Supporting Information

DNA sequencing

All genomic studies were performed on DNA extracted from blood, saliva, or sperm samples. DNA capture and sequencing of exomes was carried out as previously described by Hansen, et al at either the Baylor Genetics (BG) laboratories or at the Baylor College of Medicine Human Genome Sequencing Center (HGSC), through the Baylor-Hopkins Center for Mendelian Genomics initiative. Briefly, pre-capture libraries were prepared according to a modified manufacturer's protocol, pooled into either 4-plex library pools and hybridized in solution to the HGSC-designed Core capture reagent (52Mb, NimbleGen), or 6-plex library pools using the custom VCRome 2.1 capture reagent (42Mb, NimbleGen) according to the manufacturer's protocol with minor revisions. The sequencing run was performed in paired-end mode using either the Illumina HiSeq 2000 or 2500 platforms. In parallel to the exome workflow an Illumina Infinium Human Exome v1-2 array was generated for a final quality assessment, including orthogonal confirmation of sample identity and purity using the Error Rate In Sequencing (ERIS) pipeline developed at the BCM-HGSC. Using an "e-GenoTyping" approach, ERIS screens all sequence reads for exact matches to probe sequences defined by the variant and position of interest. A successfully sequenced sample must meet quality control metrics of ERIS SNP array concordance (>90%) and ERIS average contamination rate (<5%).

NGS analysis

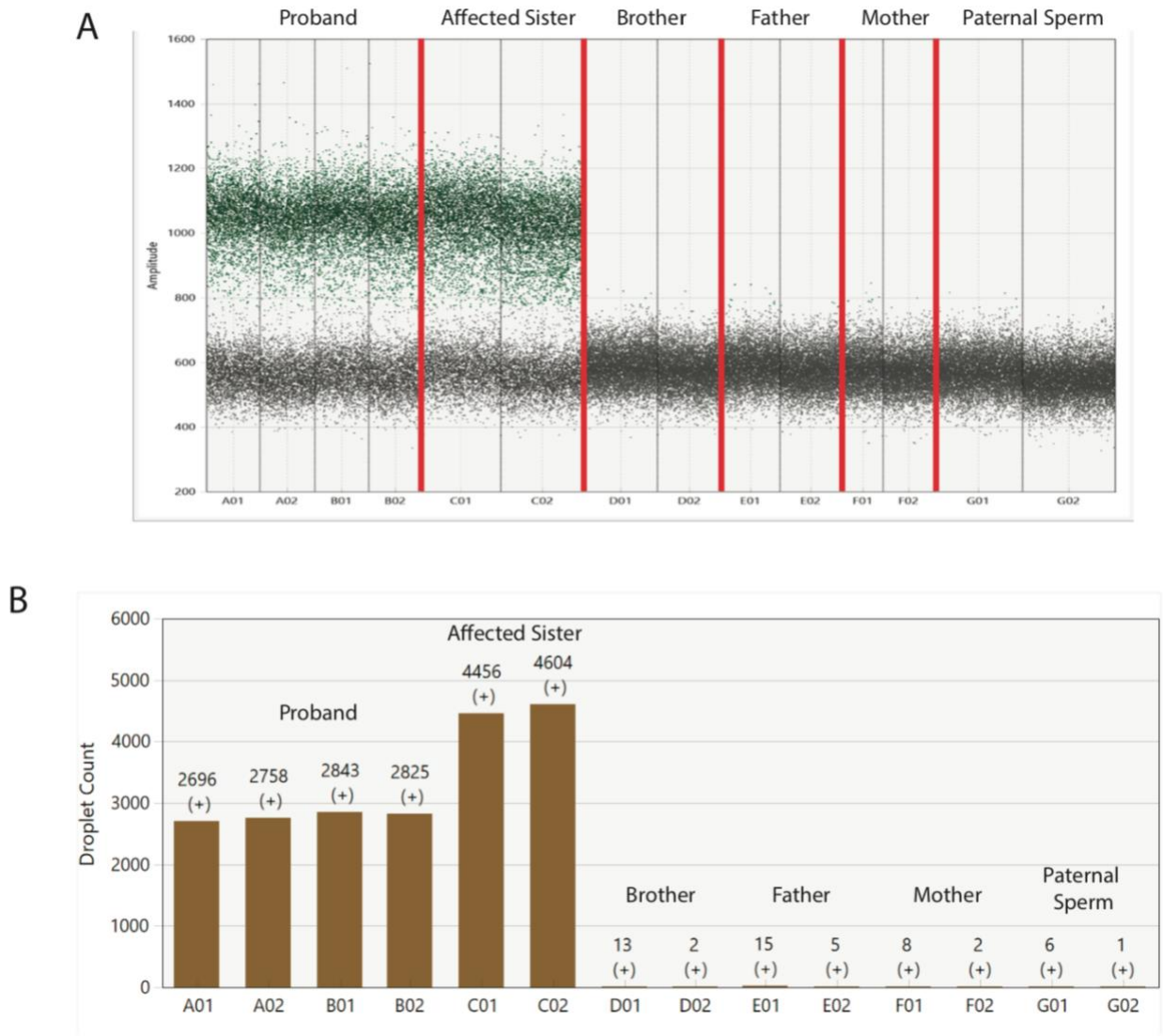
Fastq files were aligned to hg19. Variants were called with Atlas2 (v1.4.3) and annotated with ANNoVAR. High quality homozygous variants, compound heterozygous variants, and ultra-rare (MAF < 1/1,000) or novel variants shared between index family affected siblings and absent from unaffected family members were prioritized. For the index family, IBD was estimated

using PLINK (Purcell et al., 2007). Familial relationships were reconstructed and confirmed by PRIMUS (Staples, Nickerson, & Below, 2013).

Droplet Digital PCR (ddPCR)

We utilized ddPCR on the Bio-Rad QX200 system (Hercules, CA) to assess possible mosaicism for the variant in the parental samples as well as germline mosaicism in a paternal sperm sample. A TaqMan probe with a HEX fluorescent reporter and accompanying forward and reverse primers were designed to selectively amplify in presence of the variant and not amplify the wildtype position and ordered through the Sigma-Aldrich custom oligo system (St. Louis, MO). Droplets were generated containing approximately 40 ng of genomic DNA and subsequently run on a standard thermocycler to endpoint (95°C for 10 min, 40 cycles of 95°C for 30 sec and 62°C for 60 sec, 95°C for 10 min). The presence or absence of a HEX fluorescent signal was quantified for each droplet with the QuantaSoft software suite.

Supplementary Figures and Tables



Supp. Figure S1 – ddPCR reveals no low-level mosaicism in parental blood samples or paternal sperm. A) Separation of positive and negative droplets for the mutation-specific HEX labeled fluorescent TaqMan probe. Green droplets represent positive amplification of the target variant and grey droplets represent no amplification across all sample replicates. B) Quantification of droplets positive for the target variant across all samples tested.

Index #	Variant	Origin	CNS involvement	Type of CNS involvement	Spasticity	Motor Domain	Missense	*De novo*
1	R13H	Baylor	Yes	Delayed motor milestones, specific learning disabilities, ADHD, cognitive alterations	Yes	Yes	Yes	Yes
2	R13L	Baylor	Yes	ID, cognitive behavioral alterations	No	Yes	Yes	Yes
3	T99M	Baylor	Yes	Global developmental delay, brain abnormalities, seizures, cerebral palsy, optic atrophy, ID	No	Yes	Yes	Yes
4	G102S	Baylor	Yes	Global developmental delay, insensitivity to heat and pain	No	Yes	Yes	Yes
5	R216C	Baylor	Yes	Delayed motor milestones	No	Yes	Yes	Yes
6	R316Q	Baylor	Yes	Global developmental delay	Yes	Yes	Yes	Yes
7	N272del	Peru proband	Yes	ID, cerebellar atrophy	Yes	Yes	No	Yes
8	N272del	Peru sister	Yes	ID, cerebellar atrophy, pale optic disks	No	Yes	No	Yes
9	S58L	Lee et al.	Yes	Mild ID, language delay, cerebellar atrophy, neuropathy	Yes	Yes	Yes	Yes
10	T99M	Lee et al.	Yes	ID, walks independently, no optic nerve atrophy	Yes	Yes	Yes	Yes
11	T99M	Lee et al.	Yes	Severe ID, non-verbal, optic nerve atrophy, microcephaly, epilepsy	Yes	Yes	Yes	Yes
12	G102D	Lee et al.	Yes	Mild ID, abnormal EEG, neuropathy, cerebellar atrophy	Yes	Yes	Yes	Yes
13	V144F	Lee et al.	Yes	Moderate ID, mild optic atrophy,	Yes	Yes	Yes	Yes
14	V144F	Lee et al.	Yes	Moderate ID, mild optic atrophy,	Yes	Yes	Yes	Yes
15	R167C	Lee et al.	Yes	Mild ID, neuropathy	Yes	Yes	Yes	Yes
16	A202P	Lee et al.	Yes	ID, non-ambulatory, non-verbal, optic nerve atrophy, microcephaly, cerebral atrophy	Yes	Yes	Yes	Yes
17	S215R	Lee et al.	Yes	ID, non-ambulatory, optic atrophy, microcephaly, epilepsy, neuropathy, cerebellar and cerebral atrophy	Yes	Yes	Yes	Yes
18	R216P	Lee et al.	Yes	ID, abnormal EEG, mild optic nerve atrophy, cerebellar atrophy	Yes	Yes	Yes	Yes
19	L249Q	Lee et al.	Yes	ID, Optic nerve atrophy,	Yes	Yes	Yes	Yes
20	E253K	Lee et al.	Yes	Severe ID, non-ambulatory, non-verbal, optic nerve atrophy, seizures, cerebellar atrophy	No	Yes	Yes	Yes
21	E253K	Lee et al.	Yes	Severe ID, non-ambulatory, non-verbal, optic atrophy, seizures NA, cerebellar atrophy	No	Yes	Yes	Yes

22	R316W	Lee et al.	Yes	Mild ID, Optic atrophy, cerebellar atrophy	Yes	Yes	Yes	Yes
23	E148D	Ohba et al.	Yes	Nystagmus, mild ID, cerebellar atrophy	No	Yes	Yes	Yes
24	R254W	Ohba et al.	Yes	Mild ID, cerebellar atrophy, optic atrophy	Yes	Yes	Yes	Yes
25	R254Q	Ohba et al.	Yes	Mild ID, cerebellar atrophy	Yes	Yes	Yes	Yes
26	R307Q	Ohba et al.	Yes	Severe ID, progressive cerebellar atrophy, optic atrophy, nystagmus	Yes	Yes	Yes	Yes
27	R316W	Ohba et al.	Yes	Severe ID, progressive cerebellar atrophy, optic atrophy, nystagmus	Yes	Yes	Yes	Yes
28	T99M	Hamdan et al.	Yes	Severe ID, cerebellar atrophy	Yes	Yes	Yes	Yes
29	T99M	Esmaelii Nieh et al.	Yes	Severe developmental delay, cortical visual impairment, cerebellar atrophy	Yes	Yes	Yes	Yes
30	T99M	Esmaelii Nieh et al.	Yes	Severe developmental delay, seizures, cerebellar atrophy	Yes	Yes	Yes	Yes
31	E253K	Esmaelii Nieh et al.	Yes	Severe developmental delay, cortical visual impairment, microcephaly	Yes	Yes	Yes	Yes
32	R316W	Esmaelii Nieh et al.	Yes	ID, ASD, microcephaly	Yes	Yes	Yes	Yes
33	R216C	Esmaelii Nieh et al.	Yes	ID	Yes	Yes	Yes	Yes
34	R216H	Esmaelii Nieh et al.	Yes	Developmental delay, cerebellar atrophy	Yes	Yes	Yes	Yes
35	P305L	Spagnoli et al.	Yes	Mild ID, microcephaly, bilateral optic atrophy, cerebellar atrophy	Yes	Yes	Yes	Yes
36	R13C	Kurihara et al.	Yes	Tonic seizures, ASD, ADHD, intellectual disability, cerebellar and corpus callosum atrophy, left optic atrophy.	Yes	Yes	Yes	Yes
37	T99M	Okamoto et al.	Yes	Intellectual disability, progressive cerebellar atrophy, corpus callosum hypoplasia, nystagmus, optic atrophy, tonic clonic seizures, microcephaly, neurogenic bladder	Yes	Yes	Yes	Yes
38	R13H	Tomaselli et al.	Yes	Axonal neuropathy, autism, delayed motor milestones	Yes	Yes	Yes	Yes
39	R307Q	Hotchkiss et al. (patient 1)	Yes	ID, progressive cerebellar atrophy, optic nerve atrophy, seizures, peripheral neuropathy,	Yes	Yes	Yes	Yes
40	G199R	Hotchkiss (patient 2)	Yes	ID, cerebellar atrophy, optic nerve atrophy	Yes	Yes	Yes	Yes
41	G102S	Citterio et al. (P67809)	Yes	Mild language and motor delay, IQ: 80	Yes	Yes	Yes	No

42	S69L	Citterio et al. P7814 V-6 (proband)	Yes	Mild psychomotor retardation	Yes	Yes	Yes	No
43	S69L	Citterio et al. P7814 IV-4 (mother)	No	NA	No	Yes	Yes	No
44	S69L	Citterio et al. (P7814 III-8)	No	NA	Yes	Yes	Yes	No
45	S69L	Citterio et al. (P7814 III-6)	No	NA	Yes	Yes	Yes	No
46	R167C	Citterio et al. (P116909)	No	NA	Yes	Yes	Yes	Yes
47	I1127T	Citterio et al. (P22413)	Yes	Normal IQ, subcortical and periventricular hyperintensities frontoparietal regions and pons	Yes	No	Yes	No
48	T258M	Cheon et al. Patient I.1 (mother)	Yes	Mild ID, language delay, optic nerve atrophy, scoliosis polyneuropathy sensorimotor in lower extremities	Yes	Yes	Yes	No
49	T258M	Cheon et al. Patient II.1 (daughter)	Yes	Mild ID language delay, optic nerve hypoplasia, thinning of corpus callosum, microcephaly	Yes	Yes	Yes	No
50	T258M	Cheon et al. Patient II.2 (daughter)	Yes	Moderate ID, language delay, pale optic disks, thinning of corpus callosum	Yes	Yes	Yes	No
51	T258M	Cheon et al. Patient II.3 (son)	Yes	Mild ID language delay, pale optic disks, thinning of corpus callosum, epilepsy	Yes	Yes	Yes	No
52	A85D	Yoshikawa et al.	Yes	Cerebellar and mild cerebral atrophy, ID	Yes	Yes	Yes	No
53	M30T	Pennings et al. (Patient 1)	No	NA	Yes	Yes	Yes	No
54	Y56C	Pennings et al. (Patient 2)	No	NA	Yes	Yes	Yes	Yes
55	S69L	Pennings et al. (Patient 3)	Yes	IQ: 80	Yes	Yes	Yes	No
56	Y74C	Pennings et al. (Patient 4)	No	NA	Yes	Yes	Yes	Yes
57	G78S	Pennings et al. (Patient 5)	No	NA	Yes	Yes	Yes	Yes
58	T106N	Pennings et al. (Patient 6)	No	NA	Yes	Yes	Yes	No
59	R167H	Pennings et al. (Patient 7)	No	NA	Yes	Yes	Yes	No
60	L173P	Pennings et al. (Patient 8)	Yes	IQ: 80	Yes	Yes	Yes	Yes
61	S252R	Pennings et al. (Patient 9)	No	NA	Yes	Yes	Yes	No
62	S252R	Pennings et al. (Patient 10)	No	NA	Yes	Yes	Yes	Yes
63	T258M	Pennings et al. (Patient 11)	No	NA	Yes	Yes	Yes	Yes

64	T258M	Pennings et al. (Patient 12)	Yes	Thin corpus callosum	Yes	Yes	Yes	No
65	T258M	Pennings et al. (Patient 13)	Yes	Thin corpus callosum	Yes	Yes	Yes	Yes
66	R350W	Pennings et al. (Patient 14)	No	NA	Yes	Yes	Yes	No
67	A460G	Pennings et al. (Patient 15)	No	NA	Yes	No	Yes	Yes
68	Q623*	Pennings et al. (Patient 16)	No	NA	Yes	No	No	Yes
69	R843C	Pennings et al. (Patient 17)	No	NA	Yes	No	Yes	No
70	R843C	Pennings et al. (Patient 18)	No	NA	Yes	No	Yes	Yes
71	N859K	Pennings et al. (Patient 19)	No	NA	Yes	No	Yes	Yes
72	Y1325*	Pennings et al. (Patient 20)	No	NA	Yes	No	No	No
73	D1369fs	Pennings et al. (Patient 21)	No	NA	Yes	No	No	Yes
74	P1431fs	Pennings et al. (Patient 22)	No	NA	Yes	No	No	No
75	Y1581fs	Pennings et al. (Patient 23)	No	NA	Yes	No	No	Yes
76	Contiguous Gene deletion	Pennings et al. (Patient 24)	Yes	Moderate ID	Yes	No	No	Yes
77	S69L	Ylikallio et al. (proband)	Yes	Attention deficit disorder	Yes	Yes	Yes	No
78	S69L	Ylikallio et al. (father)	No	NA	Yes	Yes	Yes	Yes
79	T99M	Langlois et al.	Yes	Optic atrophy, infantile spasms, developmental delay, cerebellar atrophy	No	Yes	Yes	Yes
80	E253K	Samanta et al.	Yes	Seizure, optic nerve pallor, epileptic spasms, developmental delay, progressive cerebellar and cerebral atrophy	No	Yes	Yes	Yes
81	G321D	Dobkowska et al.	Yes	Cognitive decline	Yes	Yes	Yes	No
82	G102S	Lu et al.	Yes	Intellectual deficiency, Nystagmus and pes cavus Cerebellar atrophy, axonal polyneuropathy	NA	Yes	Yes	No
83	S58L	Megahead et al.	Yes	ID, seizures, Wide-based staggering gait cerebellar and cerebral cortex atrophy	NA	Yes	Yes	Yes

84	S215R	Raffa et al.	Yes	Developmental delay, hypoplastic-atrophic optic discs, epilepsy, spastic paraparesis	Yes	Yes	Yes	Yes
85	S69L	Roda et al.	No	NA	Yes	Yes	Yes	No
86	R216H	Travaglini et al.	Yes	Developmental delay, progressive cortical cerebral and cerebellar atrophy with thin corpus callosum	Yes	Yes	Yes	Yes
87	R216H	Urtiaga et al.	Yes	Developmental delay, neurogenic bladder, axonal sensitive polyneuropathy, cerebellar atrophy, ADDH	Yes	Yes	Yes	Yes
88	V8M	Iqbal et al	No	NA	Yes	Yes	Yes	No
89	I27T	Iqbal et al	No	NA	Yes	Yes	Yes	No
90	Y74C	Van den Warrenburg et al.	No	NA	Yes	Yes	Yes	No
91	Q632*	Van den Warrenburg et al.	No	NA	Yes	No	No	No
92	R380W	A.E Van Beusichem et al. (patient 1)	Yes	Severe intellectual deficit, epilepsy with prominent pes planovalgus	Yes	No	Yes	NA
93	R216C	A.E Van Beusichem et al. (patient 2)	Yes	ID, cerebellar atrophy	Yes	Yes	Yes	NA
94	T99M	VanEyk et al.	Yes	Partial dysgenesis of the corpus callosum, developmental delay, epilepsy, scoliosis, optical atrophy, blindness	Yes	Yes	Yes	Yes
95	R316W	VanEyk et al.	Yes	CP with intermittent hypertonicity, microcephaly, developmental delay and cortical visual impairment	Yes	Yes	Yes	Yes
96	C92*	Wang et al.	Yes	Developmental delay, microcephaly, Rett syndrome features, normal brain MRI, abnormal breathing patterns	No	Yes	No	Yes
97	L249P	Demily et al.	Yes	22q duplication syndrome + *KIF1A* mutation. ID, cerebellar ataxia, spastic paraparesis, saccadic pursuit eye movement and horizontal nystagmus, cerebellar atrophy, seizures	Yes	Yes	Yes	Yes
98	E253K	Muir et al.	Yes	Infantile spasms, ID, cerebellar atrophy, optic atrophy, hypotonia	NA	Yes	Yes	NA
99	S217F	Kashimada et al.	Yes	Optic atrophy, cerebellar atrophy, ataxia	NA	Yes	Yes	NA

Supp. Table T1 – Curation of pathogenic *KIF1A* variants previously described in the literature. Each row represents a case

reported in the literature with a presumably pathogenic variant in *KIF1A*. In addition to variant information, additional information provided includes reference and high-level phenotypes (i.e. involvement of central nervous system).