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Supplemental Data

Bi-allelic *ADPRHL2* Mutations Cause Neurodegeneration with Developmental Delay, Ataxia, and Axonal Neuropathy

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SUPPLEMENTAL NOTE: CLINICAL CASE REPORTS

The proband of Family F1, F1:II.3 is described in full in the manuscript. This proband has two affected siblings; two older brothers are preterm fraternal twins both affected with spastic diplegia which has been attributed to perinatal brain damage. One of them (F1:II.1) deceased at the age of 23 years while the other one was still alive the age of 27 years (F1:II.2). No material was available to test the carrier status of individual F1:II.1 and we were unable to receive detailed clinical reports to evaluate clinical signs suggestive of ADPRHL2 deficiency.

Likewise the clinical information available for F1:II.2 was limited. According to the mother individual F1:II.2 had a spastic diplegia and learning disability. He was born as the second of dizygotic twins at 31 weeks of gestation. He showed a motor developmental delay with spasticity and hypertonia since the 6th month of life which has been attributed to a history of perinatal hypoxia. He started to walk at 3 years of age. At the age of 6 years an Achilles tendon extension was performed and he was subsequently able to walk without support. Nevertheless, due to kyphosis his independent ambulation was severely impaired and further declined. From the age of 26 years on he suffered from cramping in the area of the thighs upon exposure to cold. To our knowledge no further neuroimaging or electrophysiological investigations have been performed and the proband was not available for clinical follow-up. It thus remains currently unclear to which extend his clinical presentation can be explained by presumed perinatal hypoxia or might represent a manifestation of ADPRHL2 deficiency and whether he has any additional signs specific for his genetic disease.

Individual F3:II.1, a female, is a currently 12 year old girl born to healthy consanguineous parents. She presented with global developmental delay, a history of seizures, hypertonia, decreased muscle bulk, ataxia, axonal sensorimotor neuropathy, and bilateral sensorineural hearing loss. Seizures were controlled with valproic acid. She never did not have spoken language, but used signs. Brain MRI revealed small corpus callosum and cerebellar volume loss. Sural nerve biopsy at age 2 years revealed axonal neuropathy with Wallerian degeneration. The subject has astigmatism and intermittent esotropia. Dysmorphism included plagiocephaly, hypertelorism, somewhat coarse hypotonic facies. Growth defects

manifested in acquired microcephaly with OFC < 2 % (Z < -2.05), acquired short stature, and weight in the 3rd centile.

Individual F4:II.3, a male, was born as the first of dizygotic twins to healthy unrelated parents by caesarean section. His birth weight was 2400 g, Apgar score 8 at five minutes. His older brother and sister are healthy; his twin sister is similarly affected. His neonatal period, infancy, and childhood were uneventful. At the age of 13 years he first presented with three episodes of ataxia triggered by an infection. At the age of 14 years first sings of muscular weakness became evident. He had walking difficulties, memory problems, nystagmus, and developed a squint. Neurological examination showed hypotonia, high tendon reflexes, positive Babinski's sign, and he developed a pes cavus. Brain MRI at age X years was normal. Plasma lactate concentration was at the borderline or normal (2.01, 16.6, ref. <2.0 mmol/L). He is now aged 32 years and in quite stable condition with dysarthria and balance disturbances.

Similar clinical features were seen in his twin sister (individual F4:II.4) including signs of weakness from the age of 11 years on. Her muscular atrophy was more severe and she developed respiratory insufficiency requiring continuous mechanical ventilation from the age of 14 years on. A brain MRI showed atrophy of the cerebellum and she was diagnosed with an ataxic pyramidal / extrapyramidal syndrome. Extensive laboratory and genetic testing excluded various differential diagnoses (e.g. borreliosis, Friedreich ataxia, Wilson disease, hypobetalipoproteinemia, Refsum disease). Due to suspicion of mitochondrial disorder a muscle biopsy was performed at the age of 16 years. There were features of neurogenic impairment with regeneration signs (muscle fibers grouping). Activities of mitochondrial respiratory chain complexes were normal in muscle and fibroblasts homogenates. She remained dependent on mechanical ventilation until she died aged 30 years.

Individual F5:II.2, a male, was born after normal pregnancy to healthy unrelated parents from Kosovo. He is the second child of his parents with one healthy older sister. He has three healthy older maternal half-brothers. One older maternal half-sister suffered from epileptic seizures starting at 6 months of age, she

had global developmental delay and visual impairment; she eventually died at 8 years of age. No further information was available for this half-sister.

The proband's early motor development was normal. At the age of 3 years, single short episodes of dystonic posturing were noted by the parents, followed by the development of gait abnormalities with weakness, ataxic-dystonic posturing in a bended trunk and head position and choreatic arm movements in association to a prior febrile illness two days before. Weakness and movement disorder slowly improved over several months; however, parents noted general weakness during infections and unprovoked episodes of ataxia lasting for one to two days. Additionally, evolving clinical features included a putative external ophthalmoplegia with ptosis, impaired saccades and bulbar elevation as well as nystagmus. Ophthalmologic examination was indicative of retinal pigment epithelium anomalies. Electrophysiological testing revealed an isolated axonal motor neuropathy at 7 years of age. Cognitive development was delayed from infancy, but showed continuous progress and was not adversely affected during periods of motor weakness or episodic ataxia, He currently shows mild cognitive impairment. Initial brain and spinal MRI and an MR spectroscopy at age 3 years were without pathological findings; at 7 years mild cerebellar atrophy was observed on MRI. At last examination at 7 years and 7 months he displayed acquired microcephaly (OFC 49.5cm, < 1^{st} percentile, SDS -2.7) with his other anthropometric measures being within the lower range. His gait was mildly weak and ataxic, with normal deep tendon reflexes.

Individual F6:II.1, a female, was born after normal pregnancy to healthy unrelated parents with body weight of 3,380 g and Apgar score of 9. She is the only child of her parents. Her cognitive and motor development was normal. In the 2^{nd} year of life she presented with febrile seizures (upper respiratory tract infection with 39.5^{o} C) followed by several afebrile episodes of ataxia and abnormal bended head position. Cranial MRI at that time was normal. Her condition returned to normal. At the age of 4 years episodes of ataxia with abnormal vocalization and head posture recurred. Treatment with anticonvulsants resulted in partial improvement. Significant gait problems developed when she was 9 years old. She had position and intention tremor, weakness and atrophy of her lower and upper extremity muscles, with foot drop. Deep tendon reflexes were preserved. There were no oculomotor abnormalities. Electrophysiological testing revealed an axonal motor neuropathy. Her cranial MRI at age nine years did not show any abnormalities. Extensive laboratory workup was not contributory. She died at the age of 11 years.

Individual F7:II.2, a female, was born after normal pregnancy to healthy consanguineous parents from China. Her early development was normal. At the age of 1 year and 6 months she was able to walk independently and to speak single words. From the age of 2 years on, she had recurrent episodes of one-sided dystonia, triggered by fever or infection. At the age of 3 years, she manifested with episodes of limb jitter during sleep, especially shortly after falling asleep. At the age of 4 years and 7 months, her condition worsened with new episodes of dystonic posturing after asleep and during sleep, progressive muscular weakness, fatigue, and ataxia, leading to gait abnormalities and loss of independent walking and standing. She had a high-arched palate. Brain MRI showed cerebellar atrophy. Spinal cord MRI showed no specific changes. Cerebrospinal fluid analysis showed normal cell count, glucose, chloride, and lactate, but mild elevation of protein (468 mg/L, normal range: 250-450 mg/L). Electrophysiological testing was suggestive of axonal sensorimotor peripheral neuropathy. Nerve biopsy showed a decrease in the number of nerve fibers, with significant axonal degeneration and atrophy. Histopathological studies and testing of activities of mitochondrial respiratory chain complexes performed on a skeletal muscle specimen were normal. Extensive laboratory workup was not contributory. Abdominal ultrasound showed polycystic kidneys. Chronic inflammatory demyelinating polyneuropathy (CIDP) could not be excluded, therefore treatment with methylprednisolone was started with slight improvement of symptoms. At the age of 4 years and 11 months she manifested intermittent fevers, renal dysfunction, and a suspicion of paralytic ileus. Her condition deteriorated with progressive muscular weakness, speech abnormalities, and respiratory insufficiency requiring mechanical ventilation. She received intravenous immunglobulin and plasma exchange treatment without effect and died at the age of 5 years. Her elder sister (F7:II.1) had a similar clinical presentation of abnormal gait, progressive muscular weakness, and seizures 4-5 times when she was around 1 year of age. She died at the age of 12 years and 10 months. A younger brother (F7:II.3) was reportedly healthy.

Patient F8:II.1, a male, was the first child of healthy first cousin parents of Turkish origin. He was born at term after normal pregnancy with birth measures within normal range. He developed normally up to 15 months, when cognitive and motor delay and ataxia became evident. As the disease progressed, he lost walking ability and due to muscular hypotonia also head control until the age of 23 months. Comprehensive metabolic, endocrine, and infectiological investigations were unremarkable. Thoracic X-

Ray, ECG, abdominal and muscular ultrasound showed normal findings. Cerebral MRI showed normal structures and myelinization and an arachnoid cyst (3 cm in diameter) on the right temporal lobe. After admission for further investigations he came down with chickenpox, fell into a comatose state, and had an asystolic episode requiring ICU surveillance. Extensive neurometabolic work-up including muscle biopsy and investigations for neuronal ceroid lipofuscinosis type 1 and 2 were inconspicuous. Brain MRI showed bilateral signs of limbic encephalitis in the hippocampal area. After exclusion of a paraneoplastic cause, further investigations revealed antibodies against neuropil of hippocampus and other cerebral areas. Cortisone treatment (two periods of 5 days each) stabilized his neurological state, and he finally showed limited communication, was able to sit without help, and regained crawling.

At the age of 4 years he deteriorated again in the context of two generalized febrile seizures, losing especially his ability to sit and his social skills. Severe EEG changes and further generalized epileptic seizures were treated with antiepileptic medication (valproic acid) without improvement. Brain MRI now showed extensive hippocampal sclerosis. Few weeks later he was found apneic at home and needed resuscitation for 60 minutes. For 18 days he had no spontaneous breathing, lack of pupillary reflexes with fixed pupils, and lack of all brainstem reflexes and deep tendon reflexes. He recovered poorly and died 90 days after the apneic episode in multiorgan failure.

As limbic encephalitis was postulated, the patient was included in the following manuscript: E Haberlandt, *et al.* Limbic encephalitis in children and adolescents. Arch Dis Child 2011;96:186–191. PMID: 20959359.

Individual F8:II.3 is a currently 22 months-old female, third child of her parents, and has one healthy 12 years-old sister. Individual F8:II.1 was her elder brother. She was delivered spontaneously after regular pregnancy at gestational age of 41 weeks. Her birth weight was 3.224 g (centile 10-25), length 52 cm (centile 25-50), and head circumference 33 cm (centile 3-10). The neonatal period and early infancy were initially unremarkable. At the age of 14 months recurrent episodes of sudden loss of head control were noticed. She started walking at age 15 months, but soon thereafter she presented regression in her motor functions after an episode of pneumonia. Subsequently, ataxia became evident and she was unable to walk and stand alone. Neurological exam displayed muscular hypotonia with normal tendon reflexes and no pyramidal signs. In the clinical investigations a failure to thrive and acquired microcephaly were seen

(head circumference 43.5 cm, 1 cm below centile 3). No indicative dysmorphisms were present. Ophthalmologic investigations, EEG, and abdominal ultrasound were without pathological results. Brain MRI at the age of 15 months displayed spotted white matter lesions in biparietal areas showing no progression during the next 5 months. At the age of 18 months she presented with status epilepticus. Screening for metabolic disorders including serological as well as neurooncological antibodies in serum and cerebrospinal fluid investigations at the age of 20 months were within reference ranges. Neurotransmitters were not analyzed. Electron microscopy of a skin biopsy did not show mitochondrial degeneration or pathological lysosomal storage. Currently, seizures are well-controlled with levetiracetame, but motor functions continue to deteriorate and mood swings are frequent.