Response to Reviewer 3 Comments

Qian et al. identified the Novel variants in *PPP2R1A* and also compared the genotype-phenotype relations in NDD patients having PPP2R1A variants. Overall, the study is well-written and concise.

Poin 1. Did the author check novel variants in "Bravo" and "all of US" genome browsers?

Response 1: Thanks for the question. We double-checked these variants in the online genome browsers of "Bravo" (https://bravo.sph.umich.edu/freeze8/hg38/) and "all of US" (https://databrowser.researchallofus.org/variants/chr19:52715973). No records for these five variants.

Point 2. What was the cut-off value for allelic frequency during exome analysis?

Response 2: The cut-off value of the allelic frequency we set is 2%.

Point 3. Did the author check compound het variants in exome data?

Response 3: Thanks for the suggestive comment. Patients 2 and 3 were tested trio whole exome sequencing (Trio-WES), Patients 1, 4 and 5 were tested proband-only clinical exome sequencing. We checked all compound het variants in our exome data. Except for the variants of ***PPP2R1A,*** other genetic-causing variants were excluded in these five cases.
The *de novo* and compound heterozygous variants detected from Trio-WES, and the candidate variants from proband clinical exome sequencing are listed in the following table.

Table: The candidate variants detected in the five patients

ID	Gene	location	Variant	Zygo	OMIM	Inherit	Source	Comments
Patient 1	*ANKRD11*	chr16: 89357415	NM_013275:exon5: c.397+6C>T	Het	KBG syndrome, [MIM:148050]	AD	NA	1 normal individual in in-house dataset
	PPP2R1A	**chr19: 52715973**	**NM_014225:exon5: c.538A>G(p.M180V)**	**Het**	**Houge-Janssen syndrome 2 [MIM:616362]**	**AD**	***De novo***	**Pathogenic variant matches the patient's phenotype**
	PRSS12	chr4: 119234489	NM_003619:exon7:c.1356C>T(p.D452D)	Het	Intellectual developmental disorder, autosomal recessive 1, [MIM:249500]	AR	Maternal	Synonymous variant, homozygous in-house dataset without intellectual disability feature
	PRSS12	chr4: 119273715	NM_003619:exon1:c.161C>G(p.P54R)	Het	Intellectual developmental disorder, autosomal recessive 1, [MIM:249500]	AR	Paternal	homozygous carrier in in-house dataset without intellectual disability feature
Patient 2	*DHX58*	chr17: 40259777	NM_024119:exon8: c.841_842delinsAA(p.A281K)	Het	.		*De novo*	Non OMIM disease gene
	NUP210	chr3: 13383363	NM_024923:exon23: c.3113A>G(p.D1038G)	Het	.		Paternal	Non OMIM disease gene
	NUP210	chr3:13420488	NM_024923:exon8: c.977-8A>G	Het	.		Maternal	Non OMIM disease gene
	PPP2R1A	**chr19:52715974**	**NM_014225:exon5: c.539T>C(p.M180T)**	**Het**	**Houge-Janssen syndrome 2 [MIM:616362]**	**AD**	***De novo***	**Pathogenic variant matches the patient's phenotype**
	SLC27A3	chr1:153749134	NM_001317929:exon2: c.808C>T(p.P270S)	Het	.		Maternal	Non OMIM disease gene
	SLC27A3	chr1:153747984	NM_001317929:exon1: c.11_23delinsT (p.P8_L11del)	Het	.		Paternal	Non OMIM disease gene
Patient 3	*AMER1*	chrX:63412601	NM_152424:exon2: c.566A>G(p.Q189R)	Hemi	Osteopathia striata with cranial sclerosis, [MIM:300373]	XLD	Maternal	From normal mother
	MYO5B	chr18:47431204	NM_001080467:exon20: c.2415-6C>G	Het	Microvillus inclusion disease, [MIM:251850]	AR	Maternal	Phenotype not match
	MYO5B	chr18:47429121	NM_001080467:exon21: c.2654A>G(p.D885G)	Het	Microvillus inclusion disease, [MIM:251850]	AR	Paternal	Phenotype not match

	PPP2R1A	chr19:52719066	NM_014225:exon7:c.842delinsCA (p.D282Rfs*14)	Het	Houge-Janssen syndrome 2 [MIM:616362]	AD	*De novo*	**Likely pathogenic variant matches the patient's phenotype**
Patient 4	*ARHGAP31*	chr3:119134635	NM_020754:exon12:c.3859A>G(p.T1287A)	Het	Adams-Oliver syndrome 1, [MIM:100300]	AD	NA	6 individuals without intellectual disability were in the in-house dataset
	CHRNA4	chr20:61981756	NM_000744:exon5:c.1007G>A(p.R336H)	Het	Epilepsy, nocturnal frontal lobe, 1, [MIM:600513]	AD	NA	Phenotype not match
	COL2A1	chr12:48369765	NM_001844:exon50:c.3578C>T(p.S1193L)	Het	Achondrogenesis, type II or hypochondrogenesis, [MIM:200610]; [MIM:156550]; Stickler syndrome, type I, [MIM:108300]; et al	AD	NA	Phenotype not match
	GJB2	chr13:20763612	NM_004004:exon2:c.109G>A(p.V37I)	Het	Deafness, autosomal dominant 3A, [MIM:601544]; Deafness, autosomal recessive 1A, [MIM:220290]; et al	AR	NA	Likely pathogenic variant, carrier
	HARS1	chr5:140056390	NM_002109:exon10:c.1043G>A	Het	Charcot-Marie-Tooth disease, axonal, type 2W, [MIM:616625]; Usher syndrome type 3B, [MIM:614504]	AD/AR	NA	Phenotype not match
	HTT	chr4:3208331	NM_002111:exon43:c.5827G>A	Het	Huntington disease, [MIM:143100]; Lopes-Maciel-Rodan syndrome, [MIM:617435]	AD/AR	NA	Phenotype not match
	PPP2R1A	**chr19:52724277**	**NM_014225:exon12:c.1409T>C(p.V470A)**	**Het**	**Houge-Janssen syndrome 2 [MIM:616362]**	**AD**	**NA**	**Uncertain significance variant matches the patient's phenotype**
	VCAN	chr5:82837289	NM_004385:exon8:c.8467C>G(p.Q2823E)	Het	Wagner syndrome 1, [MIM:143200]	AD	NA	Phenotype not match
Patient 5	*DDX41*	chr5:176938856	NM_016222:exon17:c.1786_1804del (p.P596Rfs*)	Het	{Myeloproliferative/lymphoproliferative neoplasms, familial (multiple types),	AD	NA	Phenotype not match

					susceptibility to}, [MIM:616871]			
	GJB4	chr1:35227495	NM_153212:exon2:c.640A>G(p.M214V)	Het	Erythrokeratodermia variabilis et progressiva 2, [MIM:617524]	AD	NA	Phenotype not match
	PPP2R1A	**chr19:52724361**	**NM_014225:exon12:c.1493G>T(p.R498L)**	**Het**	**Houge-Janssen syndrome 2 [MIM:616362]**	**AD**	***De novo***	**Likely pathogenic variant matches the patient's phenotype**
	RHOBTB2	chr8:22864996	NM_001160036:exon7:c.1304T>C(p.V435A)	Het	Developmental and epileptic encephalopathy 64, [MIM:618004]	AD	NA	9 individuals without intellectual disability in the in-house dataset
	TRIO	chr5:14498209	NM_007118:exon52:c.8059T>A(p.F2687I)	Het	Intellectual developmental disorder, autosomal dominant 44, with microcephaly, [MIM:617061]; Intellectual developmental disorder, autosomal dominant 63, with macrocephaly, [MIM:618825]	AD	Paternal	From no feature father

4. Are all the variants mentioned in Supplementary Table 1, de novo? if yes please mention it in the table or legend.

Response: Thanks for the suggestive comment. We added the inheritance information in Supplementary Table 1 in the revised version. NA indicates cases without parental origin.