Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A

Individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Age/Sex	11y/F	4y/M	18y/M	18y/F	12y/M	4y/M	3.5y/F	5m/F	27y/M
Variant	c.96C>G, p.I32M	c.421T>A, p.F141I	c.455C>T, p.S152F	c.532A>T, p.T178S	c.533C>A, p.T178N	c.536C>T, p.P179L	c.536C>T, p.P179L	c.536C>A, p.P179H	c.538A>G, p.M180V
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Birth size	N	N	N	/	/	N	N	N	N
Height	N	N	N	N	N	-3SD	N	N	N
Head circumference	N	macro	N	+5.1SD	+2SD	micro	micro	micro	+2SD
DD/ID	ID, moderate	ID, severe	IQ 86	ID, moderate	ID, severe	ID, severe	ID, severe	ID/DD	ID, moderate
Language delay	moderate	Moderate	Mild	mild	Severe	Severe	No words	/	Mild
Motor delay	-	+ (walk 4y9m)	-(walk 15m)	+(walk 2y)	+(walk 5y)	+ (can't walk)	+ (can't walk)	/	+ (walk 2y)
Epilepsy	Moderate	-	-	-	-	moderate	-	-	-
Brain MR	N	N	N	/	N	CCA, ventriculomegaly	CCA	CCA, white matter diffuse thinning, et al	N
Behavior problem	ASD, self-injury	ADHD	ASD	anxious	ADHD, destructive	/	/	/	ASD
Hypotonia	+	+	-	+	+	+	+	+	Moderate
Feeding problem	-	GR	-	-	+	TPN, G-tube	/	+	-
Hearing loss	-	-	-	-	+	/	/	-	-
Extremities/ spine	Hyperm.	Hyperm.	Hyperm.	cubitus valgus, kyphoscoliosis, et al	Hyperm. scoliosis, et al	hip & knee dislocations, kyphoscoliosis	/	/	Hyperm., thoracolumbar scoliosis
Heart	N	N	N	/	/	PDA	/	ASD, PFO	/
PMID	33106617	33106617	33106617	33106617	33106617	33106617	26168268	36209351	33106617

Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A (continue)

Individual	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Age/Sex	20y/M	16m/M	3y9m/M	6y/F	2y/F	23y/F	2y/M	10m/F	9y/F	6y/F
Variant	c.538A>G, p.M180V	c.538A>G, p.M180V	c.539T>C, p.M180T	c.539T>C, p.M180T	c.539T>C, p.M180T	c.539T>C, p.M180T	c.539T>C, p.M180T	c.539T>A, p.M180K	c.539T>G, p.M180R	c.544C>T, p.R182W
Inheritance	NA	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Birth size	N	N	N	/	N	N	N	N	SGA	N
Height	N	-2.6SD	N	/	N	N	N	N	N	N
Head circumference	+3SD	+5.2SD	+2SD	macro	+3.4SD	+2.5SD	+3.9SD	N	-2.4SD	N
DD/ID	ID, moderate	DD	ID, severe	ID, moderate	ID, moderate	ID, moderate	ID, moderate	ID, severe	ID, mild	ID, severe
Language delay	Mild	/	Moderate	/	/	mild	mild	/	Mild	severe
Motor delay	-	-	+ (walk 3y)	/	/	+ (walk 3y)	+ (walk 2y)	/	+ (walk 2.5y)	+ (can’t walk)
Epilepsy	-	-	-	/	-	-	-	+	-	Mild
Brain MR	N	olfactory bulbs no	/	/	CCA	PVLM	N	N	hippocampal dysplasia	CCA, ventriculomegaly
Behavior problem	ASD	-	/	/	-	ASD, anxious	/	/	-	/
Hypotonia	Moderate	+	+	+	severe	moderate	severe	severe	+	+
Feeding problem	+	-	/	/	+	mild	+	-	mild	/
Hearing loss	-	-	+	/	+	-	-	/	-	-
Extremities/spine	scoliosis	N	/	/	N	/	Hyperm.	/	Genu recurvatum	scoliosis
Heart	asymmetric LVH	/	/	/	PDA	N	N	/	N	PDA, PFO
PMID	33106617	33106617	33106617	33106617	33106617	33106617	33106617	33106617	33106617	33106617

Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A (continue)

Individual	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Age/Sex	4y/M	3y/F	7m, F	4y/F	11y/M	1y/F	2y/M	Neonate/M	Neonate/M
Variant	c.544C>T, p.R182W	c.544C>T, p.R182W	c.544C>T, p.R182W	c.544C>T, p.R182W	c.544C>T, p.R182W	c.544C>T, p.R182W	c.547C>T, p.R183W	c.548G>A, p.R183Q	c.548G>A, p.R183Q
Inheritance	*De novo*		NA	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	Not maternal
Birth size	N	SGA	N	N	N	N	N	N	small
Height	N	N	N	+2SD	N	N	-2.5SD	N	/
Head circumference	N	N	42 cm (25%)	N	micro	macro	N	macro	macro
DD/ID	ID, severe	ID, severe	ID/DD	ID, severe	ID, severe	ID, severe	ID, severe	/	/
Language delay	severe	severe	+	No words	No words	/ (no word)	severe	/	/
Motor delay	+ (can't walk)	+ (can't walk)	+(can't sit 4.5y)	+ (can't walk)	+ (walk 6y)	/ (no walk)	+	/	/
Epilepsy	-	severe	+	+	+	+	+	+	+
Brain MR	CCH	CCA	CCA, brain stem hypoplasia, et al	CCH	CCH	CCA	ventriculomegaly, hydrocephalus, pachygyria et al	bilateral ventriculomegaly, enlarged third ventricle, et al	CCA, hypoplastic brainstem, et al
Behavior problem	/	Hand chews	/	/	/	/	/	/	/
Hypotonia	severe	severe	/	+	+	+	+	/	+
Feeding problem	/	+	+	/	+	/	PEG	+	+
Hearing loss	/	-	/	/	/	/	/	/	/
Extremities/spine	/	/	/	scoliosis	Scoliosis, hyperm.	/	scoliosis	/	/
Heart	/	N	PS, PFO	/	/	/	/	+	Multiple abnormal
PMID	33106617	25533962	36209351	26168268	26168268	26168268	33106617	31687265	36209351

Individual	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**
Age/Sex	Neonate/M	20y/M	2y4m/M	7y/M	9m/M	4y/F	7y/F	3y/M	4y/M	13m/M
Variant	c.548G>A, p.R183Q	c.656C>T, p.S219L	c.656C>T, p.S219L	c.656C>T, p.S219L	c.656C>T, p.S219L	c.658G>A, p.V220M	c.658G>A, p.V220M	c.658G>A, p.V220M	c.658G>A, p.V220M	c.772C>A, p.R258S
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	De novo	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Birth size	N	N	N	N	N	N	SGA	SGA	N	N
Height	/	N	N	N	SS	N	/	N	N	N
Head circumference	macro	-2SD	N	N	Micro	/	-2.5SD	N	N	-3.1SD
DD/ID	/	ID, moderate	ID, moderate	DD	/	ID, mild	ID, moderate	ID, mild	ID, moderate	DD
Language delay	/	Mild	moderate	mild	/	Severe	severe	Moderate	moderate	/
Motor delay	/	+	+	+	/	/	+	+(walk 2.5y)	+ (walk 23m)	/
Epilepsy	-	Moderate	moderate	-	+	severe	severe	-	-	-
Brain MR	Partial CCA, et al	CCH	CCH	/	CCA, et al	CCA	CCA	CCH	CCH, PVLM	CCH
Behavior problem	/	ADHD, aggressive	stereotypic	ADHD	/	/	Self-injury	-	ASD	/
Hypotonia	/	+	+	+	-	+	/	+	+	-
Feeding problem	+	-	+	-	+	/	+	-	+	-
Hearing loss	/	-	-	-	+	-	-	-	+	-
Extremities/spine	/	/	N	N	N	+	/	Hyperm.	Hyperm.	/
Heart	PDA, VSD, PFO, et al	/	N	N	N	BAV, DAA	N	/	N	/
PMID	36209351	33106617	33106617	33106617	31531803	33106617	33106617	33106617	33106617	33106617

Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A (continue)

Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A (continue)

Individual	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Age/Sex	5y/M	3y/M	4y/M	1y6m/M	2y/M	16m/M	/	/	/	/
Variant	c.773G>A, p.R258H	c.773G>A, p.R258H	c.773G>A, p.R258H	c.773G>A, p.R258H	c.773G>A, p.R258H	c.773G>A, p.R258H	c.536C>A, p.P179H	c.544C>T, p.R182W	c.547C>T, p.R183W	c.773G>A, p.R258H
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	NA	*De novo*	*De novo*	NA
Birth size	N	N	SGA	N	N	SGA	/	/	/	/
Height	N	N	N	N	SS	SS	/	/	/	/
Head circumference	micro	micro	-3.5SD	-3SD	micro	micro	micro	micro	micro	micro
DD/ID	ID, severe	severe	ID, moderate	DD	DD	ID/DD	ID	ID	ID	ID/DD
Language delay	+ (30 word)	+	Severe	+	+	+ (No word)	/	/	/	/
Motor delay	+(walk 3y)	/	+ (walk 18m)	/	+	+ (can't crawl/sit)	/	/	/	/
Epilepsy	+	-	Mild	-	/(Once febrile seizure)	-	/	/	/	/
Brain MR	CCH	/	CCH	CCH	CCA	lateral ventriculomegaly, et al	CCA	CCA	CCA	CCH
Behavior problem	hyperactivity	autistic, stereotypy	ADHD	-	/	/	/	/	/	Autistic behavior
Hypotonia	+	+	-	+	+	+	/	/	/	+
Feeding problem	/	/	-	-	+	/	/	/	/	/
Hearing loss	/	/	/	-	/	/	/	/	/	/
Extremities/spine	/	/	/	/	/	scoliosis	/	/	/	/
Heart	/	/	/	/	N	/	/	/	/	/
PMID/clinvar	26168268	30755392	33106617	33106617	Chinese paper	34716204	VCV001064777	VCV000190312	VCV000376505	VCV000217458

Supplementary Table S1. The clinical features of the patients carried likely pathogenic/ pathogenic variants of PPP2R1A (continue)

Individual	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
Age/Sex	?/F	?/M	?/M	?/M	?/M	?/F	?/F	?/F	?/F	?/F	?/F	?/F
Variant	c.2T>C, p.M1T	c.2T>C, p.M1T	c.533C>G, p.T178S	c.533C>A, p.T178N	c.536C>T, p.P179L	c.544C>T, p.R182W	c.544C>T, p.R182W	c.544C>T, p.R182W	c.656C>T, p.S219L	c.658G>A, p.V220M	c.658G>A, p.V220M	c.772C>A, p.R258S
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Birth size	/	/	/	/	/	/	/	/	/	/	/	/
Height	/	/	/	/	/	/	/	/	/	/	/	/
Head circumference	macro	/	/	/	/	/	/	/	/	/	micro	micro
DD/ID	DD, moderate	DD, moderate	DD, moderate	DD	ID	/	/	DD	DD	/	DD, severe	DD, severe
Language delay	/	/	/	+	/	/	/	/	/	/	+	/
Motor delay	/	/	/	/	/	/	+	/	/	/	+	/
Epilepsy	/	/	/	/	+	+	/	/	/	+	+	/
Brain MR	/	/	/	/	CCA	CCH	CCA	CCA	CCH	CCA	CCA,	CCH
Behavior problem	autistic	/	/	/	/	/	/	/	/	/	/	ADHD
Hypotonia	+	/	/	+	/	+	/	+	/	/	+	/
Feeding problem	/	GR	/	/	/	/	/	/	/	/	+	/
Hearing loss	/	+	/	+	/	/	/	/	/	/	/	/
Extremities/spine	+	/	/	/	+	+	/	+	/	+	+	/
Heart	/	/	/	/	/	/	/	PDA	/	/	/	/
Decipher patients	274685	275646	457603	268550	259358	258589	263907	304649	428704	280097	379691	286623

Note: N, normal; -, not present the feature; +, present the feature; /, no data or inapplicability; hyperm., hypermobility; F, female; M, male; ADHD, Attention deficit hyperactivity disorder; CCA, corpus callosum agenesis; CCH, hypoplastic corpus callosum; GR, gastroesophageal reflux; LVH, left ventricular hypertrophy; PVLM, periventricular leukomalacia; ASD, BAV, bicuspid aortic valve; DAA, dilated aorta ascendens; PS, pulmonic stenosis; PFO, patent foramen ovale; VSD, micro, microcephaly; macro, macrocephaly; NA, indicates cases without parental origin.

Supplementary Table S2. Twenty-one P/LP variants of PPP2R1A gene in 66 patients

Groups ID	cDNA change	Amino acid change	Evidence code combinations based on ACMG	Cases (n)	Functional characterization
	c.2T>C	p.Met1Thr	PS2+PP2+PM2_PP	2	NA
	c.96C>G	p.Ile32Met	PS2+PP2 +PM2_PP	1	NA
	c.421T>A	p.Phe141Ile	PS2+PP2 +PS3+PM2_PP	1	Impair binding with B56α, B56β, B56γ1, B56ε, PR71, C subunit
	c.455C>T	p.Ser152Phe	PS2+PP2 +PM2_PP	1	Decreased number of dendritic spines in hippocampal neurons
Group 1	c.532A>T	p.Thr178Ser	PS2+PM1+PP2 +PM2_PP	2	NA
	c.533C>A	p.Thr178Asn	PS2+PM1+PP2 +PS3+PM2_PP	2	Impair binding with B56α, B56β, B56γ1, B56ε
	c.536C>T	p.Pro179Leu	PS2+PM1+PP2 +PS3+ PM2_PP	3	Impair binding with B55α, B56α, B56β, B56γ1, B56ε, C subunit
	c.536C>A	p.Pro179His	PM1+PM2_PP+PM5+PP2+PP3	2	NA
Group 2	c.538A>G	p.Met180Val	PS2+PM1+PP2+PS3+PS4+PM2_PP	4	Impair binding with B56α, B56β, B56γ1, B56ε, PR71
	c.539T>C	p.Met180Thr	PS2+PM1+PP2+PS3+PS4+PM2_PP	6	Impair binding with B56α, B56β, B56γ1, B56ε, PR71
	c.539T>A	p.Met180Lys	PS2+PM1+PP2 +PS3+PM2_PP	1	Impair binding with B55α, B56α, B56β, B56γ1, B56ε
	c.539T>G	p.Met180Arg	PS2+PP2 +PM2_PP	1	NA
Group 3	c.544C>T	p.Arg182Trp	PS2+PM1+PP2+PS3+PS4+PM2_PP	12	Impair binding with B55α, B56α, B56β, B56γ1, B56ε, PR71, C subunit; increased phosphorylation of GSK-3β Ser^{9}
	c.547C>T	p.Arg183Trp	PS2+PM1+PP2+PS3+PM2_PP	3	Impair binding with B55α, B56α, B56β, B56γ1, B56ε, PR71, C subunit
	c.548G>A	p.Arg183Gln	PS2+PP2 +PM2_PP	3	hyperphosphorylation of p70S6K Thr^{389} and S6 $Ser^{235/236}$, and GSK3β Ser^{9}, and Akt Thr^{308}
Group 4	c.656C>T	p.Ser219Leu	PS2+PP2 +PS3+PS4+PM2_PP	5	Impair binding with B55α, B56β, B56γ1, B56ε, C subunit
	c.658G>A	p.Val220Met	PS2+PP2 +PS3+PS4+PM2_PP	6	Impair binding with B55α, B56β, C subunit
Group 5	c.772C>A	p.Arg258Ser	PS2+PP2 +PS3+PS4+PM2_PP	2	Impair binding with B55α, PR71, C subunit

	c.773G>A	p.Arg258His	PS2+PP2 +PS3+PS4+PM2_PP	7	Impair binding with B55α, PR71, C subunit
	c.843dupA	p.Asp282Argfs*14	PVS1+PS2+PS3+PM2_PP	1	Truncating protein, impair binding with endogenous B56δ and C subunit
	c.1493G>T	p.Arg498Leu	PM6+PS3+PM2_PP+PP2+PP3	1	Impaired binding with endogenous B56δ and C subunit

Note: n, number.

Supplementary Table S3. Four clinical features of the patients detected six common site variants

Variants	**Macrocephaly/microcephaly**	**CCA/CCH**	**Hypotonia**	**Epilepsy**	**Individuals (n)**
c.536C>T, p.Phe179Leu & c.536C>A, p.Phe179His	Microcephaly 100% (4/4)	100% (5/5)	100% (3/3)	50% (2/4)	5
c.538A>G, p.Met180Val & c.539T>C, p.Met180Thr	Macrocephaly 100% (10/10)	25% (2/8)	100% (10/10)	0% (0/9)	10
c.544C>T, p.Arg182Trp	Microcephaly 44.4% (4/9) Normal head circumference 44.4% (4/9) Macrocephaly 11.1% (1/9)	100% (12/12)	87.5% (7/8)	87.5% (7/8)	12
c.656C>T, p.Ser219Leu	Microcephaly 50% (2/4) Normal head circumference 50% (2/4)	100% (4/4)	75% (3/4)	75% (3/4)	5
c.658G>A,p.Val220Met	Microcephaly 50% (2/4) Normal head circumference 50% (2/4)	100% (6/6)	100% (4/4)	66.7% (4/6)	6
c.772C>A, p.Arg258Ser & c.773G>A, p.Arg258His	Microcephaly 100% (9/9)	87.5% (7/8)	75% (6/8)	33.3% (2/6)	9

Note: CCA, corpus callosum agenesis; CCH, hypoplastic corpus callosum; n, number.