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Microcephalic Primordial Dwarfism in an Emirati Patient with *PNKP* Mutation

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Microcephaly is a rare neurological condition, both in isolation and when it occurs as part of a syndrome. One of the syndromic forms of microcephaly is microcephaly, seizures and developmental delay (MCSZ) (OMIM #613402), a rare autosomal recessive neurodevelopmental disorder with a range of phenotypic severity, and known to be caused by mutations in the polynucleotide kinase 3′ phosphatase (*PNKP*) gene. The PNK protein is a key enzyme involved in the repair of single and double stranded DNA breaks, a process which is particularly important in the nervous system. We describe an Emirati patient who presented with microcephaly, short stature, uncontrollable tonic-clonic seizures, facial dysmorphism, and developmental delay, while at the same time showing evidence of brain atrophy and agenesis of the corpus callosum. We used whole exome sequencing to identify homozygosity for a missense c.1385G > C (p.Arg462Pro) mutation in *PNKP* in the patient and heterozygosity for this mutation in her consanguineous parents. The Arg 462 residue forms a part of the lid subdomain helix of the P-loop Kinase domain. Although our patient's phenotype resembled that of MCSZ, the short stature and evidence of brain atrophy distinguished it from other classic cases of the condition. The report raises the question of whether to consider this case as an atypical variant of MCSZ or as a novel form of microcephalic primordial dwarfism. © 2016 Wiley Periodicals, Inc.

Key words: *PNKP*; mutation; MCSZ; microcephaly; primordial dwarfism

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INTRODUCTION

Microcephaly is a condition in which the head size, as measured by the occipito-frontal circumference, is smaller by at least two SD than the age- and sex-related population mean. In most cases, microcephaly is synonymous with microencephaly (small brain), and is used as a marker for it. Congenital microcephaly is broadly classified as either microcephaly vera or microcephaly with abnormal or simplified gyral pattern. The former is characterized by intellectual disability in the absence of any gross structural abnormality of the brain, while the latter is associated with structural brain malformations, such as gyrification abnormalities and agenesis of corpus callosum, along with possibly perturbed abnormalities in neuronal migration [Mochida and Walsh, 2001; Gilmore and Walsh, 2013]. There is a certain amount of ambiguity in this differentiation, however, and some forms of microcephaly have features that overlap between the two. For instance, the phenotypic spectrum of MCPH2 extends from true microcephaly in some families to that associated with simplified gyral patterns with cortical malformations in others [Nicholas et al., 2010; Yu et al., 2010]. Primary microcephaly is a genetically heterogeneous condition. Apart from the 13 genetic loci associated with autosomal recessive primary microcephaly (MCPH), there are also a number of syndromic and non-syndromic forms of microcephaly. Almost all of these are etiologically linked to mutations in single genes that are involved either in centrosomal function, cell division, or DNA damage and/or repair response [Alcantara and O'Driscoll, 2014].

Although seizures are not a common feature of the autosomal recessive primary microcephalies, they form an important component of the clinical spectrum of other congenital microcephalic syndromes such as microcephaly, seizures and developmental delay (MCSZ) (OMIM #613402), postnatal progressive microcephaly with seizures and brain atrophy (OMIM #613668), progressive microcephaly with seizures and cerebral and cerebellar atrophy (MSCCA) (OMIM #615760), Pseudo-TORCH syndrome (OMIM #251290) and others. On the other hand, microcephaly is also

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associated with some forms of primordial dwarfism (PD), a group of disorders displaying wide clinical heterogeneity, but characterized overall by severely stunted growth of a prenatal onset. Subtypes of PD associated with microcephaly include the genetically heterogeneous Seckel syndrome (see OMIM #210600 for review), microcephalic osteodysplastic primordial dwarfism (MOPD) Types I, II, and III (OMIM #210710. 210720 and 210730), and Meier–Gorlin syndrome (OMIM #224690) [Alkuraya, 2015].

Here, we report an Emirati female patient with microcephaly in association with primordial dwarfism, homozygous for a missense mutation in the polynucleotide kinase 3′ phosphatase (*PNKP*) gene.

MATERIALS AND METHODS

Blood samples were obtained from the patient and her parents for whole exome sequencing. Parental consent was obtained using IRB guidelines. Exome capture and sequencing were facilitated by the Agilent QPCR NGS Library Quantification Kit and the Illumina Platform using TruSeq v3 chemistry to a mean target coverage of 70x. Reads were mapped in the hg/19/b37 build of the human genome using the Burrows–Wheeler Aligner package. The Genome Analysis Tool Kit (GATK) was used to locally realign the mapped reads around potential indels and the covariance recalibration function was used to recalibrate the Base quality (Phred scale) scores. SNP and indel variations were called using the GATK Unified Genotyper for each sample, and SNP novelty was determined against dbSNP. Variants were annotated with gene and gene function data from Ensembl, and the variant data files were anlaysed using an annotation and prioritization pipeline. The patient's exome data were initially filtered for genes involved in Mendelian inheritance in humans. Subsequently, this subset of the data was analyzed for mutations that could explain the patient's phenotype, based on literature review and expression profile. Special emphasis was placed on homozygous variants, considering the presence of consanguinity in the family. The clinically relevant variant thus identified was confirmed by Sanger sequencing. The conservation of the amino acid residues was evaluated using Homologene (www.ncbi.nlm.nih.gov/homologene), and multiple sequencing alignments of the protein amino acid sequences were generated using Clustal (www.clustal.org).

RESULTS

The patient (IV.2, Fig. 1A) was born at 40 weeks gestation via spontaneous vaginal delivery. Her birth weight (1.8 kg), length (41 cm), and head circumference (26.5 cm) were all below the 3rd centile. She was noted to have intrauterine growth retardation, microcephaly, shoulder dystocia, and dysmorphic facial features. She was the first child born to healthy first cousin parents. The mother had previously had a spontaneous abortion at 4 weeks gestation. A detailed pedigree analysis (Fig. 1A) revealed that a common first cousin of the parents had three sons born of a consanguineous union who died of juvenile neurodevelopmental regression before the age of 15 years. Their medical reports were not available for study.

At 3 months of age, she was admitted with seizures, and was started on sodium valproate. She was also found to have mild gastroesophageal reflux and was put on anti-reflux medication. Developmental delay was noted at 7 months. At 16 months of age, she produced incomprehensive sounds and was unable to focus or follow, to elevate her head when prone or to turn to the side. She was not creeping, not reaching towards objects, not recognizing her mother, nor responding to her name. Her height, length, and head circumference continued to be under the 3rd centile at 7, 10, and 16 months follow-up visits (Fig. 2F). No hyperactivity in behavior was observer or reported.

The patient's clinical examination at 16 months of age revealed an alert girl. Her vital signs were as follows: temperature—36.5°C, heart rate—102/min, respiratory rate—30/min, blood pressure—92/56 mmHg. Dysmorphic craniofacial features included microcephaly, sloping forehead, exophthalmos, long eyelashes, depressed nasal bridge, short and bulbous nose, micrognathia, bilateral periauricular skin tags (right larger than left), and short neck (Fig. 2A and B). Ear, nose, and throat exam was normal. Heart sounds were normal with no murmur. Chest examination revealed good bilateral air entry with no additional sounds. The abdomen was soft with no organomegaly. A perianal skin tag with fissure at the 12 o'clock position and another anal fissure with open anal verge at the 6 o'clock position were detected. She had normal external female genitalia. Anterior fontanelle was closed. Neurologic examination noted central hypotonia and peripheral hypertonia with brisk deep tendon reflexes. She had spindle shaped fingers, with hypoplasia of the right thenar muscle, and a proximally placed right thumb. The patient had dry skin with a Mongolian spot on the lower back and a small café-au-lait macule at the dorsum of the left thumb.

Her investigations including TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes simplex, and HIV) screen, metabolic work-up, karyotype, and chromosome microarray all yielded normal results. Abdominal ultrasound showed mild gastroesophageal reflux, and her skeletal survey revealed generalized decrease in bone density with a small cranial vault, indicating microcephaly. Brainstem evoked response audiometry showed moderate hearing loss in the right ear and normal hearing in the left ear, while tympanogram revealed bilateral otitis media with effusion. Her echocardiogram was normal.

Generalized epilepsy with some degree of burst suppression was noted on the electroencephalogram (EEG). Computerized tomography (CT) of the brain showed hypo-attenuation mainly involving the frontal and occipital lobes of both cerebral hemispheres. This may represent diffuse ischemic changes or features of prematurity. A rim of hyper-attenuation was seen in the retrocerebellar space which may represent subdural hematoma or prominent sinuses space. Lateral ventricles appeared widely separated and parallel, representing dysgenesis of corpus callosum. Oronasopharynx CT showed left sided choanal atresia, with left maxillary and left ethmoidal sinusitis. Brain MRI revealed moderate symmetrical widening of the supra and infratentorial cerebral ventricles, with no deformity or midline shift. This was accompanied by prominent cortical sulci, widened sylvian fissures, basal cisterns, and extra-axial CSF spaces (Fig. 2C–E). These results were suggestive of brain atrophy.

Whole exome sequencing detected a likely causative variant in *PNKP*, which was the only variant identified by the annotation and

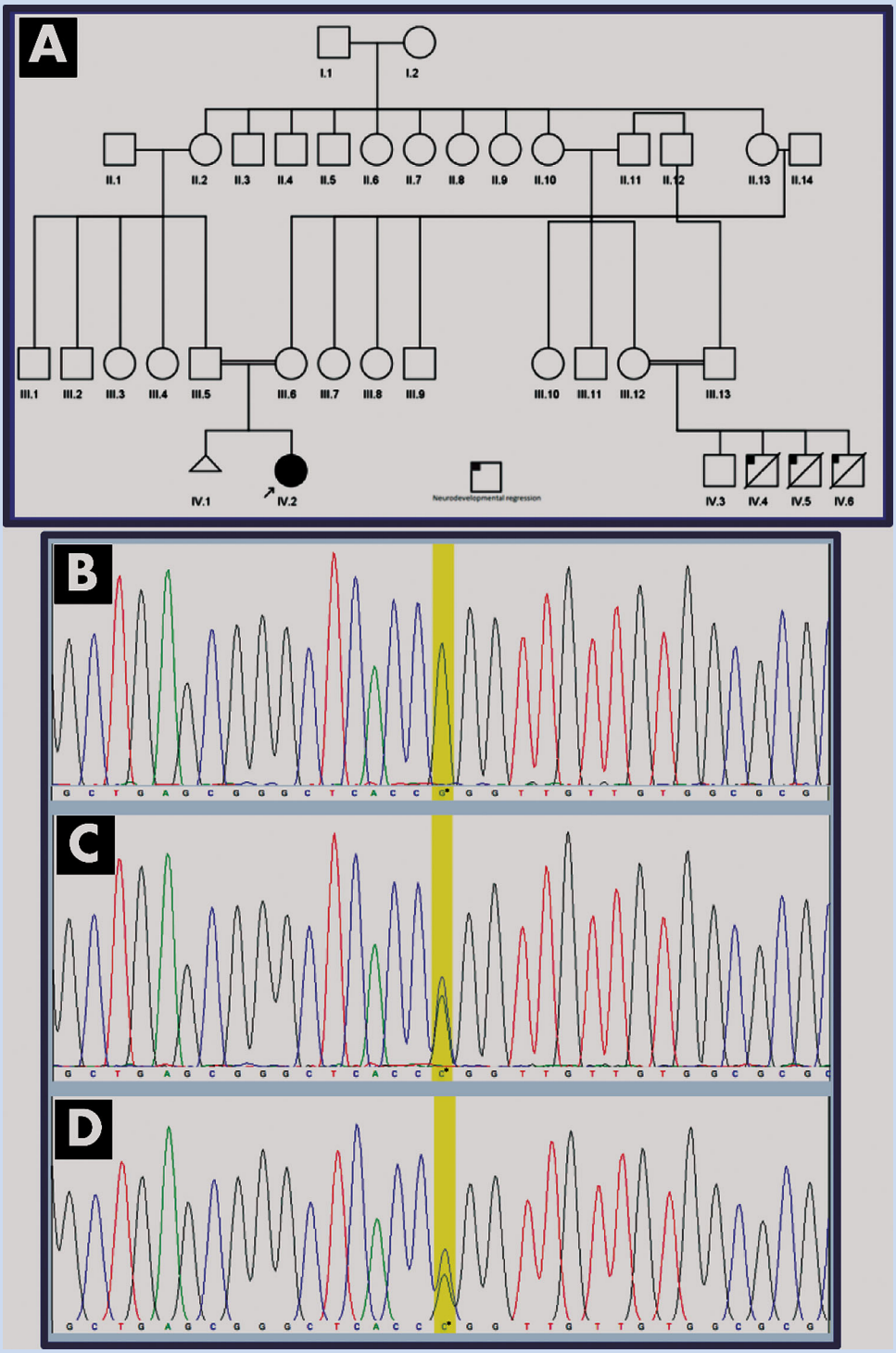

FIG. 1. (A) Family pedigree. Arrowhead denotes the patient. Symbols filled only on the upper left corner denote members with neurodevelopmental regression. Chromatograms showing the c.1385G > C mutation. (B) is the sequence of the patient showing the homozygous mutation, (C) and (D) are sequences from the parents that show the heterozygous condition. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

prioritization pipeline. The variant, located at genomic position 19;50,364,866, was a c.1385G > C mutation in exon 15, was predicted to result in a p.Arg462Pro amino acid change in the PNK protein. The mutation was found in homozygous form in the patient, and in heterozygous form in both parents. The presence of the mutation in the patient and the parents was confirmed by Sanger sequencing (Fig. 1B–D). This variant has been reported in the Exome Aggregation Consortium (ExAC) database with an allele

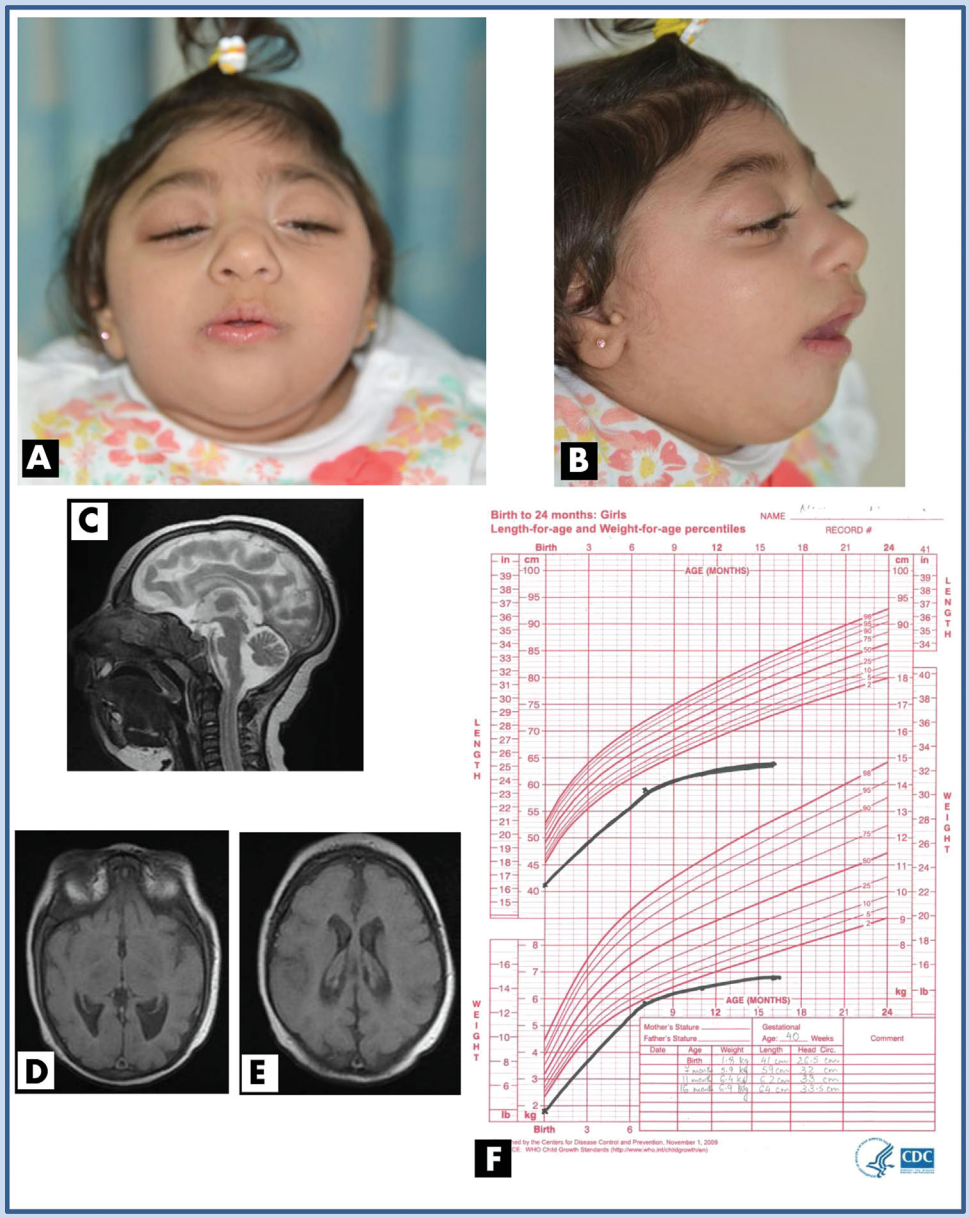

FIG. 2. (A) and (B) Overview of dysmorphic facial features. The patient shows the presence of a sloping forehead, short nose with a depressed nasal bridge, short neck, and preauricular tags. MRI brain of patient show prominent cortical sulci and widened sylvian fissures, basal cisterns, and extra-axial CSF spaces, (C) is a T2 weighted sagittal view, (D) and (E) are T2 weighted coronal views. (F) Patient's body weight and length plotted on percentile charts showing both parameters to be consistently below the 3rd centile. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

frequency of 0.0001057. The arginine residue at p.462 was found to be fairly conserved across species (Fig. 3). The mutation was classed as "probably damaging" by PolyPhen-2. I-Mutant2.0 predicted a Gibbs free energy change of −1.63 with the mutation, indicating a decrease in protein stability.

DISCUSSION

The Polynucleotide kinase 3′ phosphatase (PNK) protein is a key enzyme involved in the repair of single strand breaks (SSB), which are toxic DNA lesions that arise endogenously during normal cellular metabolism and impair genomic integrity. Since the enzyme contains both 3′ phosphatase as well as 5′ kinase activities, it is capable of carrying out end processing of damaged termini at the SSBs [Jilani et al., 1999], as well as double stranded break repair by being a functional part of both non-homologous end joining (NHEJ) and base excision repair (BER) pathways. These breaks particularly affect neurons, due to the high transcriptional demand in these cells and the high level of oxidative stress to which the nervous system is exposed [Reynolds and Stewart, 2013].

FIG. 3. Multiple alignment of PNKP protein sequences using HomoloGene showing the conserved nature of the Arg 462 residue, which is underlined.

Mutations in *PNKP* were found to be causal for MCSZ in three Palestinian families [Shen et al., 2010]. Apart from MCSZ, defects in *PNKP* are also implicated in the development of other neurodegenerative disorders, as pathogenic mutations in this gene resulting in ataxia-oculomotor apraxia 4 (OMIM #616267) [Bras et al., 2015]. In addition, a functional deficiency of the enzyme has been etiologically linked to at least one other hereditary neurological disorder, Machado–Joseph disease (OMIM #109150) [Chatterjee et al., 2015].

Microcephaly, seizures, and developmental delay (MCSZ; OMIM #613402) is a rare autosomal recessive disorder. The few patients reported so far show a considerable degree of phenotypic heterogeneity. This phenotype ranges from a classical form with no evidence of brain atrophy or clinical regression [Shen et al., 2010] to a neurodegenerative phenotype, exemplified by progressive cerebellar atrophy and sensorimotor peripheral neuropathy [Poulton et al., 2013]. The epileptic phenotype is also variable. Patients with the classic form of the condition were described to have severe early-onset infantile epileptic encephalopathy, while the ones affected with the progressive neurodegenerative form had a less severe epileptic form with occasional seizures that were found to improve with time. Our patient's phenotype overlaps both forms of the condition (Table I). In her case, the seizures were severe, in the form of tonic-clonic convulsions, and lasted for about 45 min. However, unlike other patients with the classic form of MCSZ, our patient showed evidence of brain atrophy and agenesis of the corpus callosum.

Interestingly, none of the reported patients with MCSZ have displayed a phenotype of retarded physical growth. The association of microcephaly with intrauterine growth retardation continuing into growth deficiency in our patient points to the possibility of a novel form of microcephalic primordial dwarfism syndrome. This hypothesis is supported by recent studies that have linked mutations in genes involved in the NHEJ pathways, such as *XRCC4* and *LIG4*, to certain forms of PD [IJspeert et al., 2013; Shaheen et al., 2014; Murray et al., 2015].

TABLE I. Overview of PNKP Mutations and Their Varied Phenotypes Reported in MCSZ

Mutation	Seizures	Behavioural phenotype	Cerebral structure	Neuropathy	Primordial dwarfism	Study
c.1250_1266dup	Febrile seizures that reduced in frequency with time	Hyperactivity	Severe cerebellar atrophy	Motor and sensory demyelinating	No	Poulton et al. [2013]
c.975G > A (p.E326K)	Frequent refractive seizures	Hyperactivity	Grossly normal cerebral structures, with only slight thinning of corpus callosum	None	No	Shen et al. [2010]
c.1250_1266dup and g.5645_5662del	Frequent refractive seizures	Hyperactivitys	No structural malformations reported, white matter volume slightly reduced	None	No	Shen et al. [2010]
c.526C > T (p.L176F) and c.1250_1266dup	Seizures controlled by medication	Hyperactivity	Simplified gyral pattern, slightly thinned corpus callosum	None	No	Shen et al. [2010]
c.1385G > C	Severe tonic-clonic convulsions	No hyperactivity reported	Cerebellar atrophy, agenesis of corpus callosum	No evidence of neuropathy	Yes	This study

The *PNKP* mutation identified in our patient is predicted to cause a p.Arg462Pro change in the protein. This is a highly conserved residue that lies in the kinase domain, and specifically in a P-loop containing nucleoside triphosphate hydrolase fold of the protein. Of the four other MCSZ-related *PNKP* mutations that have so far been reported, two missense mutations, Glu326Gln and Leu176Phe lie in the phosphatase domain; while the two frameshift mutations, c.1253_1269dup, and g.5645_5662del, lie within the kinase domain. All four mutations have been experimentally shown to have significantly reduced stability in vivo [Reynolds et al., 2012]. It is likely that the mutation described by us also reduces stability, as predicted by I-Mutant 2.0 [Capriotti et al., 2005]. In the wild-type protein, the Arg 462 residue forms a part of the lid subdomain helix of the P-loop kinase, which interacts with bound ATP. Although this residue is positioned away from the nucleotide binding site [Bernstein et al., 2005], the introduction of a proline residue into the helix is likely to affect the structure of the protein. Since the mutation occurs at the 3' edge of exon 15, we tried to assess its impact on the splice site. SplicePort (http://spliceport.cbcb.umd.edu/) computational scores of 0.198 for the wild-type versus 1.08 for the mutant sequence indicate that the mutation does not alter the splice site.

In conclusion, we have identified a mutation in *PNKP*, leading to a phenotype of microcephaly with primordial dwarfism. Although her phenotype resembles that of MCSZ, our patient's phenotype differed from that of other reported cases of MCSZ by showing evidence of brain anomalies and agenesis of corpus callosum in the presence of severe seizures, absence of hyperactivity, and more importantly, being associated with primordial dwarfism. PD has been associated with microcephaly in only a few clinical conditions and never in association with *PNKP* mutations. We, therefore, suggest a novel phenotype of microcephalic primordial dwarfism associated with *PNKP* gene mutation.

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