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Supplemental Data

Mutations in Histone Acetylase Modifier *BRPF1* Cause an Autosomal-Dominant Form of Intellectual Disability with Associated Ptosis

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Supplemental Figures

Figure S1.

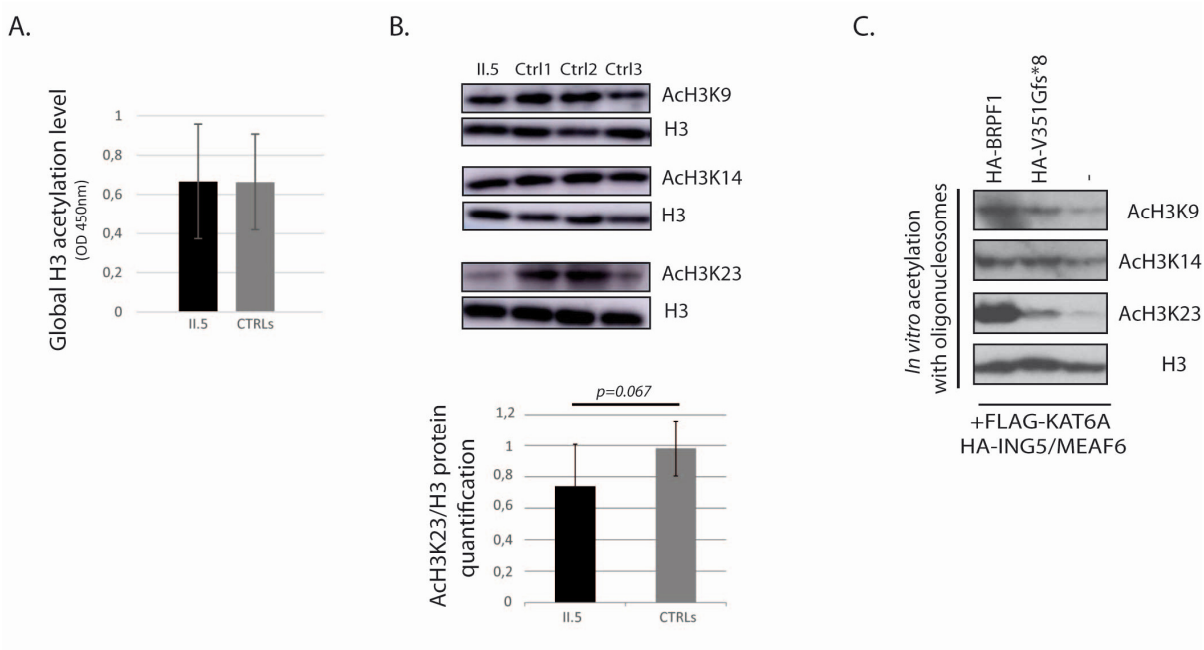

Figure S1. Effect of the c.1052_1053del variant on histone H3 acetylation level. (A) Global histone H3 acetylation level was compared between individual II-5 and three unrelated control individuals. Histone were extracted from fibroblasts and H3 acetylation level was measured (experiments performed in triplicate, error bars indicate the SD) using the EpiQuik Global Histone H3 kit. (B) Immunoblot performed on histone extracts from fibroblasts of individual II-5 and three unrelated control individuals (four extractions per individual) with specific anti-H3K9ac (Abcam, ab4441), anti-H3K14ac and anti-H3K23ac (cell signaling, #9674) antibodies and global H3 antibody (upstate, cat 06755, lot 31949). No difference was observed in H3K9 or H3K14 acetylation intensity between patient II-5 and controls when normalized by the intensities obtained for global H3. A non-significant decrease of H3K23 acetylation level was however observed for the patient II-5 compared to the three unrelated controls (experiments performed on four histone extractions per individuals) (C) HeLa cells were cotransfected with MOZ/KAT6A, ING5 and MEAF6 cDNAs with or without wild-type or mutant BRPF1 cDNA. Oligonuclesomes were extracted and *in vitro* histone acetylation assays were performed as previously described[7]. Acetylation levels were analyzed by immunoblotting using antibodies against histone H3 and its acetylated forms.

Figure S2.

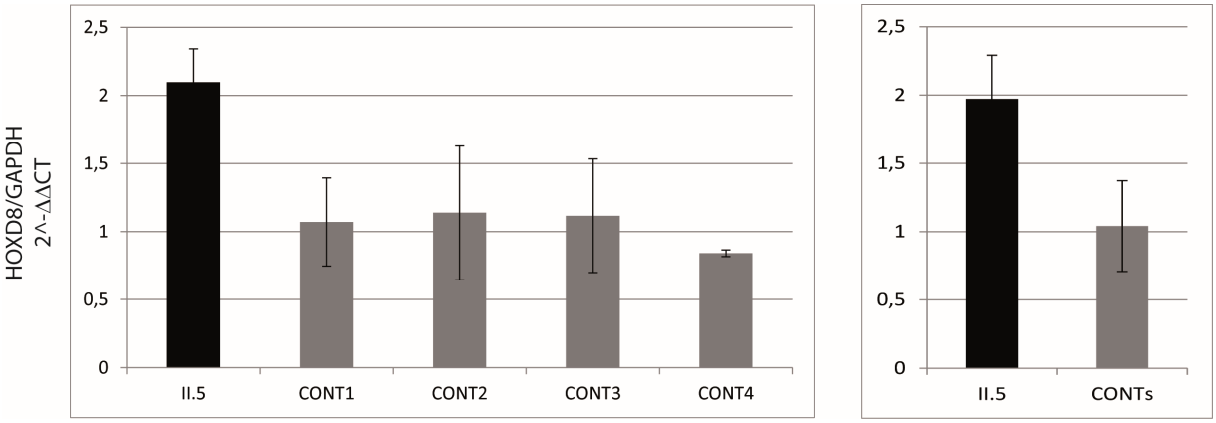

Figure S2. Significant increase in HOXD8 expression level was observed in individual II-5 cells. Quantitative real-time PCR was performed on reverse-transcribed mRNA extracted from fibroblasts of patient II-5 and of four unrelated controls (experiments performed on four different extractions per individual). The relative expression of HOXD8 *v.s*. GAPDH was calculated using the $2^{-(\Delta\Delta Ct)}$ method. Error bars indicate the SD of four independent experiments. A t-test was performed and showed a significant increase of *HOXD8* mRNA level in patient's fibroblasts.

Supplemental Tables

Table S1.

Gene	Variation	Status	Type of variation	Consequensis	At the protein level	dbSNP	ExAC	SIFT	Polyphen2
CDCA5	NM_080668.3:c.650G>A	heterozygous	substitution	missense	p.Arg217His	rs771200184	1 heterozygous	Deleterious (0)	POSSIBLY DAMAGING (0.828)
CHRNG	NM_005199: c.143C>T	heterozygous	substitution	missense	p.Ser48Leu	rs370022034	-	Deleterious (0.04)	BENIGN (0.108)
TBC1D10C	NM_198517: c.1279C>T	heterozygous	substitution	missense	p.Pro427Ser	rs756681324	15 heterozygous	Tolerated (0.12)	BENIGN (0.005)
BRPF1	NM_001003694: c.1052_1053del	heterozygous	deletion	frameshift	p.Val351Glyfs*8	-	-	NA	NA

Table S1. Rare non-synonymous variants identified by WES and common to the three affected family members sequenced.

Table S2.

Chrom	Position	Reference	Alternate	Protein Consequence	Transcript Consequence	Annotation	Allele Count	Allele Number	Number of homozygous	Population	Comments
3	9783787	C	T	p.Gln645Ter	ENST00000383829.2:c.1933C>T	stop gained	1	121 384	0	African	The variation is present in 14/65 reads. It could be a mosaic variant
3	9785260	A	C	p.?	ENST00000383829.2:c.2312-2A>C	splice acceptor	1	112 398	0	European (Non-Finnish)	It affects the acceptor splice site of exon 8 (324 nts length), might create an in frame deletion of 108 a.a (Ala771 to Lys878)
3	9785263	A	G	p.?	ENST00000469066.1:n.217-2A>G	splice acceptor	6	113 094	0	LATINO	It affects a known processed transcript, not coding for a protein
3	9786193	G	A	p.?	ENST00000383829.2:c.2920+1G>A	splice donor	1	105 458	0	LATINO	It affects the donor splice site of exon 9 (285 nts length), might create an in frame deletion of 95 a.a (Gly879 to Met973). Rs191236303
3	9787255	AG	A	p.?	ENST00000383829.2:c.3069-1delG	splice acceptor	1	121 324	0	European (Non-Finnish)	It affects 137 nts, might create a frameshift the acceptor splice site of exon 11 (137 nts length), might cause a frameshift

Table S2. Splice and nonsense variants identified in *BRPF1* in the ExAC general population

Table S3.

	Group1	**Group 2**				**Group 3**
Reference	*this report*	*Grozeva et al. 2014*	*Pinto et al. 2014*	*Kuechler et al. 2015* (Patient 1, 2, 3, Riess et al. 201, Kellogg et al. 2013)	*Ellery et al. 2014*	*Kuechler et al. 2015* (Patient 4, 5, 6, Gunnarsson et al., Peltekova et al.)
Type of mutation	*BRPF1* Lof or deletions w/o *SETD5*	*SETD5* LoF	*SETD5* deletion	*SETD5* LoF and deletions w/ot *BRPF1*	*SETD5* deletion	3p25 deletions encompassing both *SETD5* and *BRPF1*
Sex	6M, 1F	7M	M	4F, 1M	M	4F, 1M
ID severity:	7/7	7/7	1	5/5	1	5/5
severity:						
mild	3/6		1	4/5		1/5
moderate	3/6			1/5		1/5
severe	0/6			0/5		3/5
General characteristics:						
Uneventful pregnancy (born at term)	2/6	4/7	1	4/5	0	2/5
low birth weight-height/growth retardation	2/6	0/7	0	1/5	n.a.	0/4
Small stature	3/7	1/7	0	1/5		4/4
Microcephaly/smaller head size	6/6	1/7	0	0/5	n.a.	4/4
Development						
Mild walking delay (>18mo <3y)	6/7	6/7	0	4/4		0/5
Severe delay at walk (>3y)	0/7	2/7	0	0/4		5/5
speech delay	6/7	7/7	1	3/4	1	5/5

no speech	0/7	1/7	0	0/3	0	5/5
Neurological features						
Behavioral anomalies	5/7	5/7	1	4/5	n.a.	1/5
Brain anomalies (MRI)	2/3	0 (6n.a.)	n.a.	0/3	n.a.	1/4
Hypotonia	4/6	n.a.	n.a.	3/5	1	4/4
Seizures	2/7	0/7	0	2/5	1	4/5
Facial dysmorphisms						
Strabismus	4/5	1/7		4/5		3/5
Ptosis and/or blepharophimosis	7/7	1/7	0	0/5		5/5
Limbs						
Hand anomalies	5/7	2/7	0	4/5	1	4/5
Feet anomalies	4/7	1/7	0	1/4		2/5
Others features:						
Congenital heart defects	0/7	2/7	0	0/5		2/5

Table S3. Clinical features of patients reported in literature with mutations/deletions of *SETD5* (Group 2) or deletions encompassing both *SETD5* and *BRPF1* (Group 3)

Supplemental References

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