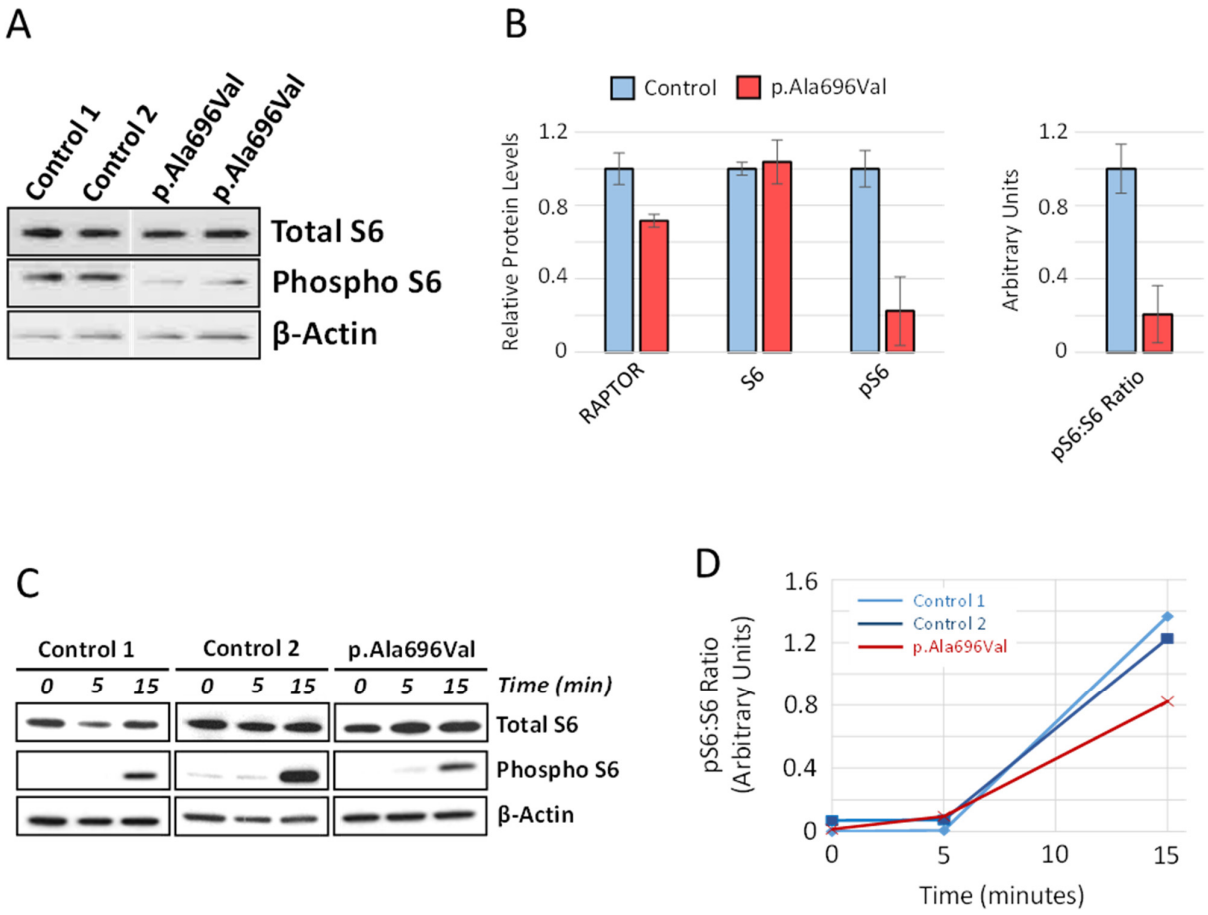


Figure S16. Fibroblast cell lines harbouring p.Ala696Val variant show defective mTOR signalling. A-B. Cells grown in presence of 10% foetal calf serum and protein harvested for analysis. A. Western-blot analysis of control and p.Ala696Val cell lines (from 2 unique individuals, brothers). B. Quantification of blots in A and calculation of ratio of phosphorylated S6 (pS6) to total S6 levels. Loss of pS6:S6 ratio indicates loss of mTOR signalling. C-D. Cells grown for 16 hours in absence of serum. Serum was returned and protein lysates collected over 15 minute time course. C. Western blot analysis showing kinetics of pS6 induction. D. Quantitation of western-blots in C.

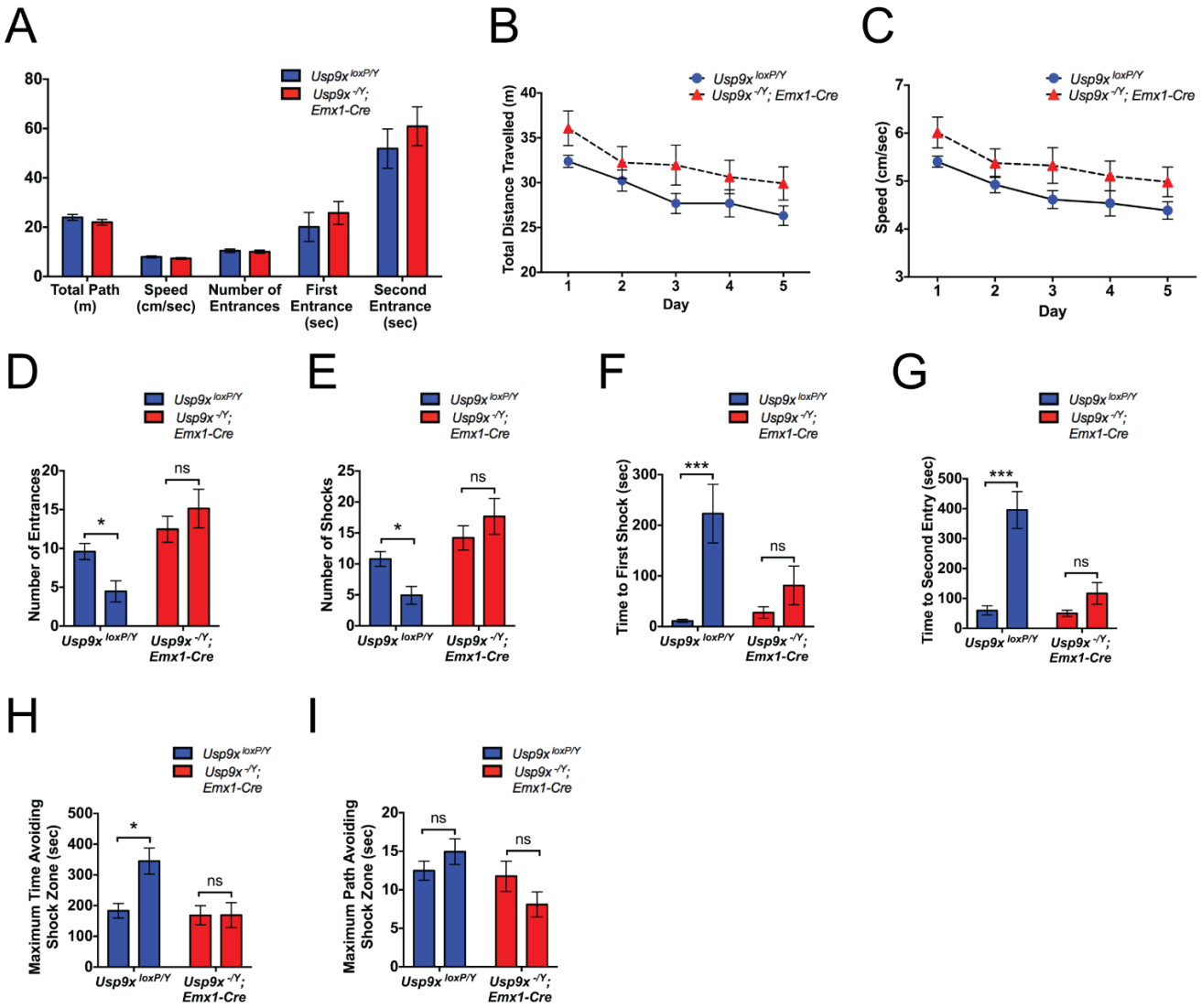


Figure S17. A. *Usp9x* knockout mice do not show altered locomotory behaviour within the APA test arena, and do not learn within the APA task. A. Analysis of mouse movement patterns within the habituation phase of the APA test revealed no significant difference between wild-type (blue bars) and *Usp9x* knockout (red bars) mice. Similarly, there were no significant differences in the total distance travelled (B) and speed (C) between control and knockout animals over the five days of the APA protocol. D-I. Intra-genotype analysis revealed that, whereas wild-type mice performed better on day 5 than day 1, indicative of learning within the APA task, knockout mice did not perform better on day 5 versus day 1 of the task. * $p < 0.05$; *** $p < 0.001$, two way ANOVA.

SUPPLEMENTAL TABLES S1-S7

	Family	Subjects	Variant Details			Platform	Segg#	Number and Frequency in gnomAD			Prediction Algorithms				
			Chromosome X GRCh37(hg19)	cDNA NM_001039590.2	Protein NP_001034679.2			Het	Hem	Frequency	Polyphen2 Score	Pred	PROVEAN Score	Pred	CADD Score
Likely Pathogenic	USA 6	1	40988391A>G	c.235A>G	p.Ile79Val	tWES	Yes				0.837	P	-0.77	N	23.7
	USA 5	1	40994047T>A	c.392T>A	p.Ile131Asn	WES	De novo				0.999	D	-6.13	D	33
	France 2	2	41025226C>T	c.2087C>T	p.Ala696Val	WES	n/a				0.287	B	-2.18	N	25.3
	UK1	1	41027299C>T	c.2464C>T	p.Arg822Cys	tWES	De novo				0.991	D	-6.47	D	35
	USA 14	1	41029757A>G	c. 2912A>G	p.Asn971Ser	tWES	De novo				0.999	D	-6.99	D	29.3
	France 3	1	41057806C>T	c.4406C>T	p.Pro1469Leu	tWES	De novo				0.963	D	-7.13	D	33
	USA 13	1	41060427A>T	c.4718A>T	p.Gln1573Leu	tWES	De novo				0.102	B	-1.39	N	22.8
	USA 18	1	41073847C>A	c.5216C>A	p.Thr1739Asn	tWES	De novo				0.83	P	-2.95	D	28.9
	USA 1	1	41075423T>A	c.5603T>A	p.Val1868Glu	tWES	De novo				0.732	P	-5.48	D	31
	USA 19	1	41075854T>A	c.6034T>A	p.Phe2012Ile	tWES	De novo				0.961	D	-4.71	D	29.5
	Spain 1	1	41077669G>A	c.6254G>A	p.Arg2085His	tWES	De novo				0.974	D	-4.14	D	34
	Portugal 1	1	41082601T>C	c.6697T>C	p.Ser2233Pro	DES	Yes				0.918	D	-3.85	D	29.2
Unknown Significance	USA 7	1	40994022G>T	c.367G>T	p.Gly123Trp	tWES	n/a				0.996	D	-6.18	D	32
	Netherlands 11	2	40996160C>A	c. 539C>A	p.Pro180His	tWES	Yes				0.999	D	-7.76	D	31
	USA 9	1	40999955T>C	c.701T>C	p.Phe234Ser	tWES	n/a				0.999	D	-6.86	D	27.2
	USA 3	1	41000406A>G	c.958A>G	p.Arg320Gly	WES	n/a				0.903	P	-4.95	D	24.8
	Netherlands 9	1	41007713C>T	c.1511C>T	p.Ala504Val	tWES	Yes				0.955	D	-3.05	D	31
	Netherlands 12	1	41007751A>C	c.1549A>C	p.Ser517Arg	tWES	Yes				0.982	D	-3.46	D	26.4
	USA 12	2	41029255C>T	c.2644 C>T	p.Arg882Cys	tWES	Yes				1	D	-6.37	D	34
	Canada 2	1	41029345A>G	c.2734A>G	p.Ile912Val	tWES	n/a	1	0	5.60E-06	0.001	B	-0.49	N	11.99
	Netherlands 10	1	41043278C>A	c.3176C>A	p. Ala1059Asp	tWES	Yes				0.186	B	-1.05	N	25.1
	Netherlands 1	1	41043370T>C	c.3268T>C	p.Tyr1090His	tWES	Yes				0.999	D	-4.2	D	27.9
	Netherlands 7	1	41045786A>G	c.3575A>G	p.His1192Arg	tWES	n/a				0.039	B	-2.02	N	13.96
	USA 21	1	41045833C>T	c.3622 C>T	p.Pro1208Ser	tWES	n/a				0.05	B	-3.01	D	24.8
	USA 11	1	41048705C>G	c.3954C>G	p.Asp1318Glu	tWES	Yes				0.083	B	-2.63	D	20.1
	USA 22	1	41057869C>T	c.4469C>T	p.Pro1490Leu	tWES	n/a				0.973	D	-9.26	D	34
	Spain 2	1	41060527C>A	c.4818C>A	p.Asp1606Glu	tWGS	n/a				0.003	B	-0.58	N	20
	USA 17	1	41064568C>A	c.4837C>A	p.Pro1613Tyr	tWES	n/a				0.014	B	-0.66	N	25
	USA 26	2	41064704G>A	c.4973G>A	p.Arg1658Gln	tWES	Yes	3	2	1.70E-05	0.004	B	-0.27	N	23.3
	Canada 6	1	41077774T>C	c.6359T>C	p.Ile2120Thr	WES	n/a	1	0	5.59E-06	0.474	P	-3.46	D	27.4
	USA 28	1	41084144A>C	c.6901A>C	p.Lys2301Gln	tWGS	n/a				0.36	B	-2.29	N	24.4
	Netherlands 3	2	41089041dupA	c.7440dupA	p.Ala2481fs*17	WES	Yes	7	4	6.21E-05	n/a	n/a	n/a	n/a	n/a
	Canada 1	1	41089041dupA	c.7440dupA	p.Ala2481fs*17	DES	Yes	7	4	6.21E-05	n/a	n/a	n/a	n/a	n/a
	Canada 3	2	41089041dupA	c.7440dupA	p.Ala2481fs*17	tWES	n/a	7	4	6.21E-05	n/a	n/a	n/a	n/a	n/a
	Netherlands 5	1	41091697C>T	c.7633C>T	p.Pro2545Ser	WES	n/a	0	1	5.60E-06	0.986	D	-0.7	N	20.1
Likely Benign	Netherlands 2	1	41000604G>C	c.1081G>C	p.Val361Leu	WES	No				0.739	P	-1.99	N	24.3
	Netherlands 6	1	41002691G>A	c.1309G>A	p.Ala437Thr	WES	No				0.69	P	-3.32	D	25.2
	Swiss 1	1	41031160A>G	c.3097A>G	p.Met1033Val	WES	No				0.001	B	-0.48	N	13.33
	Norway 1	1	41043275G>A	c.3173G>A	p.Arg1058Lys	tDES	No				0.002	B	0.03	N	23.4
	Canada 5	1	41043684C>A	c.3314C>A	p.Pro1105His	WES	No				0.906	P	-4.83	D	24.3
	Netherlands 4	1	41043792T>C	c.3422T>C	p.Met1141Thr	WES	No	1	1	1.12E-05	0.001	B	-2.01	N	8.661
	Netherlands 8	1	41055921A>G	c.4163A>G	p.Asn1388Ser	tWES	n/a	16	4	1.03E-04	0.013	B	-0.36	N	23.1
	Belgium 1	1	41064560A>G	c.4829A>G	p.Asn1610Ser	DES	n/a				0.137	B	-1.71	N	23.3
	France 1	1	41075489G>A	c.5669G>A	p.Gly1890Glu	tWES	No	2	0	1.12E-05	0.002	B	-0.33	N	15.6
	USA 4	1	41075489G>A	c.5669G>A	p.Gly1890Glu	WES	No*	2	0	1.12E-05	0.002	B	-0.33	N	15.6
	USA 29	1	41077775A>G	c.6360A>G	p.Ile2120Met	tWES	No*	17	6	1.15E-04	0.754	P	-1.65	N	24.2
	Germany 1	1	41077775A>G	c.6360A>G	p.Ile2120Met	WES	No	17	6	1.15E-04	0.754	P	-1.65	N	24.2
	Denmark 1	1	41082482A>G	c.6578A>G	p.Lys2193Arg	WES	No				0.078	B	-1.35	N	24.1
Reported	Paemka 1	1	41031097T>C	c.3034T>C	p.Ser1012Pro	WES	De novo				0.943	D	-3.84	D	28.5
	Homan 1	1	41077693T>A	c.6278T>A	p.Leu2093His	XES	De novo				0.984	D	-4.99	D	29.1
	Homan 2	2	41078388C>A	c.6469C>A	p.Leu2157Ile	XES	n/a	1	0	5.68E-06	0.245	B	-1.18	N	22.9
	Homan 3	3	41089848delA	c.7574delA	p.Gln2525fs*18	XES	Yes				n/a	n/a	n/a	n/a	n/a

Table S1. USP9X variants associated with NDDs. Likelihood of pathogenicity assigned using American College of Medical Genetics and Genomics Guidelines (ACMG). Cases refers to number of affected individuals in the family. Subject UK 1 has Decipher ID: 260068. Subject Netherlands 2 has Decipher ID: 323395. Subject Homan 1 has Decipher ID: 318087. Platform refers to sequencing approach: WES, Whole Exome sequencing; WGS, Whole Genome Sequencing; DES Disease gene panel Exome sequencing; XES, X-chromosome Exome Sequencing; prefix t is for trio-based approach. Segg#: segregation studies: yes: segregates beyond trio analysis; n/a: not conducted beyond trio analysis; No, found in healthy male relative (*recurrent variant found in a healthy male relative in an unrelated family). Results of prediction algorithms colour coded: Red, deleterious/pathogenic; pink potentially deleterious / likely pathogenic.

	Gene	Variant	Inheritance	Genotype	ACMG	Relevant Associated Genetic Disease (MIM)	Notes
Netherlands 1	ARID1B	Chr6 g.157528688T>G; NM_020732.3:c.6413T>G; p.Leu2138Arg	De novo	Heterozygous	VUS	Coffin-Siris Syndrome (135900)	Pathogenicity stems from LOF mutations. No missense mutations reported in OMIM. Pateint not displying hallmark Coffin-Siris Syndromic features (e.g. 5th finger abnormalities). Varinat absent in gnomAD.
Netherlands 5	FBXO28	Chr1 g.224345411_224345414delCTCT; NM_015176.3:c.1070_1073delCTCT; p.Ser357Leufs*28	De novo	Heterozygous	LB	n/a	4x heterozygous LOF alleles in gnomAD
USA 5	PHKA1	ChrX g.71864208T>C; NM_002637.3:c.1459+4A>G (IVS14+4A>G)	Maternally Inherited	Hemizygous	LB	Muscle glycogenosis (300559)	Phenotypes do not match. Variant found 1x heterozgous in gnomAD; 4 other varaints affecting same splice site found 4x hemizygous in gnomAD; 4x hemizygous LOF alleles found in gnomAD.
USA 9	POLG1	Chr15 g.89870178C>A; NM_002693.2:c.1550G>T; p.Gly517Val	De novo	Heterozygous	LB	Progressive external ophthalmoplegia (157640)	Phenotypes do not match. 30 LOF heterozygous alleles in GnomAD. Varaint absent in gnomAD.
USA 11	CDK11A	Chr1 g.1650770C>T; NM_024011.3:c.352G>A; p.Gly118Arg Chr1 g.1636016C>T; NM_024011.3:c.1537G>A; p.Glu513Lys	Inherited	Compound Heterozygous	LB	n/a	10 LOF homozygous alleles found in gnomAD. Both variants absent in gnomAD
USA 13	MECP2	ChrX g.153296048G>A; NM_004992.3:c.1231C>T; p.Pro411Ser	Maternally Inherited	Hemizygous	VUS	Severe congenital encephalopathy with early death (300673) and Mental retardation with spasticity and other features (300055)	Missense varinats have been associated with male neurodevelopmental disorders. Pateint without characteristic Rett Syndromic features (e.g. epilepsy, spastcity). Varinat absent in gnomAD.
	KDM2B	Chr12 g.121947486C>T; NM_032590.4:c.1531G>A; p.Glu511Lys	Inherited	Compound Heterozygous	LB	n/a	p.Glu511Lys allele found 4x heterozygous in gnomAD. p.Lys492Arg is absent in gnomAD. >800 LOF alleles in heterozygous state, and 4 LOF alleles in homozygous state in gnomAD
		Chr12 g.121947542T>C ;NM_032590.4:c.1475A>G; p.Lys492Arg	Denovo				
USA 17	SORCS1	Chr10 g.108432707C>A; NM_001206572.1:c.1977G>T; p.Glu659His	De novo	Heterozygous	LB	Alzheimer disease 6 (605526)	Phenotypes do not match. Varaints absent in gnomAD. 55 LOF heterozygous alleles in gnomAD.
	Duplication 9q21.2	525.93kb region spanning 5 genes.	De novo	Heterozygous	VUS	n/a	n/a
USA 22	RPS6KA3	ChrX g.20179844G>A; NM_004586.2:c.1877C>T; p.Pro626Leu	Maternally inherited	Hemizygous	VUS	Coffin-Lowry syndrome (303600) and XLID (300844)	Varaint has not been reported in these disorders. Variant absent in gnomAD. Pateint not displaying hallmark Coffin-Lowry Syndomic features (e.g. short stature, skeletal abnormalitites, hearing deficit, digital features); but may overlap with milder non-syndromic XLID also reported for missense variants.

Table S2. Additional genetic variants of note in USP9X cohort.

Table S3. Clinical features of individuals with likely pathogenic USP9X missense variants. Please see accompanying Microsoft Excel File.

Table S4. Clinical features of individuals with USP9X missense variants of unknown significance. Please see accompanying Microsoft Excel File.

Test	Genotype						Statistical Analysis		
	Usp9x+/Y			Usp9x-/Y			Levene's Test	t-test	
	Mean	SEM	N	Mean	SEM	N		Equal Variance Assumed	Equal Variance Not Assumed
of.1	1366.34	104.84	27	1760.75	111.58	19	0.464	0.015	0.014
of.2	814.28	75.39	27	1038.33	67.84	19	0.444	0.041	0.032
of.3	619.72	71.51	27	878.23	58.38	19	0.222	0.012	0.008
of.4	468.7	59.87	27	680.38	74.67	19	0.398	0.031	0.033
of.5	501.01	64.19	27	583.86	86.68	19	0.286	0.436	0.447
of.6	391.69	57.57	27	524.01	79.49	19	0.306	0.173	0.186
Body Length	3.7	0.1	30	3.4	0.1	26	0.088	0.037	0.039
Spont	2.5	0.1	30	2.6	0.1	26	0.692	0.599	0.598
Respiration	2	0	30	2	0	26	N/A	N/A	N/A
Tremor	2	0	30	2	0	26	N/A	N/A	N/A
Urination in Jar	0.3	0.1	30	0.5	0.1	26	0.062	0.263	0.277
Defecation in jar	0.8	0.2	30	0.9	0.2	26	0.842	0.767	0.765
Weight	26.6	0.6	30	24.4	0.4	26	0.193	0.004	0.003
Transfer.arousal.	3.8	0.2	30	3.5	0.2	26	0.274	0.2	0.204
Locomotor Activity	21.5	1.1	30	19.1	1.3	26	0.247	0.169	0.173
Palpebral Closure	2	0	30	2	0	26	0.029	0.287	0.327
Piloerection.	1	0	30	1	0	26	0.029	0.287	0.327
Gait	2.8	0.1	30	2.6	0.1	26	0	0.039	0.044
Pelvic Elevation	2	0	30	2	0	26	0.029	0.287	0.327
Tail Elevation	1	0	30	1	0	26	0.029	0.287	0.327
Touch Escape	2.5	0.1	30	2.2	0.1	26	0.177	0.07	0.077
Positional Passivity	4	0	30	4	0	26	N/A	N/A	N/A
Trunk.Curl	1	0	30	1	0	26	0.007	0.196	0.169
Limb Grasping	1	0	30	1	0	26	0.059	0.357	0.326
Visual Placing	2.8	0.1	30	2.4	0.1	26	0.358	0.024	0.025
Grip Strength	2.9	0.1	30	2.8	0.1	26	0.044	0.359	0.38
Body Tone	1.1	0	30	1.1	0.1	26	0.771	0.884	0.885
Pinna.Reflex	0.1	0.1	30	0.1	0.1	26	0.807	0.944	0.943
Corneal Reflex	1	0	30	1	0	26	0.306	0.137	0.163
Toe.Pinch	2.7	0.1	30	2.7	0.1	26	0.899	0.831	0.831
Skin Colour	1.6	0.1	30	1.4	0.1	26	0.176	0.083	0.084
Heart Rate	1.5	0.1	30	1.6	0.1	26	0.078	0.287	0.285
Limb Tone	2	0.1	30	1.9	0.2	26	0.466	0.485	0.487
Abdominal Tone	1	0	30	1	0	26	0.059	0.357	0.326
Lacrimation	1	0	30	1	0	26	0.84	0.92	0.92
Salivation	1.8	0.1	30	1.8	0.1	26	0.412	0.985	0.985
Provoked Biting	0.2	0.1	30	0.2	0.1	26	0.972	0.821	0.821
Righting Reflex	3	0	30	3	0	26	0.059	0.357	0.326
Negative Geotaxis	3.7	0.1	30	3.3	0.2	26	0.033	0.136	0.147
Wire Manoeuvr	3.9	0.1	30	3.8	0.1	26	0.649	0.723	0.72
Contact Righting.	1	0	30	1	0	26	N/A	N/A	N/A
Fear	0.4	0.1	30	0.3	0.1	26	0.98	0.951	0.951
Irritability	0.9	0	30	0.9	0	26	0.691	0.843	0.842
Aggression.	0.7	0.1	30	0.7	0.1	26	0.819	0.806	0.805
Vocalization	0.6	0.1	30	0.8	0.1	26	0.023	0.09	0.087
Grip Strength 1	1.34	0.08	30	1.09	0.05	26	0.026	0.011	0.009
Grip Strength 2	1.29	0.06	30	1.02	0.03	26	0	0	0
Grip Strength 3	1.2	0.06	30	1.06	0.04	26	0.022	0.079	0.07
Average Grip Strength	1.28	0.06	30	1.06	0.04	26	0.001	0.005	0.004
Hot Plate 1	7.7	1.3	3	9	1.2	6	0.685	0.522	0.487
Hot Plate 2	6.9	1.2	3	12.9	2.1	6	0.258	0.095	0.039
Hot Plate 3	7.4	1.2	3	11.9	1.8	6	0.345	0.142	0.073
Avergae Hot Plate	7.3	0.94	3	11.24	1.41	6	0.336	0.11	0.053

Table S5. Results of SHIRPA neurological screen of mice lacking USP9X in the developing and adult forebrain structures. Tests highlighted in green identify statistical difference.

Antibodies for Western Blot

Antigen	Species	Dilution	Source
USP9X	rabbit	1:500	Bethyl Laboratories, USA
Raptor	rabbit	1:1000	Cell Signaling Technology, USA
SMURF1	mouse	1:250	Abcam
Phospho-S6	rabbit	1:1000	Cell Signaling Technology
S6	rabbit	1:1000	Cell Signaling Technology
SMAD4	mouse	1:200	Santa Cruz Biotechnology
ITCH	rabbit	1:1000	Cell Signaling Technology
MIB1	rabbit	1:1000	Abcam
CTNNB1	mouse	1:1000	BD Transduction Laboratories, Australia
activated CTNNB1	mouse	1:300	Millipore, Merck, Australia
MCL1	mouse	1:250	BD Pharmingen, Australia
ACTB	mouse	1:20000	Sigma-Aldridge

Antibodies for Immunofluorescence

Antigen	Species	Dilution	Source
USP9X	rabbit	1:500	Bethyl Laboratories, USA
SMAD4	mouse	1:200	Santa Cruz Biotechnology, USA
activated CTNNB1	mouse	1:500	Millipore, Merck, Australia

Table S6. Antibodies used in this study.

Family	Approving Institutional Review Board
USA 6	Women's and Children's Health Network Human Research Ethics Committee, South Australia.
USA 5	University of Michigan Review Board, MI, USA
France 2	French Law on Genetic Tests (Law of Bioethics)
UK1	UK Research Ethics Committee approvals 10/H0305/83 and GEN/284/12.
USA 14	Western Institutional Review Board, Puyallup, WA, USA
France 3	French law on genetic tests (law of Bioethics)
USA 13	Women's and Children's Health Network Human Research Ethics Committee, South Australia, Australia
USA 18	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
USA 1	UPMC Children's Hospital of Pittsburgh, PA, USA
USA 19	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
Spain 1	Women's and Children's Health Network Human Research Ethics Committee, South Australia.
Portugal 1	Medical Genetics Unit, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.
USA 7	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
Netherlands 11	Clinical Exome Sequencing study University Medical Center Utrecht, The Netherlands
USA 9	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
USA 3	Cook Children's Medical Center IRB, TX, USA
Netherlands 9	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Netherlands 12	Clinical Diagnostic Exome Sequencing Study, Erasmus University Medical Center Hospital, The Netherlands
USA 12	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
Canada 2	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
Netherlands 10	Clinical Diagnostic Exome Sequencing Study, Utrecht University Medical Center Hospital, The Netherlands
Netherlands 1	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Netherlands 7	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
USA 21	National Human Genome Research Institute, USA
USA 11	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
USA 22	Mayo Clinic Institutional Review Board, MN, USA
Spain 2	Hospital Universitario Quirónsalud de Madrid Ethics Board, Spain.
USA 17	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
USA 26	Duke University Medical Center, NC, USA
Canada 6	The Hospital for Sick Children Research Ethics Board (REB#1000054798), Canada.
USA 28	Nationwide Children's Hospital Ethics Board (IRB11-00215), OH, USA
Netherlands 3	Clinical Diagnostic Exome Sequencing Study, Utrecht University Medical Center Hospital, The Netherlands
Canada 1	Children's Hospital of Eastern Ontario, Canada
Canada 3	Cedars Sinai Institutional Review Board (Protocol #00046408), CA, USA
Netherlands 5	Clinical Diagnostic Exome Sequencing Study, VU University Medical Center, The Netherlands
Netherlands 2	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Netherlands 6	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Swiss 1	Cantonal Ethical Board Zurich, Switzerland.
Norway 1	University Hospital of North Norway, Tromsø, Norway
Canada 5	Children's Hospital of Eastern Ontario, Canada
Netherlands 4	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Netherlands 8	Clinical Diagnostic Exome Sequencing Study, Radboud University Medical Center Hospital, The Netherlands
Belgium 1	French Law on Genetic Tests (Law of Bioethics)
France 1	French Law on Genetic Tests (Law of Bioethics)
USA 4	The University of Texas Health Science Center at Houston, HSC-MS-09-0057, TX, USA
USA 29	Nationwide Children's Hospital IRB, OH, USA
Germany 1	Ethics Board of the Medical Faculty of the University of Heidelberg, Germany
Denmark 1	University Hospital Copenhagen, Denmark

Table S7. Patient consent protocols.

SUPPLEMENTAL CLINICAL DATA DESCRIPTION

USA 6

Variant: ChrX GRCh37(hg19) g.40988391A>G; NM_001039590.2 c.235A>G, NP_001034679.2 p.Ile79Val.

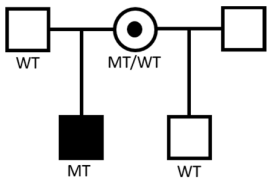
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Pathogenic

Authors: Tyler Pierson, Elizabeth Bhoj, Stephanie Byers

Pedigree:



Clinical Notes:

Patient now 19 years old has intellectual disability, developmental delay, absent speech, seizures, hypotonia, severe motor disability (non-ambulatory), short stature, relative macrocephaly. Patient uses gastric tube for feeding and has gastroesophageal reflux. Facial dysmorphisms include short palpebral fissures, large incisors, full eyebrows. Fingers are short and trident-shaped.

Brain MRI revealed progressive cerebral and cerebellar volume loss, hypodensity in the left basal ganglia, unchanged and consistent with a lacune infarct (remote). There is a less conspicuous area of hypodensity on the contralateral side. There are hypodense white matter changes along the periventricular white matter and bilateral centrum semiovale.

USA 5

Variant: ChrX GRCh37(hg19) g.40994047T>A; NM_001039590.2 c.392T>A; NP_001034679.2 p.Ile131Asn.

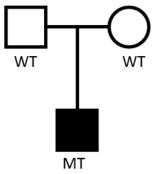
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No; Variant of unknown significance in PHKA1 [c.1459+4A>G (IVS14+4A>G)]

ACMG Classification: Likely Pathogenic

Authors: Catherine E. Keegan

Pedigree:



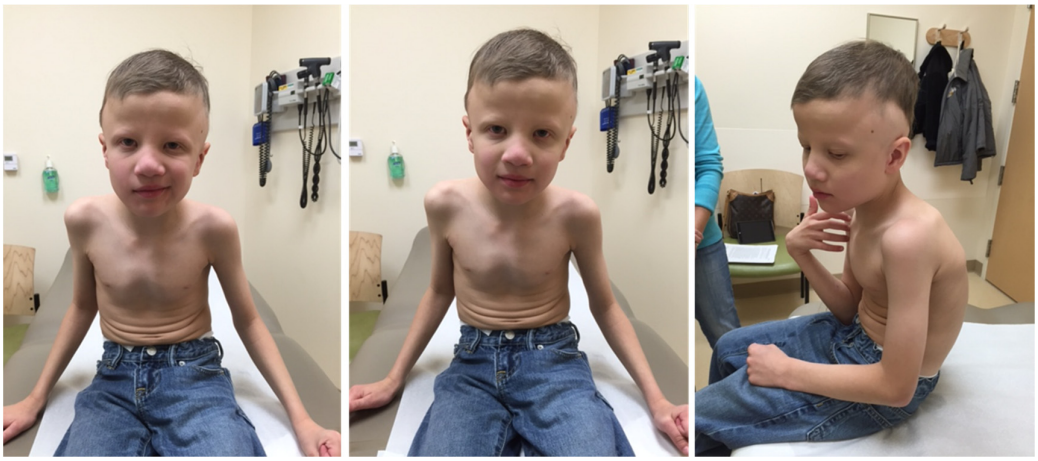
Clinical Notes:

A 9 year old male initially referred for evaluation at 9 months of age due to a history of hypotonia and left clubfoot requiring casting. He was the product of an uncomplicated pregnancy, delivered at 39 weeks of gestation, and weighing 8 lbs 3 oz. The clubfoot was identified while in utero. Following delivery, it was noted that he had hypotonia and feeding problems due to a poor suck. A brain MRI revealed a thin posterior corpus callosum and midbrain at the lower limits of normal in size. Physical findings on exam were a prominent anterior fontanelle and right-sided posterior plagiocephaly, mild frontal bossing, somewhat low-set ears that were normally rotated. He had epicanthal folds and inferior orbital creasing, a flat nasal bridge, wide nose, a sacral dimple with a visualized base, small toenails, and significant head lag on pull to sit.

The patient was referred for re-evaluation at age 9 after having been lost to follow up due to persistent hypotonia, generalized weakness, speech apraxia and dysarthia. A recent brain MRI redemonstrated mildly enlarged trigones with thinning of the posterior aspect of the corpus callosum and superimposed generalized low white matter volume. An arachnoid cyst versus a cisterna magna in the posterior fossa was also identified. He had a neurological evaluation for possible absence-type seizures; his EEG was normal. He had had a normal cardiology evaluation including a normal EKG and echo. He was evaluated by Pediatric Ophthalmology where he was noted to have a mild astigmatism and exotropia. He had been diagnosed with obstructive sleep apnea and subsequently had a tonsilloadenoidectomy. Other past medical history included constipation, eczema, alopecia involving his posterior scalp, pitted and ridged nails, and hypodontia (two missing teeth).

His examination at age 9 revealed a weight of 20.4 kg (0.8th percentile), a height of 122.5 centimeters (4.5th percentile), and a head circumference of 51.9 centimeters (38th percentile), consistent with relative macrocephaly. He had a tall, broad forehead. He had mild ptosis. Skin exam was unremarkable. He had ridging of his fingernails. He had reduced muscle bulk in both the upper and lower extremities and hypotonia. He had joint laxity notable at the fingers, wrists, elbows and knees, as well as pes planus.

The patient was reported to have a significant history of developmental delay in both motor and speech and language, although he has continued to make slow developmental progress. His motor milestones include sitting at 10 months, crawling at 12 to 13 months, and walking independently at 3 years of age. He has apraxia of speech. His receptive language was reported to be more advanced than his expressive language. He is presently in a moderately cognitively impaired program receiving multiple services and therapies.



FRANCE 2

Variant: ChrX GRCh37 (hg19) g.41025226C>T; NM_001039590.2 c.2087C>T; NP_001034679.2 p.Ala696Val

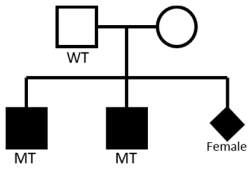
Discovery Platform: Whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Pathogenic

Authors: Martine Raynaud, Laurent Pasquier

Pedigree:



Clinical Notes:

The clinical picture was very severe and was identical in two brothers separated by two years, who died very early after birth. Prenatally they displayed intrauterine growth restriction and increased echogenicity of the fetal bowel. Postnatally they had meconium ileus, pancytopenia, punctuata epiphysis. MRI revealed malformation of cortical development (reduced gyration with thickening of the cerebral cortex, thin corpus callosum, features of double cortex on temporal lobes) Extended metabolic explorations were normal.

UK1

Variant: ChrX GRCh37(hg19) g.41027299 C>T; NM_001039590.2 c.2464C>T; NP_001034679.2 pArg822Cys

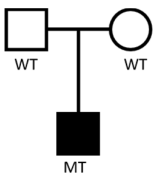
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Pathogenic

Authors: Henrietta Lefroy, Usha Kini.

Pedigree:



Clinical Notes:

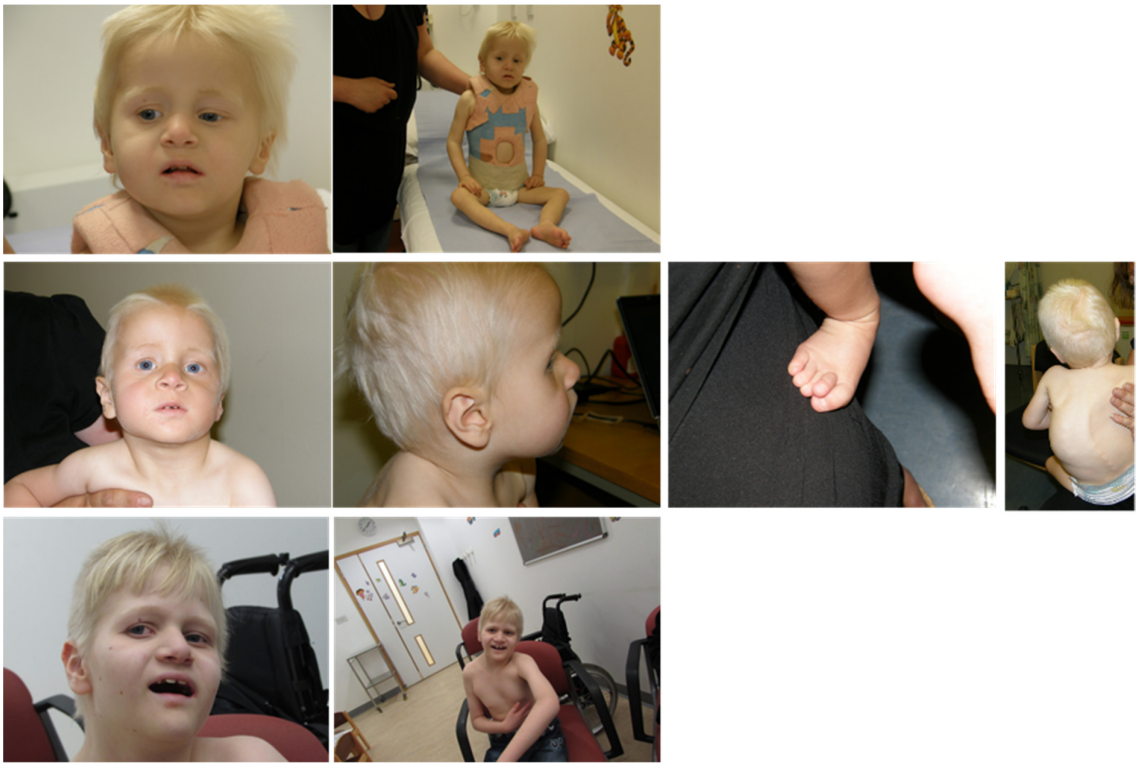
9 year old boy referred due to developmental delay, thoracolumbar scoliosis, hypotonia and microcephaly. He is the fourth child of unrelated parents. He has three older brothers, the oldest of whom has speech and language difficulties.

His parents became concerned at 6 months as they felt he was quite floppy. His motor milestones were delayed; sitting unsupported at 11 months and standing with support at 13 months. He is unable to walk at 9 years of age but can use a standing frame and walker. He is also able to mobilise via bottom shuffling. In terms of his speech, he began making sounds such as 'mmm' by 2.5 years. At 9 years he has no words but makes non-specific noises. His understanding is limited but he does recognise familiar people and objects.

He was diagnosed with significant thoracolumbar scoliosis from 6 months which was treated with a spinal plaster cast and subsequently magec rods from T2 to L4. He had positional plagiocephaly. He was found to have a Duane anomaly affecting his left eye. He had grommets inserted for glue ear. He has a persistent defect in his tympanic membrane and ongoing discharge.

On examination he has striking coarse blonde hair and blue eyes. He has microcephaly (< 0.4th centile, -2 SD). He has facial asymmetry with deep set eyes and broad anteverted nares. He has overlapping 2/3rd toes bilaterally and thin arms and legs.

His investigations include an MRI brain which revealed mildly dilated, mildly dysmorphic ventricles and an 'impression' of reduced white matter which was normally myelinated. His genetic testing included an array-CGH which showed a paternally inherited 7q31 deletion. This only contained one gene; IMMP2 and was felt less likely to be significant. He underwent testing for Prader-Willi syndrome which did not find an abnormality. He was then enrolled into the DDD study which revealed a de novo USP9X mutation.



USA 14

Variant: ChrX GRCh37(hg19) g. 41029757A>G; NM_001039590.2 c. 2912A>G; NP_001034679.2 p.Asn971Ser

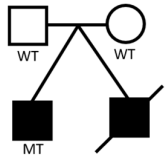
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No.

ACMG Classification: Likely Pathogenic.

Authors: Keri Ramsey

Pedigree:



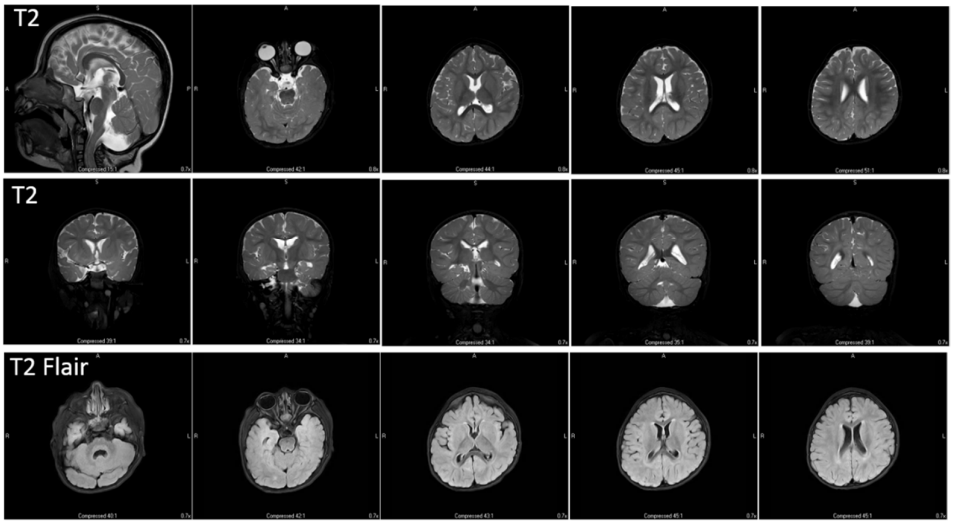
Clinical Notes:

Patient has Global developmental delay, severe intellectual disability, speech delay (babbling) spastic quadriparesis (non-weight bearing; tight abductors in his legs, non ambulatory), hypotonia (truncal, head lag, slips through at shoulders, joint hypermobility). Brain MRI shows possibly delayed myelination or gliosis in the posterior periventricular white matter.

Has severe growth retardation (receives growth hormone), and is of short stature. Brachycephaly, prominence of the temples (on either side of the orbits), shallow orbits, mid face hypoplasia, flat nasal bridge, prominence of midline forehead resembles slightly the Kleeblattshadel deformity, intermittent nystagmus with lateral gaze. Had Bi-lateral hip dysplasia requiring surgery. Fed completely by G-tube because of aspiration. Is hypoglycemic.

Had a twin brother that died at 1 year of respiratory illness. Twins thought to be monozygotic because they were in the same sac. Twin diagnosed with congenital heart disease.

MRI at 20 months



France 3

Variant: ChrX GRCh37(hg19) g.41057806C>T; NM_001039590.2 c.4406C>T; NP_001034679.2 p.Pro1469Leu

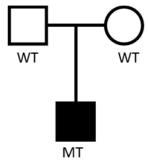
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No.

ACMG Classification: Likely Pathogenic.

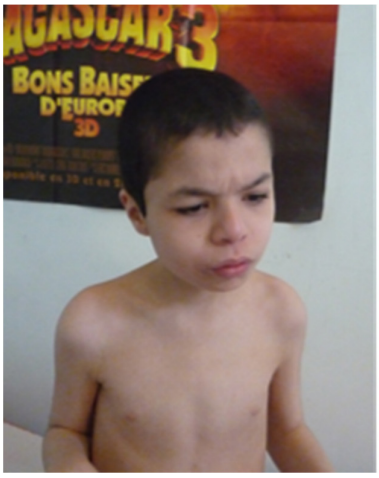
Authors: Boris Keren, Alexandra Afenjar, Thierry Billette de Villemeur

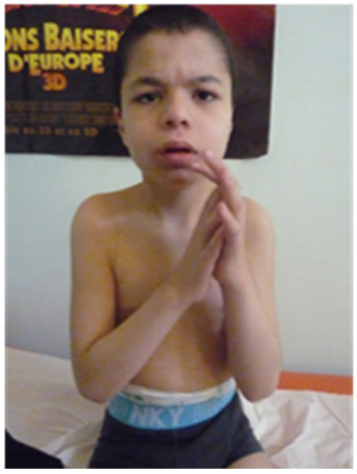
Pedigree:

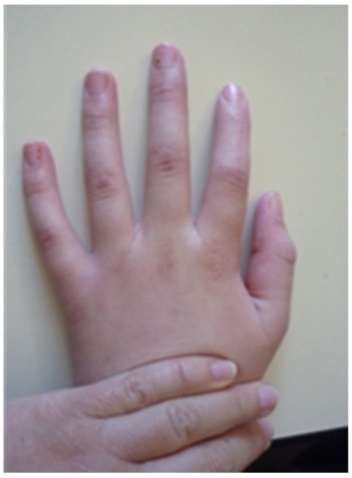


Clinical Notes:

Severe developmental delay and intellectual disability. Started walking at 6 years old. Has hypotonia and motor disability and broad based gait. Has autistic, obsessive and aggressive behaviors. Displayed growth retardation and is of short stature. Has joint hypermobility. Dysmorphisms include broad thumbs, broad nasal tip, palpebral fissure oblique down. Has feeding difficulties and gastro-esophageal reflux. Is hyperglycemic.







USA 13

Variant: ChrX GRCh37(hg19) g.41060427A>T; NM_001039590.2 c.4718A>T; NP_001034679.2 p.Gln1573Leu

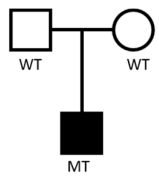
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No. *MECP2* maternally inherited (c.1231C>T; p.Pro411Ser) – previously reported in hemizygous state in 2 individuals in gnomAD; *KDM2B* – 2 heterozygous variants (c.1531G>A;p.Glu511Lys – maternally inherited) and (c.1475A>G;p.Lys492Arg – de novo) – of unknown significance.

ACMG Classification: Likely Pathogenic.

Authors: Carey Mcdougall, Elaine Zackai

Pedigree:



Clinical Notes:

Global developmental delay, speech delay, hypotonia and motor disability. Slight myopia. Facial dysmorphisms include mild upslant to palpebral fissures, mildly low set ears, short philtrum and bulbous nasal tip. Has slow weight gain and requires soft foods. Has hyperextensibility of joints and skin.

USA 18

Variant: ChrX GRCh37(hg19) g.41073847C>A; NM_001039590.2 c.5216C>A; NP_001034679.2 p.T1739N

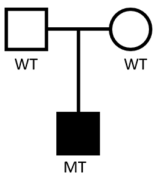
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely pathogenic

Authors: Tyler Pierson, Naomi Yachelevich

Pedigree:



Clinical Notes:

Subject is a former full-term 5yo boy with a history of a diagnosis of autistic spectrum disorder. He has also been found to have diffusely low tone. He had normal growth parameters at births and was note to have mild neonatal jaundice. He spent some time in the NICU to gain some extra weight, as well as having some difficulty regulating his temperature, which corrected over the next few days. He had significant infantile hypotonia with subsequent delayed motor milestones. He started walking at 2 years of age. Has wide based gait and pseudoataxia in gait may be due to low tone - wears SMOs, has external rotation of lower extremities with excessive pronation of both feet. Parents state that he is generally happy and eats well, and is affectionate. He has challenges with communication and sensory issues. He has some self-stimulatory behaviour, and often clenches his hands and gets very excited by anything to do with numbers or clocks. He tends to have certain interests such as exit signs and red lights, as well as anything with numbers. He has alternating exophoria. They feel he can follow 2-step commands, but anything more is quite challenging for him. He knows his letters and numbers, and can count up to 100. He has a little wide-based gait as well. He had some mildly diffuse low tone, which involved both his axial and appendicular musculature. His bulk was normal. His strength was within normal limits. Deep tendon reflexes were hypoactive at 1/5 throughout. Plantar responses were flexor bilaterally. Mildly decreased sensation with regard to light touch, temperature and vibratory sense, but that could be secondary to his cooperation waning at this point. He had an MRI, which suggested some mild colpocephaly. Brain MRI shows Periventricular Leukomalacia; Periventricular Leukomalacia; 'mild FLAIR hyperintensity along the periventricular white matter most prominent in parietal regions bilaterally which show mild thinning'; also small cystic foci in periatrial white matter bilaterally. Dysmorphisms include deep set eyes, bilateral epicanthus, flat nasal bridge, mild hypertelorism, full eyebrows. Has calcaneovalgus feet, joint hypermobility, tight heel cords. Metabolic testing revealed low free carnitine, high lactate, low thyroxine.

USA 1

Variant: ChrX GRCh37(hg19) g.41075423T>A; NM_001039590.2 c.5603T>A**;** NP_001034679.2 p.V1868E

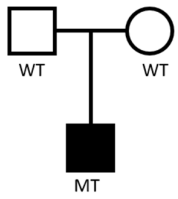
Discovery Platform: Trio-based whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Pathogenic

Authors: Elena Infante, Amy Goldstein, Suneeta Madan-Khetarpal

Pedigree:



Clinical Notes:

At 6 years, patient with intellectual disability, global delay (non-verbal, cannot walk independently), cortical visual impairment and hypotonia (Tone decreased centrally). Displays occasional chorea and dystonic posturing of hands but not when in use. Ankle dystonia when walking. Displays some flapping/stereotypies. Has a history of febrile seizures with normal EEG.

Has some dysmorphic facial features including ears which are very small, malformed, and posteriorly rotated.

Repeated Brain MRI was stable, showing a thin corpus callosum with associated colpocephaly of the lateral ventricles, a suggestion of bilateral polymicrogyria along the sylvian fissures, bilateral hippocampal malrotation, stable, bilateral hypoplastic olfactory bulb, stable, and a left middle cranial fossa arachnoid cyst, which is stable.

At 7 years, walks independently with very wide-based gait. Has major issues with anxiety/overstimulation. He gets very fearful, tearful and stiff. Seizures present. A recoded event revealed Epileptiform discharges noted as generalized spike and slow wave discharges. Electroclinical seizures were recorded as numerous brief typical absences recorded, clinically presenting with staring, unresponsiveness and eye flutter, and associated with 3 Hz generalized spike and slow wave activity on the EEG, lasting approximately 12-16 seconds each. Treatment initiated with Depakote.

USA 19

Variant: ChrX GRCh37(hg19) g.41075854T>A; NM_001039590.2 c.6034T>A; NP_001034679.2 p.F2012I

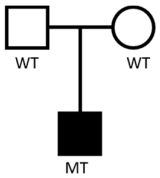
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely pathogenic

Authors: Tyler Pierson, Angela E. Lin, MD, Marcie A. Steeves, Mohammed Ali Almuqbil

Pedigree:



Clinical Notes:

Prenatal US suggested trisomy 13 or 18 with ventriculomegaly, bilateral clubfeet, bilateral polydactyly, unusual facial features.

Patient now 3 ½ years.

Global developmental delay, severe ID, non-verbal, chronic epilepsy, hypotonia, motor disability, growth restriction (weight <3rd, height 3rd (OFC not measured)). Anomalies have included: Laryngeal cleft, type 1, postaxial polydactly type B of hands, Striking diffuse joint hypermobility, retroflexed hips, clubbed feet bilaterally and feeding difficulties (needs G-tube).

Brain MRI #1 at birth showed evidence of infarcts in bilateral temporal, occipital, parietal lobes, partial agenesis of corpus collosum, cerebellar hypoplasia, ventriculomegaly, piriforma apetrure stenosis and probable hypoplasia of the pituitary gland. MRI #2 at 15 months: Unchanged appearance of callosal dysgenesis, left cerebellar hypoplasia and probable hypoplasia of the pituitary gland. Unchanged size and configuration of the dilated ventricles.

5 ½ year old sister has absence seizures, normal brain MRI.

Spain 1

Variant: ChrX GRCh37(hg19) g.41077669G>A; NM_001039590.2 c.6254G>A; NP_001034679.2 p.R2085H

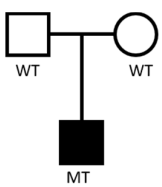
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Pathogenic.

Authors: Carlos López-Otín, Olaya Santiago-Fernández

Pedigree:



Clinical Notes:

Patient has intellectual disability, developmental delay, speech delay, hypotonia, motor disability, visual impairment (myopia, strabismus). Brain MRI revealed hypoplasia of cerebellum vermis and Dandy Walker malformation, dysplasia of corpus callosum, ventriculomegaly, and white matter loss. Dysmorphisms including Bilateral epicanthus, Flat Nasal Bridge, clinodactyly IIIth to Vth toes, ear fissure, bilateral equinovarus feet and hyperextensibility of joints and skin.

Portugal 1

Variant: ChrX GRCh37 (hg19) g.41082601T>C; NM_001039590.2 c.6697T>C; NP_001034679.2 p.Ser2233Pro

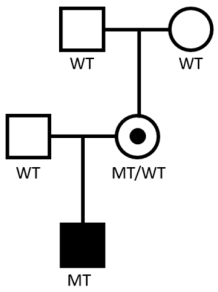
Discovery Platform: TrusightOne disease exome.

Other variants of significance: No

ACMG Classification: Likely Pathogenic

Authors: Cláudia Falcão-Reis, Joaquim Sá

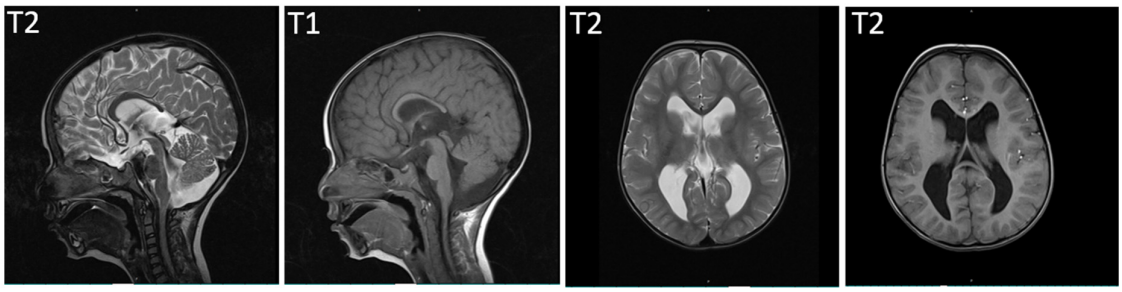
Pedigree:



Clinical Notes:

Patient delivered at 39 weeks with normal growth parameters, APGAR score 6,8,9. During gestation: transient placental abruption during 2nd trimester and oligohydramnios after amniocentesis (advanced maternal age – 36YO; prenatal karyoptype 46,XY), no abnormalities noted on ultrasound. Sent to the NICU after birth because of suspected sepsis (10 days duration), bilateral hip dysplasia and cryptorchidism were noted and managed.

At 3 years of age patient presented with severe developmental delay (non-verbal) with global hypotonia (non-ambulant) and sometimes auto-aggression (very slow progress since age 3, no regression). Dysmorphic facial features include low anterior hairline, synophrys, broad and depressed nasal tip, spaced teeth. Patient has broad and large great toes, hyperextensibility of joints and skin, small ovoid scalp aplasia cutis (secondary to birth trauma?). Growth has been normal (weight, stature and OFC between 25th and 50th centile). Brain MRI revealed lateral ventriculomegaly, peripheral T2 hypersignal areas, hypoplasia of corpus callosum.



USA 7

Variant: ChrX GRCh37(hg19) g.40994022G>T; NM_001039590.2 c.367G>T; NP_001034679.2 p.G123W

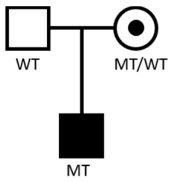
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance.

Authors: Tyler Pierson, Yezmin Perilla-Young, Laurie Smith

Pedigree:



Clinical Notes:

Patient with hypotonia, profound developmental and intellectual impairment and seizures of unknown etiology. Has speech delay (communicates by picture book, some sign language. uses only two words), autism, partial idiopathic epilepsy, hypotonia, motor disability, repetitive behaviors hand flapping and is irritable. Has motor disability, with waddling gait and requires ankle-foot orthosis for walking. Displays macrocephaly, pectus carinatum, and bilateral coxa valga. Brain MRI shows delayed maturation of white matter, thinning corpus collosum, small pituitary gland, and posterior positioning of the cerebral arteries. Dysmorphisms include turricephalic, tall forehead, arched eyebrows, down slanted palpebral fissures, slightly posteriorly rotated ears with small cartilaginous nodule on posterior caudal aspect, flat foot, persistent finger pads, 5th finger clinodactyly, cryptorchidism.

Netherlands 11

Variant: ChrX GRCh37(hg19) g.40996160C>A; NM_001039590.2 c.539C>A; NP_001034679.2 p.Pro180His

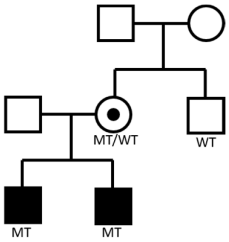
Discovery Platform: whole exome sequencing

Other variants of significance: ?.

ACMG Classification: Unknown significance

Authors: Renske Oegema, Bert van der Zwaag, E. van Binsbergen

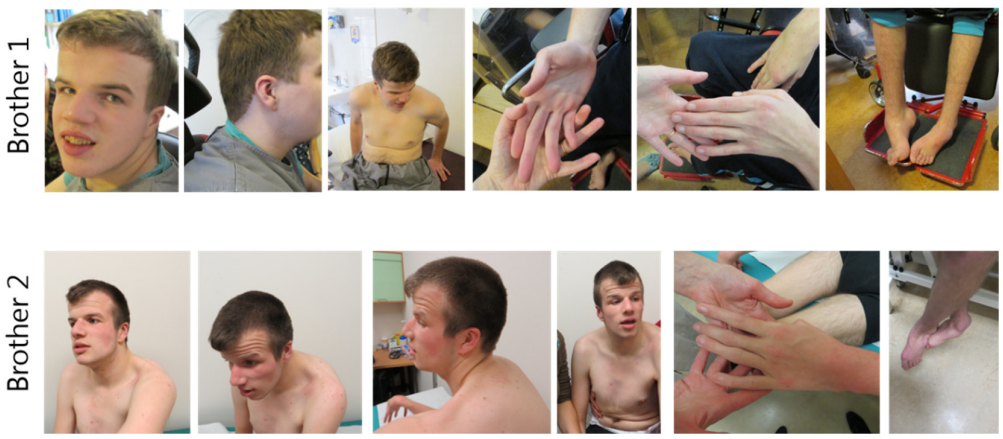
Pedigree:

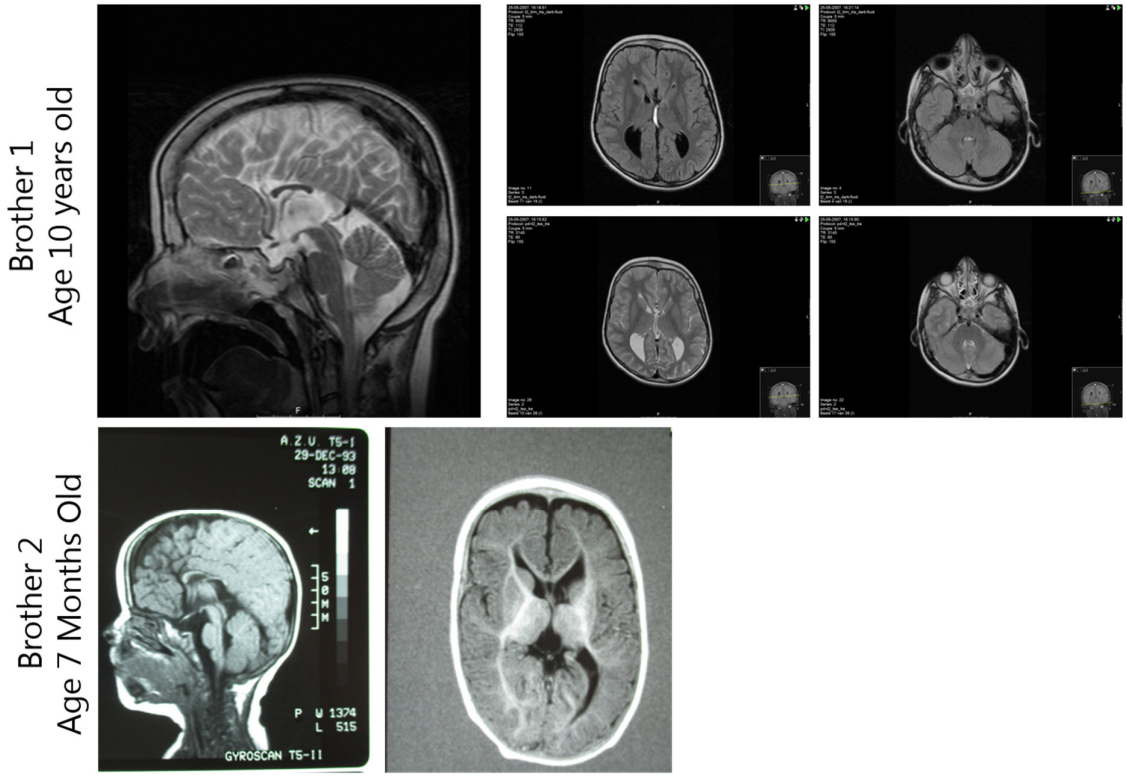


Clinical Notes:

Proband is a 21-year old male with moderate to severe ID, epilepsy and spasticity, and on brain MRI thin corpus callosum. He has a sacral dimple, hip dysplasia. In childhood he had hypotonia and recurrent upper respiratory tract infections. He was born at 37 weeks of gestation, in breech position. He underwent surgery for bilateral inguinal hernia at 6 weeks of age. His development was delayed from birth. He started crawling after his second birthday and walking at 4 years of age. Epilepsy was diagnosed at age 3. Generally he has a friendly and cheerful personality. Occasionally he has temper tantrums. Clinical genetic examination: speaks a few words, makes noises. OFC 59 cms (+0.75 SDS). He has a broad forehead, deep set eyes with full upper eyelids. Broad base to the nose, broad mouth. Caries dentition. Small, posteriorly rotated ears. Large hands with long fingers (hand 21.5 cm, third finger 9.5 cm). Thumbs cannot be fully extended. There is an abnormal shape of the thorax with low set nipples. He has pes equines, hammer toes and calf muscle atrophy.

His brother is more severely disabled, he never gained independent walking and his behaviour is more challenging. He can crawl and ride a (special) bicycle. He speaks 7-8 words and used speech computer and signing. He is not diagnosed with epilepsy. He was born after 33 weeks of gestations with postaxial polydactyly. Pregnancy was complicated by HELLP syndrome. BW 1880 grams (normal), Apgar 4/8. He was admitted to the neonatal care unit, and treated for respiratory insufficiency and hyperbilirubinemia. He suffers from constipation. He has scoliosis. OFC 56.7 cm (- 0.5 SD).





USA 9

Variant: ChrX GRCh37(hg19) g.40999955T>C; NM_001039590.2 c.701 T>C; NP_001034679.2 p.F234S

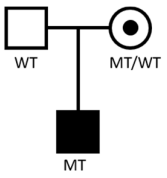
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No. De-novo Heterozygous *POLG1* c1550 G>T; pGly517Val of unknown significance

ACMG Classification: Unknown significance.

Authors: Tyler Pierson, John M. Graham, Christine Shieh.

Pedigree:



Clinical Notes:

Patient with intellectual disability, developmental delay, speech delay, autistic and obsessive behavior. Has hypotonia, athetoid movements, and is ataxic with a wide based gait. He wears ankle-foot orthoses and pronates badly when he walks. Sleep is disturbed with frequent awakenings. Has mild scoliosis. Has growth retardation and short stature. Dysmorphisms include broad forehead, flat nasal bridge, small nose, overhanging columella, up-slanted palpebral fissures, high palate, hyperextensible dislocatable thumbs, overcrowded upper teeth, low set and posterior rotated ears, prognathic lower jaw. Metabolic testing revealed low cysteine, mildly elevated asparagine, mildly elevated lactate, low 'free' carnitine.

USA 3

Variant: ChrX GRCh37(hg19) g.41000406A>G; NM_001039590.2 c.958A>G; NP_001034679.2 p.R320G

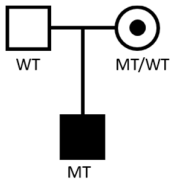
Discovery Platform: Whole exome sequencing.

Other variants of significance: No

ACMG Classification: Unknown Significance

Authors: Scott Perry

Pedigree:



Clinical Notes:

Patient with global developmental delay, speech delay, epilepsy, autistic mannerisms, and hypotonia.

Netherlands 9

Variant: ChrX GRCh37(hg19) g.41007713C>T; NM_001039590.2 c.1511C>T; NP_001034679.2 p.Ala504Val

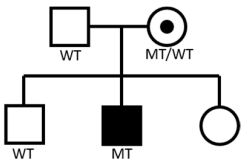
Discovery Platform: Whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: David Koolen, Tjitske Kleefstra

Pedigree:



Clinical Notes:

Severe ID, Non verbal, Automutilation, Scoliosis

Netherlands 12

Variant: ChrX GRCh37(hg19) g.41007751A>C; NM_001039590.2 c.1549A>C; NP_001034679.2 p.Ser517Arg

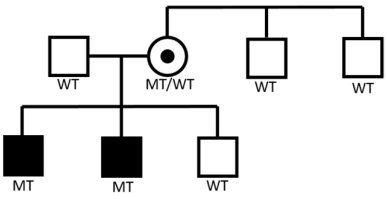
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

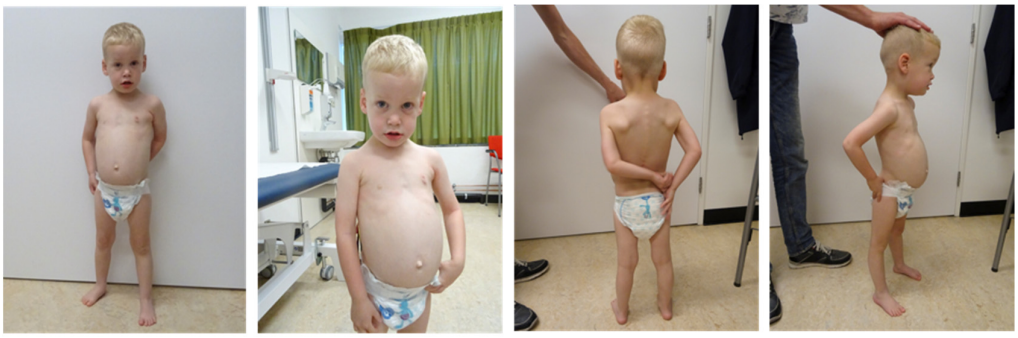
Authors: Serwet Demirdas

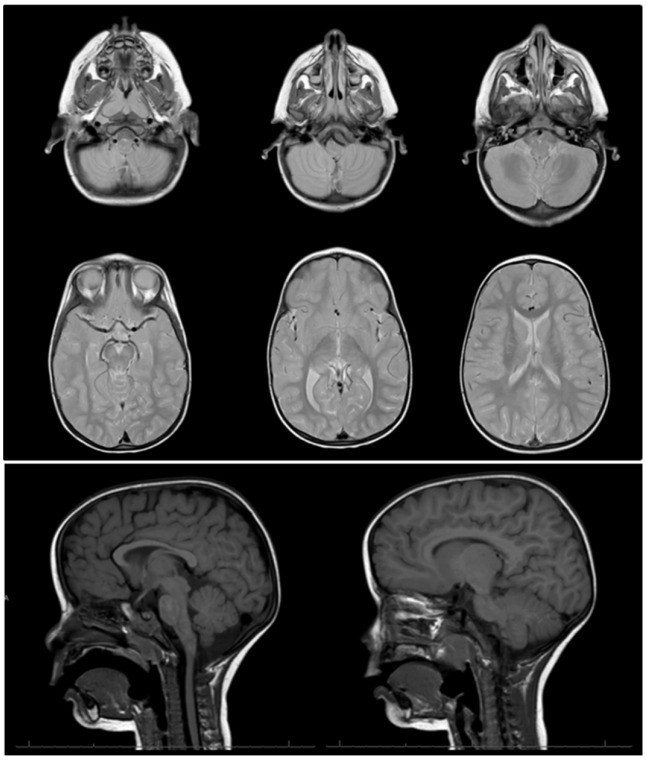
Pedigree:



Clinical Notes:

2-year-old boy with developmental delay, specifically a speech-/language delay and walking difficulties. The walking difficulties are clinically difficult to distinguish between peripheral or central problem. His walking is broad-based with overstretching, foot lifters weakness and he has some extrapyramidal. Dysmorphic features are apparent: deepset eyes, frontal bossing, hypoplastic nails, inverted nipples etc. His growth parameters are within range. MRI of both the brain and the spine were unremarkable. Metabolic screening in plasma and urine, array results and sequencing of CGG-repeats in the FMR1 gene were normal. WES of a panel of 1174 genes contributing to intellectual disability showed a VOUS class 3 in the USPX9 gene, inherited from his healthy mother. The patient has a brother with autism whom also carries the variant, and another brother and two healthy maternal uncles who do not carry the variant.





USA 12

Variant: ChrX GRCh37(hg19) g.41029255C>T; NM_001039590.2 c.2644 C>T; NP_001034679.2 p.R882C

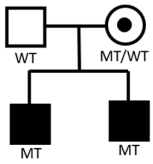
Discovery Platform: Quartet based whole exome sequencing

Other variants of significance: No.

ACMG Classification: Unknown significance.

Authors: Tyler Pierson, Bradley Schaefer, Noelle R Danylchuk.

Pedigree:



Clinical Notes:

Patient 1. Developmental delay, intellectual disability, speech delay but responded to therapy, obsessive about food, has ADHD, mild growth retardation and short stature. Has keratitis' on palms/soles.

Patient 2. Developmental delay, intellectual disability, obsessive about food, has ADHD. Has keratosis.

Mother with learning disability and psychiatric disorder.

Canada 2

Variant: ChrX GRCh37(hg19) g.41029345A>G; NM_001039590.2 c.2734A>G; NP_001034679.2 p.Ile912Val

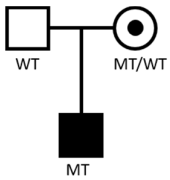
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: Tyler Pierson, Margot Van Allen, Tracy Oh.

Pedigree:



Clinical Notes:

Patient with global developmental delay, intellectual disability, speech delay and regression of language skills, autism, ADHD and has frequent tantrums. Dysmorphisms include broad forehead, dimpled chin and 5th finger brachydactyly.

Netherlands 10

Variant: ChrX GRCh37(hg19) g.41043278C>A; NM_001039590.2 c.3176C>A; NP_001034679.2 p.Ala1059Asp

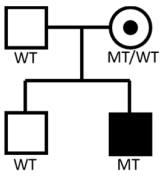
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No. Maternally inherited variant in the RANBP2 gene (RANBP2 (NM_006267.4): c.[7418A>G];[=] p.[(Glu2473Gly)];[(=)] (Chr2(GRCh37):g.[109384413A>G];[=]) of unknown significance

ACMG Classification: Unknown significance

Authors: Renske Oegema, Bert van der Zwaag, E. van Binsbergen

Pedigree:



Clinical Notes:

This boy was born with cesarean section at 37 weeks of gestation. The last week of the pregnancy were complicated by preeclampsia and polyhydramnios. Birth weight 2400 grams. He had feeding difficulties during the first months with nasal

regurgitation. There was excessive drooling till 8 months. His motor development was delayed, he walked independently at 23 months. He also had a speech delay, started speaking at almost 3 years of age, with hypernasality and articulation difficulties. Intensive speech therapy was beneficial. He is very shy in communication with strangers. At 3 years of age he was diagnosed with velopharyngeal insufficiency. He was successfully operated at 4 years. He is now 5 years of age and attends regular education.

Clinical genetic examination (age 4): OFC -1 sds, height -1 sds, weight +1 SDS. A boy with a prominent forehead, full upper eyelids, low nasal bridge, broad mouth. He has broad thumbs.

At 3years 10 months he presented with an epileptic seizure - generalized tonic-clonic. Two weeks later he presented with status epilepticus and was started on anti-epileptic drug. Brain MRI was normal. SNP array.

Netherlands 1

Variant: ChrX GRCh37(hg19) g.41043370T>C; NM_001039590.2 c.3268T>C; NP_001034679.2 p.Tyr1090His

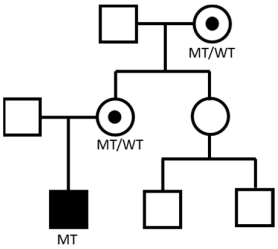
Discovery Platform: whole exome sequencing.

Other variants of significance: ARID1B. Chr6 (GRCh37): g.157528688T>G; NM_020732.3: c.6413T>G p.Leu2138Arg (de-novo)

ACMG Classification: Unknown Significance

Authors: Tjitske Kleefstra and Margot Reijnders

Pedigree:



Clinical Notes:

Patient has ID, epilepsy, no speech, scoliosis (not progressive), thin hair, facial dysmorphisms: hypertelorism, macrostomia, full eyebrows, low frontal hairline. Skin abnormalities in Blaschkolines. MRI: absent distal part corpus callosum. N.B. There is no skewed X-inactivation present in mother and grandmother. Mother has epilepsy.

Netherlands 7

Variant: ChrX GRCh37(hg19) g.41045786A>G; NM_001039590.2 c.3575A>G; NP_001034679.2 p.His1192Arg

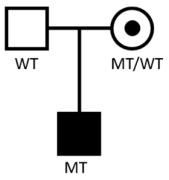
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance.

Authors: Tjitske Kleefstra, Margot Reijnders

Pedigree:



Clinical Notes:

Patient with severe intellectual disability, global developmental delay, speech delay (only few words), autistic behaviour, motor disability (wheelchair for long distances), ataxia and anxiety. Patient has microcephaly. Facial dysmorphisms including macrostomia, large ears, small and high nasal bridge. Patient has constipations.

USA 21

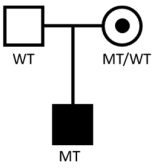
Variant: ChrX GRCh37(hg19) g.41045833C>T; NM_001039590.2 c.3622 C>T; NP_001034679.2 p.Pro1208Ser

Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: Kelly Schoch, Loren Pena, Undiagnosed Disease Network
Pedigree:



Clinical Notes:

4 year old Hispanic male with global developmental delay, moderate intellectual disability, speech delay (only a few words), many febrile seizures from 19-28 months of age (mild diffuse slowing on EEG), hypotonia, motor disability (assisted walking), ataxia, visual impairment (intermittent downbeat nystagmus). Patient had intrauterine growth restriction and post-nataly had growth retardation, short stature and microcephaly. Brain MRI showed mild prominance of sulci in the frontal poles bilaterally at 14 months and possible T2 signal hyperintensity involving the periventricular WM in the frontal lobes and possible mildly delayed myelination at 3 years old. Dysmorphisms include upslanting palpebral fissures, almond shaped and mildly wide spaced eyes, sacral dimple, round and cupped ears with simple helices and mild hypertelorism. Has constipation, and has osteopenia with recurrent bone fractures (may be due to rickets of prematurity).

USA 11

Variant: ChrX GRCh37(hg19) g.41048705C>G; NM_001039590.2 c.3954 C>G; NP_001034679.2 p.D1318E

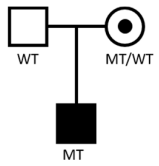
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No. Compound heterozygous CDK11A variants (both inherited) E513K, G118R. Unknown significance.

ACMG Classification: Unknown significance.

Authors: Tyler Pierson, Kamer Tezcan.

Pedigree:



Clinical Notes:

Developmental delay and regression, intellectual disability, speech delay, autism and ADHD, impaired hearing (corrected by surgery). Brain MRI was normal. Frequent vomiting independent of food intake.

USA 22

Variant: ChrX GRCh37(hg19) g.41057869C>T; NM_001039590.2 c.4469C>T; NP_001034679.2 p.Pro1490Leu

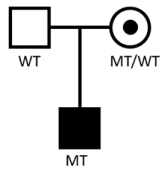
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No; ChrX(GRCh37):g.20179844G>A; NM_004586.2(RPS6KA3):c.1877C>T; p.(Pro626Leu) of unknown significance.

ACMG Classification: Unknown significance

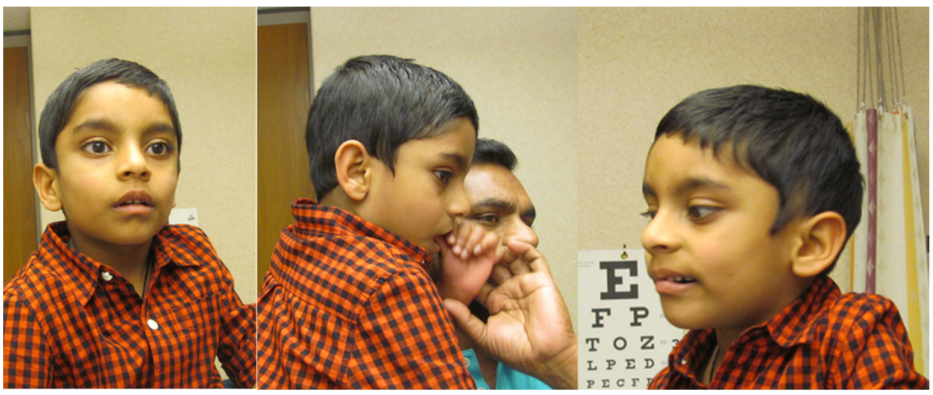
Authors: Filippo Pinto e Vairo, Pavel N. Pichurin, Sarah A. Ewing, Sarah S. Barnett, Eric W. Klee

Pedigree:



Clinical Notes:

6-year-old male with a history of autism, speech delay, abnormal EEG and Focal versus generalized epilepsy of unknown etiology. Also, his HC is at p5, he has mild medial flaring of eyebrows, nonspecific white matter abnormalities, hypertrichosis, and generally nondysmorphic. EEG showed mild diffuse slowing, bifrontal spikes and sharp waves, and intermixed bursts of generalized atypical spike and wave discharges occurring with the bifrontal discharges.



Spain 2

Variant: ChrX GRCh37(hg19) g.41060527C>A; NM_001039590.2 c.4818C>A; NP_001034679.2 p.Asp1606Glu

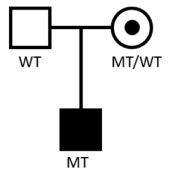
Discovery Platform: Trio based whole genome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance.

Authors: Alberto Fernandez-Jaén.

Pedigree:



Clinical Notes:

Patient clinical features include unilateral deafness (hypoplasia of cochlear nerve), microcephaly, short stature, mild intellectual disability, speech delay, and no dysmorphic features. Other studies (metabolic, serologies, ocular, muscular) were normal.

USA 17

Variant: ChrX GRCh37(hg19) g.41064568C>A; NM_001039590.2 c.4837C>A; NP_001034679.2 p.P1613T.

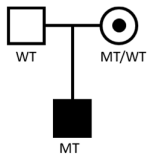
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: SORC21 c.1977G>T p.Q659H (de novo; may contribute). Duplication 9q21.2; 525.93kb, 5 genes – unknown significance.

ACMG Classification: Unknown significance.

Authors: Tyler Pierson, Alexander Asamoah, Kelly Jackson.

Pedigree:



Clinical Notes:

Subject is a 6yo male former full-term child intrauterine growth retardation, short stature with macrocephaly. He has a history of motor delay and speech delays, episodes of hypoglycemia, and cyclic vomiting. The hypoglycemia/vomiting episodes have resolved for the most part, except when he is ill and he can get hypoglycemic quite easily. He has had GERD as well and is thought to possess a genetic marker for Crohn disease (he does not have it). He is non-dysmorphic and has left exotropia. No family history of childhood onset neurological/developmental disease. Family is non-consanguineous.

At ~2yoa he had several episodes that seemed like staring spells/absence seizures, but EEG was normal and he has not had any further episodes since. He had an MRI brain, that father thought was normal, but did not provide a report. He was a late walker at 18mos, but currently does not exhibit any motor issues. He was also a late talker and still has some minor cognitive and speech issues.

USA 26

Variant: ChrX GRCh37(hg19) g.41064704G>A; NM_001039590.2 c.4973G>A; NP_001034679.2 p.Arg1658Gln

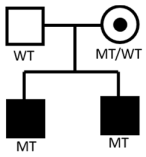
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: Vandana Shashi, Jennifer A. Sullivan, Ioana Cutcutache

Pedigree:



Clinical Notes:

6 year old male with epilepsy with history of prolonged status epilepticus, acquired microcephaly, and global developmental delays. Seizure control is good with phenobarbital. Walked independently at 4.5 years. Profound Speech delay with Expressive > receptive. He is functionally non- verbal. 9 year old full brother is significantly delayed with reading skills and mildly delayed with math skills. His developmental milestones were normal except for a mild speech delay. He has ADHD. Father required special education in school.

Canada 6

Variant: ChrX GRCh37(hg19) g.41077774T>C; NM_001039590.2 c.6359T>C; NP_001034679.2 p.I2120T

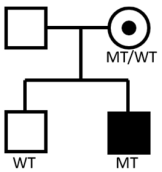
Discovery Platform: Whole exome sequencing.

Other variants of significance: No

ACMG Classification: Unknown Significance

Authors: Saadet Mercimek-Andrews

Pedigree:



Clinical Notes:

At age of 9 ¾ years phenotype consisted of ID, hypotonia from his first year, fine motor delay, speech language delay, history of developmental regression, febrile seizures (onset 21 months) and after age 3 refractory epilepsy (of different types, generalized tonic clonic, atonic, myoclonic, absence) refractory to multiple anti-epileptic medications (Phenobarb, valproate, clobazam, lamotrigine, keppra) and the ketogenic diet. Non dysmorphic apperance. Brain MRI was normal at the age of 2 years, whereas showed increased T2 signal intensity in the left middle frontal gyrus, raising the question of low-grade glioma at the age of 6 years old. His neuropsychological assessment showed moderate ID with markedly restricted functions for speaking, walking and mental functions necessary for everyday life.

USA 28

Variant: ChrX GRCh37(hg19) g.41084144A>C; NM_001039590.2 c.6901A>C; NP_001034679.2 p.Lys2301Gln

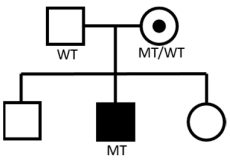
Discovery Platform: Trio based whole genome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: Daniel Koboldt

Pedigree:



Clinical Notes:

Patient is a currently 4-year-old male who was born at full term after an uncomplicated pregnancy weighing 6lbs, 9oz. He had some issues with feeding in the neonatal period, including poor latching for breastfeeding. His general health was good, and he first came to attention at age 9 months when concern was raised about hypotonia and motor delay. At age 4, he still cannot walk independently though has been cruising since about 18 months. He knows 2-3 single words and a few signs. Other features include chronic drooling and tremor that has been described by his pediatric neurologist as a cerebellar tremor. Cranial MRI obtained at 3 years was unremarkable. Acanthocytes were reportedly seen on CBC smear, but a repeat smear was normal. *MTTP* gene sequencing and deletion/duplication testing was negative. Whole exome sequencing was performed and 3 variants of unknown significance were identified: paternally inherited variants in *CACNA1A* and *COL6A2*, and maternally inherited *USP9X* variant. Deletion/duplication testing for *COL6A2* was performed and negative. Whole genome sequencing was performed on a research basis, and the patient was found to carry other variants of unknown significance in several genes. It is unclear at this time whether or not any of these could be contributing to this patient's phenotype.

Netherlands 3

Variant: ChrX GRCh37(hg19); g.41089041dup;

Long isoform: NM_001039590.2 c.7440dup; NP_001034679.2 p.Ala2481fs
Short isoform: NM_001039590 c.[0]; NP_001034679 p.[0]

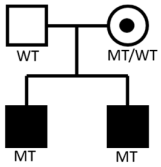
Discovery Platform: whole exome sequencing (both affected brothers and both parents)

Other variants of significance: No

ACMG Classification: Unknown Significance

Authors: Peter VanHasselt

Pedigree:



Clinical Notes:

Both patients displayed intrauterine growth restriction. Both present with global developmental delay, intellectual disability, autistic behaviour. Both had microcephaly. Both had transient (after birth) hypoglycaemia and elevated tyrosine. Both have feeding difficulties and one diagnosed with gastro-esophageal reflux.

Canada 1

Variant: ChrX GRCh37(hg19); g.41089041dup;

Long isoform: NM_001039590.2 c.7440dup; NP_001034679.2 p.Ala2481fs
Short isoform: NM_001039590 c.[0]; NP_001034679 p.[0]

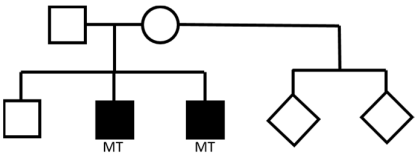
Discovery Platform: Whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown Significance

Authors: Mathew Lines

Pedigree:



Clinical Notes:

Family History

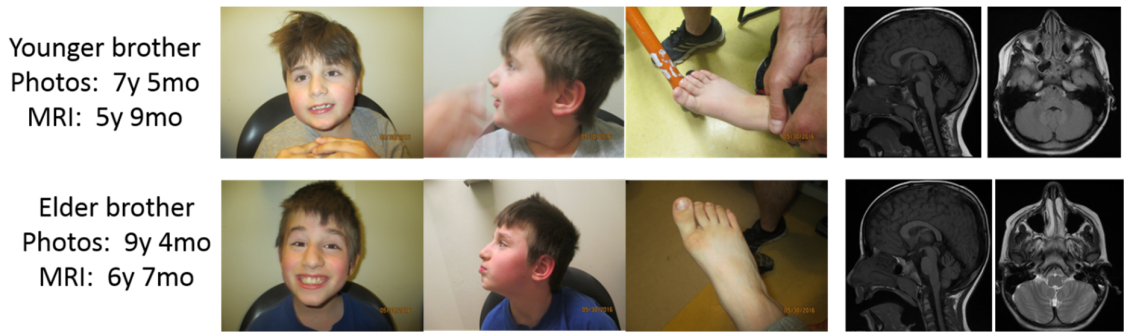
Paternal family history: (non-contributory) aortic valve surgery (for aortic regurgitation). Cerebellar stroke. Attention deficit. Brother (paternal uncle to probands) with cerebral palsy and had severe developmental delay ("toddler level" according to family). Father's ethnic background is 'Caucasian' (paternal) and Austrian/Hungarian (maternal). Maternal family history: Mother denies learning problems; did have seizures as a child, but these resolved at age seven, and she has required no medication since. Mother has several family members with bipolar disorder. She is of mixed French Canadian/Italian/Irish/First Nations ancestry.

Younger Brother

Born at term weighing 6 lbs 12 oz. Mother was a 30 year old G5P4A1 at the time of delivery. He was delivered by repeat C-section and had an uncomplicated neonatal course. Boy with Global developmental delay. (1) Gross motor: Sat unsupported at 16 months; Prone ('commando') crawl at 2 years. At 5 ½ years: Pull to stand, ambulate with walker, eat with spoon, scribble with fist grip, no writing, some speech (dysarthric), not toilet trained. (2) Cerebellar ataxia (truncal, postural, extremities) – needs walker for ambulation (3) Hypotonia (4) Generalized tonic-clonic seizures (six episodes, always in context of febrile illness) requiring no medications. Physical Exam: Growth (age 7 years); Height: 118 cm (24 %, Z = -0.70, Source: WHO Growth Chart for Canada); Weight: 30 kg (97 %, Z = 1.86, Source: WHO Growth Chart for Canada); Head circumference: 52.8 cm (62nd centile). Appearance is nondysmorphic and in keeping with parents. The major exam finding is marked limb and gait ataxia, which expressed itself both in terms of dramatic gait instability, marked dysmetria and dysdiadochokinesis, and dysarthria. He was able to sit on the edge of the exam table unassisted. Extraocular movements were full. Tone was reduced with no ankle clonus; reflexes were normal; plantar responses were flexor. Brain MRI: Enlarged vermian fissures and CSF between cerebellar lobes, in keeping with atrophy of the anterior vermis (region from the lingula to the tuber) without evidence of hypoplasia. The cerebellar hemispheres are within normal limits. The corpus callosum has a slightly thick appearance. All of the following were normal: Microarray (SNP), SCA panel (repeat enumeration SCAs 1-3, 6-8, and 17), Transferrin isoelectric focusing, AFP, VLCFAs, Monogenic ataxia NGS panel (U. Chicago) (negative for mutations in any of the 346 genes included in the panel) (*done as an exome slice, see below). The USP9X diagnosis was made upon inspection of the remaining (off-panel) exome variants from the U. Chicago ataxia panel.

Elder Brother

Born at term by (repeat) Caesarean section, weighing 6 lbs 12 oz. Mother was a 30 year old G5P4A1 at the time of delivery. Neonatal course was uncomplicated. Boy with (1) Global developmental delay, (2) central hypotonia, (3) Cerebellar ataxia, (4) Strabismus (s/p surgery) No seizures. Development: Delays recognized ~6mo. Is considered to be less cognitively affected than his brother. Roll over 14mo. Sit unsupported 2.5 years. At 7y 11mo: Attends a modified Grade 3 program. Can walk a few independent steps, or for long distances in his walker. He is able to feed himself with a knife, fork, or spoon, and can write his name with some errors. His speech consists of complete sentences, including pronouns and plurals, and his speech is slow but relatively understandable. He exhibits normal eye contact and is toilet-trained. Growth (7y 11mo): Height: 127.1 cm (21 %, Z = -0.80, Source: WHO Growth Chart for Canada); Weight: 29 kg (61 %, Z = 0.29, Source: WHO Growth Chart for Canada); Head circumference: 53.1 cm (58th centile). The main findings on exam are marked gait and limb ataxia. Reflexes are normal. Plantar responses are down-going. Investigations: EMG / Nerve conduction studies normal; Brain MRI findings similar to those seen in brother (anterior vermis atrophy; thick corpus callosum); Microarray, TIEF, AFP, VLCFAs, monogenic ataxia panel (U. Chicago) – all normal.



Canada 3

Variant: ChrX GRCh37(hg19); g.41089041dup;

Long isoform: NM_001039590.2 c.7440dup; NP_001034679.2 p.Ala2481fs
Short isoform: NM_001039590 c.[0]; NP_001034679 p.[0]

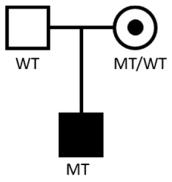
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Unknown significance

Authors: Tyler Pierson, Christine Shieh.

Pedigree:



Clinical Notes:

Patient with global developmental delay, intellectual disability, speech delay, autism, primary generalized epileptiform activity on EEG but no clinical seizures. Also with anxiety and some obsessions. Has macrocephaly. Dysmorphisms include borderline brachydactyly, sacral dimple, broad forehead, dimpled chin, very mild asymmetry of face. Also has hyperextensibility of metacarpophalangeal joints displays and genu valgum. Has feeding difficulties requiring tube feeding.

Netherlands 5

Variant: ChrX GRCh37(hg19) g.41091697C>T; NM_001039590.2 c.7633C>T; NP_001034679.2 p.P2545S

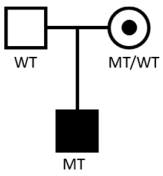
Discovery Platform: Whole exome sequencing.

Other variants of significance: No. Variant of unknown significance NM_015176.3 FBXO28 c.1070_1073delCTCT p.(S357Lfs*28) de novo and heterozygous.

ACMG Classification: Unknown Significance

Authors: Janneke Weiss, Petra Zwijnenburg

Pedigree:



Clinical Notes:

Patient had intrauterine growth restriction. Postnataly, growth within target height range, with no dysmorphisms, global developmental delay, absent speech and autism. Brain MRI revealed delayed myelinisation, otherwise no abnormalities.

Netherlands 2

Variant: ChrX GRCh37(hg19) g.41000604G>C; NM_001039590.2 c.1081G>C; NP_001034679.2 p.Val361Leu

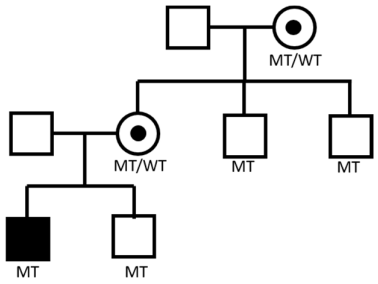
Discovery Platform: whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Benign – Found in healthy male relative

Authors: Tjitske Kleefstra and Margot Reijnders

Pedigree:



Netherlands 6

Variant: ChrX GRCh37 (hg19) g.41002691G>A; NM_001039590.2 c.1309G>A; NP_001034679.2 p.Ala437Thr

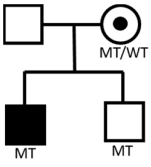
Discovery Platform: Whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Benign – Found in healthy male relative (maternal uncle – not shown)

Authors: Tjitske Kleefstra and Margot Reijnders

Pedigree:



Swiss 1

Variant: ChrX GRCh37(hg19) g.41031160A>G; NM_001039590.2 c.3097A>G; NP_001034679.2 p.Met1033Val

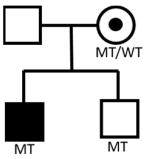
Discovery Platform: Whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Benign – Found in healthy brother

Authors: Pascal Joset

Pedigree:



Severe global DD, Dandy Walker formation and hypoplasia of cerebellum.

Norway 1

Variant: ChrX GRCh37(hg19) g.41043275G>A; NM_001039590.2 c.3173G>A; NP_001034679.2 p.Arg1058Lys

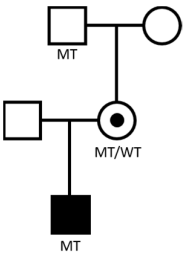
Discovery Platform: Trio based Disease exome sequencing, (4813 gene Illumina TruSightOne “Mendeliome” panel).

Other variants of significance: No

ACMG Classification: Likely Benign (variant found in healthy male relative)

Authors: Marie Falkenberg Smeland

Pedigree:



Canada 5

Variant: ChrX GRCh37(hg19) g.41043684C>A; NM_001039590.2; c.3314C>A; NP_001034679.2 p.Pro1105His

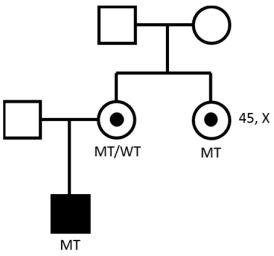
Discovery Platform: Whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Benign (Found in unaffected maternal aunt who has Turner syndrome)

Authors: Mathew Lines

Pedigree:



Netherlands 4

Variant: ChrX GRCh37(hg19) g.41043792T>C; NM-001039590.2 c.3422T>C; NP_001034679.2 p.M1141T

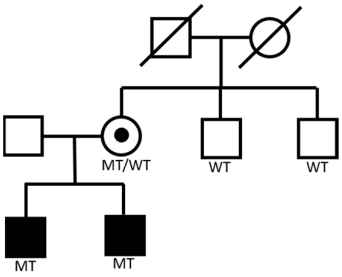
Discovery Platform: whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Benign – Found in healthy male relative

Authors: Tjitske Kleefstra and Margot Reijnders

Pedigree:



Netherlands 8

Variant: ChrX GRCh37(hg19) g.41055921A>G; NM_001039590.2 c.4163A>G; NP_001034679.2 p.Asn1388Ser

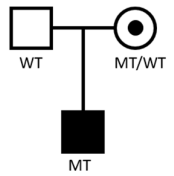
Discovery Platform: Trio based whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Benign. –Hemizygous alleles found in gnomAD.

Authors: Tjitske Kleefstra, Margot Reijnders

Pedigree:



Belgium 1

Variant: ChrX GRCh37(hg19) g.41064560A>G; NM_001039590.2 c.4829A>G; NP_001034679.2 p.Asn1610Ser

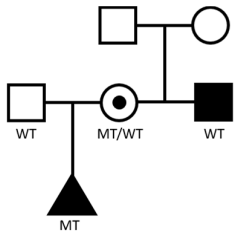
Discovery Platform: Trio based Disease exome sequencing (3989 genes, SeqCap EZ Choice XL, NimbleGen Roche)

Other variants of significance: No.

ACMG Classification: Likely benign

Authors: Lionel Van Maldergem, Julie Désir, Martina Marangoni

Pedigree:



FRANCE 1

Variant: ChrX GRCh37 (hg19) g.41075489G>A; NM_001039590.2 c.5669G>A; NP_001034679.2 p.Gly1890Glu

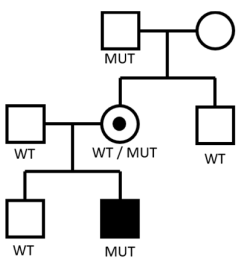
Discovery Platform: Trio-based whole exome sequencing.

Other variants of significance: Missense change in *H3F3A* [Chr1(GRCh37):g.226259121G>C NM_002107.4:c.352G>C p.(Val118Leu)] considered likely pathogenic.

ACMG Classification: Likely Benign – Found in healthy male relative.

Authors: Sebastien Kury, Sandra Mercier

Pedigree:



USA 4

Variant: ChrX GRCh37(hg19) g.41075489G>A; NM_001039590.2 c.5669G>A; NP_001034679.2 p.Gly1890Glu

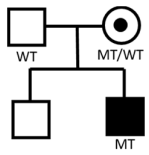
Discovery Platform: Whole exome sequencing

Other variants of significance: No

ACMG Classification: Likely Benign (Found in healthy male in case France 1)

Authors: Mary Kay Koenig

Pedigree:



Germany 1

Variant: ChrX GRCh37(hg19) g.41077775A>G; NM_001039590.2 c.6360A>G; NP_001034679.2 p.Ile2120Met

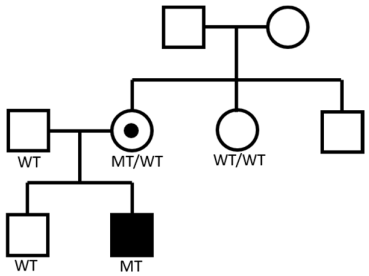
Discovery Platform: whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Benign – Variant found in healthy male relative, Hemizygous alleles found in gnomAD

Authors: Nicola Dikow, Ute Moog

Pedigree:



USA 29

Variant: ChrX GRCh37(hg19) g.4107775A>G; NM_001039590.2 c.6360A>G; NP_001034679.2 p.Ile2120Met

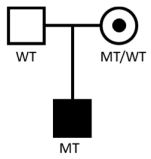
Discovery Platform: Trio based whole genome sequencing

Other variants of significance: No.

ACMG Classification: Likely Benign. Variant found in healthy a male in family Germany 1, Hemizygous alleles found in gnomAD

Authors: Scott E. Hickey

Pedigree:



Denmark 1

Variant: ChrX GRCh37(hg19) g.41082482A>G; NM_001039590.2 c.6578A>G; NP_001034679.2 p.Lys2193Arg

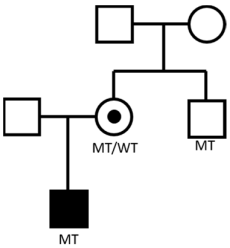
Discovery Platform: whole exome sequencing.

Other variants of significance: No

ACMG Classification: Likely Benign – Found in healthy male relative

Authors: Sabine Grønborg

Pedigree:



Supplemental References

1. Zhang Q, Dong A, Walker JR, Bountra C, Arrowsmith CH, Edwards AM, et al. (2018): *Crystal structure of a peptidase*. http://www.rcsb.org/structure/5WCH.
2. Reijnders MR, Zachariadis V, Latour B, Jolly L, Mancini GM, Pfundt R, et al. (2016): De Novo Loss-of-Function Mutations in USP9X Cause a Female-Specific Recognizable Syndrome with Developmental Delay and Congenital Malformations. *Am J Hum Genet*. 98:373-381.