PRUNE Syndrome Is a New Neurodevelopmental Disorder: Report and Review

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Abstract
PRUNE syndrome, or neurodevelopmental disorder with microcephaly, hypotonia, and variable brain anomalies (OMIM#617481), is a new rare autosomal recessive neurodevelopmental disease that is caused by homozygous or compound heterozygous mutation in PRUNE1 on chromosome 1q21. Here, We report on 12-month-old and 30-month-old girls from 2 unrelated Saudi families with typical presentations of PRUNE syndrome. Both patients had severe developmental delay, progressive microcephaly, and dysmorphic features. Brain magnetic resonance imaging showed slight thinning in the corpus callosum, mild frontal brain atrophy, and delayed myelination in one of the patients. Both patients had the same missense mutation in PRUNE1 (c.383G>A, p.Arg128Gln), which was not reported before in a homozygous state. We compared our patients to previously reported cases. In conclusion, We suggest that clinicians consider PRUNE syndrome in any child presenting with dysmorphic features, developmental delay, progressive microcephaly, central hypotonia, peripheral spasticity, delayed myelination, brain atrophy, and a thin corpus callosum.

Keywords
PRUNE syndrome, PRUNE1, progressive microcephaly, spasticity, developmental delay

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The PRUNE1 gene is a member of the DHH (Asp-His-His) phosphoesterase group, which are the enzymes that break phosphoester bonds in a wide range of substrates.1 Additionally, it has a role in cell migration.2 Recently, it was shown that PRUNE plays a role in neuronal migration and proliferation, which was related back to its regulatory effect on microtubule polymerization. The change in tubulin dynamics could be related to the patients’ microcephaly and other neurological derangements.3

PRUNE syndrome, or neurodevelopmental disorder with microcephaly, hypotonia, and variable brain anomalies (OMIM#617481), is a new rare autosomal recessive neurodevelopmental disease that is caused by homozygous or compound heterozygous mutation in the PRUNE1 gene on chromosome 1q21.4 It was first described by Karaca et al, who reported 4 patients from 4 different families. The patients had a distinct form of dysmorphic features and global developmental delay associated with microcephaly, central hypotonia, spastic quadriplegia, cerebral atrophy, and cerebellar atrophy. The main dysmorphic features were sloping forehead, flat nasal bridge, abnormal dentition, widely spaced teeth, micrognathia,

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and low-set ears. Several patients were noted to have seizures. This report was followed by 2 additional reports in 2017. The first reported 14 patients from different countries: Turkey, Saudi Arabia, United States, Oman, India, Iran, and Italy. Two of the 14 patients were reported by the same group in the previous study. Costain et al reported an additional patient with PRUNE1 mutation. These recent reports confirmed that PRUNE syndrome is pan-ethnic. The diagnosis was confirmed in all reported patients by finding a homozygous or compound heterozygous mutation in the PRUNE1 gene. As this is a newly described disease, there are scarce data in the literature about clinical and radiological phenotypes. In this case report, We elaborate more on the clinical, radiological, and molecular findings of 2 unrelated Saudi probands with the PRUNE syndrome and compare our results to the previously reported cases. To the best of our knowledge, this is the first report in the literature to confirm a homozygous missense mutation (p.Arg128Gln) in the PRUNE1 gene and the fourth report in the literature to examine this new neurogenic disorder. In this report, We expanded the genotype of PRUNE syndrome.

Methods

Patients

This is a retrospective chart review of patients from genetics and neurology clinics at King Abdulaziz Medical City and Prince Sultan Medical City at Riyadh, Saudi Arabia.

Molecular Studies

This study was done at accredited laboratories using the following methods.

Patient 1. Targeted exome sequencing using genomic DNA from the patient’s specimen, the exonic regions, and flanking splice junctions of the gene were sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100 base pair or greater paired-end reads. Reads were aligned to human genome build GRCH37UCSC hg19 and analyzed for sequence variants in the greater paired-end reads. Reads were aligned to human genome build GRCH37UCSC hg19 and analyzed for sequence variants in the selected genes or regions of interest using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing was used to confirm all potentially pathogenic variants identified. Segregation analysis was done to both parents after discovering the mutation in the index case.

Patient 2. Whole-exome sequencing was done to the index case and both parents: Approximately 37 Mb (214 405 exons) of the consensus coding sequences were enriched from fragmented genomic DNA by >340 000 probes designed against the human genome (Nextera Rapid Capture Exome, Illumina) and the generated library sequenced in an Illumina platform to an average coverage depth 70-100×. An end-to-end in-house bioinformatics pipeline including base calling, primary filtering of low quality reads and probable artifacts, and annotation of variants was applied. All disease-causing variants reported in HGMD and ClinVar as well as all variants with minor allele frequency of less than 1% in ExAC database are considered. Evaluation is focused on exons and intron boundaries ±20.

Literature Review

A thorough review of the literature for all reported PRUNE disease cases or mutations in PRUNE1 gene was done using a systematic search in PubMed database.

Case Report

Patient 1

Patient 1 was a full-term baby girl born by normal spontaneous vaginal delivery. She had a birth weight of 2.7 kg (−1.1 standard deviation [SD]), was 46 cm in length (−1.5 SD), had a head circumference of 33 cm (−0.5 SD), and had an Apgar score of 8 and 9 at 5 and 10 minutes, respectively. After delivery, she stayed in the hospital for 1 day due to jaundice. The first concern by the parents was at 1 year of age due to global developmental delay. At that time, she was unable to walk or crawl or even sit; she just laid in the bed and had no speech. Then, she was referred to a genetics service at 16 months of age for further evaluation, where her examination showed the following growth parameters: weight 8.8 kg (−0.8 SD), length 46 cm (−0.6 SD), and head circumference 44.5 cm (−1 SD). She had dysmorphic features ( plagiocephaly, macrocephaly, epicanthal folds, hypertelorism, flat nasal bridge, abnormal dentition, widely spaced teeth, micrognathia, low-set ears, and hirsutism; Figure 1A). A neurological examination showed central hypotonia and spastic quadriplegia with hyperreflexia and clonus. Other systemic examinations were unremarkable. Currently, on examination at 30 months, she continued to have progressive microcephaly (head circumference 45 cm [−2.1 SD], length 83 cm [−2.1 SD], and weight 10 kg [−2 SD]). Development was severely delayed as she could only sit with support, had inconsistent hand grasp, and produced sound without any words. She is functioning at the level of 6 months of age and continues to have central hypotonia and spastic quadriplegia with hyperreflexia and clonus. A study of her upper GI showed moderate to severe gastroesophageal reflux with no malrotation. A milk scan showed significantly delayed gastric emptying. The auditory brainstem response test was normal. An ophthalmology examination showed bilateral rudimentary iris strands. Brain magnetic resonance imaging (MRI) showed delayed myelination, slightly abnormal shape of the corpus callosum, and mild frontal cerebral atrophy (Figure 1B and C). A skeletal survey and echocardiogram were unremarkable. Extensive biochemical and molecular genetics investigations, including ammonia level, lactic acid, creatine kinase level, total homocysteine, acylcarnitine profile, urine organic acids, and chromosomal analysis, were unremarkable. CGH microarray showed multiple blocks of homozygous sequence encompassing 46 Mb (1.6%) of autosomal genomic length with no apparent chromosomal derangements. Targeted exome sequencing for these blocks showed a previously reported homozygous missense mutation in PRUNE1 gene (c.383G>A, p.Arg128Gln; NM_021222.1). Both parents were homozygous for this mutation.
Patient 2 was a full-term baby girl born by normal spontaneous vaginal delivery. Her birth weight was 2 kg (−3 SD), her length was 45.5 cm (−2 SD), her head circumference was 33 cm (−0.5 SD), and her Apgar scores were 8 and 9 at 5 and 10 minutes, respectively. Family history showed positive parental consanguinity. Additionally, 1 cousin died with similar phenotype. The patient showed severe global developmental delay, mainly in motor function: At 12 months of age, the patient was smiling and fixating but could not turn from side to side and had no grasp. There was no history of seizures. On examination, the growth parameters were as follows: weight 6.7 kg (−2.5 SD), length 67 cm (−2.5 SD), microcephaly was present, and head circumference 43 cm (−2.3 SD). Apart from the microcephaly, she did not have any other dysmorphic features. She was fixating, following, and smiling. There was axial hypotonia and appendicular spasticity with deep tendon reflexes +3. The ophthalmology examination was unremarkable.

The MRI brain showed a slightly abnormally shaped corpus callosum and slightly prominent CSF spaces anteriorly with normal myelination. Magnetic resonance spectroscopy was unremarkable.

Whole-exome sequencing showed homozygous mutation in the PRUNE1 gene: c.383G>A p.(Arg128Gln). Both parents were heterozygous for this mutation.
Discussion

Our report showed similar findings delineated by previous reports. Table 1 compared the clinical findings in this report with the former ones. In 19 children, all had the following cardinal features: severe global developmental delay, no speech, the patients just laid in the bed and did not acquire any milestones, appendicular hypotonia, and spastic quadriplegia, in addition to hyperreflexia, which was reported in the majority of the patients. Dysmorphic features, as mentioned above, were reported in all previous cases. Plagiocephaly and progressive microcephaly ranging between −2 and −6 SD were detected in 79% of the cases. Eye findings including congenital cataracts and optic atrophy were reported in approximately 21% of the cases. In this study, patient 1 had rudimentary iris strands, which was not reported previously. Further studies are needed to prove the correlation of PRUNE1 mutation with the described ocular phenotype. Approximately 50% of the patients had seizures, including generalized tonic-clonic seizures, myoclonic seizures, and

Table 1. Demographic and Clinical Data of the Presented Cases Compared to Previously Reported Cases.

|                           | Patient 1 | Patient 2 | Previously Reported Cases | Total (%) |
|---------------------------|-----------|-----------|---------------------------|-----------|
| Demographic               |           |           |                           |           |
| Number of patients        | 1         | 1         | 17                        | 19        |
| Number of families        | 1         | 1         | 9                         | 11        |
| Age at evaluation (year)  | 2.5       | 1         | 0.3-21                    | 0.3-21    |
| Sex (M:F)                 | 0:1       | 0:1       | 7:10                      | 7:12      |
| Origin                    | Saudi Arabia | Saudi Arabia | Turkish, Saudi Arabia, United States, Oman, India, Iran, and Italy |
| Racial ethnic backgrounda | Arab      | Arab      | Arab, Turkish, and Oji-Cree |
| Consanguinity             | No        | Yes       |                           | 12/17     |
| Clinical phenotypes       |           |           |                           | 13/19 (68.4%) |
| Dysmorphic features       | Yes       | No        | 17/17                     | 18/19 (94.7%) |
| Plagiocephaly             | Yes       | Yes       | 13/17                     | 15/19 (78.9%) |
| Congenital cataract       | Yes       | No        | 3/17                      | 4/19 (21%) |
| Optic atrophy             | No        | No        | 2/17                      | 2/19 (10.5%) |
| Rudimentary iris strands  | Yes       | No        | 0/17                      | 1/19 (5.2%) |
| Global developmental delay| Yes       | Yes       | 17/17                     | 19/19 (100%) |
| Nonverbal                 | Yes       | Yes       | 17/17                     | 19/19 (100%) |
| No independent ambulation | Yes       | Yes       | 17/17                     | 19/19 (100%) |
| Progressive microcephaly  | Yes       | Yes       | 16/17                     | 18/19 (94.7%) |
| Axial hypotonia           | Yes       | Yes       | 17/17                     | 19/19 (100%) |
| Spastic quadriplegia      | Yes       | Yes       | 16/17                     | 18/19 (94.7%) |
| Hyperreflexia             | Yes       | Yes       | 13/17                     | 15/19 (78.9%) |
| GER                       | Yes       | No        | NA                        |           |
| Seizure                   | No        | No        | 9/17                      | 9/17 (47.3%) |
| Brain MRI                 |           |           |                           |           |
| Delayed myelination       | Yes       | No        | 15/17                     | 16/19 (84.2%) |
| Thin corpus callosum      | Abnormal shape | Abnormal shape | 6/17                     | 8/19 (42.1%) |
| White matter disease      | No        | No        | 6/17                      | 6/19 (31.5%) |
| Cerebral atrophy          | Yes       | Yes (mild)| 6/17                      | 8/19 (42.1%) |
| Cerebellar atrophy        | No        | No        | 6/17                      | 6/19 (31.5%) |
| Molecular findings        |           |           |                           |           |
| Mutation in PRUNE1 gene   | p.Arg128Gln | p.Arg128Gln | p.Asp30Asn, p.Pro54Thr, p.Asp106Asn, p.Arg297Trp, p.Gly174X |
| Types of mutation         | Homozygous missense | Homozygous missense | Homozygous or compound heterozygous, missense or nonsense, in addition to one splice site mutation |

Abbreviations: F, female; GER, gastroesophageal reflux; M, male; MRI, magnetic resonance imaging; NA, not available.

aThe racial ethnic background of the Italian, Iranian, and Indian patients are not available in the original report.
infantile spasms. An electroencephalogram (EEG) was performed in 1 patient who had infantile spasms and showed a higher degree of hypsarrhythmia on the right posterior temporal region. The patients presented herein had no seizures, and their EEGs were normal. One of our patients had gastroesophageal reflux, which was not reported previously.

Radiological results display that most of the patients have delayed myelination, which is reported in 84.2% of the cases, and approximately one-third of the patients have a thin corpus callosum, white matter disease, and cerebral and cerebellar atrophy. It is clear that PRUNE syndrome features are mainly neurological in nature, and this is partly explained by the fact that PRUNE1 is highly expressed in the brain and plays a significant role in neuronal cell proliferation, motility, and migration. The significant similarity of the clinical phenotype indicates that all of the above features are distinctive components of this novel neurodevelopmental disorder. Several novel genes have been recently described with the following main features: intellectual disability, progressive microcephaly, spasticity, delayed myelination, and a thin corpus callosum. For example, the SLC1A4 gene defect was reported in 4 Ashkenazi Jews and the RUSC2 gene defect was detected in 3 children from Saudi Arabia. The newly described diseases expand the differential diagnosis of this phenotype (progressive microcephaly, intellectual disability, thin corpus callosum, and axial hypotonia with peripheral spasticity), which might affect the protein features.

Most of the previously reported cases have homozygous missense variants (90%). Only 1 patient had a splice site mutation, and another patient had compound heterozygous for missense and nonsense mutations. The high rate of homozygous mutations can be explained by the elevated rate of consanguinity in the reported cohort. Interestingly, there have been no reported deletions, duplications, or homozygous nonsense mutations so far. Such observation may be due to the small sample size or the severity of the mutation may not be compatible with survival until birth. Subsequent larger studies are needed to determine a proper genotype–phenotype correlation. Most of the reported mutations were clustered in the DHH domain of the protein, except the p.Arg297Trp mutation, which is located in DHHA2 domain (Figure 2).

In conclusion, the authors want to alert clinicians to consider PRUNE syndrome in any child who presents with dysmorphic features, profound developmental delay, progressive microcephaly, central hypotonia, peripheral spasticity, delayed myelination, brain atrophy, and a thin corpus callosum. Animal model research are vital to understanding the pathophysiology of PRUNE syndrome, which may result in a better understanding of the disease in order to pave the way for future discovery of a treatment for this disorder.
Author Contributions

MA and BT contributed to conception and design; contributed to acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. MN contributed to acquisition, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. KH contributed to conception, contributed to acquisition, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. AAH contributed to conception, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. AA contributed to design, contributed to acquisition, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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Ethical Approval

The study was approved by the IRB office at King Abdullah International Research Center (RC 16/113/R).

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