Expanding the Phenotype of *PPP1R21*-Related Neurodevelopmental Disorder

Mohammed Almannai*[1,2], Dana Marafi[3, 4,] Maha S. Zaki[5,6], Reza Maroofian[7], Stephanie Efthymiou[7], Nebal Waill Saadi[8], Bilal Filimban[9], Hormos Salimi Dafsari[10,11,12,13], Fatima Rahman[14], Shazia Maqbool[14], Eissa Faqeih[9], Fuad Al Mutairi[1,2], Hind Alsharhan[4,15,16,17], Omar Abdelaty[15], Saadoun Bin-Hasan[15], Ruizhi Duan[3], Mahmoud M. Noureldeen[18], Alaa Alqattan[15], Henry Houlden[7], Jill V Hunter,[19,20], Jennifer E. Posey[3], James R. Lupski[3,19,21,22], Ayman W. El-Hattab*[23,24]

1. Genetics and Precision Medicine department (GPM), King Abdullah Specialized Children's Hospital (KASCH), King Abdulaziz Medical City, Ministry of National Guard Health Affairs (MNG-HA), Riyadh, Saudi Arabia
2. Medical Genomics Research Department, King Abdullah International Medical Research Center, Ministry of National Guard Health Affairs, King Saud Bin Abdulaziz University for Health Sciences, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia
3. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, 77030, USA
4. Department of Pediatrics, College of Medicine, Kuwait University, P.O. Box 24923, 13110 Safat, Kuwait
5. Clinical Genetics Department, Human Genetics and Genome Research Institute National Research Centre, Cairo, Egypt
6. Genetics Department, Armed Forces College of Medicine (AFCM), Cairo, Egypt.
7. Department of Neuromuscular disorders, UCL Queen Square Institute of Neurology, University College London, London, WC1N 3BG, UK
8. College of Medicine, University of Baghdad, Pediatric Neurology, Children Welfare Teaching Hospital, Baghdad, Iraq.

9. Section of Medical Genetics, Children's Hospital, King Fahad Medical City, Riyadh, Saudi Arabia.
10. Department of Pediatrics, Center for Rare Diseases, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany
11. Randall Centre for Cell and Molecular Biophysics, Faculty of Life Sciences and Medicine (FoLSM), King's College London, London, United Kingdom
12. Max-Planck-Institute for Biology of Ageing, Cologne, Germany.
13. Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), Cologne, Germany.
14. Department of Developmental - Behavioral Pediatrics, University of Child Health Sciences & The Children's Hospital, Lahore, Pakistan.
15. Department of Pediatrics, Farwaniya Hospital, Ministry of Health, Sabah Al-Nasser, 92426, Kuwait
16. Kuwait Medical Genetics Center, Ministry of Health, Sulaibikhat, 80901, Kuwait
17. Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
18. Department of Pediatrics, Faculty of Medicine, Beni-Suef University, Beni-Suef, Egypt
19. Texas Children Hospital, Houston, Texas, 77030, USA
20. Department of Radiology, Baylor College of Medicine, Houston, Texas, 77030
21. Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, 77030, USA
22. Department of Pediatrics, Baylor College of Medicine, Houston, Texas, 77030, USA

23. Department of Clinical Sciences, College of Medicine, University of Sharjah, Sharjah, United Arab Emirates
24. Genetics Clinics, University Hospital Sharjah, Sharjah, United Arab Emirates

*__Correspondence:__ Mohammed Almannai; almannaimo@ngha.med.sa, Ayman W. El-Hattab; elhattabaw@yahoo.com

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ABSTRACT

PPP1R21 encodes for a conserved protein that is involved in endosomal maturation. Biallelic pathogenic variants in *PPP1R21* have been associated with a syndromic neurodevelopmental disorder from studying 13 affected individuals. In this report, we present 11 additional individuals from nine unrelated families and their clinical, radiological, and molecular findings. We identified eight different variants in *PPP1R21*, of which six were novel variants. Global developmental delay and hypotonia are neurological features that were observed in all individuals. There is also a similar pattern of dysmorphic features with coarse faces as a gestalt observed in several individuals. Common findings in 75% of individuals with available brain imaging include delays in myelination, wavy outline of the bodies of the lateral ventricles, and slight prominence of the bodies of the lateral ventricles**.** *PPP1R21*-related neurodevelopmental disorder is associated with a consistent phenotype and should be considered in highly consanguineous individuals presenting with developmental delay/intellectual disability along with coarse facial features.

INTRODUCTION

Neurodevelopmental disorders (NDD) are among the most common conditions referred for genetics evaluation[1]. Intellectual disability (ID) is associated with deficits in intellectual and adaptive functioning and has an estimated prevalence of 1% to 3% of the population[2]. There are numerous causes for ID including environmental and genetic factors. With recent genetics and genomics advances, and increased availability of genetic testing, the molecular genetic basis of ID is being expanded on a continuous basis[3]. In countries with populations that have a high rate of consanguinity, autosomal recessive (AR) monogenic disorders are the leading genetic cause of ID[4].

In 2017, Anazi et al. reported one individual with global developmental delay (GDD), hypotonia, and coarse facial features with homozygous nonsense variant in *PPP1R21* (c.2089C>T:p.Arg697*)[3]. Suleiman et al. reported three children, including the one previously published in Anazi et al, with homozygous loss of function variants in *PPP1R21* who presented with a similar phenotype characterized by profound GDD, hypotonia, dysmorphic facial features, and brain abnormalities (cerebellar vermis hypoplasia, ventricular dilatation, and reduced white matter volume)[5]. Rahman et al. reported six affected individuals from four unrelated families presenting with the same phenotype and all harbored biallelic loss of function variants in *PPP1R21*. Furthermore, they showed localization of PPP1R21 protein to the early endosome suggesting a role for PPP1R21 in the endosomal maturation[6]. Recently, four more individuals were reported, including one asymptomatic newborn diagnosed with exome-based carrier screening, making the total reported cases with *PPP1R21*-related NDD with hypotonia, facial dysmorphism, and brain abnormalities (OMIM #619383) to date 13 cases[7–10].

In this report, we present clinical, radiological, and molecular details of 11 previously unpublished individuals with *PPP1R21*-related NDD from nine unrelated families with six novel variants in *PPP1R21*.

MATERIALS AND METHODS

We recruited 11 individuals from 9 unrelated families from diverse ethnic backgrounds and countries of origin including Saudi Arabia, Egypt, Sudan, Pakistan, Iraq, and Kuwait. Written informed consent was obtained from the legal guardians of the subjects included in this study for enrollment in the research study and publication of photographs. Ethical approval was obtained by the institutional review board (IRB) for human subjects from the different participating sites including Baylor College of Medicine (BCM) IRB (protocol H-29697), King Abdullah International Medical Research Center IRB(NRC23R/613/10), and Science and Technology Development Fund (STDF), Academy of Science Research and Technology, Egypt (Grant number: 33650; Ethical approval 20105). This research was conducted in accordance with the Declaration of Helsinki.

Brain magnetic resonance imaging (MRI) of eight individuals was available for review by a single board-certified neuroradiologist (JVH). The molecular diagnosis of *PPP1R21*-related NDD was made from clinical(cES) or research exome sequencing (rES). Family 1, 4 and 5 had cES at Centogene GmbH, Rostock, Germany as previously described[11]. Family 2 and 3 had research rES analysis at BCM as previously described[12]. Family 6 and 7 had research rES analysis at University College London (IoN UCL 07/Q0512/26) as previously described[13]. Families 8 and 9 had cES at King Fahad Medical City (KFMC), Riyadh, Saudi Arabia using Illumina HiSeq 4000 as per manufacturer's instructions.

RESULTS

CLINICAL DESCRIPTION

Eleven subjects from nine unrelated families with biallelic *PPP1R21* deleterious variants are included in this study (Figure1; Table 1). Detailed clinical information is available as Supporting Information 1 and is also summarized in Table 1.

Most of our subjects were born at full term. When available, growth parameters at birth were normal. Body growth at last follow-up examination was normal for most subjects. Feeding disorders included oropharyngeal dysphagia, recurrent vomiting, drooling, swallowing difficulties, and choking, were commonly seen, reported in two-thirds of the subjects in our cohort (7/11; 64%).

The main feature of the phenotype was neurodevelopmental delay. All subjects exhibited moderate to severe GDD and ID, depending on the age of diagnosis. IQ scoring is available for two subjects in our cohort and it was 40 (Subject 6) and 50 (Subject 10). When available, hearing assessment was normal while three subjects had optic atrophy and one had nystagmus. Some neurobehavioral problems with aggression, anger, bruxism, and stereotyped behavior were reported in three subjects. Almost all of our subjects had hypotonia (10/11; 91%) with hyporeflexia in seven of them (64%). Seizures were not common, seen in only three (27%) subjects in our cohort, which were in the form of generalized clonic seizures. The response to antiepileptic drugs was variable, with one subject requiring four antiepileptics drugs (Subject 7) while subject 10 was seizure free on monotherapy.

Two subjects in this report have hepato-(splenomegaly), one has left ventricular hypertrophy. Respiratory problems, in the form of apnea, recurrent respiratory infections and bronchitis, reported in four subjects (Table 1).

Coarse facial features were observed in most individuals in this cohort with high arched and thick eyebrows, thick lips, broad nasal bridge, and low set ears (Figure 2). Hypertrichosis was also reported in six subjects.

Two subjects (Subjects 7 and 11) in our cohort died at 22 months and 9.5 years of age, respectively. The cause of death was suspected sepsis in the 22-month-old girl and it was not clear in the other. (Supplementary file 1)

NEURORADIOLOGICAL FINDINGS

Brain MRI images were available for review in eight subjects. Common neuroradiological findings in 75% of the subjects (as seen on images of subjects 1, 4, 5, 6, 10 and 11) include delays in myelination, wavy outline of the bodies of the lateral ventricles, and slight prominence of the bodies of the lateral ventricles (Figure 3). Overall, there also was slight generosity of the extra-axial spaces. There is a question of the deep grey nuclei appearing small particularly the thalamus (such as seen in subjects 4 and 10) but this needs to be assessed in a larger cohort. Half of the subjects with available imaging (subjects 1, 4, 10 and 11) have some degree of plagiocephaly and/or thinning of the corpus callosum which is a reflection of motor developmental delay and delayed myelination. Cerebellar hypoplasia was not noted in any subject from our cohort[6].

MOLECULAR FINDINGS

Eight different variants in *PPP1R21* [NM_001135629.3] were identified in the nine unrelated families. Subject 1 (Family 1) was found to be homozygous for a novel splice-site insertion-deletion variant (c.1445_1446+1delAGGinsCT). This variant is predicted to affect splicing by *in silico* prediction (SpliceAI donor loss score of 0.99) (Supplementary Table 1). Subject 2 (Family 2) was homozygous for the previously reported nonsense variant (c.2089C>T; p.Arg697*). Subjects 3 and 4 (Family 3) were found to be homozygous for a novel intronic variant (c.1935+5G>A) which is predicted to affect splicing by *in silico* prediction (SpliceAI donor loss score of 0.98) (Supplementary Table 1). Subject 5 (Family 4) was found to have a novel homozygous nonsense variant (c.1921C>T; p.Gln641*) that has a CADD score of 41. Subject 6 (Family 5) and subject 10 (Family 8) were found to have the same novel homozygous frameshift variant (c.1950del; p. Arg651Gfs*16). Subjects 7 and 8 (Family 6) were homozygous for a novel missense variant (c.2096T>C; p.Leu699Pro) and subject 9 (Family 7) was homozygous for another novel missense variant (c.224T>G; p.Leu75Arg). All of these missense variants are ultra-rare and absent from gnomAD control database, predicated to be pathogenic by most *in-silico* tools (Supplementary Table 1), had high CADD score (29.9 and 27.5respectively) and segregated with the phenotype in the families while no other potential candidate variants to explain the phenotype in these two families were identified. Subject 11 (Family 9) was homozygous for the previously reported nonsense variant (c.427C>T; p.Arg143*).

All variant alleles are ultra-rare (minor allele frequency [MAF]≤ 1/10,000) and absent in the homozygous state from the databases of BCM GREGoR (Genomics Research to Elucidate

the Genetics of Rare diseases) and from the Genome Aggregation Database (GnomAD v.2.1.1)[14,15].

Sanger dideoxy sequencing confirmed the variant alleles and segregation with the disease in all families (Figure 1).

DISCUSSION

In this report, we present detailed clinical, molecular, and radiological features of 11 individuals from 9 unrelated families with *PPP1R21*-related NDD. Our report raises the total number of reported cases with *PPP1R21*-related NDD to 24 subjects to date. Ten subjects in our cohort were of Arab ethnicity (from Saudi Arabia, Kuwait, Egypt, Sudan, and Iraq) and one was from Pakistan. Consanguinity was historically reported in all of our subjects and also in the majority of the previously reported cases as well.

Common findings in this syndrome include coarse facial features with hypertrichosis. The main feature of the phenotype is neurodevelopmental delay and all reported subjects so far exhibited moderate to severe GDD and ID depending on age of diagnosis with most of them having hypotonia and hyporeflexia. Seizures are not common; while three of subjects in this cohort and of one the previously reported cases had generalized clonic seizures[10]; myoclonic seizures have been reported in one of the previously published cases[6] and two other individuals had febrile seizures that did not recur[5].

Feeding issues are also common, reported in 16 subjects (16/23; 69%). Different ophthalmological problems, including squint, esotropia, nystagmus and optic atrophy are common, evident in more than 50% of the total reported individuals (12/23; 54%).

Several key features overlapped with lysosomal storage disorders. Five total subjects (5/23; 22%) have hepato-(splenomegaly). Cardiac problems, including left ventricular hypertrophy, hypertrophic cardiomyopathy, and left ventricular noncompaction, have been reported also in five individuals (5/23; 23%). Respiratory problems are also common. Coarse facial features were observed in most individuals (15/21;71%) with hypertrichosis seen in several of them. Out of the 24 reported subjects, four were deceased at time of last report. The consistent phenotype in this cohort further supports that *PPP1R21*-NDD is a recognizable syndrome characterized clinically by GDD, ID, hypotonia, and dysmorphic facial features; there remains the possibility that other alleles at the locus may associate with a more variable phenotype.

The most common neuroradiological features in 6/8 (75%) of the subjects in our cohort with available brain MRI images are delays in myelination, wavy outline of the bodies of the lateral ventricles, and slight prominence of the bodies of the lateral ventricles (Figure 3). Similar to our cohort, the prominence of the ventricular system has been noted in 8/11 (73%) previously published cases with available brain imaging[3,5,6,8–10]. Delays in myelination was previously observed in 5/10 (50%) while abnormalities in the corpus callosum, as an indirect reflection of abnormal myelination, were more commonly observed in 8/11 (73%) of the previously published cases[3,5,6,9,10]. In contrast, the irregular outline of the bodies of the lateral ventricles was less frequently noted in only 4/11 (36%) of the published cases compared to our cohort[6,9,10]. Interestingly, cerebellar hypoplasia, previously observed in 4/10 subjects (40%), does not appear to be a feature of all alleles in our cohort[6,9].

Eight different variants in *PPP1R21* [NM_001135629.3] were identified in the nine unrelated families including six novel variants. These novel variants include one nonsense, one frameshift, two missense, and two intronic variants predicted to affect splicing and result in

donor loss. The transcripts resulting from the nonsense variants are predicted to lead to a truncated protein or be degraded by non-sense mediated decay[16]. We proposed that null, frameshift, and splicing variants result in complete loss-of-function (LoF) while the missense variants result in partial LoF thus behaving as hypomorphic alleles. Previous reports have only reported complete LoF variants (nonsense, frameshift, and splicing variants)[5–9]. Here were report the first two subjects with *PPP1R21* missense variant alleles and neurodevelopmental phenotype which along with the other novel variant expand the allelic spectrum of the disease. PPP1R21 has two domains, an N-terminus KLRAQ domain and a C-terminus TTKRSYEDQ domain[6]. The first missense variant p.Leu75Arg localizes to the first domain, KLRAQ domain, while the other variants; p.Leu699Pro localizes to the TTKRSYEDQ domain. Future functional studies may be able to evaluate the functional consequences of these variant alleles *in vitro*. Of note, the two unrelated subjects with the novel frameshift variant (c.1950del p.Arg651Glyfs*16) belong to the same tribe from Saudi Arabia, suggesting a possible clan specific founder effect.

PPP1R21 is located on 2p16.3 and encodes for an evolutionary conserved protein that is involved in early endosomal maturation and trafficking through co-localization with the early endosomal marker EEA1. Endolysosomes are important for the degradation of dysfunctional proteins and organelles that are included in autophagosomes. Endolysosomal dysfunction has been linked to a number of neurodegenerative diseases including Alzheimer's disease and Parkinson's disease[17] as well as several monogenic disorders of autophagy and intracellular trafficking that also show similar phenotypes such as corpus callosum dysgenesis, neurodevelopmental disorders or facial dysmorphisms. PPP1R21 deficiency may lead to a stalling in endolysosomes[18,19] as evidenced from a delayed transferrin-488 clearance in the *PPP1R21* mutant cells. Rehman et al. showed this protein is expressed within the developing

mouse cortex[6]. Electron microscopy (EM) studies showed mildly increased number of myelin figures/myelin bodies in the *PPP1R21* loss of function cell line[6]. In a *ppp1r21*-mutant zebrafish model with primary biliary cholangitis, there was an increase in immune cell infiltration and liver fibrosis[20] that were rescued by TOR inhibition with rapamycin. It is unclear how any TOR inhibition may dampen TOR overactivation, rescue endosomal stall and potentially ameliorate the disease course in patients. In fibroblasts from a patient with a splice-site mutation in *PPP1R21*, immunoblot studies revealed accumulation of autophagy adaptor p62, proteomic analyses revealed an overactivation of the ubiquitin–proteasome system (UPS), and cell morphological measurements indicated spatial rearrangement in the actin cytoskeleton. The authors hypothesized overactivation of the UPS represents an attempt to counteract protein aggregates resulting from endosolysosomal stall and thereby prevent cellular degeneration [7]. While we report the first two missense variants in *PPP1R21* associated with this recognizable disorder, we did not observe any clear indication of allelism that would account for any milder spectrum of the disorders in a genotype-phenotype spectrum.

In conclusion, here we present the clinical, molecular, and radiological features of *PPP1R21*-related NDD in 11 new subjects with six novel variants and two recurrent variants. This disorder is characterized by predominant neurodevelopmental phenotype with GDD, ID, hypotonia, and dysmorphism. This genotypic and phenotypic expansion delineates the features of *PPP1R21*-related disorders and provides insights into the neurodevelopment and neurobiology of disease. While there are no specific features to make this syndrome easily distinguishable on clinical basis, storage disorders that are associated with ID are an important group to be considered in the differential diagnosis. Nevertheless, this syndrome should be considered in the differential diagnosis of autosomal recessive ID syndromes.

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COMPETING INTERESTS

J.R.L. has stock ownership in 23andMe, is a paid consultant for Genomics International, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, genomic disorders, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics (BG); J.R.L. serves on the Scientific Advisory Board (SAB) of BG. Other authors declare no conflict of interest.

Authors Contributions

Conceptualization: M.A., A.W.E. Resources: M.A., A.W.E., D.M., R.D., M.Z., J.V.J., J.E.P., and J.R.L. Formal analysis: M.A., A.W.E., D.M., J.V.J. Methodology: M.A., A.W.E. Supervision: A.W.E., J.E.P., and J.R.L. Writing-original draft: M.A., D.M. Writing-review & editing: M.A., A.W.E., D.M, R.D., B.F., H.E., M.Z., E.F., F.M.., K.S., H.S., O.A., S.H., M.N., H.A., A.A., J.V.J., J.E.P., and J.R.L.

DATA AVAILABILITY

The data that supports the findings of this study are available in the supplementary material of this article.

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Table 1. Characteristics of subjects included in this report

Subject	1 (F1-II2)	2 (F2-II9)	3 (F3-II2)	4 (F3-II4)	5 (F4-II2)	6 (F5-II2)	7 (F6-II2)	8 (F6-II3)	9 (F7-II4)	10 (F8-II1)	11 (F9-II3)	Subjects in this report (11)	Previously reported cases (13) **	Total (24)
Gender	Male	Female	Male	Male	Female	Male	Female	Female	Male	Male	Female	6 males: 5 females	5 males:8 females	11 males:13 females
Ethnicity	Arab	Arab	Arab	Arab	Arab	Arab	Arab	Arab	Pakistani	Arab	Arab			
Country of origin	Kuwait	Egypt	Sudan	Sudan	Egypt	Saudi	Iraq	Iraq	Pakistan	Saudi	Saudi			
Consanguinity	+	+	+	+	+	+	+	+	+	+	+	11/11	10/12	21/23
***PPP1R21* variant**	c.1445_1446+1delAGGinsCT	c.2089C>T p.Arg697*	c.1935+5G>A	c.1935+5G>A	c.1921C>T p.Gln641*	c.1950del p.Arg651Glyfs*16	c.2096T>C p.Lys699Pro	c.2096T>C p.Lys699Pro	c.224T>G; p.Leu75Arg	c.1950del p.Arg651Glyfs*16	c.427C>T; p.Arg143*			
Age at last evaluation	18 months	3.5 months	11 years	7 years	22 months	5 years	9.5	5.5	7 years	18 y	21 m	3.5 months-18 years	7 days-27 years	7 days-27 years
Outcome	Alive	Alive	Alive	Alive	Alive	Alive	Deceased	Alive	Alive	Alive	Deceased	2 deceased:9 alive	2 deceased: 11 alive	4 deceased: 20 alive
Growth at last evaluation **Weight in kg (SD)** **Height in Cm (SD)** **OFC in Cm (SD)**	 13.3(1.15) 76.3(-1.73) 48(0.21)	 5 (-0.87) 60(-0.44) 39(-0.86)	 28(-1.96) 150(0.4) 52(-1)	 19(-1.5) 121(-0.14) 51.5(-0.4)	 9.9(-0.9) 84(-0.2) 45(-1.36)	 24.6(1.9) 119(2.2) 55(2.8)	 28(-0.54) NA 54(1.5) *	 13(-3.4) NA NA	 13(-5.1) 115(-1.2) 49(-2)	 72.5(0.4) 177.6(0.1) 59(4.2)	 10.6(-0.35) 77(-2.44) 46.5(-0.28)			
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	11/11	12/12	23/23
Seizures	-	-	-	-	-	-	+	+	-	+	-	3/11	2/12	5/23
Hypotonia	+	+	+	+	+	+	+	+	+	-	+	10/11	12/12	22/23
Diminished reflexes	+ Areflexia	-	+	-	+ Areflexia	- Hyperreflexia	+	+	+	-	+ Hyporeflexia	7/11	5/12	12/23
Feeding Problems	+ Gastrostomy	+	+ Excessive drooling	-	-	-	+	+	+	-	+	7/11	9/12	16/23
Respiratory Problems	+ Apnea, Tracheostomy	+ recurrent infections	+ Apnea	-	-	-	+ Allergic bronchitis	-	-	-	-	4/11	8/12	12/23
Cardiac Problems	LVH	-	-	-	-	NA	NA	NA	NA	NA	-	1/6	4/12	5/18
Ophthalmological Problems	+ Unable to fixate or track	-	+ Nystagmus (Eye exam normal)	+ Optic atrophy and nystagmus	-	NA	+ Optic atrophy	+ Optic atrophy	-	NP	+ Vertical Nystagmus	6/9	9/12	15/21
Others	Medullary nephrocalcinosis, subclinical hypothyroidism, osteopenia		Hepatosplenomegaly	Hyperextensibility of the joints, splenomegaly							splenomegaly			
Hypertrichosis	+	+	+	+	+	-	-	-	-	-	+	6/11	4/12	10/23
Facial Features														
Thick and arched eyebrows	+	+ (Thick only)	NA	+	+	NA	+	+	+	+ (thick only)	+ (thick only)	9/9	4/12	13/21
Hypertelorism	-	-	NA	-	-	NA	-	-	+	-	-	1/9	4/12	5/21
Epicanthus	-	+	NA	-	+	NA	+	+	-	-	-	4/9	2/12	6/21
Broad nasal bridge	-	+	NA	+	-	NA	+	+	-	+	-	5/9	3/12	8/21
Short nose	+	+	NA	+	-	NA	-	-	-	-	+	4/9	3/12	7/21
Low hanging columella	-	+	NA	+	-	NA	-	-	-	+	+	4/9	4/12	8/21
Long philtrum	+	+	NA	+	-	NA	+	+	-	-	+	6/9	3/12	9/21
Thick lips	-	+	NA	+	-	NA	+	+	-	+	-	5/9	3/12	8/21

Micrognathia	+	-	NA	-	-	NA	-	-	-	-	+	2/9	1/12	3/21
Low set ears	-	+	NA	+	-	NA	-	-	-	-	-	2/9	9/12	11/21
Flat Occiput	+	-	NA	-	-	NA	-	-	-	-	+	2/9	3/12	5/21
Coarse face	+	+	NA	+	-	NA	+	+	-	+	-	6/9	9/12	15/21
Others	High-arched palate, large right ear, small puffy hands, tapering of fingers, deep creases over the knuckles, overriding toes.			Long face with high forehead, open mouth with protruded tongue	high forehead, overlapping toes					large head, triangular face with protruded mandible and deep eyes, drooling of saliva, large hands and feet, wide spaced gait	almond shaped eyes, depressed nasal bridge,			

Cm: Centimeters; EEG: electroencephalogram; GTC: generalized tonic clonic; Htz: heterozygous; Hmz: homozygous Kg: Kilograms; NA: not available; NP: not performed; PF: palpebral fissure; SD: standard deviation. *OFC obtained at 6.5 years of age; ** one subject not included in calculations since was 7 days old at time of report

FIGURE LEGENDS

Figure 1.a. Pedigrees of families included in this report. Family members for whom DNA testing is available are denoted with asterisks. **Figure 1.b.** The alignment of pathogenic variants on the *PPP1R21* gene (isoform 1) and protein are shown. Novel variants in this report are indicated in bold.

Figure 2.a: Frontal and lateral views of Subject 1showing coarse facial features with thick and arched eyebrows, short nose, long philtrum, micrognathia and flat occiput. **Figure 2.b:** Frontal view of Subject 2 showing coarse facial features with thick eyebrows, epicanthus, broad nasal bridge, short nose, low hanging columella, long philtrum and thick lips. **Figure 2.c:** Frontal view of Subject 4 showing coarse facial features with thick and arched eyebrows, broad nasal bridge, short nose, low hanging columella, long philtrum and thick lips. **Figure 2.d:** Frontal view of Subject 5 showing thick and arched eyebrows and epicanthus. **Figure 2.e and f:** Frontal views of Subject 7 and 8 respectively showing coarse facial features with thick and arched eyebrows, epicanthus, broad nasal bridge, long philtrum and thick lips. **Figure 2.g:** Frontal view of Subject 9 showing thick and arched eyebrows and hypertelorism. **Figure 2.h:** Frontal and lateral views of Subject 10 showing coarse facial features with thick eyebrows, broad nasal bridge, low hanging columella and thick lips. **Figure 2.i:** Frontal and lateral views of Subject 11 showing thick eyebrows, short nose, low hanging columella, long philtrum, micrognathia and flat occiput

Figure 3. Brain MRI images for subjects with biallelic variants in *PPP1R21*.

a (1,2): Axial T2 images of subject 1 at 1 month of age showing enlargement of the bodies of the lateral ventricles (yellow stars), generous extra-axial CSF spaces, bilateral symmetric under-

opercularization (blue arrow, left sided finding not visible on this cut), and mild right posterior plagiocephaly (red arrowhead)**. a(3):** Axial T1 images of subject 1 at 10 months of age showing decompression of the right lateral ventricle compared to left after right ventriculoperitoneal shunt placement, slight irregularity of the outline of the bodies of the lateral ventricles (yellow arrowheads) and worsening of the right posterior plagiocephaly (red arrowhead). **b(1,2):** Axial T2 images of subject 2 deceased sibling (F2:II8) at 1 month of age showing normal (age-appropriate) myelination of the posterior limb of the internal capsule. **c(1-3):** Axial T2 (left) image of subject 4 at 4 years of age showing a wavy outline of the bodies of the lateral ventricles (yellow arrowheads), mild dilation/prominence of the lateral ventricles (yellow stars), and small deep nuclei structure, and thinning of the splenium of the corpus callosum. Axial T2 (middle) shows a retro-cerebellar cyst (yellow circle). Mid-sagittal T1 (right) also shows the thinning of the splenium of the corpus callosum (white arrow). **D(1-3):** Axial T2 images of subject 5 at 6 months (left) and 1 year of age (middle and right) showing wavy outline of the bodies of the lateral ventricles (yellow arrowheads), symmetric under-opercularization (blue arrows), generous extra-axial spaces, and delayed myelination as evident by the lack of myelination of the anterior limb of the internal capsule (brown arrowheads). **e(1,2):** Axial T2 (left) image of subject 7 at 3 years of age dysmorphic lateral ventricles (yellow arrowheads), and under myelination of both limbs of the internal capsule (brown arrowheads). Mid-sagittal T2 showing normal corpus callosum, brainstem, and cerebellum. **f(1,2):** Mid-sagittal (left) image of subject 10 at 16 years of age showing mild thinning of the posterior body and splenium of the corpus callosum (white arrow). Axial T2 (right) shows a slight wavy outline of the bodies of the lateral ventricles (yellow arrowheads), slight prominence of the lateral ventricles (yellow stars) and hypoplasia of the thalami (small deep grey nuclei). **g(1-3):** Midsagittal T1 (left) and Axial T2 images of subject

11 at 10 months of age show a slightly wavy outline of the bodies of the lateral ventricles (yellow arrowheads), slight prominence of the lateral ventricles (yellow stars), generous extra-axial spaces, incomplete myelination of the anterior limb of the internal capsule (brown arrowheads), foreshortening of the corpus callosum (white arrow), mild right plagiocephaly(red arrowhead) and turricephaly.