The American Journal of Human Genetics, Volume *102*

Supplemental Data

A Recurrent *De Novo PACS2* Heterozygous Missense Variant Causes Neonatal-Onset Developmental Epileptic Encephalopathy, Facial Dysmorphism, and Cerebellar Dysgenesis

Heather E. Olson, Nolwenn Jean-Marçais, Edward Yang, Delphine Heron, Katrina Tatton-Brown, Paul A. van der Zwaag, Emilia K. Bijlsma, Bryan L. Krock, E. Backer, Erik-Jan Kamsteeg, Margje Sinnema, Margot R.F. Reijnders, David Bearden, Amber Begtrup, Aida Telegrafi, Roelineke J. Lunsing, Lydie Burglen, Gaetan Lesca, Megan T. Cho, Lacey A. Smith, Beth R. Sheidley, Christelle Moufawad El Achkar, Phillip L. Pearl, Annapurna Poduri, Cara M. Skraban, Jennifer Tarpinian, Addie I. Nesbitt, Dietje E. Fransen van de Putte, Claudia A.L. Ruivenkamp, Patrick Rump, Nicolas Chatron, Isabelle Sabatier, Julitta De Bellescize, Laurent Guibaud, David A. Sweetser, Jessica L. Waxler, Klaas J. Wierenga, DDD Study, Jean Donadieu, Vinodh Narayanan, Keri M. Ramsey, C4RCD Research Group, Caroline Nava, Jean-Baptiste Rivière, Antonio Vitobello, Frédéric Tran Mau-Them, Christophe Philippe, Ange-Line Bruel, Yannis Duffourd, Laurel Thomas, Stefan H. Lelieveld, Janneke Schuurs-Hoeijmakers, Han G. Brunner, Boris Keren, Julien Thevenon, Laurence Faivre, Gary Thomas, and Christel Thauvin-Robinet

SUPPLEMENTAL DATA

Case reports

Clinical, genetic/metabolic data were reviewed, as well as EEGs and MRIs data from the medical records. Each group contributed phenotypic details for affected individuals from their center, in accordance with local Internal Review Board approved protocols. De-identified primary EEG and MRI data were reviewed by neurologist/epileptologist HEO and neuroradiologist EY from Boston Children's Hospital when available. We specifically evaluated age at seizure onset, seizure types, evolution of epilepsy over time, EEG patterns, developmental phenotype, physical and neurologic exam features, and medical signs/symptoms. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and proper informed consent were obtained.

Individual 1: a 16-year-old girl was the first child of healthy unrelated parents, with a healthy maternal half sister. Born at 38 weeks of gestation after normal pregnancy, she presented normal birth measurements (weight 3270g (-0,3 SD), height 51cm (+0,6 SD), and OFC 34 cm (+0,4 SD), as well as hypotonia, metatarsus varus and increased tendon reflexes. At day 6, she presented with focal seizures. Psychomotor development was delayed: she sat without support at 8 months of age and walked at 22 months of age. She spoke her first words at 20 months of age and her first sentence just before the age of 4 years. Her stature and weight gain were normal. Her last examination demonstrated pyramidal syndrome, slender fingers and facial dysmorphism (figure 1) including hypertelorism, long and down-slanting palpebral fissures, thick eyebrows, wide mouth with downturned corners, thin vermillion of upper lip

and everted vermillion of the lower lip, prominent frontal incisors and wide spaced teeth. She also presented with asymptomatic permanent moderate neutropenia (unclear if medication related), epilepsy requiring carbamazepine treatment and a moderate ID. Brain MRI, at 5 years of age, showed mild foliar distortion of the left cerebellar hemisphere and mega cisterna magna. Standard chromosomal analysis and metabolic assessment were normal, as well as *COH1* and *STK9* Sanger sequencing.

Individual 2: a 4-year-old girl was the second child of healthy unrelated parents. Her father had 3 maternal uncles affected with ID. Prenatal ultrasound survey had been marked by dextrocardia. At 37 weeks of gestation, weight was 3295g (median). Neonatal convulsions started at day 4 with generalized tonic-clonic seizures with eye deviation, head back, opisthotonos, and facial erythrosis. Neonatal EEG showed right central and occipital spike waves. At 3 ½ months the EEG showed right and left temporal sharp waves but an otherwise normal background and no seizures. EEG was normal after 1 year of age. Psychomotor and speech delay occurred with sitting acquired at 20 months of age and first words at 26-30 months of age. She also had a poor eye pursuit, horizontal nystagmus, sleeping and behavioral disturbances with stereotypies, and agitation. At last examination at 4 years of age, she presented with facial dysmorphism including long and down-slanting palpebral fissures, synophris, low posterior and anterior hairline, wide mouth with downturned corners and thin vermillion of the upper lip, and sacral dimple. Neurological features included a wide based gait and difficulties in fine motor skills. With valproate treatment, she was seizure free since 6 months of age. She also had a mild chronic anemia with normal white count. Examination of fundus, visual and auditory evoked potentials, and electroretinogram were normal. Brain MRI, at 5 years of age, showed inferior vermian

hypoplasia with prominent foramen Magendie and cisterna magna, severe foliar distortion of cerebellar hemispheres with centrifugal orientation, and hypothalamic fusion anomaly (Figure 1B). Standard chromosomal analysis, array-CGH and metabolic assessments were normal.

Individual 3: a 15-year-old boy was the third child of healthy unrelated parents, with a healthy older brother and sister. Born at 38 weeks of gestation after normal pregnancy, he presented with macrosomia (birth weight = 4535g (+2 SD)). Neonatal convulsions started at day 4. Psychomotor development delay was normal with independent sitting at 6 months of age and walking at 18 months of age. Speech was delayed with the first word at 24 months of age. At last examination, he presented with normal growth measurements, moderate ID needing special educational school and facial dysmorphism with long and down-slanting palpebral fissures, prominent pillars of philtrum, wide mouth with downturned corners, thin vermillion of the upper lip and everted vermillion of the lower lip, prominent frontal incisors and diastemas of the teeth (Figure 1). She also had 2/3 syndactyly of toes and cryptorchidism. Neurological examination was normal and seizures are currently controlled by carbamazepine. Brain MRI showed increased subarachnoid spaces. Standard chromosomal analysis, array-CGH, FRAXA screening and metabolic assessment were normal.

Individual 4 : a 8-year-old girl was the second child of healthy unrelated parents. Born at 35 weeks of gestation, she weighted 3380g (+1,8 SD). Generalized tonic-clonic seizures with eye deviation started at day 7. Awake EEG showed excessive multifocal sharp waves, excess discontinuity, and seizures consisting of generalized attenuation followed by frontal-central spike waves. Seizures treatment was started with phenobarbital, pyridoxal phosphate, and

pyridoxine. From age 6 months she has been treated by valproate. Brain MRI, at 3 weeks of age, showed mild inferior vermian hypoplasia with a patulous foramen Magendie and mega cisterna magna, as well as mild distortion of the cerebellar folia (Figure 1). She walked independently at 18 months of age. Speech was delayed. Seizures were well controlled on medication after 2 years of age, but convulsions recurred immediatly upon withdrawal of valproate. At last examination, she presented with normal growth parameters and facial dysmorphism with long and down-slanting palpebral fissures, short philtrum, wide mouth with downturned corners, thin superior lip and everted inferior lip, broad nasal root, and low-set ears. She also had ID and mild autistic spectrum disorder. Standard chromosomal analysis, array-CGH, and metabolic assessment were normal.

Individual 5: a 19-month-old boy was born at term with mild congenital microcephaly (birth measurements: weight = 2570g (-1.7 SD); length = 48cm (-0.9 SD); OFC = 32.9 cm (-2.3 SD)). Facial dysmorphism included epicanthic folds, short mild up slanting palpebral fissures, a thin upper lip, a wide mouth, and a broad flat nasal bridge. He had variant transverse palmar creases and clinodactyly of the fifth fingers, but the latter feature was also present in his mother and maternal grandmother. Neonatal seizures, started at day 2, presented with saturation dips to 70% and stiffening of the right arm with flexion of the left arm or general hypotonia, initially treated by levetiracetam that was stopped at 5 months of age. At 2 months he showed clonics of both arms while the eyes widened and turned up, at 4 months his seizures started with a scream, the eyes widened, the arms flexed, consequently the arms showed contractions which secondarily generalisation to all limbs, at 10 months he had his last seizure which was generalized tonic-clonic with a nystagmus. Twice he has been re-admitted because of recurrence of ongoing seizures. Both times he reacted well to a loading

dose of phenytoin and was treated by carbamazepine. Brain MRI, at 7 days of life, showed a retrocerebellar arachnoidal cyst, inferior vermian hypoplasia with prominent foramen Magendie and a mega cisterna magna, as well as severe foliar distortion of the left cerebellar hemisphere with centrifugal orientation, and hypothalamic fusion anomaly (Figure 1). Initial two long lasting neonatal EEG 's were unremarkable. EEG at 4 months showed intensive multifocal epileptic discharges at the left hemisphere more than at the right, with once a clinical seizure starting left parieto-occipitally, with secondary generalization. Two days later the EEG showed frequent ictal generalized discharges, with a possible focal start tempero-occipitally at both sides. Four days thereafter, i.e. the last EEG recording, just after his seizures were stopped, showed diffuse slowing without epileptic discharges. At last examination (19 months), growth parameters were: weight -1,0 SD, height -1,8 SD, and OFC -1,9 SD. Psychomotor development was delayed but improved after phenobarbital treatment was discontinued at 14 months of age. He crawled and made a few assisted steps at 18 months of age. He spoke his first words at 19 months of age. Ophthalmologic evaluation revealed strabismus and he had a mild chronic anemia. SNP-array was normal. Whole Exome Sequencing showed, besides the variant in *PACS2*, a de novo variant of unknown significance in *CHD1*.

Individual 6: a 8 year-old boy was the fifth child of healthy unrelated parents. He was born at 40 weeks of gestation after a normal pregnancy, and initial exam demonstrated macrosomia (Birth mensurations : Weight = 4730g (+2.5 SD); Length = 54.5cm (+ 2SD); OFC = 40.5 cm (> 3SD)). Epileptic seizures occurred on day of life 2. EEG showed left temporal spikes. Psychomotor development was delayed: he sat without support at 16 months of age and walked autonomously after 22 months of age. No verbal language (except 3- 4 words).

He presented also obsessive compulsive disorder and stereotypies. Physical examination showed facial dysmorphism including long and down-slanting palpebral fissures, thin vermillion of upper lip, fine eyebrows, large ears and small mouth. Brain MRIs at 10 days and 4 months of age was normal. At 3 years and 6 months of age, he presented seizure without any fever.

Individual 7: a 13-month-old boy was born at term with normal birth mensurations (weight = 3860g (+1 SD); length 53.4cm (+ 1SD); OFC 35cm (median)). Facial dysmorphism included slight down-slanting palpebral fissures, break in the left brow, short, depressed and slightly broad nasal bridge, rounded nasal tip, wide mouth with downturned corners, and bilateral uplifted ear lobes. Initially focal seizures started at day 2 with autonomic features and consisted of isolated stiffening with apnea and bradycardia, without convulsions. Seizures later evolved to include clonic movements. He developed epilepsy controlled by phenobarbital. Brain MRI, at 2 months of age, showed inferior vermian hypoplasia and left retrocerebellar cyst causing distortion of the smaller left cerebellar hemisphere and thinning of the overlying bones. This cyst was in communication with the fourth ventricle through the widened foramen of Magendie. No cyst membrane was identified. The differential considerations included a Blake pouch cyst or arachnoid cyst. EEG recording at 7 weeks of age showed bi-temporal sharp waves with right predominance and right mixed frequencies of predominantly delta and theta waves. During sleep, the background was higher in amplitude and contains more slow activity with brief periods of relative suppression (persistent trace alternans). The last 24 hour EEG recording at 9 months of life was normal. Psychomotor development was delayed with decreased axial tone and slightly increased tone in hands: no independent sitting at 8 months of age and no walking/cruising at 16

months of age. At last examination (16 months), growth parameters were normal. He presented with undescended testicle and decreased hearing thought to be due to ear infections/fluid in ears. Ophthalmologic evaluation was normal and he had a mild chronic anemia with normal white count. Standard chromosomal analysis, array-CGH, FRAXA screening, and metabolic assessment were normal.

Individual 8: a 5-year-old girl, is the first child of healthy unrelated parents. Born at 37 weeks of gestation after pregnancy complicated by preeclampsia, she presented small for gestational age (weight = 2010g (-3 SD); length = 48cm (-1.5 SD); OFC = 32.5cm (-2.3SD)). Two weeks after birth, she presented with focal epilepsy followed by generalized seizures with sudden cramping/stiffness, turning away eyes, and no reaction. Convulsions occurred during several minutes but total recovery took several hours. Her last seizure occurred at 2 years of age. At last examination she did not need any medication. Brain Magnetic Resonance Imaging (MRI) in neonatal period was normal. Psychomotor development was delayed: she sat without support at 10 months of age and walked at 27 months of age. Speech was delayed, she spoke her first words around 2 years of age. Speech development took a giant stride when she was 3,5 years old and antiepileptic drugs were stopped. Physical examination showed facial dysmorphism including hypertelorism and a broad nasal bridge, mild down-slanting of the palpebral fissures, wide mouth with thin lips and a broad forehead. SNP-array analysis showed two paternally inherited duplications (1p13.3 and 11q22.3), as well as one maternally inherited duplication (Xq23). Sequencing of a targeted NGS panel on epilepsy revealed a variant of unknown significance in the *SLC9A6* gene (X-linked intellectual disability type Christianson), which turned out to be paternally derived as well. Whole-exome Sequencing showed, besides the variant in *PACS2*, a *de novo*

heterozygous variant of unknown significance in the *FBXO31* gene [MIM 609102], that has been one time reported in autosomal recessive mental retardation [MIM 615979].

Individual 9: a 3-year-old girl, the third child of healthy unrelated parents, was born full term by repeat c-section (weight = 3470g (median), length = 48cm (- 2SD) and OFC = 34.5cm (median)). She has congenital hypotonia, severe global developmental delay without regression, neonatal-onset epilepsy with tonic and tonic-clonic seizures including episodes of status epilepticus, oropharyngeal dysphasia, and visual dysfunction. She first presented with seizures on day of life 2, described as head thrusting back and eyes rolling up or head and eye deviation with variable progression to a generalized convulsion. The predominant early seizure type was generalized tonic. Initial EEG pattern showed excess discontinuity, multifocal sharp waves (frequent left frontal-central), and focal tonic seizures associated with generalized decrement followed by focal left fronto-central rhythmic sharp waves (age 3-5 days). Subsequent seizures continued to involved bilateral tonic activity, at times with a more complex semiology ending in myoclonic jerks associated with generalized periodic discharges (3 weeks of age), with generalized and bifrontal electrographic features. She had multifocal myoclonus, variably correlating with focal spikes on EEGs in the first month. She developed over time bilateral tonic and tonic-clonic seizures with head deviation, including episodes of status epilepticus at 5 months and 6 months. Seizures spaced in the second half of her first year to every 1-2 months, at times in clusters then as of 11 months she was seizure free for >2 years. She had recurrence of two GTCs, 1 month apart only in the setting of weaning off of phenobarbital to off at age 3.5 years. She continued on levetiracetam monotherapy. Formal developmental testing at 33 months of age evidenced an overall age-equivalent level of 10 months. She obtained the following composite scores on the Bayley-

III: 55 for cognitive, 53 for language and 46 for motor composites. She sat at 18 months, and has been cruising but not walking independently since approximately 2.5 years. She has behavioral challenges, limited understanding and communication skills (nonspecific babbling only, no communicative gestures), and sensory sensitivities. Using BITSEA and M-CHAT-R measure, she demonstrated somewhat more behavior concerns and somewhat less social/behavioral competence than expected for her age. She had twisting type hand stereotypies. On last exam, she had coarse facial features, diffuse hypotonia, limited eye contact but some tracking, unable to follow commands but responsive to mother's voice. She has oropharyngeal dysphasia requiring thickened liquids. Gastrostomy tube was used only for medications at this time. MRI brain, at 1 week of age, showed mega cisterna magna and patulous foramen Magendie, subtle cerebellar foliar distortion, and hypothalamic fusion anomaly (Figure 1B). Extensive blood, urine and CSF metabolic investigations were normal, as well as array-CGH. GeneDx infantile epilepsy panel evidenced a missense variant of unknown significance in the *NRXN1* gene (c.2310G>T, p.Gln770His).

Individual 10: a 7-year-old boy, 4th child of parents who are consanguineous (3rd cousins), was born at term with no perinatal distress. Birth mensurations were not available. He presented initially with seizures at 1 month of age. Seizure types were predominantly tonic clonic seizures especially with illness or fever. Breath-holding spells were another trigger. Initial EEG pattern was normal. Initially he had up to 5 admissions in 1 month for seizures, but gradually they spaced out to approximately 5 in one year then seizure free for 2 years at last follow-up. Last EEG showed rare generalized spikes. Brain MRI, at 1 month of age, showed mega cisterna magnaa with patulous foramen Magendie (Figure 1B). He had a diagnosis of autism spectrum disorder. He sat independently at 12 months, cruised at 18

months and walked independently at 2 years. He had a few words which are repetitive. Last examination showed mild facial dysmorphim and bilateral palmar creases, limited words and echolalia, and hand stereotypies (flapping, hitting own head). Extensive blood, urine and CSF metabolic investigations were normal, as well as array-CGH. GeneDx infantile epilepsy panel evidenced a heterozygous variant of unknown significance in the *RELN* gene (c.8005G>A - p.Val2669Ile, NM_005045.3) which is not thought to explain his symptoms.

Individual 11: a 12.5-year-old boy was the second child of healthy unrelated parents. At 39.5 weeks of gestation, after normal pregnancy, he was born by caesarian section. He presented with normal birth measurements (weight = 3,510g (median); length = 48cm (- 1 SD); OFC = 35cm (median)). At day 1, he presented with feeding difficulties, transient nystagmus, focal seizures evolving in generalized tonic clonic type, treated with valproate and vigabatrin. The last seizures occurred at 2 months of age, and he has been off antiseizure medications since 2 years of age. EEG showed focal epileptiform discharges in the left rolandic region. Growth and weight mensuration's were normal with OFC at - 1, 8 SD. He presented with language and developmental delay (walking at 2 years of age), behavioral disturbances with self-harm behavior and stereotypies. Facial dysmorphism included brachycephaly, long horizontal palpebral fissures, large nasal bridge, bulbous nasal tip, and long smooth philtrum and thin superior lip. Additional features include V finger clinodactyly, micropenis, testicular ectopia, partial hypothyroid and central precocious puberty. High resolution chromosomal, metabolic screening *SNRPN* methylation analyses were normal. *PACS1* causing gene was also clinically evocated. Brain MRI, at 12.5 years of age, revealed thick corpus callosum and inferior vermian hypoplasia.

Individual 12: a 9-month-old female was the second child of healthy, unrelated parents. The pregnancy was complicated by gestational diabetes requiring insulin and pre-term labor, treated with terbutaline. She was delivered at 34 weeks of gestation (weight = 3090 g (+0.5 SD); length = 45.7 cm (-1.5 SD)) and was kept in the neonatal intensive care unit for blood sugar monitoring. On the third day of life she had her first seizures, which started with eyelid fluttering. Intermittent EEG monitoring between 3 and 30 days of life showed excessive multifocal spikes and sharp waves especially in the bilateral temporal regions, interpreted as indicative of underlying cortical irritability with increase susceptibility for seizures. Brain MRI at 3 days showed small scattered subarachnoid and intraventricular hemorrhages. A head ultrasound was normal. Seizures became stereotyped: she would bring her arms to her chest, keep her hands fisted, head and eyes would turn to the right, and she would have shaking of the right leg. Seizures lasted less than a minute, and occurred 6-7 times a day. She was treated with levetiracetam and phenobarbital, but seizures persisted. She was discharged home at 35 days of age. At 2 months of age, she was having 3-4 seizures each day presenting as one-minute episodes of stiffening. An EEG at that time was significantly abnormal with persistent multifocal seizure discharges and an abnormal background. At 3 months of age, she had an episode of status epilepticus and was placed on oxcarbazepine, and has not had any further seizures. Brain MRI, at 3 months of age, showed mega cisterna magna and severe foliar distortion with centrifugal orientation (Figure 1B). Review of prior imaging studies showed that the cerebellar abnormality was indeed present on her initial MRI done at 3 days of age. She made slow progresses in her development. At 7 months, head control was improving, she was able to grab objects with her hands, brought her hands to her mouth, could push up in a prone position, and was attempting to roll. She was babbling and “razzing”, smiling, giggling, and playing social games. At most recent

examination, at 7 months of age, physical findings included hypertelorism, broad nasal root, with depressed nasal bridgen bulbous nasal tip, thin superior lip, wide mouth with downturned corners, brachycephaly, long philtrum, and inverted nipples. Appendicular tone was normal but she had truncal hypotonia. She had minimal head lag. She was attentive, babbled, smiled appropriately, and fixed and follows with her gaze. She performed constant tongue thrusting. Blood screen for CDGs was normal.

Individual 13 : a 3.5-year-old female was the second child of healthy, unrelated parents. Her older sister, one half-brother and one half-sister were healthy and there was no family history of epilepsy or intellectual deficiency. She was delivered at 33 weeks of gestation, after an uncomplicated pregnancy (weight = 2270 g (-0.5 SD); height = 47 cm (median); OFC = 32.5 cm (median)). She had atrial septal defect and sacral pit. Seizures started during the first week of life and were characterized by bilateral, asymmetrical tonic stiffening, leading to opisthotonus, apnea and bradycardia with erythrosis or cyanosis, eye and head deviation, followed by a right or left side hemiclonic or a bilateral clonic seizure, which could end by focal clonic shaking of one limb. Seizures were organized in clusters of 10 – 13 over 10 hours, lasting 1-5 minutes each. Seizure activity remained high during the first year, with nearly weekly seizure clusters. Then seizures frequency slowed down, mainly triggered by fever and infections with 1-5 seizures per cluster. At age of 3 years, she has seizure free intervals of 3 to 6 months. No other seizure type occurred. Initial EEG at 6 days of age was normal. At age of 2 months (43 weeks of gestation) multifocal epileptiform activity appeared with prominent high-amplitude slow spikes in both temporal regions and small rapid spikes on vertex and right, rarely left fronto-central regions. Two seizures were recorded showing prominent diffuse ictal decremental activity, which was diffuse or predominant on anterior

regions, followed rhythmical theta activity emerging from right frontocentral area, evolving then over both central regions, intermixed with spikes, with highest amplitude on the vertex. Paroxysmal activity stopped earlier on right frontocentral region, and was then more prominent over the left side, diffusing to temporo occipital region. One of the seizures were followed by a marked postictal depression. A postictal alternating pattern with amplitude attenuations of 1-2 seconds resolved after a few minutes. At evolution, interictal abnormalities became rare, background rhythm nearly normalized with normal sleep spindle development. At age 17 months, diphasic spikes occurred at vertex spreading to the right frontocentral region, enhanced in sleep. She was treated initially with Vigabatrin, Levetiracetam, Valproate and Lamotrigine, which was stopped and switched to Clobazam at age of 3 years because of significant potentiation of the right fronto-central spike wave focus becoming subcontinuous at wake with diffusion to right temporal - occipital and to left frontal - central region. In sleep there was continuous right hemispheric paroxysmal activity with maximal amplitude over the right frontocentral area, spreading significantly to left side. Some degree of regression was noticed, concomitant to electrical status epilepticus predominating on right hemisphere. After withdrawal of Lamotrigine, she recently started to make a few steps wit support. She can speak a few words. She has good visual and interactive contact but has some stereotypies (rocking and hand friction with emotion). Neurological examination showed increased tendon reflexes, without other features of pyramidal syndrome. She had some dysmorphic features: hypertelorism, broad nasal root, thin vermillon of upper lip, and wide mouth with downturned corners. Brain MRI, at 23 months of age, showed moderate cerebellar foliar distortion (Figure 1B). Metabolic screening and array-CGH were normal. A 90-genes panel for monogenic epilepsies was negative.

Individual 14: a 5.5-year-old Chinese female was the first child born to this set of healthy unrelated parents. Her 18 year-old paternal half-sister was healthy. The family history was negative for learning disabilities, intellectual disabilities, autism spectrum disorders, birth defects, multiple miscarriages, sudden death, seizures, and consanguinity. She was delivered at 39 WG with macrosomia (weight = 4.035 g (+ 0.6 SD); length = 49.5 cm (+ 0.9 SD); OFC = 34.5 cm (+ 0.1 SD). She had several episodes of tonic posturing of her upper extremities, some associated with bradycardia and duskiness around 50 hours and was placed on video EEG monitoring which demonstrated that these episodes did have electrographic correlates, suggestive of tonic seizures. She was treated with phenobarbital with resolution of the seizures. She was initially treated empirically with ampicillin and gentamicin for neonatal sepsis, aciclovir for HSV infection, which was discontinued after a negative infectious work-up. Her newborn state screen was negative. Her neurological exam was normal. Her work-up in the NICU included a MRI brain, showing mild underopercularisation and white matter heterogeneity, which was felt to be within normal limits and a small hemorrhage in the left superior parietal cortex likely due to birth trauma, magnetic resonance spectroscopy which was normal, lactate/pyruvate which was negative for infection, normal cerebrospinal fluid and serum lactate, and elevated serum pyruvate due to feeds. Due to her clinical stability and seizures, which were easily controlled, she was discharged home on day of life 9. phenobarbitol was weaned around 6 weeks of life, but at 2 months of life she presented with seizures consisting of initial eye deviation then whole body stiffening and was restarted on phenobarbitol as well as pyridoxine and levetiracetam, the later was subsequently weaned and lacosamide was added after recurrent breakthrough partial seizures. She had several recurrent partial as well as generalized seizures with viral illnesses. Her last clinical

seizure was at about 4 years old. A trial of weaning of levetiracetam was halted after demonstration of electrographic seizures, and she has been stable maintained on pyridoxine, levetiracetam, and Lacosamide. Her development was delayed as she was noted to be hypotonic with an ataxic gait. A comprehensive developmental assessment at 4 years of age demonstrated cognitive and language skills at an approximate 3 to 3.5 year level, with normal receptive but decrease expressive language skills. She continues to demonstrate low tone, normal deep tendon reflexes, and a wide based steady gait, with selective mutism. Her laboratory evaluations included comprehensive cerebrospinal fluid, urine and blood metabolic studies including cerebrospinal fluid neurotransmitters that were normal. At 31 months of age, brain MRI showed prominent cisterna magna and patulous foramen magendie with subtle foilar distortion, predominant on left side (Figure 1B). Genetic testing included a pyridoxine-dependent seizure panel (*SCN1A, ALDH7A1*), *AASA, PC6, FOXG1, CDLK5, MEF2C* del/dup studies and GeneDx Comprehensive Epilepsy panel (53 genes). All were normal except the latter returned a heterozygous missense variant of unknown clinical significance in the *PNKP* gene. Biallelic mutations in this gene are associated with microcephaly, Seizures and developmental delay. Whole exome sequencing revealed compound heterozygosity of variants of unknown significance in the *AHNAK* and *XRCC1* genes. Collaboration with Keith Caldecott and lab members Richard Hailstone and Nicholas Hoch, failed to demonsttrate a functional significance in *XRCC1* activity in lymphoblasts derived from this individual (data not shown).

Acknowledgments

The following members of the C4RCD group (listed in alphabetical order) have contributed significantly to this work: Chris Balak, Newell Belnap, Ana Claasen, Amanda Courtright, David

W. Craig, Matt de Both, Matthew J. Huentelman, Madison LaFleur, Sampathkumar Rangasamy, Ryan Richholt, Isabelle Schrauwen, Ashley L. Siniard, Szabolcs Szelinger.

Figure S1 : Alignment of the PACS1 and PACS2 proteins with location of the p.Arg203Trp and p.Glu209Lys showing protein homoly.