Bi-allelic mutations in PRUNE lead to neurodegeneration with spinal motor neuron involvement and hyperCKaemia

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We aimed to systematically investigate the neuromuscular involvement of individuals with PRUNE mutations who may have a major spinal motor neuron involvement as part of the PRUNE-associated neurodegenerative phenotype. The complex neurological phenotypes associated with Prune mutations include microcephaly with brain abnormalities, spasticity, seizures, severe developmental delay and developmental regression. We used whole exome sequencing to identify the mutation and electrophysiological and muscle biopsy studies to evaluate the signs of spinal motor neuron involvement. The affected individuals carry homozygous PRUNE mutation (NM_021222.1, c.316G>A, p.D106N), showing the signs of spinal motor neuron involvement supported by electrophysiological and muscle biopsy findings and also persistent high creatine kinase levels. We confirm that individuals with PRUNE mutations may have a major spinal motor neuron involvement as part of the PRUNE-associated neurodegenerative phenotype. The PRUNE gene should be considered in all the individuals with non-5q spinal muscular atrophy. High creatine kinase values may be a part of PRUNE disease spectrum.

Key words: PRUNE, spinal motor neuron, exome, creatine kinase, hyperCKaemia.

The PRUNE gene encodes prune exopolyphosphatase that is a member of the phosphoesterases (DHH) protein superfamily.¹ In the past two years, PRUNE gene mutations have been identified in individuals with complex neurological phenotypes. It was demonstrated that PRUNE gene is highly expressed in the human fetal brain and plays a role in the regulation of the cell migration and correct development of the central nervous system.²³ Clinical and genetic evidence supports that bi-allelic mutations in PRUNE gene is the cause of a new neurodevelopmental disorder characterized with microcephaly, cortical, cerebellar atrophy and global developmental delay.³⁴ PRUNE was firstly described by Karaca et al.³ as a candidate disease-causing gene for a new neurodevelopmental and neurodegenerative disorder. In 2015, Karaca et al.³ demonstrated PRUNE gene mutations in four families among 128 mostly consanguineous families with neurogenetic disorders including brain malformations. All reported variants were located within the catalytic phosphoesterases (DHH) domain of the PRUNE protein.
The common features of these patients were microcephaly, fronto-temporal cortical atrophy, thin or hypoplastic corpus callosum and cerebellar atrophy. Seizures, severe developmental delay and developmental regression were the cardinal features reported at the presentation. In 2017, Zollo et al. investigated 15 individuals affected by a neurodevelopmental and neurodegenerative disorder. PRUNE mutations associated with the impairment of microtubule polymerization, cell migration and proliferation were confirmed in all the individuals and defined PRUNE as a molecule foundational for normal human cortical development.

We report the second additional affected individual with a homozygous PRUNE mutation showing signs of spinal motor neuron involvement supported by electrophysiological and muscle biopsy findings. The persistent high creatine kinase levels of this patient was another striking finding.

Case Report

We report on a currently twelve-month-old Turkish boy who is under investigation and treatment of psychomotor retardation and intractable seizures in our neuropediatric clinic. After an uneventful pregnancy the patient was born at term from healthy consanguineous parents. Although the Apgar scores were 10/10, the patient developed significant respiratory difficulties just approximately one hour after birth, requiring mechanical ventilation in the neonatal intensive care unit. Myoclonic seizures also occurred frequently thus the patient was treated with levetiracetam and phenobarbital. After 80 days the patient was discharged without antiepileptic drugs and without assisted ventilation, but was re-admitted to the emergency department because of sudden onset respiratory insufficiency just two days later. At the age of 3 months, his body weight was 3.3 kg (0.5th percentile), height was 56 cm (19th percentile), and head circumference was 38 cm (14th percentile). The developmental milestones were prominently delayed; so far he could not hold his head up, smile and follow objects. His appearance was mildly dysmorphic with a wide forehead, retrognathia, hypertelorism, long philtrum and pectus excavatum (Fig. 1A). On examination, he had low muscle tone with poor sucking and had failure to thrive. Deep tendon reflexes were reduced. Babinski sign and cloni were present.

Fig. 1. Phenotypic features, electrophysiological and pathological findings of the patient: (A) Photograph of the patient at the age of 12 months; he had flask paralysis; he was feeding via nasogastric tube and required tracheostomy. (B) Interictal EEG recording slowed disorganized rhythm with multifocal epileptic activity. (C) EMG of the patient: slight effort of the right quadriceps muscle showed reduced giant motor unit potentials. (D, E) The muscle biopsy showed clustered distributed atrophic type 2 fibers (a: H&E, 20x, b: slow myosin immunostaining 20x).
In a few days after admission, deterioration became evident and the patient had significant respiratory insufficiency requiring mechanical ventilation. Tracheostomy was needed at the age of 5 months. During the clinical course, the patient had generalized tonic clonic and myoclonic seizures and epileptic spasms, which were resistant to antiepileptic drugs (levetiracetam, phenobarbital and vitamin B6). Electroencephalography showed disorganized rhythm with multifocal epileptic activity (Fig. 1B).

Laboratory studies including complete blood count and metabolic evaluations (urine organic acids, plasma acylcarnitines, plasma amino acids, ammonia, lactate, pyruvate, biotinidase, and long chain fatty acids) revealed no abnormalities. TORCH serology was negative. Creatine kinase levels ranged from 343 to 1940 U/L (normal values: 0-171 U/L). Echocardiogram and abdominal ultrasonography were normal. Ophthalmological examination was unremarkable. The patient had two MRI (magnetic resonance imaging) brain scans, performed respectively at ages of 6 weeks and 4 months and both of them showed non-specific abnormalities like mildly dilated lateral ventricles and a small posterior arachnoid cyst (data not shown). Nerve conduction studies were normal but compound muscle action potentials were low for his age. Needle electromyography revealed positive sharp waves, fibrillations, and polyphasic motor unit potentials with increased amplitude and duration (Fig. 1C). The muscle biopsy showed atrophic muscle fibers in small groups (Fig. 1D) but also randomly distributed. The atrophic fibers were shown to be type 2 fibers by fast myosin immunostaining (Fig. 1E). Type 1 fiber predominance was appreciated. Overall, the muscle biopsy was evaluated to show signs of a neurogenic atrophy.

Karyotype analyses and microarray CGH were normal. Subtelomeric FISH analysis for Prader-Willi syndrome was negative. Diagnostic sequencing of GAA did not show any pathogenic mutations. SMN1 mutations were excluded by repeated testing in independent diagnostic laboratories. Epileptic encephalopathy next generation sequencing panel testing including SCN1A, SCN1B, GABRG2 did not unravel any pathogenic mutations. Thus, we proceeded with whole exome sequencing using the Agilent V6 whole exome kit on the IlluminaHiSeq 4000 with 2x75bp reads and a mean coverage of x83 fold. Data analysis and variant filtering for rare autosomal recessive inheritance, as well as for de-novo dominant or X-linked using the internal Varbank pipeline (https://varbank.ccg.uni-koeln.de/) as in detailed described earlier. We found the homozygous PRUNE mutation (NM_021222.1, c.316G>A, p.D106N) which was described earlier. The parents confirmed to be heterozygous. Despite extensive filtering, we could not find any additional disease causing mutation in a neuromuscular disease gene that might have explained the hyperCKaemia and spinal motor neuron involvement. Informed consent was received from the family for publication.

Discussion

Prune is an exopolyphosphatase that is a member of the phosphoesterases (DHH, Asp-His-His) protein superfamily, consisting of two motifs DHH and DHHA2. Prune was identified as a glycogen synthase kinase 3 (GSK-3β)-binding protein. GSK-3β is a crucial inhibitory regulator of many neuronal functions including neurite outgrowth, synapse formation, neurogenesis and survival, which indicates indirectly a role of Prune in brain development. Zollo et al. showed mutations in both DHH and DHHA1 impair cell differentiation, proliferation and migration properties, also impair microtubule polymerization activity. However, mutation in DHH such as p.D30N revealed a delay in microtubule formation affecting mainly growth phase, while the mutation in DHHA2 p.R297W negatively influences the early growth rate of microtubule polymerization processes.

Most patients with PRUNE gene mutations reported by Zollo et al. had severe psychomotor delay, progressive microcephaly, spasticity and seizures. Respiratory insufficiency was not reported for any individual in this study. In 2017, Iacomino et al. reported a 9 month-old Italian child with homozygous PRUNE mutation who suffered a complex respiratory insufficiency requiring early intubation at
birth. On examination, he had dysmorphic appearance and respiratory distress with diaphragmatic weakness. The remarkable findings noticed on his neurological evaluation were severe muscular hypotonia with diffusely reduced tendon reflexes, poor spontaneous movements, distal joint arthrogryposis and bilateral talipes equinovarus. Muscle biopsy findings revealed signs of a neurogenic muscle atrophy with fibrotic replacement and electromyography displayed polyphasic motor unit potentials with increased amplitude and duration. Clinical signs, muscle biopsy and electrophysiology features pointed to a lower motor neuron disease. Here, we report confirming affected patient with the p.D106N PRUNE mutation presenting with similar overt signs of spinal motor neuron involvement. He had global developmental delay and profound muscular hypotonia. He could not hold his head up, smile and follow objects. He had flak paralysis with diffusely reduced deep tendon reflexes. Feeding was via nasogastric tube and he required tracheostomy at the age of five months because of respiratory insufficiency. Needle electromyography findings were compatible with neurogenic involvement. The muscle biopsy showed the atrophy of type 2 fibers and the predominance of type 1 fibers were also consistent with findings reported in some SMA patients since denervation may cause single fiber atrophy. Small atrophic groups were observed in some areas (Fig. 1D, 1E) in our patient’s muscle biopsy, supporting the impression of a neurogenic muscle atrophy.

In previous reports, we found two other affected individuals with PRUNE gene mutation showing signs of spinal motor neuron involvement. Costain et al. reported one Ojibwe-Cree male patient with PRUNE gene mutation who required intubation in the first hours of life despite being well with normal Apgar scores in the postpartum period. An additional affected individual with PRUNE gene mutation reported by Karakaya et al. developed respiratory insufficiency at 8 months of age. He required permanent mechanical ventilation through tracheostomy. The individual reported by Costain et al. had neonatal hypotonia, progressive thoracolumbar scoliosis and bilateral talipes equinovarus and the patient reported by Karakaya et al. had neonatal generalized muscular hypotonia, severe scoliosis and electromyography findings that were compatible with neurogenic involvement. All these four cases had clinical findings and imaging results consistent with common features of PRUNE gene mutation associated phenotypes. We summarized clinical features, electrophysiology, muscle biopsy findings of these four individuals with PRUNE mutations and neuromuscular involvement in Table I.

Recently, two unrelated Saudi families with PRUNE gene mutations reported by Alfadhel et al. had psychomotor delay, progressive microcephaly, spasticity and dysmorphic features. However, neither clinical nor laboratory evidences suggesting spinal motor neuron involvement were mentioned in this report. Zollo’s study confirmed the interaction between PRUNE and tubulin, which was identified by mass spectrometry-based interaction screen. Tubulopathies are severe neurodevelopmental and neurodegenerative disorders caused by the multiple mutations in the genes encoding tubulin proteins. Mutations in tubulin-specific chaperones associated with tubulin folding and polymerization lead to a neurodevelopmental disorder affecting both central and peripheral nervous system resembling the phenotype of our patient. These data may provide a correlation and show a clinical overlap between tubulin (TUBB)-related an early-onset and progressive neurodegenerative encephalopathy with spinal muscular atrophy. The reported mutation p.D106N is located in the active side of PRUNE and is involved in the binding of Mn^{2+}, the co-factor essential for the enzymatic activity.

We also emphasize that the individual presented here, has elevated creatine kinase (CK) levels ranging from 343 to 1940 U/L (normal values: 0-171 U/L). The patient reported by Iacomino et al. also had elevated CK values (976 U/L, normal values <150 U/L) and was also homozygous for the p.D106N mutation as our patient. Mild to moderately elevated CK activity is a frequent biochemical finding in proximal spinal muscular atrophy.
| Characteristics                                      | I Costain et al. 2017 | II Karakaya et al. 2017 | III Iacomino et al. 2017 | IV Index Patient |
|------------------------------------------------------|-----------------------|-------------------------|--------------------------|-----------------|
| Age / gender                                         | 2-year-old/male       | 3-year-old/male         | 9-months-old/male        | 12-months-old/male |
| Consanguinity                                        | +                     | +                       | -                        | +               |
| Age onset / clinical presentation                    | Neonatal/severe hypotonia | Neonatal/severe hypotonia | Neonatal/severe hypotonia | Neonatal/severe hypotonia |
| Microcephaly                                         | -                     | + (secondary microcephaly) | -                        | -               |
| Dysmorphic appearance                                | +                     | +                       | +                        | +               |
| Generalized muscle weakness with hypotonia           | +                     | +                       | +                        | +               |
| Deep tendon reflexes                                 | N/A                   | N/A                     | Reduced deep tendon reflexes | Reduced deep tendon reflexes |
| Respiratory insufficiency/required mechanic ventilation | +/ +                 | +/ +                   | +/+                      | +/+             |
| Feeding difficulties                                 | + (feeding via gastrostomy tube) | + (feeding via nasogastric tube) | + (absent suction) | + (feeding via nasogastric tube) |
| Spasticity                                           | -                     | +                       | -                        | -               |
| Severe global developmental delay                    | +                     | +                       | +                        | +               |
| Seizures                                             | +                     | +                       | +                        | +               |
| Other physical findings                              | Scoliosis, equinovarus | Kyphoscoliosis, flexion contractures | Distal joints arthrogryposis, talipes equinovarus, bell-shaped thorax | Flask paralysis |
| Electromyogram/nerve conduction studies              | Neurogenic discharges/signs of denervation | Neurogenic discharges/signs of denervation | Neurogenic discharges/signs of denervation | Neurogenic discharges/signs of denervation |
| Brain MRI                                            | Cortical atrophy, thinning of the corpus callosum, T2 hyperintensity in the cerebral white matter | Cerebral and cerebellar atrophy with delayed myelination and inferior vermis hypoplasia | Diffuse cortical atrophy, thinning of white matter, signal changes in the periventricular white matter and pons | Non-specific abnormalities |
| Muscle biopsy                                         | N/A                   | N/A                     | Neurogenic atrophy       | Neurogenic atrophy |
| Creatine kinase (CK) level                           | N/A                   | N/A                     | 976 U/L                  | 343 to 1940 U/L  |
| Molecular genetic analysis                           | Homozygous in *PRUNE1* (c.521-2A>G) | Homozygous frameshift *PRUNE1* c.874_875insA, p.H292Qfs*3 | Homozygous missense mutation in *PRUNE1* c.316G>A (p.D106N) | Homozygous missense |

MRI: magnetic resonance imaging. N/A: not available.
In a study on all types of childhood and juvenile onset SMA, CK activity of 504 SMA patients were analyzed by Rudnik-Schöneborn et al. This was the first study to correlate the CK activity with different prognostic aspects on a large number of SMA patients including all clinical spectrum of types I–III. It was reported that the CK level was significantly higher in SMA type III patients than in the two other groups (SMA type I and type II). Elevated creatine kinase values were striking findings in both, our patient and the other individual reported by Iacomino et al. with a homozygous PRUNE mutation. However, these individuals resembled the phenotype of SMA type I. This data was challenging with the consequences reported by Rudnik-Schöneborn et al. study. Therefore, it will require further investigations to understand completely PRUNE's role in elevated creatine kinase values in these patients. Although Karaca et al. and Zollo et al. also reported patients harboring the homozygous p.D106N mutation but did not report on CK levels nor on detailed neuromuscular investigations. Thus, we assume that the neuromuscular involvement with lower motor neuron signs and hyperCKaemia might not be well appreciated in the context of the severe epilepsy and secondary microcephaly. It also shows that careful and very detailed neurological investigations are required to fully understand the pathophysiological consequences of novel neurogenetic disorders.

In conclusion, PRUNE mutations might be associated with a significant neuromuscular involvement. We aim to underline that spinal motor neurons involvement should be considered in patients with PRUNE mutations and the PRUNE gene should be investigated in all individuals with spinal muscular atrophy phenotypes.

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