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Supplemental Data

Mutations in the Chromatin Regulator Gene *BRPF1* Cause Syndromic Intellectual Disability and Deficient Histone Acetylation

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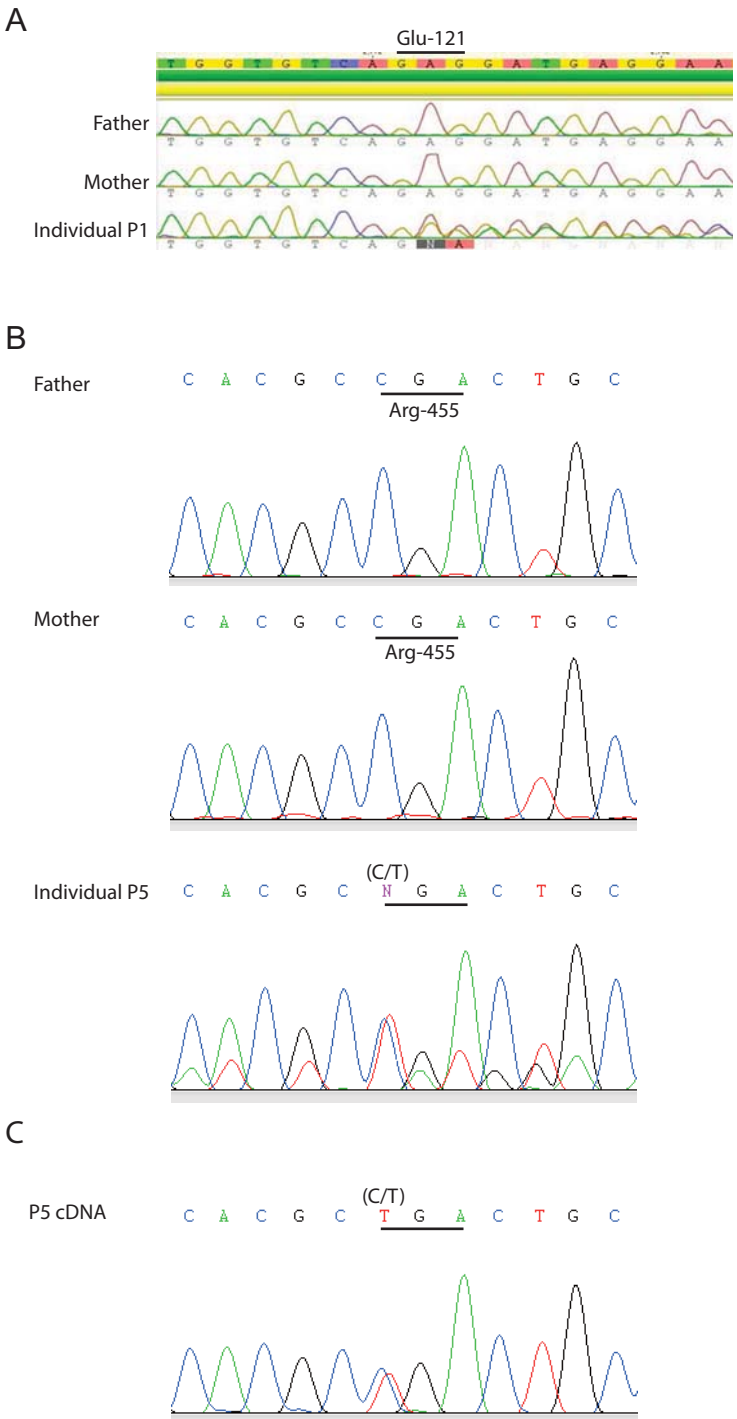

Figure S1. Confirmation of two de novo BRPF1 mutations by Sanger sequencing

(A) Sanger sequencing chromatograms of genomic DNA from individual P1 and her parents. Only the region around the mutation site is displayed here. Unlike her parents, she contains an AG deletion (c.362_363delAG, Table 1), which introduces a reading-frame shift starting at the codon for Glu-121 of BRPF1.

(B) Sequencing chromatograms of genomic DNA from individual P5 and her parents. Only the region at the mutation site is shown here. Unlike her parents, she is heterozygous for the mutation c.1363C>T (Table 1). In the mutated allele, the mutation introduces a TGA stop codon that replaces the codon for Arg-455 of BRPF1.

(C) Sanger sequencing chromatogram of fibroblast cDNA from individual P5. The results not only confirm that she is heterozygous for the mutation c.1363C>T, but also show that the normal and mutant alleles are expressed to similar levels.

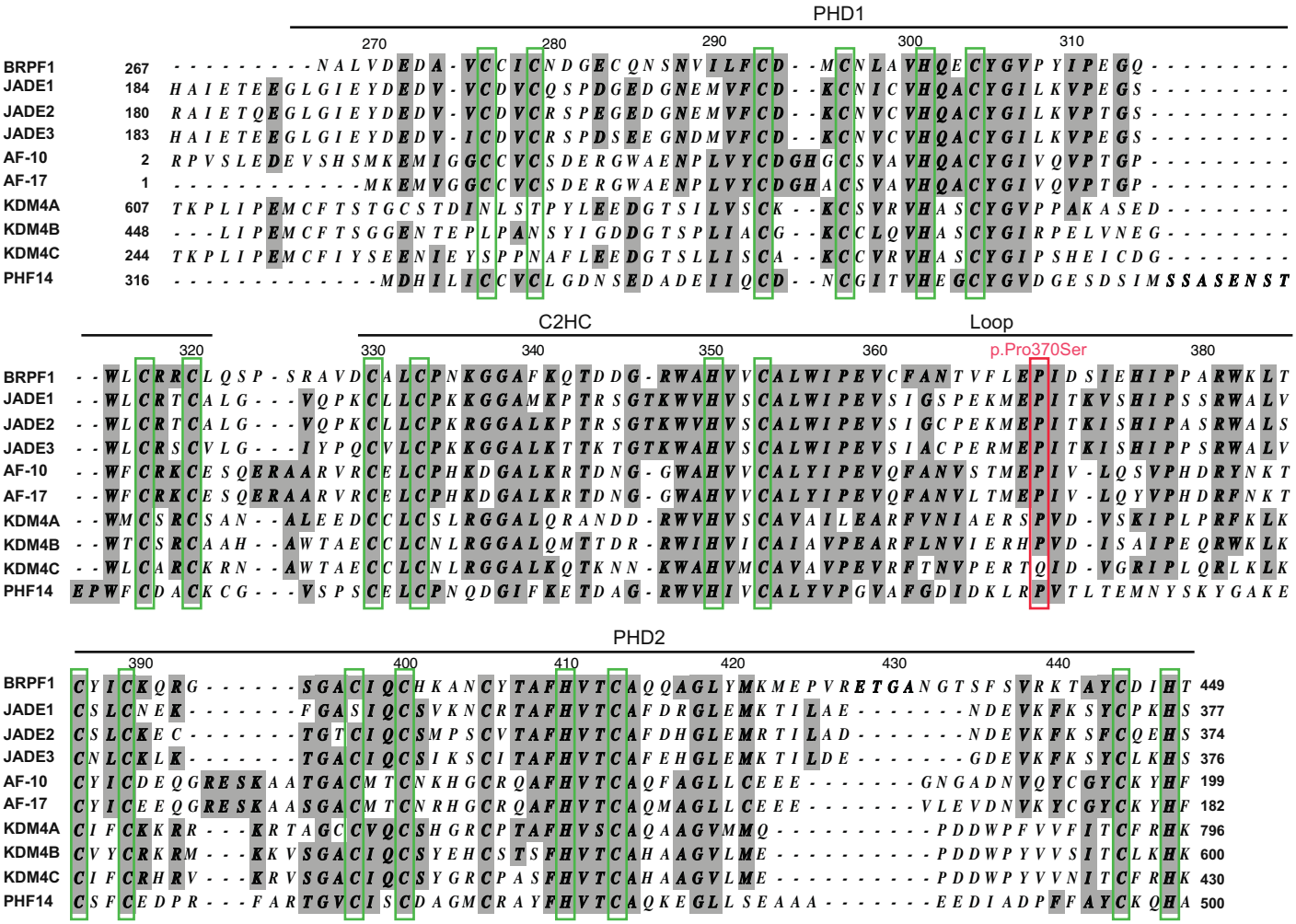

Figure S2. Sequence comparison of PZP domains in BRPF1 and other human proteins

The sequence of human BRPF1 (residues 267-449) was aligned with homologous regions of three JADE proteins (JAED1, JADE2 and JADE3), two leukemia translocation partners (AF-10 and AF-17), three histone demethylases (KDM4A, KDM4B and KDM4C) and PHD finger protein 14 (PHF14). Pro-370 of BRPF1 and its corresponding residues in other PZP proteins are boxed in red. Cysteine and histidine residues involved in zinc chelating to form zinc fingers are boxed in light green. To generate the alignment, the sequence of human BRPF1 (corresponding to residues 267-449) was used as bait for BLAST search against the human protein sequence database at the NCBI BLAST search website. Homologous sequences were then downloaded to MacVector for alignment of residues 267-449 of BRPF1 to other PZP domains. The region corresponding to residues 420-440 was subject to manual editing to align the last two cysteine and histidine residues of different PZP domains. The GenBank accession numbers used are: NP_001003694 (BRPF1), NP_955352 (JADE1), XP_016864771 (JADE2), NP_055550 (JADE3), NP_001182555 (AF-10), NP_005928 (AF-17), XP_005271413 (KDM4A), XP_011526118 (KDMT4B), XP_016869994 (KDM4C) and XP_016868304 (PHF14). Not shown here is the histone methyl-transferase KMT3B (formerly known as NSD1), which also contains a PZP domain but is less conserved.[1]

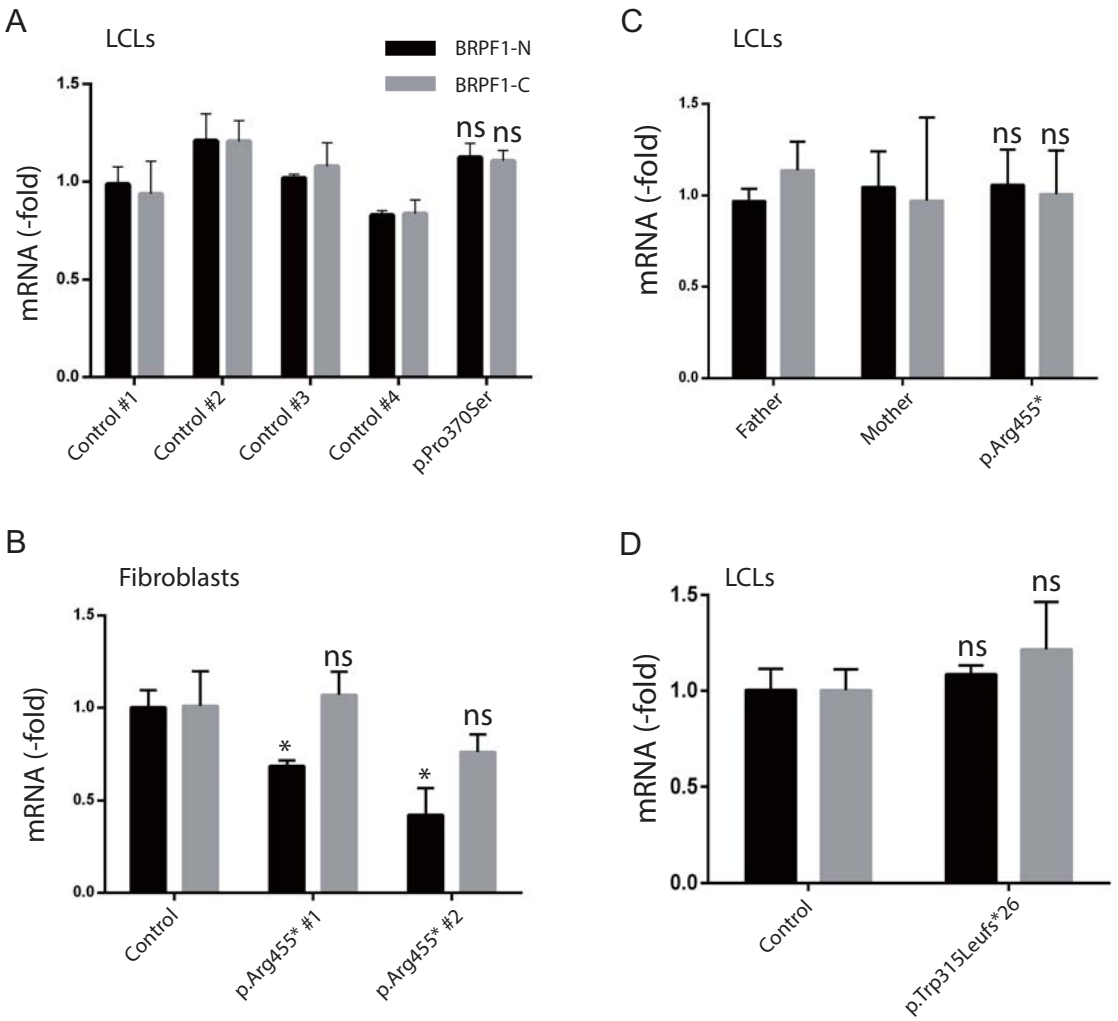

Figure S3. *BRPF1* mRNA levels in normal and mutant cells

(A) RT-qPCR analysis of *BRPF1* mRNA in control and p.Pro370Ser lymphoblastoid cell lines (LCLs). BRPF1-N, RT-qPCR using primers amplifying a region encoding an N-terminal part of BRPF1; BRPF1-C, RT-qPCR using primers amplifying a region encoding a C-terminal part of BRPF1.

(B) Analysis of *BRPF1* mRNA in control and p.Arg455* fibroblasts. Two different batches of fibroblasts were tested. RT-qPCR primers were the same as those used in (A).

(C) RT-qPCR analysis of *BRPF1* mRNA in LCLs prepared from individual P5 (encoding the variant p.Arg455*) and her parents.

(D) Analysis of *BRPF1* mRNA in control and p.Trp315Leufs*26 LCLs. RT-qPCR primers were the same as those used in (A). The experiments in (A-D) were repeated three times and mean values are presented with standard deviations. ns, not statistically significant when compared to the corresponding control(s); *, $p<0.05$.

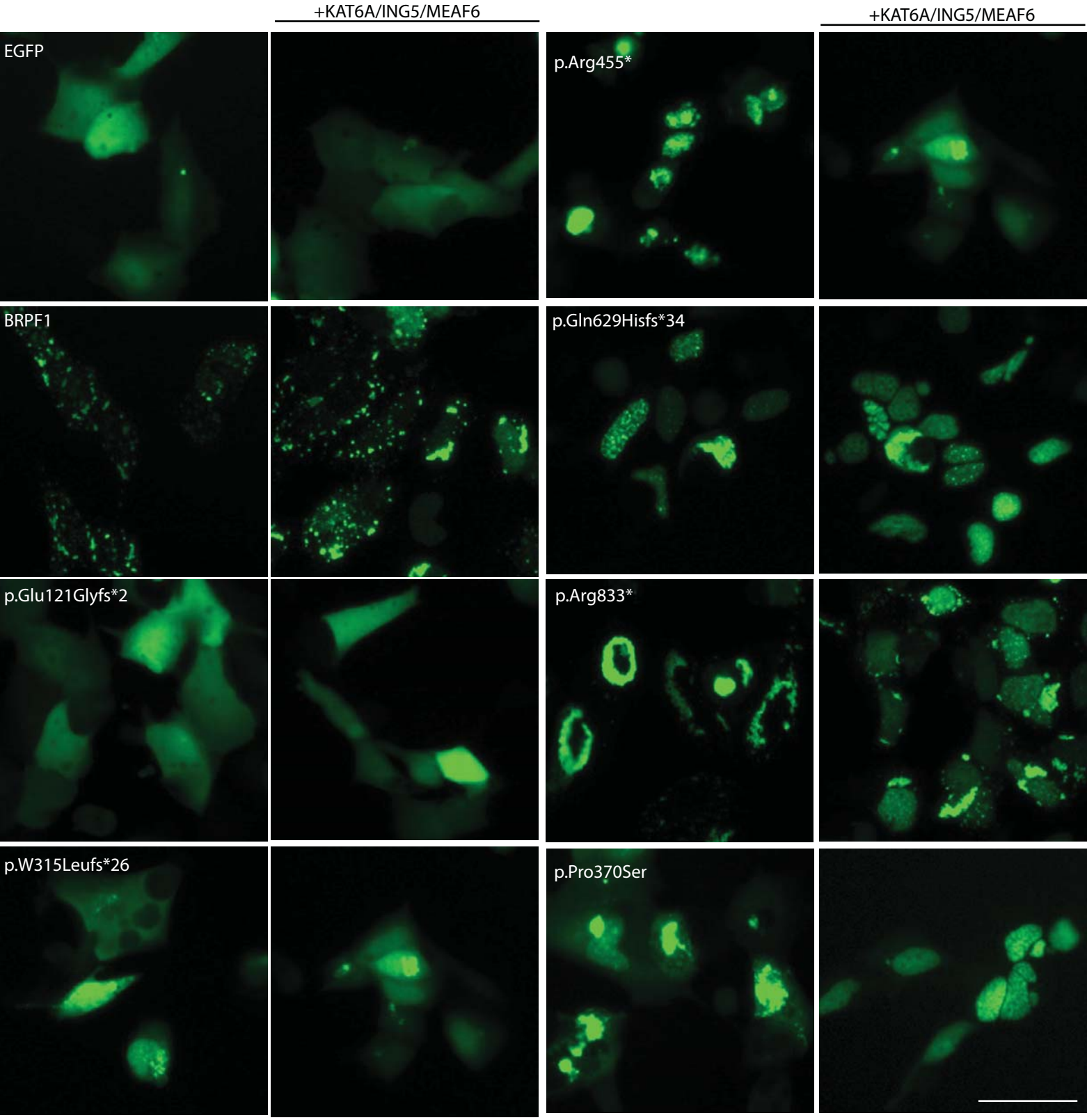

Figure S4. Subcellular localization of BRPF1 and its variants

BRPF1 and its variants were produced as EGFP-tagged fusion proteins in HEK293 cells, with or without exogeneous KAT6A, ING5 and MEAF6 as indicated. EGFP itself was used as the negative control. About 18 h post-transfection, GFP signals were determined by live fluorescence microscopy. For NIH3T3 fibroblasts, it is known that BRPF1 formed cytoplasmic dots but became nuclear when KAT6A was also produced.[2] Images shown here were taken 18 h after transfection. In the presence of exogeneous KAT6A, ING5 and MEAF6, BRPF1 was mainly cytoplasmic at that time (as shown here), but became mainly nuclear in 50% cells at 36 h after transfection (data not shown). No such changes were found when BRPF1 was produced alone (data not shown). Scale bar, 50 μm.

Table S1. Detailed features of 10 individuals with heterozygous *BRPF1* mutations

Individuals	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Gender	Female	Male	Female	Male	Female	Male	Male	Female	Female	Female
Mutation in NM_001003694.1	c.362_363 delAG	c.942_955 del	c.942_955 del	c.1108C>T	c.1363C>T	c.1688_ 1689del	c.1883_ 1886dup	c.2497C>T	c.2915dup C	c.3298C>T
Alteration in BRPF1	p.Glu121Gly fs*2	p.Trp315Leu fs*26	p.Trp315Leu fs*26	p.Pro370Ser	p.Arg455*	p.His563 Profs*8	p.Gln629 Hisfs*34	p.Arg833*	p.Met973 Asnfs*24	p.Arg1100*
Mode of transmission	*De novo*	Parental samples not available	Parental samples not available	Mosaic in unaffected mother (mosaicism, 7% in blood DNA, based exome sequencing reads)	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Family history	2 older healthy maternal half-siblings. Mother with history of meningioma. Father with joint hypermobility. No consanguinity.	Similarly affected full sibling (P3). Biological mother with intellectual disability.	Similarly affected full sibling (P2). Biological mother with intellectual disability.	2 sisters in good health. Brother has dyslexia and learning difficulties. No history of birth defects, learning difficulties, mental retardation or inherited disorders. No consanguinity.	Nil significant	One brother IQ 120, normal development, ADHD. Parents both ADHD but with university degrees.	Nil significant	Paternal uncle has Tourette	Nil significant	Healthy younger sister
Pregnancy issues	Polyhydramnios, subjective	Uncertain prenatal history.	Uncertain prenatal history.	No	Maternal diabetes	No	Early twin loss	None	1st trimester bleed, nil	Ultrasound at 20 weeks soft-

	decreased fetal movements								else	marker at heart
Delivery issues (specify gestational age)	42 weeks, SROM, SVD	38 weeks. Tracheomalacia, Placed in foster care within first week of life as mother with ID. Father with cerebral palsy. An older sister had been adopted out.	Uncertain, though no known birth complications. Adopted at age of 17 months.	No, delivered at 39 weeks gestation	37w, SVD forceps, apgar 4-8, features consistent with Erb's palsy noted	Born at term Amniotic liquor, needed some extra oxygen Discharged from hospital within hours	Post-term; induced; NVD	40/40	37/40	34 5/7 weeks
Birth weight	7lb 13oz	7lb 12oz	NA	8 lb 3 oz	3955 g	3500 g	3.54 kg	2.7 kg	5lb 70z	2145 g
Birth length	NA	uncertain	NA	21 inches	NA	49 cm	NA	NA	NA	44 cm
Birth head circumference	NA	50th percentile	NA	NA	NA	NA	NA	NA	NA	32.6 cm
Neonatal issues	No major concerns	Tracheomalacia, hypothyroid. GERD, mild RAD, FTT	NA	Failure to thrive but resolved (had esophageal ulcer, peptic esophagitis and patulous gastroesophageal junction)	Neonatal hypertrophic cardiomyopathy, resolved	Little sleep, did not ask for feeding but no real feeding difficulties	Poor feeding	None	none	Hypotonia and tube feeding necessary
Gross motor delay	Hypotonia, delay (sat 12 months; walked at 27 months)	Crawled 14m; walked 16months; GDD now ID. Toe walked	Not recognized or reported	Hypotonia	Yes	Did not turn around or crawl, needed physiotherapy, could crawl at 15 months	First walked ~2 years old	None	Walked at the age of 3, never more than short distances achieved	Hypotonia, yes, walked when 2.5 years old

						and walk (unsteady) at 18 months				
Fine motor delay	mild	Yes	Yes, problems with significant bilateral fine motor coordination	NA	Yes	Normal	NA	Delayed	Palmar grip only	NI
Language delay	Yes, Expressive> receptive	Yes	Yes	Yes and speech apraxia	Yes	Delayed, spoke few words, language understanding and production was delayed. Has a low vocabulary	Speech delay	Moderate	2 single words, 1st at 3	yes
Developmental delay or intellectual disability (please specify severity)	Global developmental delay; discrepant results on cognitive assessment: overall IQ 23rd % ile (WPPSI-III) but low skills in general language index (9th %ile), and extremely low in visual	Yes: GDD noted during infancy. At 7y10m: significant developmental problems in the area of language, visual perceptual fine motor problem solving and gross motor areas. He continues to	At 11 years old, intellectual functioning was assessed with the WISC-IV and found to be in the low average range with a Full Scale IQ of 88. Evaluation by psychiatry at the age of	global development delay (more prominent in expressive language), intellectual functioning disability	DD and ID	IQ 80-100 (brother and parents ≥120) Severe autism, Gilles de la Tourette, ADHD	Mild (main-stream school with extra help)	Mild-moderate ID	Severe ID	Mild ID IQ 56

	perception skills (1st %ile)	have his weakest area in the gross motor area. General developmental age is around 3 to 3-1/2 years of age.	14 years. documents more concerning history: defiant, oppositional, odd, episodes of encopresis at school. No autism. Functions lower than cognitive testing.							
Seizures	no	Yes; diagnosed with epilepsy with generalized tonic clonic seizures infrequently, now seizure free x2 years. Has been off AEDs for several months.	Yes, localization related epilepsy (partial seizures with secondary generalization, diagnosed at age 8y. Rx with AEDs x4years. Now off meds and. Seizure free	No. EEG abnormal (treated with Trileptal and 30lb weight gain, but no clinical seizures).	Episodes of staring spells, consistent with absence seizures. However, EEG only showed high-voltage hypnagogic hypersynchrony, without epileptiform activity.	No	Yes (from 5/52)	None	Multiple types, not controlled by medication, evolving	No
Other neurological abnormalities	Hypotonia, decreased muscle bulk with hyperextensible joints	Mild optic nerve hypoplasia. Mild Central sleep apnea.	Optic nerve slightly small.	NA	ADHD, dyspraxia, social processing disorder. hypotonia	Hypotonic in infancy	Sleep disturbance (melatonin)	Diagnosed with autistic spectrum disorder age 12	Progressive leg contractures secondary to muscular tightening	

Brain MRI	(June 2012): Hyper-intensities of the cortical white matter of the frontal lobes bilaterally.	Paucity of WM, thinning of corpus callosum. Small focus of gliotic change, possibly secondary to remote insult, in the anterior portion of right centrum semi-ovale.	Normal	Normal	Normal	Not done	NA	Not done	Global lack of white matter bulk only	At ultrasound 2 small supependymal cysts. MRI in conclusion after revision normal
Age at last follow-up	6.5 y	13y 3m	13y 10m	11y 2m	8	13	15 y	8 y	17 y	12 y
Weight at last follow-up	33kg (97th % ile)	37.4kg (10th; Z=-1.28)	Weight 73rd percentile	49.5kg (91st %), general appearance overweight	48.1k	45,4 kg (+0,2 SDS)	33.5kg/75-91 [aged 9 on 23.8.06]	32.6kg	43.5kg	57 kg
Height at last follow-up	126 cm (85-97th % ile)	144cm (4th % ; Z=-1.81)	Height 21st percentile	146 cm (58th %)	131.5cm	159 cm (-0,3 SDS)	137.7/50-75 [aged 9 on 23.8.06]	136.3cm	155cm	152 cm
Head circumference at last follow-up	53.5 cm (+1 to +2 SD)	54.5cm (54th %; Z=0.11)	FOC 83rd percentile	52.3 cm	51cm	56 cm (+0,5 SDS) Measured at age 13 yrs 7 months	54.0/25-50 [aged 9 on 23.8.06]	54cm	49cm at age 12	normal
Cranial shape	Normal	Normal	Normal	Mild dolicho-cephaly	Narrow bifrontal diameter	Normal	Normal	Normal	Shape normal but markedly micro-cephalic	normal
Forehead	Narrowing of forehead towards the apex	Normal	Broad, large/tall (Without frank bossing)	Frontal bossing	Normal	broad	Normal	Broad	Normal	Broad
Face, general	Round face, fontal	See below	See below	See below	See below	Sparse facial	See below	Round, dysmorphi	Mildly dysmorphi	Dysmorphic features

	upsweep of hairline,					expressions		c, small chin	c	
Hair	Normal	Upsweep of furontal hair line; normal texture and distribution	Upsweep of frontal hair line. Normal texture and distribution.	Texture: mild hair on upper lip area. Pattern and color: central and hair whorls, some tapering of the posterior hairline laterally along the neck but hairline is not low overall.	Normal	cowlick	Normal	High frontal hairline	Low frontal hairline	normal
Eye dysmorphisms	Right-sided ptosis, hooded eyelids, upslanting palpebral fissures, broad and sparse lateral eyebrows	Hyperteloric. IPD: 6.2 (~97%ile); IC: 3.5/ OC: 9.5/ PF: 3.0) Mild down-slant of PFs Full and robust eyebrows	Appears to have ptosis	Narrow palpebral fissures Synophrys. Straight eyebrows. Narrow palpebral fissures seen in earlier age.	Blepharop himosis, ptosis, medial flaring of eyebrows, hypertelori sm, upslanting palpebral fissures	Ptosis	Down-slanting palpebral fissures	Hypertelori c	Normal	Epicanthal folds, short palpebral fissures, hypertelori sm
Vision	Farsighted	Hyperopia since early childhood// in addition has become more myopic with age	At age 13: Nystagmus, likely congenital. good vision with both eyes open Optic nerves are slightly small in size. Compound hyperopic astigmatism,	Normal	Normal	60%, amblyopi a, +4 dpt glasses. No inner eye abnormal ities	Normal	Has a tendency to squint but no visual deficiency identified	Has a tendency to squint but no visual deficiency identified	normal

Ear dysmorphisms	Normal in form with thick, fleshy ear helices.	Ears look small but measure between 25-50 percentile (5.5/5.8cm) Right ear (5.5cm) is minimally low set. (see photo)	Normal	Small right-sided Darwinian tubercule	Simple superior helices	Prominent conchae, small earlobes	Relatively small ears	Simple, low set, posteriorly rotated	Large, mildly low set	Relatively simple formed
Hearing	normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Nose		Fleshy nose. Small nostrils.	Fleshy nasal tip with small nostrils.	Full nasal ridge. Lateral buildup to the nose. Bulbous tip. Somewhat pointed nasal tip and narrow nares.	Flat nasal root, relatively small	Normal		Normal	Normal	Flat nasal bridge
Mouth	cupid's bow upper lip,	Short philtrum. Small mouth/ (was micrognathic as younger child). Currently with malocclusion /"cross bite". Teeth extracted for crowding. Needed to have primary teeth extracted as they did not fall out when the adult teeth came	Short philtrum. Mouth normal.	Small mouth, upper lip is somewhat thin and overhanging there is a cupids bow, mildly smooth philtral pillars. Micrognathia	Relatively small, downturned corners of the mouth	Normal		Full upper lip, widely spaced teeth	Wide mouth, upturned upper lip, short philtrum, widely spaced teeth	Normal, full lips

		in. Now has orthodontics in place.								
Palate	Widely spaced teeth, prominent upper incisors, high-arched palate,	Narrow. Normal arch. Normal uvula.	Normal	Teeth and gums overjet	Normal	High arched	Bifid uvula	normal	normal	normal
Hands	Hyperextensible joints of the hands: thumbs at MCPs, distal and proximal IP joints except for 5th MCPs. Hand lengths normal (75th %ile). Normal hand creases.	Normal	Normal	Slightly squared thumbs, minimal degree cubitus valgus. Bridged transverse palmar creases bilaterally seen at age 5.	Left digitalized thumb with small extra bone (phalanx?). Bilateral 5th finger clino-dactyly	Normal	Fifth finger clino-dactyly	Short, broad fingertips	Long, tapering fingers	Normal, atypical palmar creases
Feet	Relatively short feet (25th %ile in length) with 5th toe medial clinodactyly, bilateral metatarsus adductus	Normal	Normal	Mild two-three toe syndactyly	Normal	overpronation of ankles	NA	Normal	Normal	normal
Heart	Normal examination	Normal examination	Normal examination	Normal	Small patent ductus arteriosus, biventricular hypertrophic	Normal examination	Normal examination	Normal	Normal	Mild pulmonary stenosis

					cardiomyopathy thought to be secondary to maternal diabetes (resolved), atrial septal defect					
Kidneys	NA	Normal RUS	Normal RUS/MRI Normal VCUG (workup for enuresis)	NA	NA	Not evaluated	NA	NA	Normal	normal
Feeding difficulties	No	FTT as infant with severe GERD. Had G-tube placed (with fundo) (has since been removed). Major feeding/GI issues as a younger child with gastroparesis, dysmotility. Prompted anal manometry (normal).	None known.	Neonatal period	No	Poor oral motor function (burping, munching, making a mess). Normal appetite	Yes	Reflux, nil else	None	Neonatal and as a baby
Other genetic findings (see note 2)	None	Hetero-zygous pathogenic variant of *MPDZ,* but no signs of	Hetero-zygous pathogenic variant of *MPDZ,* but no signs of	*De novo* duplication of ARID1B gene. Inactivating *ARID1B*	BRAF c.956C>T p.Ser319 Phe BRAF mutations in Noonan	No other pathogenic mutatons were found on	None	Compound mutations in *AIPL*, but one is benign. c.805G>A	Possibly pathogenic *de novo* mutation in *CBL* (c.1145A>	*PIK3R1*: NM_18152 c.1290del, p.Lys430 Asnfs*14, hetero-

		severe congenital hydro-cephalus, caused by *MPDZ* mutations.	severe congenital hydro-cephalus, caused by *MPDZ* mutations.	mutations in Coffin-Siris syndrome, but impact of *ARID1B* duplication remains unknown.	syndrome, but this mutation is benign.	exome. SNP-array was normal		(p.Gly269Arg) & c.401A>T (p.Tyr 134Phe). *AIPL* mutations in leber congenital amaurosis (retinal dystrophy)	T p.Lys382Ile.) but no signs of Noonan syndrome. Compound mutations in *TTI2*: c.1274A>C (p.His416 Pro) & c.1100C>T (Pro367Leu), but the first is probably benign. TTI2 forms a trimeric complex with two other proteins, one of which is linked to syndromic ID, but with much more severe clinical features.	zygous, a*nd de novo.* But there are no signs of SHORT syndrome, which is known to be caused by *PIK3R1* mutations.
Other clinical features	C spine fusion (C2/C3); Premature adrenarche without central puberty (age 7y); Constipation;	Hypothyroidism at age 2 months, currently with normal TSH off synthroid. C2/C3 fusion. Congenital anomaly of	C2/C3 fusion. Mild disk bulges at C4-C5 and C5-C6	Bruises easily. Medications: albuterol sulphate, amoxicillin, cefdinir, fluticasone, oxcarbazepine, prednisone. Has a few	Joint hypermobility. Enlarged left S1 pedicle, abnormal contour to C2 and 3 with apparent underlying	Hypermobile in large joints of the lower extremities, Delayed toilet training (encopresis,	None	Extremely hypermobile at elbows. Puberty at 9, menarche at 10	Thelarche from 7, two bleeds only, found to be in ovarian failure, with post-menopausal hormone levels at 17	Idiopathic pediatric discus calcification at T11-T12

		T9 with butterfly vertebra. Muscle biopsy: Mild variation in fiber size, focal fiber grouping, increased subsarcolemmal oxidative activity (NADH and COX), increased mitochondria (some enlarged and abnormally shaped), increased neutral lipid. Normal ETC enzyme activities except for mild decrease in NADH:ferricyanide dehydrogenase.		scattered nevi.	segmentation abnormality.	constipation), Asthma eczema				

Notes:

1) Abbreviations: NA, not available; Nl: Normal; DD: developmental delay; ID: Intellectual disability; ADHD: attention deficit hyperactivity disorder.
2) Besides *BRPF1* variants, some individuals harbor mutations in other genes. For example, individual P4 carries a duplication of *ARID1B*, individual P8 expresses two compound *AIPL* variants, individual P9 possesses mutations in the *CBL* and *TTI2* genes, and individual P10 carries a pathogenic *PIK3R1* mutation (Table S1). Most of such genetic variants do not appear to be responsible for the major clinical features that we observed in the individuals (Table 2 and S1). For example, *PIK3R1* mutations have been linked to SHORT syndrome[3] and this individual's dysmorphisms do not resemble those reported in the literature. Cognition is typically normal for individuals with SHORT syndrome,[3] so the intellectual disability and speech delay in individual P10 are very likely due to the *BRPF1* mutation (Tables 1-2 and S1). One possible exception is

the *TTI2* mutation in individual P10. Alterations of TTI2 itself and a close partner have been linked to syndromic intellectual disability,[4,5] but the resulting clinical features are much more severe than those in individual P10. Thus, an interesting possibility to be investigated is whether combined effects of different mutations in *BRPF1* and another gene may contribute to phenotypic variations among the 10 individuals (Tables 2 and S1).

Supplemental References

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