

# Final exon frameshift biallelic *PTPN23* variants are associated with microcephalic complex hereditary spastic paraplegia.

## Supplemental Paper

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**Table S1. Candidate *PTPN23* gene variants identified in patients with severe neurological impairment and seizures.**

Reference	Alazami[1]	Trujillano[2]	Sowada[3]	Smigiel[4]	Bend[5] Patient 4
Genotype (NM_015466.4)	Homozygous c.3995G>T: p.(Arg1332Leu)	Homozygous c.904A>G p.(Met302Val)	c.1595C>T p.(Pro532Leu)   c.3586C>T p.(Arg1196*)	c.1902C>G p.(Asn634Lys)   c.2974delC; p.(Leu992Tyrfs*168)	Homozygous c.2568_2594del27; p.(Val857_Pro865del)
Family history	Parents are cousins Two similar deceased siblings	NK	Isolated case	Isolated case	
Ethnicity	Saudi Arabian	NK	Northern European	Northern European	Syrian
Sex	M	NK	F	F	F
Age	5y	NK	Died 4y10	4y	7y
OFC (cm) [SD]	NK	Microcephaly	Primary microcephaly	46 cm (3 cm below 3rd percentile) Primary microcephaly	47cm
<b>Development</b>					
Developmental impairment	✓ - global developmental delay	✓ - global developmental delay with developmental regression	✓ - profound, no cognitive and motor development	✓ - profound	✓ - profound, no milestones
<b>Neurology</b>					
Findings	“Cerebral palsy”	Spasticity abnormality of movement	progressive spasticity	severe spasticity of all four limbs	Spasticity and contractures; wheelchair bound, little purposeful movement
<b>Neurology - other</b>					
Seizures	✓	✓	✓ - from 5m, infantile spasms then polymorphic and treat- ment resistant	✓ - from 2y electroencephalog- raphy showed multifocal discharges	(✓) Febrile
Optic atrophy		NK	✓	✓ and abnormal visual evoked potentials (VEP).	Significant visual impairment
<b>Neuroimaging</b>					
<i>MRI brain</i>	Severe white matter volume loss with enlarged ventricles  Mild cerebellar atrophy Thin corpus callosum Cystic encephalomalacia	brain atrophy	Severe white matter volume loss with enlarged ventricles, Absent myelination, Hypoplastic corpus callosum	Severe white matter volume loss with enlarged ventricles, Absent myelination, Cerebellar atrophy Thin corpus callosum	(reported by parents) Brain atrophy and lissencephaly
<b>Other</b>					
	Gastroesophageal reflux dis- ease				

**Table S2. Candidate *PTPN23* gene variants, proposed by Bend *et al.* 2020 [5].**

	GRCH38 nomenclature NM_015466.4:	gnomAD v2.1.1 non-neuro (AF)	gnomAD v3.1 non-neuro (AF)	SiFT	Polyphen2 HumVar	Rationale for exclusion
Patient 1	Chr3:g.47410478C>T c.2680C>T p.(His894Tyr)	1 het (<0.01%)	4 het (<0.01%)	Tolerated	Benign	-
	Chr3:g.47410545A>G c.2747A>G p.(Gln916Arg)	7 het (0.003%)	4 het (<0.01%)	Tolerated	Benign	
Patient 2	Chr3:g.47411546G>A c.3748G>A p.(Glu1250Lys)	20 het (<0.01%)	18 het (0.01%)	Tolerated	Possibly damaging	-
	Chr3:g.47410663 CCAGCCCCATCCT>C c.2878_2889del12; p.(Gln960_Pro963del)	321 het (0.14%)	260 het (0.2%)	-	-	Poor amino acid conserva- tion / repeti- tive region (a)
Patient 3	Chr3:g.47408451C>T c.1291C>T p.(Arg431Trp)	36 het (0.01%)	25 het (0.01%)	Damaging	Possibly damaging	-
	Chr3:g.47410284C>T c.2486 C > T p.(Pro829Leu)	2 het (<0.01%)	3 het (<0.01%)	Damaging	Benign	-
Patient 4	Chr3:g.47410366 AGTT- GCAGGTCTCC CCTCGGCCCCACC > - c.2568_2594del27; p.(Val857_Pro865del)	Absent <sup>b</sup>	Absent	-	-	-
Patient 5	Chr3:g.47411681GAGA>G c.3884_3886delAGA; p.(1295_1296delLys)	113 het (0.05%)	50 het (0.03%)	-	-	Ashkenazi Jewish gno- mAD v2.1.1 frequency 1.4% <sup>c</sup>
Patient 6	Chr3:g.47412926C>CCC c.4651_4652dup; p.(Leu1552Hisfs*33)	Absent	Absent	-	-	-
Patient 7	Chr3:g.47409268A>G c.1748A>G p.(Lys583Arg) <i>in cis</i>	20 het (<0.01%)	50 het (0.03%)	Damaging	Possibly damaging	<i>in cis with</i> <b>Gln1017His</b>
	Chr3:g.47410849G>C c.3051G>C p.(Gln1017His) <i>in cis</i>	59 het (0.03%)	49 het (0.04%)	Tolerated	Benign	<i>in cis with</i> <b>Lys583Arg</b>
	Chr3:g.47406548G>A c.695 G > A p.(Arg232Gln) <i>in trans</i>	10 het (<0.01%)	14 het 1 hom (0.01%)	Damaging	Benign	<b>Homozygous individual in gnomAD 3.1.1</b>

Abbreviations: AF, allele frequency; het, heterozygous individual; hom, homozygous individual

(a) Note that this deletion can also be annotated as c.2866\_2877del12 p.(Gln956\_Pro959del) as it actually is a deletion of a once-repeated 12 nucleotide sequence (repeat QPHP), thus making the position of the change potentially ambiguous to mapping software. This region of the protein is poorly conserved (Fig S4) and although we can not conclude that the variant is benign we would consider it a variant of uncertain significance with limited evidence supporting causality.

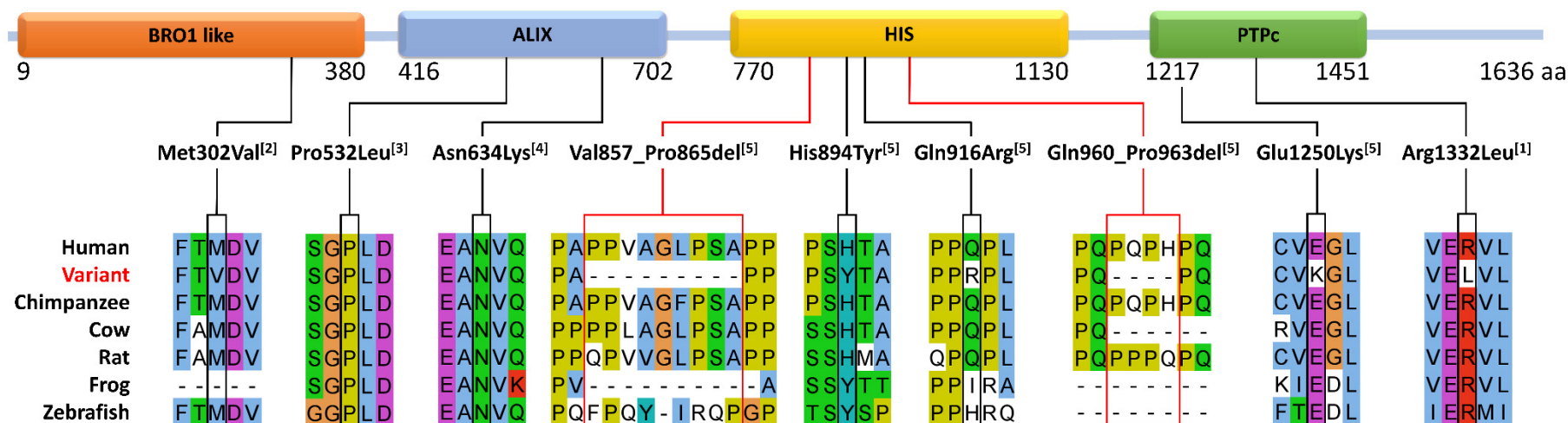
(b) An alternative allele also resulting in the same p.Val857\_Pro865del is present in one individual in gnomAD v2.1.1

(c) Assuming Mendelian ratios then it is expected that 1 in every 20,408 Ashkenazi Jewish individuals would be homozygous, suggesting the existence of about 600 such people

**Table S3. Candidate *PTPN23* gene variants identified in patients with non-specific developmental impairment by Bend *et al.* 2020 [5] not excluded by allele frequency data in population databases**

Reference	Patient 1[5]	Patient 2[5]	Patient 3[5]
Genotype (NM_015466.4)	c.2680C>T p.(His894Tyr)   c.2747A>G p.(Gln916Arg)	c.3748G>A p.(Glu1250Lys)   c.2878_2889del12; p.(Gln960_Pro963del)	c.1291C>T; p.(Arg431Trp)   c.2486C>T; p.(Pro829Leu)
Family history			
Ethnicity	Kuwaiti	European	North American
Sex	M	M	M
Age	14m	3y	6y
OFC (cm) [SD]	normal	normal	normal
<b>Development</b>			
Developmental impairment	✓ “Gross and fine motor skills at 12.5m corrected age were at 9m”	✓ “late to walk independently (~18 months)”	✓
<b>Neurology</b>			
Findings	nil	bilateral intention tremor and shuffling gait	Apraxia
<b>Neurology - other</b>			
Seizures	✗	✗ but abnormal EEG	✗
Optic atrophy	anomalous looking optic disc	NA	✗
<b>Neuroimaging</b>			
<i>MRI brain</i>	Ventriculomegaly with mild decrease in cerebral volume	Ventriculomegaly suspicious for brain volume loss	unremarkable
<b>Other</b>			
	Dysmorphic features, tracheo-oesophageal fistula, mild left pelviectasis	Dysmorphic features, Hypopigmented 4cm patch	VSD/PDA, autism, dyspraxia, hyperlexia, Sensory processing disorder

**Supplementary Figure S4. Position of previously published *PTPN23* candidate missense variants and small in-frame deletions in relation to known *PTPN23* protein domain architecture.**



## References for Supplemental paper

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