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Supplemental Data

High Rate of Recurrent *De Novo* Mutations in Developmental and Epileptic Encephalopathies

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Supplemental Note: Case Reports

1) Individuals with *de novo* variants in *NTRK2* (NM_006180.4):

HSC0103; *NTRK2* (c.1301A>G: p.Tyr434Cys): This is a 3.5 years old male. He was born full term, 41 weeks by vaginal delivery. At 3 days of life, he developed episodes of extension epileptic spasms involving both his arms and legs with his eyes rolling up, lasting for less than 1 sec. At one month of age, the episodes became more frequent, with 3-4 clusters per day, each of them lasting 2-3 mins. They were now described as extension of both arms and legs together with flexion of the trunk and upward rolling of his eyes. He was diagnosed with infantile spasms, which subsequently progressed to multiple seizure types. At his baseline, he experiences 4-5 spasms per day as well as approximately ten episodes of upward tonic eye deviation with fluttering of the eyelids. He failed multiple AEDs: vigabatrin, ACTH, levetiracetam, clobazam, topiramate, valproic acid. He subsequently developed other types of seizures. He has problem swallowing and is fed through an NG tube. On examination his weight is 13.4, below the 3%. He is microcephalic with a head circumference at 46.5 cm, far below the 3%. He has severe global developmental delay. He is non-ambulatory and nonverbal. There is no dysmorphism. He has truncal hypertonia with increased tone of the extremites and exaggerated deep tendon reflexes at all levels. His pupils are symmetrical and reactive but he is not following, not fixating and there is no nystagmus. His multiple EEGs showed modified hypsarrythmia and abnormal visual evoked potential.

CGH microarray was normal. Ecocardiogram was normal. Recent MRI showed normal brain signal intensity with no evidence of focal lesion or diffusion restriction, but with hypoplasia of the optic chiasm and both optic nerves. Trio WGS sequencing identified a *de novo* missense variant in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys).

indvSLIJ; *NTRK2* (c.1301A>G: p.Tyr434Cys): This male individual was born by caesarean section due to transverse lie. His birthweight was 3.8 lbs 5oz, and birth length was 21.1 inches. Head ultrasound was done as part of his workup, which was normal. He had his first seizure within 12 hours of birth which resolved by day 10 of life. Details are unclear, however by parental report these presented as startle like events. He had some difficulties with breastfeeding after birth. However, no problems with bottle feeding are reported. The parents noted that he was not able to sit unassisted at 6 months of age. By 1 year, he was seen by Neurology service for global developmental delay. He was also referred to an ophthalmologist then and was diagnosed with optic nerve hypoplasia bilaterally. He has significant visual impairment. ECI intervention was started and he began receiving OT, PT, and speech therapy. At the age of 4 years, he was diagnosed with autism. Regarding specific developmental milestones, the parents reported that he sat up at 12 months, walked at 14 months, and said his first word at 16 months of age. He has on examination hyper-reflexia in legs, Babinski signs, and hypertonia in ankles, findings consistent with spastic diplegic cerebral palsy. Brain MRI at the age of 5 years showed moderate loss of the bilateral optic nerves and optic chiasm. At the age of 5 years he was admitted to the hospital with prolonged status epilepticus. He developed several months later focal seizures with impaired awareness, sometimes progressing to bilateral tonic clonic seizures. The events were described as zoning out, drooling, tremor-like shaking of the body, and fall if not held. They lasted 20-30 seconds each. He is on oxcarbazepine therapy 30 mg/kg/day, with no further seizures for 1 month. The wake and sleep EEGs reveal diffuse background slowing for age without discernable posterior rhythms and right temporal intermittent rhythmic delta frequency activity (TIRDA) that might indicate an underlying epileptic focus. His current problems include: GDD, hypotonia, ASD, severe ID, speech delay (says 15 words at the age of 5y7mo), visual impairment and seizures. Previous investigations were normal and included plasma amino acids, array CGH, Fragile X, and lactate of 1.8. Clinical trio WES (GeneDx) revealed a *de novo* mutation in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys).

T25821; *NTRK2* (c.1301A>G: p.Tyr434Cys): Concerns about individual T25821, a 4.5 year old girl and the fourth child of unrelated Ashkenazi Jewish parents, arose at 6 weeks when her head control remained poor. She has made little developmental progress subsequently. She can sometimes smile with a spasm but does not smile responsively and does not laugh. She only started fixing transiently at 2 years and has cortical visual impairment. Currently, she has no voluntary movement other than putting her hand in her mouth, she smiles but not always responsively, she has no reliable communication and is dependent for all activities of daily living. This individual developed epileptic spasms, both flexor and extensor, at 4 months with series lasting a few minutes. At 4 months, prednisolone controlled spasms within 2 days but they subsequently relapsed and she remained refractory to anti-epileptic therapy including: vigabatrin, levetiracetam, topiramate, lacosamide, valproate, rufinamide, zonisamide, diazepam, phenytoin, cannabis oil and the ketogenic diet. At 2

years, spasms continued to occur frequently with 3-5 spasms every 15 minutes. They comprised multiple types with eye rolling up and to either side or to the midline lasting 1-2 seconds but sometimes longer and could involve extension of the upper limbs. By 2 years, isolated spasms had reduced to 3 times per day. She also has tonic seizures every few days. At 6 months, she lost the ability to feed and required nasogastric feeding until 16mths when she had a percutaneous endoscopic gastrostomy. Episodes of uncontrollable crying occurred nightly at age 2 years, often followed by vomiting. On examination, she was hypotonic with poor head control, had noisy respiration and a persistent cough and had subtle choreoathetosis, also involving her tongue. MIP-targeted sequencing of *NTRK2* identified a *de novo* missense in this gene (NM_006180.4:c.1301A>G: p.Tyr434Cys).

HF303; *NTRK2* (c.1301A>G: p.Tyr434Cys): The case was the first child of a 28 year old woman who suffered from migraines, heartburn and iron deficiency during the pregnancy. The case was born at term by spontaneous vaginal delivery at 2.8 Kg without significant complications. At approximately 2 weeks of age, abnormal eye movements developed and a dilated eye exam demonstrated a small optic nerve. At 4 months of age, he developed epileptic spasms that responded to ACTH, but other seizures developed. Other AEDs were started with vigabatrin helping the most. He had storms of dystonia and hyperventilation that responded to diazepam. A lumbar puncture was only significant for low 5-HIAA 173 (nl=179-711) and HVA 358 (nl=450-1132). Genetic testing showed a negative array CGH, *CDKL5* sequencing epilepsy gene panel, Williams panel, DYT1 and 15q13 methylation profile. Seizures continue with the development of focal seizures with impaired awareness and atonic seizures. Other AEDs were tried, including zonisamide, clonazepam and diazepam. He was also started on taurine and hemp oil has alternative therapies. He remains non-verbal with global developmental delay despite aggressive treatment of his seizures. Feeding problems started as an infant with severe gastroeosophageal reflux, requiring NG-tube placement. MRI at 6 months old demonstrated hypoplasia of the optic nerves, tracts and chiasm. He was diagnosed with ASD at 3 years of age by a developmental pediatrician using the DSM criteria. He was later seen in an autism clinic where his diagnosis was verified by a pediatric neurologist examination and scores on the Social Responsiveness Scale (Total Raw Score 109; T-Score 80) and Autism Symptoms Questionnaire (Total Score 11) that were well within the range for autistic disorder. His cardiac evaluation revealed a normal echocardiogram with normal cardiac anatomy and biventricular systolic function. Family history was significant for mother having restless leg syndrome and chronic constipation, and father having chronic diarrhea. On the parental side of the family, there was a history of learning disabilities, ADHD and hypertension. His examination demonstrated a thin boy with BMI on the 2%ile. He was non-verbal with poor social interactions. Cranial nerves were unremarkable but eye movements were roving with nystagmus. Motor examination demonstrated appendicular hypotonia, reduced bulk but normal strength. His deep tendon reflexes were reduced but symmetric. Sensory, gait and coordination examinations were unremarkable. Trio exome sequencing done independently at 2 sites (GeneDx; Hudson-alpha) identified a *de novo* missense variant in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys). No other likely pathogenic variants were reported in the exome of this individual.

HSJ0335; *NTRK2* (c.2159C>T:p.Thr720Ile): Individual HSJ0335 is a 11 years old girl and the fourth child of non-consanguineous parents from Guatemala. She was born at term after an uneventful

pregnancy, by iterative C-section. The perinatal evolution was unremarkable (BW 2690g, APGAR 5-9-9) apart from physiological jaundice treated with phototherapy. She presented with global developmental delay and poor weight gain at 4 months of age. She has remained with a moderate global delay and evolved towards moderate-severe intellectual deficiency. She started holding her head at 6 months, could sit with support at 9 months but without support only at 18 months. She started rolling front-back at 15 months, never crawled but could walk with support at 27 months. She started walking independently at 3 years of age. She remains clumsy with a tendency for anteropulsion when standing. She said her first words at 2 years of age but with a slow progression (3 words at 3 years, 15 words at 6.5 years). She was oblivious of others, has little interests for toys, presents stereotypic rocking of the trunk and wriggling of the hands. She was diagnosed with autism spectrum disorder at 3 years of age, and with moderate-severe intellectual deficiency at 6 years of age without formal neuropsychological evaluation. When last seen at 9.5 years, she could walk short distances but used a wheelchair for longer distances, she could climb stairs while holding the railing and could drive a tricycle. She used approximately 50 words, could juxtapose 2 words, understood simple commands and could count up to 5. She could grasp objects and throw them but could not draw or dress herself. She presented 2 febrile generalized tonic-clonic seizures at 23 and 26 months. She developed rare afebrile focal seizures around 2.5 years old: she would scream, look terrified with behavioral arrest for a few seconds and 1-2 hours post-ictal fatigue. The parents did not seek medical attention for these episodes. At 5.5 years of age, she developed tonic-clonic seizures proceeded by a scream, lasting 1-3 minutes and occurring 2-4 times per week. The seizures were refractory to clobazam, were aggravated by levetiracetam but were partially controlled by topiramate (from daily seizures to monthly seizures). She still presented occasional prolonged generalized tonic-clonic seizures (status epilepticus), with or without fever, occurring every 2 months. Valproic acid was added to topiramate at 6.5 years of age and she was seizure-free for 10 months. Unfortunately, the seizures recurred despite increased doses and the valproic acid was replaced by carbamazepine at 7 years of age. She had only one short recurrence 2 years ago and has remained seizure-free since then, on carbamazepine monotherapy. 4 EEGs were done between ages 15 months and 6 years and were normal. Another EEG at 6.5 yo post-status epilepticus showed diffuse slowing, more marked at the left temporal area. She had been followed initially by the genetics and dietary services for failure to thrive and swallowing difficulties. She was fed through NG tube for a few months. Her weight generally followed the 10^{th} percentile from the age of 1 to 2.5 years of age (with a height around the 3^{rd} centile). However, she suddenly started gaining weight around 3 years of age, eventually reaching the 95^{th} percentile at 9.5 years of age (with a height at the 50^{th} percentile). She is now described as hyperphagic and must be supervised to prevent overeating. On last examination, she was smiling but could not maintain eye contact. She had stereotypic movements of the hands with flapping when excited. She had no dysmorphic traits apart from obesity. The motor exam was unremarkable. She could walk with help and had a wide-based stance. She has had an extensive investigation which was negative, including an array cGH and Fragile X screen, serum sialic acid and amino acid screen and urinary organic acid screen, urinary purines/pyrimidines, GUAC/creatine, *MECP2* sequencing. Her lactate was initially mildly increased (3.4) but normalized on four other tests. Pyruvate was normal. Her brain MRI at 20 months revealed delayed myelination (corresponding to that of a 13 months old child), mild reduction of global white matter tracts with mildly enlarged lateral ventricles, thin corpus callosum (posteriorly), normal spectroscopy. A brain

MRI at 6 years of age revealed bilateral atrophy of the hippocampi, decreased volume of the anterior commissure and mild increased subarachnoid spaces. The myelin was felt to be normal. An ophtalmological examination at 20 months of age was unremarkable (no optic nerve hypoplasia). Her genome sequencing revealed a *de novo* missense variant in *NTRK2* : NM_006180.4: c.2159C>T: p.Thr720Ile.

2) Individuals with *de novo* variants in *GABRB2* (NM_021911.2):

1242500; *GABRB2* (c.236T>C: p.Met79Thr): This 9 year female was the only child of non-consanguineous parents. The family history was unremarkable. The pregnancy, screening ultrasounds, delivery and birth parameters were normal. Neonatal examination was notable for hypotonia and dysmorphic features with round face and hypertelorism, everted lower lip, fifth finger clinodactyly and short broad great toenails. Her subsequent development was globally delayed. She walked at 4 years. Her language acquisition was also very late, slow and limited. She has severe ID. Ophthalmologic examination showed hypermetropia and astigmatism. Brain MRI and EEG were normal. This individual presented clinical seizures with altered consciousness (mostly absences), which started at birth (diagnosed at 11 months) and responded to levetiracetam. Clinical WES identified a *de novo* pathogenic truncating variant in *CHAMP1* (NM_021911.2: c.1876 1877delAG: p.Ser626Leufs (case published as case F3-II.1 in Isidor et al. 2016 PMID: 26751395). A recent revision of the exome data flagged a missense variant in *GABRB2* as likely pathogenic (NM_021911.2: c.236T>C: p.Met79Thr) and was subsequently confirmed by Sanger sequencing to be *de novo*.

K.02591; *GABRB2* (c.373G>A: p.Asp125Asn): This 10 year old female has moderate intellectual disability. She sat at 12 months and walked at 23 months. First words were at about 23 months. She babbled at 2.3 years and progressed to single words over next 2-3 years. She is hypermobile in her joints. She experienced febrile seizures and intermittent tonic clonic seizures from 6 years of age. She was treated with valproate and seizures stopped after several years. She is now seizure free on no AEDs. Brain MRI is normal. EEG was not done. Trio exome sequencing done as part of the DDD Study revealed a de novo missense in GABRB2 (NM_021911.2:c.373G>A: p.Asp125Asn).

CNSA01; *GABRB2* (c.908A>G: p.Lys303Arg): This is a 4 years old boy who presented with neonatal seizures on the first day of life. He was born after a normal pregnancy at 39 weeks' gestation. He had unremarkable familial and prenatal history. Birth weight, length, and head circumference were within normal ranges. We initially recorded focal seizures and then multifocal seizures (seizures recorded from both sides at 14 days of age). EEG background consisted of bilateral abnormalities. Neonatal MRI revealed diffuse white matter abnormalities on T2 weighted sequences. Feeding difficulties were observed during the neonatal period. Dystonic movements were observed at 3 months of age. Head growth slowed at 3 month-old and is now below the first percentile. Several antiepileptic drugs were tried. A combination of valproate, lamotrigine and topiramate was associated with a decrease in seizure frequency and intensity. Rare seizures were observed after 18 months of age. Dystonic movements became the prominent motor symptom. Gabapentin seemed to have a good efficacy on this symptom. It was difficult to distinguish clinically whether the motor symptoms were

seizures or dystonic movements. Repeat EEGs were conducted to elucidate this question that would impact the choice in treatment. This individual has axial hypotonia from early life. Feeding difficulties remained present. Perendoscopic gastrostomy was performed to provide adequate nutritional requirements. He has severe intellectual disability. At age of 4, he is seizure free but dystonic movements are observed. He is not ambulatory and non-verbal. Targeted gene panel sequencing revealed a de novo variant in GABRB2 (NM_021911.2:c.908A>G: p.Lys303Arg).

T21213B; *GABRB2* (c.911C>T: p.Ala304Val): T21213B is a 14 year old girl with profound ID who is nonverbal and cannot walk independently. Her parents are unrelated and her paternal grandfather had focal epilepsy. She was born vaginally at term following a normal pregnancy with normal growth parameters. She had mild initial feeding difficulties as she did not suck well. She was otherwise developmentally normal until 6 months of age when she was not able to sit or hold her head appropriately. Due to continued developmental concerns an EEG was performed at 15 months of age, which was severely encephalopathic approaching hysparrythmia in sleep. There were no seizures at this time. Treatment of the EEG with clobazam resulted in some improvement in both EEG and development. Vigabatrin caused a deterioration both electrographically and clinically. Hydrocortisone resulted in improvement on EEG and development. Multiple attempts to wean the hydrocortisone over the next 2 years resulted in regression of development. She had no seizures until 3.5 years of age when she presented with regression associated with multiple seizure types. Recurrent periods of deterioration were associated with weaning of steroids and would begin with her becoming unsteady and not feeding for a day followed by the onset of absence, myoclonic and atonic seizures with an encephalopathic EEG. If her hydrocortisone dose was not increased at that stage then over several days the seizures became frequent and she developed non-convulsive status epilepticus. These periods of regression and seizures lasted 3 to 14 days. During these she would have two types of absence seizures: typical brief absence seizures with no motor semiology as well as absence seizures with a significant myoclonic component. These seizures, captured on video-EEG, began with brief eyelid flickering and eyes rolling upward followed by loss of tone with low amplitude rhythmic myoclonic movements of arms. The EEG during these showed 3 Hz GSW. The episodes of non-convulsive status did not respond to AED therapy and only resolved after several days of increased hydrocortisone. Multiple trials of AEDs to prevent these periods of deterioration and allow weaning of the hydrocortisone failed resulting in her receiving hydrocortisone at varying doses continuously for 12 years. She has had no seizures occurring outside the episodes of deterioration which occur several times per year correlating with the hydrocortisone being weaned to a low level. On examination between periods of regression she is a happy child. She has no dysmorphic features but her height, weight and head circumference all below the 3rd percentile. She has generalised hypotonia with normal strength and reflexes. Metabolic investigations and MRI imaging were unremarkable.

HSJ0753; *GABRB2* (c.730T>C:p.Tyr244His): Individual HSJ0753 is a 4 years old girl and the 2nd child of non-consanguinous French-Canadian parents. She was born at term after an uneventful pregnancy (BW 4035g, HC 35cm, APGAR 9-9-9). She was hospitalized for one week at 3 days of life for irritability and feeding difficulties (chocking episodes). On examination, she had no visual contact,

major axial hypotonia and spasticity. She has profound ID. Cortical blindness was confirmed following normal ophtalmological examination and normal visual evoked potentials and electroretinogram. She developed an early-onset myoclonic epilepsy with initial seizures at 4 months of age. Her seizures consist of erratic myoclonic jerks involving the eyelids and limbs, sometimes with nystagmoïd eye movements, lasting seconds to minutes but with recurrent episodes of prolonged myoclonic status epilepticus. Her seizures have been refractory to levetiracetam, valproic acid, topiramate and a bolus of pyridoxine (100 mg). They responded partially to benzodiazepine, phenobarbital and phenytoin. They were aggravated by cannabidiol. They did not respond to the ketogenic diet, which she attempted twice with incomplete compliance. At 14 months of age, she has developed continuous erratic myoclonus when she is awake that disappears during sleep. She occasionally present with episodes of tonic limb extension. In the last year, she has had a few brief generalized tonic-clonic seizures. She is currently treated with phenobarbital, clonazepam and topiramate. Her initial EEG at 4 months of age revealed generalized 2 Hz spike-wave discharges, predominant in both frontal areas. At 6 months of age, the EEG showed rhythmic high amplitude slow waves (2.5Hz) and high-amplitude poly-spike and slow wave discharges at 2-3Hz for 3-15 seconds. The EEG evolved towards hypsarrhythmia with electrodecremental responses at 7 months of age but she never presented clinical epileptic spasms (and no clinical correlates were observed on video-EEG monitoring). Since the age of 26 months, the EEGs have revealed diffuse continuous spike-waves at 2Hz. On last examination at 4 years of age, she had no eye contact, had severe axial hypotonia, limb hypertonia with diffuse hyperreflexia. A progressive microcephaly was noted at 17 months of age (42.5 cm, $<2^{nd}$ percentile)**.** She had 2 brain MRIs (9 days and 1 year) that were normal. Her blood lactates were found to be elevated at two occasions (2.9 and 4.4 mmol/L) but have repeatedly been normal since then. An extensive metabolic screen was normal including ammonia, serum amino acid screen, urinary organic acid screens, glycosylation, long chain fatty acids, acylcarnitines, free and esterified carnitine, urinary purines/pyrimidines, GUAC/creatine, CSF lactate, amino acids, neurotransmitters). Sequencing of a panel of 126 epileptic encephalopathy genes (MNG) and of the mitochondrial DNA were non-diagnostic. Genome sequencing revealed *a de novo* missense in *GABRB2* (NM_021911.2: c.730T>C:p.Tyr244His).

T23211; *GABRB2* (c.730T>C:p.Tyr244His): Individual T23211 is a 6 year old girl and the fifth pregnancy of consanguineous Iraqi parents. Four previous pregnancies include a first trimester miscarriage, a boy who died at 20 days of age due to complications of maternal gestational diabetes and a boy who died at 18 months attributed to meningitis in Iraq. She presented at 5 months with global developmental delay, failure to thrive and microcephaly. On admission she was observed to have previously unrecognised tonic seizures. She was admitted and treated with phenobarbitone and levetiracetam, reducing the tonic seizures from 7 per day to 2 per day. She had choreoathetoid movements which gradually resolved after ceasing phenobarbitone. She became encephalopathic within 24 hours of starting vigabatrin at 8 months for persistent seizures. She had increased drooling and tonic seizures with eye flickering associated with stertorous breathing, desaturation and tachycardia. Seizures escalated over time and culminated in admission due to status. At 10 months she developed focal autonomic seizures and focal tonic seizures requiring multiple hospital admissions. She was admitted to hospital for 6 weeks at 15 months due to repeated vomiting and escalation of seizure frequency leading to an episode of status epilepticus. With treatment she settled back to having

5-10 brief seizures per day comprising tonic extension followed by eyelid flickering. She required nasogastric tube feeding from 6 months until she had a percutaneous endoscopic gastrostomy at 2.5 years. She has profound global intellectual impairment and cannot roll over or sit, is non-verbal and has cortical visual impairment. Investigations done and found negative include aCGH, Mito and POLG panel of mitochondrial mutations (Victorian Clinical Genetics Service). Targeted MIPs sequencing of *GABRB2* identified a missense in this gene that was confirmed by Sanger sequencing to be *de novo* (NM_021911.2:c.730T>C:p.Tyr244His).

G64518; *GABRB2* (c.830T>C: p.Leu277Ser): This ten year old girl is the second child of unrelated parents; 3 siblings are well and there is no significant family history. She was born after an uncomplicated pregnancy, with a birth weight of 2.5 kg. There were no neonatal problems, and she breast fed well for 4 months. There were no early concerns about development, she sat at 7 months. Although she was noisy, her vocalisations did not contain vowel sounds. She pulled to stand at thirteen months, but has never walked alone, although she does ambulate with hand held or a walking frame. She has only two words, used occasionally and communicates her wants by tapping. She developed a generalised epilepsy at age two; this was worsened with treatment with valproate. A small dose of lamotrigine was effective in controlling her seizures. Off treatment, she has mild absence seizures and febrile tonic-clonic seizures. Her general health is good; she has episodic panting respiration. She is generally happy and content. She has developed a mild microcephaly, her growth is otherwise normal. Testing (normal results) has included; microarray, MECP2 and TCF4 mutation testing, very long chain fatty acids, transferrin iso-electric focussing, white cell enzymes, routine haematology, biochemistry and thyroid function testing. Cerebral MRI showed mild ventricular dilatation at age 2, but was normal at age 3. Whole exome sequencing as part of the DDD study identified a de novo missense variant in *GABRB2* (NM_021911.2; c.830T>C: p.Leu277Ser).

HA076; *GABRB2* (c.830T>C: p.Leu277Ser): This 15-year-old male is the only child of non-consanguineous white Welsh parents. He was conceived by IVF. There was no family history of learning disability or epilepsy. Hewas born by emergency caesarean section for breech position at 38 weeks gestation. Birth weight was 4.12Kg (75-91st centile), length 56cm (98-99.6th centile) and OFC 39cm (slightly above 99.6 centile). He breast fed well. However, at 8-9 months there were concerns about poor feeding, lack of weight gain, and slow development. A Paediatric Neurologist diagnosed global developmental delay. He sat at 11-12 months and walked just before 2 years. At 4 years and 8 months He presented with clusters of myoclonic seizures. He was diagnosed with epileptic encephalopathy. The onset of seizures was associated with loss of language skills. He went from using several words and animal noises to no speech. The seizures responded rapidly to Valproate but within 2 weeks he began having head nods and clusters of myoclonic seizures with loss of posture. He was noted to have some mild left leg weakness and increased leg tone bilaterally. He had brisk but symmetrical reflexes. Topiramate and Clonazepam were started. The Valproate and Clonazepam were stopped at 6 years 4 months and Levetiracetam was started. Reviewed at 9 years his antiepileptic medications were still Levetiracetam and Topiramate. He was having occasional absences. The drop attacks had stopped. His speech development was limited (2-3 words). His fine motor skills were poor

and he had a mildly broad-based gait. He could run, jump and climb, but could not ride a bike. He could finger feed and just about use a spoon. This individual's height was 124.5cm (9th centile), weight 26Kg (25th centile) and OFC 54.5cm (75th centile). He was subtly dysmorphic with an alternating convergent squint, full and everted lower lip and small epicanthic folds. He had two symmetrical hair whorls on his scalp. The rest of his neurological examination was normal. At age 9 years he had a 10 minute episode of dystonia following an intramuscular dose of prochlorperazine (given to treat an episode of vomiting). His Topiramate was stopped, but Levetiracetam alone was unable to control seizures. Clobazam was restarted. At 11 years the Clobazam was replaced with Lamotrigine. At 13 years of age he had 3 generalised tonic-clonic seizures following an attempt to stop his Levetiracetam. Reviewed recently (at 15 years and 8 months of age) this individual has severe intellectual disability. He attends a specialist school for children with severe learning difficulties. He has minimal speech with only 2-3 recognisable words and 3-4 hand signs. He communicates what he wants by pointing or taking a person to the object. He makes some sounds (e.g. car noises). He is not toilet trained. He has poor concentration and is very active. He has no sense of danger. He is sociable and has good eye contact. He has occasional brief emotional outbursts, usually due to frustration. He is on Levetiracetam and Lamotrigine which controls his seizures well (one observed absence in the past year). Investigations: At 2 years his EEG was normal. Repeated at 4y8m (2 weeks after seizure onset and on treatment) his EEG showed marked slow background (2-3Hz delta along with rhythmical 3-4Hz slow components diffusely but more marked in anterior quadrants) frequent runs of higher amplitude rhythmical 2-3Hz delta (maximal in temporal to occipital regions, more marked on right) on four occasions becoming generalised and prolonged (8-10s) showing 'notched morphology' and more marked on right. During these four electroclinical events he leant forward, raised his arms to side and shuffled in chair. Sleep EEG at 6y0m showed abnormal sleep - mostly slow wave sleep. On waking he removed the electrodes but the background appeared low amplitude and slow. MRI at 2 years of age showed multiple small focal areas of abnormal increased signal in the periventricular and deep white matter mainly within the frontal lobes. MRI scan was repeated at 4y10m and 9y3m with no significant change. Extensive biochemical investigation was normal. Genetics testing included basic karyotype, subtelomeric screening with FISH, array CGH, Fragile X syndrome, ARX, Angelman methylation and Severe Infantile Epilepsy 35 gene Panel - were all normal. Trio-based whole-exome sequencing identified a *de novo GABRB2* missense variant (NM_021911.2; c.830T>C: p.Leu277Ser) in this individual.

31841; *GABRB2* (c.851C>A: p.Thr284Lys): Individual 31841 was the third pregnancy of unrelated parents. He was born quickly at 38+5/7 weeks by normal vaginal delivery with no concerns at birth. He has two older siblings, both in good health. There is unconfirmed family history of a paternal uncle having a childhood seizure disorder. On day 5, individual 31841 presented to hospital at the suggestion of the community nurse as he was not opening his left eye and was having difficulty feeding. His mother had been manually pulling his tongue down to get a bottle in and he had been feeding quite well while she did this. He examined normally on day 5 and was sent home with referral for ophthalmological review. On day 7 the community nurse raised further concerns regarding abnormal hand movements and "jerkiness". His parents had noticed decreased activity, poor feeding and jitteriness that day. The individual was admitted to hospital and on examination he was noted to be hypotonic with back arching and jitteriness. He was having tonic seizures captured on amplitude-

integrated EEG. He was loaded with phenobarbitone leading to a burst-suppression pattern on EEG. Individual 31841 required intubation and ventilation due to increasing apnoeic episodes. He was started on levetiracetam as well as phenobarbitone. Formal EEG showed epileptiform bursts associated with myoclonic jerks, along with suppression, consistent with early myoclonic encephalopathy. He was started on biotin, folinic acid and pyroxidine, all to no effect and all metabolic screens were normal. He was started on IV midazolam due to increasing seizure activity but continued to have seizures with multifocal myoclonus (diaphragm, feet and hands), right sided tonic episodes and lip smacking. These episodes lasted up to 10min with upwards of 20 episodes per day. On day 9 of this admission the decision was made to palliate the individual and he passed away at 17 days old. Clinical aCGH was found normal. WES identified a de novo missense in *GABRB2* (NM_021911.2: c.851C>A:p.Thr284Lys)

3001866; *GABRB2* (c.946G>A: p.Val316Ile): This 21 month old female presented with focal seizures progressing to become bilateral tonic clonic at 12months of age. She also has apneic/cyanotic episodes which have become more frequent. The seizure semiology consists of staring with eyes fluttering followed by left arm twitching and subsequent progression to all extremities. She stopped breathing and turned blue. The seizure lasted 2-3 minutes and then she was apneic for 4 minutes. Parents performed CPR (mouth breathing) for 4 minutes and then she became limp and subsequently slept for hours. There was no triggering factors. She was evaluated in the emergency department and blood work was normal. She has had no myoclonic or atonic seizures. Her motor development is normal but she has probable language delay (uses only 2 words at 21 months of age). Echo, EKG and Holter monitoring were all normal. Chest CT which showed NEHI (Neuroendocrine hyperplasia of infancy) which can cause hypoxemia and she is using oxygen during sleep. MRI brain at 1 yr of age show few punctate foci of low signal on the hemosiderin sensitive sequence within the cerebellum, which may reflect foci of tiny prior hemorrhage. EEGs and video-EEGs were normal. A Sleep study at14 months of age showed mild obstructive sleep apnea and periodic Legs movements. Sleep study (21 months of age): There were no apneas for more than 15 sec and no bradycardias. There was one oxygen desaturation (5 sec). The average oxygen saturation was 98% on RA; normal study on room air. Clinical microarray testing was negative. Clinical WES (BCM-Miraca) identified a private missense in *GABRB2* (NM_021911.2: c.946G>A: p.Val316Ile) that was shown by Sanger sequencing to be *de novo*.

3) Individuals with *de novo* variants in *CLTC* (NM_004859.3):

PBSD; *CLTC* (c.977_980delCATG: p.Ser326Cysfs*8): This girl was first seen in Genetic and Neurology clinics for delayed development, hyperactivity and impulsivity at age 5 years. Birth weight was 2.9 kg. at full term. The family history was negative for delays or genetic problems. A younger sister was normal. Parents noted that she said a few words early, but her language did not progress and she did not begin to talk until age 2. She did not put words together until age 3. She crawled late, at 14 months of age, and walked by 18 months. Further motor progress was slow, and she was late in running and feeding herself. She struggled with poor fine motor skills and was always clumsy. Intensive

interventions with speech, occupational and physical therapies were helpful, but she fell further behind her peers. As she got older, poor social skills became more of an issue. Although generally kind and affectionate, she would grab objects from other children, played poorly with her peers, and demonstrated poor understanding of social rules and cues. This improved with behavioral therapies, but continued to be a problem. IQ testing at age 5 showed Full Scale IQ of 79 and Verbal Comprehension Index 80, Perceptual Reasoning Index 82, Processing Speed 78. She had learning gaps and it was difficult for her to remember and learn new things. At age 5 she knew letters of the alphabet, but could not read or identify single words. She was poor at math, and had superficial concept formation. She had a nice personality, followed directions, and did not have behavioral problems, other than hyperactivity and social difficulties. Issues with gross motor delays continued. A physical examination at age 5 showed mildly large size (95 pc) attributed to tall parents, minimal epicanthic folds, mild hypotonia, trace deep tendon reflexes and poor fine motor skills. Her gait was mildly wide based. An MRI, at age 5 demonstrated "gray-white matter junction signal abnormality seen in the bilateral temporal, frontal and periatrial white matter regions. These findings most likely represent hypomyelination." A metabolic disorder was suspected, but an acylcarnitine profile showed only mild increase in glutaryl, hexanoyl, and decanoyl acylcarnitines, and was normal on repeat testing. Urine organic acids were normal. A microarray was not done. Stimulant therapy for ADHD was begun, and she had good response with decreased hyperactivity and better focus, but learning continued to lag. At age 11 her academic skills in math and language arts were assessed at the 3rd grade level. Trio exome sequencing (GeneDx) showed a c.989_992delCATG (p.Ser330Cysfs*8) in *CLTC* (NM_004859.3), a frameshift, not found in either parent. No other likely pathogenic variants were identified.

5289183; *CLTC* (c.1660_1668del: p.Met554_Tyr556del): This is a 20 year old male. He was born full term with a birth weight of 3.3 kg following an uncomplicated pregnancy. His neonatal course was unremarkable. He had mild gross motor delays and began sitting at 8 months and walking at 15 months. As a toddler, he fell frequently and was clumsy. Around age 6 years old, he began showing signs of a gait abnormality characterized by lower limb spasticity, mild truncal instability, and a slight hand tremor bilaterally. For the next 6 years, he experienced progressive spasticity with weakness in the lower extremities, brisk reflexes, intermittent myoclonic jerks, ataxia and a mild resting hand tremor. He underwent bilateral tendon lengthening at age 11 years old with no improvement in gait. By the age of 13, his gait stabilized and has remained unchanged since with no further progression. He is able to jump up on two feet, but cannot jump or balance on one foot. At age 14 years old, he had a single seizure event and video EEG showed some discharges, but no seizure activity. He was treated with anti-epileptic drugs for 2 years, after which the medication was discontinued since there was no further seizure activity. He has a history of cognitive delays. Delays were initially noticed when he entered school and presented with difficulties with reading and counting. A neuropsychological evaluation in 2003 and was revealing with a full scale IQ score of 69 and a second one in 2007 was revealing for a full scale IQ score of 72, consistent with low-average cognitive function. He began receiving special education at age 7 and graduated from high school. He has difficulty with abstract thoughts and complex reasoning and his cognitive functioning remains in the low-average range. He is one of 15 siblings and is of Ashkenazi Jewish descent with an unremarkable family history. He had a medical evaluation with normal results for his brain MRI, c-spine MRI, temporal bone CT, EEG,

electromyelogram, nerve conduction studies, echocardiogram and EKG. He had normal genetic and metabolic testing including chromosomes, Fragile X, mtDNA, lactate, pyruvate, urine mucopolysaccharides and oligosaccharides, creatine kinase, very long chain fatty acids and plasma amino acids. Trio whole exome sequencing done at GeneDx was revealing for a heterozygous *de novo* nonframeshift mutation in *CLTC* (NM_004859.3:c.1660_1668del: p.(Met554_Tyr556del).

indvAA; *CLTC* (c.2669C>T:p.Pro890Leu): This 4-year-old boy was born at term following a pregnancy with oligohydramnion in the last weeks and an uneventful delivery. After birth he shortly needed oxygen, but recovered quickly and was send home at day two. At three weeks of age he was admitted to the hospital for 5 days because of an RSV-infection. He underwent an adenoidectomy at two years of age and grommets were inserted because of multiple ear infections and delayed speech development. He has a global developmental delay. Speech development is more delayed than motor development. He started walking at 19 months of age and he falls easily. At three years of age he spoke several single words. At four years of age he sporadically uses two word sentences and starts to imitate words and behavior. He understands simple assignments. There are no overt behavioral problems, but he is easily distracted and under-aroused regularly. He loves water. His developmental age was tested at 18,5 months at an actual age of 35,5 months. On exam at the age of three years and two months his weight was 18.8 kg (+1.4 SD), height 97.6 cm (-0.91 SD). His head circumference is just below the 50th percentile. He has a rather long philtrum, a full lower lip with open mouth behavior, and a high palate. He drools continuously. He has mildly lax ligaments. At three years of age the child neurologist observed mild ataxic movements and a myoclonic jerk. EEG and MRI brain were normal. Chromosomal SNP array showed a likely benign paternal deletion 8p23.3p23.2, without OMIM-genes in the deletion. There were no homozygous regions. FMR1 analysis was normal. Trio whole exome sequencing (Radboud UMC; Nijmegen) showed only a *de novo* missense variant in *CLTC* (NM_004859.3:c.2669C>T: p.Pro890Leu).

CAUSES-1; *CLTC* (c.2669C>T: p.Pro890Leu): This 5-year-old boy was born at 39 weeks to non-consanguineous parents and has one brother with spina bifida and another brother with iris heterochromia. During the pregnancy maternal serum PAPP-A level was low at 15 weeks. There was a maternal short cervix, and contractions were present at 33 weeks gestation. Maternal steroids were administered and bedrest from 33-37 weeks was advised. Delivery was unremarkable and APGARS were 8 (1 min), 9 (5 min), and 9 (10 min). He rolled at 6 months, sat unsupported at 8 months and took his first steps were at 2 years 11 months. He had his first words at 2.5 years. At 4.5 years he had 6 words. He has never had seizures. Formal IQ testing at 4.5 years noted intellectual disability/GDD but the severity was unspecified as the assessments differed between home, school and clinic. He has oral and motor apraxia, poor attention and suspected ADHD. MRI brain and spine was normal. Formal ophthalmological and cardiological assessments were normal. On exam at the age of 4.5 years and two months his weight was 16.2 kg, height 96 cm. His head circumference was 52 cm. Blood pressure was 107/78 mmHg. He was alert and friendly and made excellent eye contact. He had an immature gait. He had an immature pincer grasp but could scribble. He knew his body parts and some colors. Cranial nerve functions were normal. He had mildly reduced tone with normal strength. He had normal reflexes with down-going plantar reflexes. Chromosomal SNP array and 15q13 methylation was normal. Whole

exome sequencing in trio with his non-affected parents as part of the CAUSES study demonstrated a *de novo* missense variant in *CLTC* (NM_004859.3: c.2669C>T:p.(Pro890Leu).

18052017; *CLTC* (c.2669C>T: p.Pro890Leu): This 30 year-old female was born from nonconsanguineous parents after an uneventful pregnancy and delivery. Two younger brothers (24 and 20 year-old) were healthy. Psychomotor delay and behavioral features (aggressiveness and impaired social interaction) were noticed during the first years of life. From the age of 4 years, oculo-manual and gross motor incoordination, and proximal limb rigidity became evident; persistent impairment of social skills was reported. Nevertheless, at that age, her cognitive development was reported as normal. During the following years, a cognitive decline was noticed, and at the age of 11, when she was fist investigated for diagnostic purposes, mild intellectual disability, drooling and gait incoordination were reported. Brain MRI, and neurophysiological studies were normal. Extensive neurometabolic work-up provided normal results except for a mild persistent hyperphenylalaninemia (180-240 microM; normal values: 60-120) associated with marginal reduction of urinary neopterin (0.15 mmol/mol creat; n.v. 0.2-1.7). PAH mutation analysis disclosed compound heterozygosity for the c.453T>A (p.Asp151Glu) and c.1139C>T (p.Thr380Met) variants. A similar biochemical alteration and the same PAH genotype was documented in one of the two unaffected brothers. In the following years, hypo- and bradykinesia emerged in association with dysphagia, hyporexia and weight loss with an intermitting course. At the age of 13, tetrahydrobiopterin (BH4) loading test proved to normalize blood Phe/Tyr ratio, and the reduced pteridine derivative was added to the therapy with a stabilization of the clinical condition. Looking for an alternative molecular cause explaining the individual's condition, BH4 synthetic pathway was further explored by molecular analysis of PTS, GCH1, and SPR genes, but no functionally relevant variant was identified. Similarly, possible involvement of FMR1 was excluded. On the last examination at the age of 30, she exhibited bradykinesia and bradypsychism, hypomimia and clumsiness, moderate intellectual disability (WAIS IQ 45), attention instability and verbal reiteration with relatively good adaptive skills. Brain MRI and DaTSCAN were normal. Trio-based WES identified a de novo missense change, (NM_004859.3: c.2669C>T: p.Pro890Leu), in *CLTC* as the only excellent candidate underlying the trait.

indvPAR; *CLTC* (c.3140T>C: p.Leu1047Pro)

This 16 years old boy is the third and only affected child of unrelated Caucasian parents. Family history was unremarkable. Pregnancy was uncomplicated. BW: 2860 g (-1.24 SD), BL: 48 cm (-0.81 SD), BHC: 34 cm (-0.93 SD). Hypotonia was recorded at birth and remained prominent till now. Feeding was difficult because of poor sucking, was and complicated by a severe gastroesophageal reflux (GER) that persisted through infancy, causing reported inhalation pneumonias. At the age of 10 years, he had Nissen fundoplication and gastrostomy. Development milestones were severely delayed: he controlled head position at age 9 months, could sit unsupported at age 13 years old. He had severe to profound intellectual disability, with limited interaction, no speech, no purposeful use of the hands. Seizures probably begin during the first months, but were initially considered to be vagal malaise related to his GER. Treatment with VPA was initiated at 2 years of age and resulted in the disappearance of clinical seizure. EEG showed nonspecific irritative pattern, without foci. He was noted to have spasticity of the lower limbs in infancy, but this stiffness evolved to choreo-athetotic movements clearly noted at age of

6 years, with some myoclonic jerks. Eye tracking was abnormal, with saccades but no clear oculomotor apraxia. Hypotonia lead to progressive kyphoscoliosis that required surgical arthrodesis T2 to sacrum at 14 years of age. MRI in infancy showed thin, short corpus callosum, with hypoplasia of its posterior part, wide Virchow-Robin spaces, and normal gyration, cerebellum and brainstem. Diffuse hypersignals in the frontal, temporal and parietal white matter was noted at age 3 years, that did not evolved between the age of 3-6 years. Syringomyelia T4-T7 was observed at the time of spine surgery. ERG and evoked visual potentials were normal in infancy. When examined at 13 years of age, he was 135 cm tall(-2.72SD), weighted 33 kg (-1.84SD) and had a HC: 49 cm (-3.64SD). Oval-shaped face, upslanted palpebral fissures, long nose with bulbous tip, tented upper lip, wide mouth, big central incisors, microretrognathia, and pointed chin. In infancy: ridged metopic suture was noted, but this anomaly vanished with time. Trio clinical exome sequencing identified a de novo missense in CLTC

273692; *CLTC* (c.3322T>C: p.Trp1108Arg): At 12 weeks of pregnancy, screening risk indicated a high risk of Down's and CVS was performed which gave a normal chromosome result (karyotype). Delivery was at term complicated by cord around the neck. At day one, he was noted not to cry for feeds and was sleepy. A few hours later he became jittery. He went home at three days of age. He was reviewed in the Genetics Clinic aged seven months. For the past two weeks, he had required NG tube feeding. He was on treatment with Baclofen to prevent stiffness and on antireflux medication. Developmentally he could smile at his mother when her face was close to his. He had a head circumference of 44cm between 2nd and 9th centile, length 65cm and weight 7.25 kilos. He did not have any dysmorphic features helpful for making a diagnosis. He was fairly hairy at birth but no longer hirsute. He had relatively small jaw. Posture was abnormal with arching of his back and generalised stiffness. He had right convergent squint but no nystagmus. Brain MRI scan had been reported as showing a small cerebellum. Nerve conduction studies were normal, EMG had some features of a myopathic process. Ophthalmology assessment showed normal structural eye features. Seizures have become more of a problem. He was suspected at two years of age to have myoclonic jerks and was referred for a further neurological opinion regarding seizures. Seizures have been confirmed and now settled on levetiracetam. On review at age four years, his phenotype was that of the quadriplegic cerebral palsy, significant intellectual disability, bilateral convergent squint and cortical visual immaturity. Head circumference aged was at 48.5 cm (9th centile). He was continuing with gastro-oesophageal reflux and was gastrostomy fed.

261801; *CLTC* (c.3595C>T: p.Gln1199*): This 10 years/7month old male is the middle of three children to a non-consanguineous Caucasian couple. He was born at term after an uncomplicated pregnancy. His father has joint hypermobility but there is no other relevant family history. Hypotonia and poor head control were noted in the neonatal period. Although ptosis was not recognised at that stage, it is believed to be congenital. Early investigations concentrated on congenital myasthenic syndrome in view of the hypotonia, ptosis and easy fatigability. Repetitive stimulation testing and Tensilon testing both gave normal results, as did Ach receptors antibodies and a metabolic screen including lactate and CK levels. DYT1, RAPSN, DOK7 and array CGH analysis were all normal. He first presented to Genetics at 5 years and 4 months, at which point, he was repeating his reception year

in school because of developmental delay and intellectual disability. He was able to make an attempt at writing his first name but only recognised the letters within it and could only count to 5. There were also concerns about speech delay and limited vocabulary. When reviewed again at 10 years and 7 months, he was estimated to be working at 6 – 7 year-old level and Special Needs input was being planned for secondary education. His hypotonia and hypermobility are still present but less pronounced and neurological examination is otherwise normal. He has frequent injuries, when unable to protect his face in a fall. He becomes easily fatigued and his parents often discover he has put himself to bed. When tired, both the ptosis and slurring of speech worsen. He has some obsessions, for example requiring smart, matching clothes, with exactly the correct leg length and wanting to wear a tie at all times. He also has some ritualistic behaviour such as needing a new book and pencil after a single use. His social skills are poor and he is a sensitive child, who is prone to stress and anxiety. He has had a normal MRI scan of his head and has never had any seizures. He has zygomatic hypoplasia and often has peri-orbital puffiness and discoloration. Trio exome sequencing as part of the DDD study identified a *de novo* nonsense variant in *CLTC* (NM_004859.3:c.3595C>T: p.Gln1199*).

indvMB; *CLTC* (c.3621_3623del: p.Asp1207del): indvMB is the second child of healthy, non-consanguineous parents with unremarkable family history. She has two healthy sisters. She was born at term, following uneventful pregnancy and delivery, with a weight of 3140 g, length 49.5 cm and head circumference 34.5 cm. She had severe global developmental delay since her first months of life: she sat at 3.5 years, stood up with support at 4, walked a few steps at 6, and did not develop any understandable language. The first years of life were also complicated by feeding difficulties, because of a severe gastroesophageal reflux, significantly improved after antireflux surgery at 5 years. The epilepsy of indvMB started at 3 years. She first had febrile tonico-clonic seizures, then febrile and afebrile seizures.When she was 4 years old, daily myoclonic jerks of her four limbs appeared, as well as tonic seizures with upgaze. The epilepsy was pharmacoresistant, partly because of a poor tolerance to many treatments: lamotrigin induced vomiting and worsened myoclonias, the introduction of clonazepam or clobazam was associated with behavioral troubles, valproate induced asthenia and levetiracetam acute pancreatitis. Finally, the epilepsy was partially controlled by a combination of topiramate and lacosamide, but her EEG remained pathologic. First EEG showed interictal multifocal spikes and spike – waves with bifrontal predominance, generalised spike-waves during myoclonic jerks. EEG at 7 years old was characterized by bifrontal slow-waves throughout the recording, without spikes. At the age of 8 years, she was unable to speak, she could understand very simple commands, had some autistic features with fluctuating eye contact. She could grab objects but had poor fine motor skills. Her weight was 27 kg (+0,5 DS), her height was 120 cm (-1 DS), her head circumference was 49 cm (-2 DS) with acquired microcephaly. She had no malformation but mild facial dysmorphic features: upslanting palpebral fissures, long filtrum with thin upper lip, prominent ears, scattered and slow growing hair. Neurological examination revealed severe hypotonia, ataxia and weak osteotendinous reflexes. Brain MRI at 2 years, 3 years and 6 years showed thin corpus callosum, hypersignal of the subcortical white matter (T2), and widened lateral ventricles. Metabolic studies (plasma lactate, pyruvate, ammonia, plasma amino acids and urin organic acids chromatographies, plasma acylcarnitine profile, isoelectric focusing of serum transferrine, urinary guanidinoacetate and creatine, lumbar punction, urine purines/ pyrimidins measurements) were normal. The following studies were

also normal: study of the mitochondrial respiratory chain on skin sample, karyotype, array-CGH, study of the methylation of the Prader-Willi/Angelman locus on chromosome 15, study of MECP2 and TBC1D24 genes, and an optical microscope examination of the hair. Finally, a trio based WES was performed and revealed a heterozygous de novo 1 amino acid inframe deletion in *CLTC* (NM_004859.3: c.3621_3623del: p.Asp1207del).

HSC0054; *CLTC* (c.4575dupA: p.Glu1526fs*18): This is a 23 years old female. She is the product of an uncomplicated pregnancy and delivery. Her parents are non-consanguineous. Family history is unremarkable. She had global developmental delay. She walked at the age of 4 years. She currently has moderate intellectual disability and can recognize letters but cannot read. She has perseverative behavior, characterized by some obsessive compulsive symptoms. She also has a somewhat dysmorphic face with abnormally long teeth. She has flat feet, scoliosis and had low muscle tone as a child (normal tone now). Limited neurological examination was normal. She has a history of 4 seizure types. Her epilepsy began at 5 months of age with seizures characterized by abnormal eye movements. Before 1 year, she developed absence seizures and another type of seizure that could be interpreted as myoclonic or tonic seizures. These occurred 50-60 times per day until puberty. She had her first GTCS at 11 years and then continued to have one GTCS every 3 months. She has had focal seizures from the age of 12 or 13 years characterized by panic, a terrified look on her face and she would pace back and forth and scream for 1-2 minutes. Past treatments include Clobazam, Valproic acid, Acetazolamide and the Ketogenic diet. She is currently on Levetiracetam and Lamotrigine. Her last known seizure was at the age of 20 yrs and has been seizure free since. EEGs have shown generalized spike and wave and polyspike and wave discharges and independent inter-ictal epileptiform discharges at F4 and over both mid temporal regions. MRI showed immature myelination in both temporal lobes and thinning of the corpus callosum as a child; a later scan as an adult was reported as normal and a neuroglial cyst noted in the right posterior centrum semiovale. Trio WGS identified a *de novo* frameshift variant in *CLTC* (NM_004859.3: c.4575dupA: p.Glu1526fs*18)

LDKQS; *CLTC* (c.4605+2T): This individual is currently a 12-year-10-month-old male whose neonatal history was remarkable for difficult feeding. Breastfeeding was originally attempted, however he was unable to latch and was eventually bottle fed. Originally the difficulty feeding was attributed to a short frenulum although concerns for hypotonia were also noted. Developmental milestones were delayed throughout infancy. He started to walk around age 2.5 years of age and began to talk around 3.5 years of age. In early childhood he was diagnosed with sensory motor difficulties including auditory hypersensitivity, tactile hypersensitivity, and visual hypersensitivity to light. At approximately seven years of age, he was diagnosed with a "cookie bite" sensorineural hearing loss, and was subsequently fitted for hearing aids. The hearing aids improved his hearing, but did not noticeably improve his speech ability. Also at age seven, it was discovered that he has left ventricular noncompaction (LVNC) of the left ventricular apex. This is felt to be a mild asymptomatic form of LVNC. Throughout his life, has had hypotonia, particularly noticeable in his core. He has also struggled with chronic constipation. At age 11, the child was seen by a neuropsychologist for intellectual disability. Full scale IQ was 44 on the WISC IV. Testing showed him functioning significantly below average in the areas of verbal comprehension, perceptual reasoning, working

memory, and processing speed. He has a speech and language disorder. He struggles with anxiety, which is controlled through medications. At age 12, he is able to read some books and his able to ride a bike. His gait and run are normal. Overall, he is in good general health. Trio clinical exome sequencing (GeneDx) revealed a de novo variants in CLTC (NM_004859.3: c.4605+2T

DDD00280; *CLTC* (c.4663C>T:p.Gln1555*): This female individual now aged 6 years/8month was referred to Clinical Genetics for assessment aged 3.5 years. She was the 2nd child of nonconsanguinous parents and her elder brother has a diagnosis of autism. Her mother has 3 healthy children from a previous relationship. There is no other family history of note. She was born at 36 weeks gestation after an uneventful pregnancy. She was in good condition at birth and weighed 7lb 14oz. She was noted to be hypotonic in the neonatal period and had gastro oesophageal reflux. At the Age 3.5 years, there were concerns regarding developmental delay and speech delay. She walked at 19 months and by 3 ½ was described as clumsy with joint hypermobility. She was provided with ankle supports and required a buggy for distances. She was beginning to put 2 or 3 words together. She was drooling, had glue ear and was described as a snorer with a poor sleep pattern. She had a sleep study suggestive of obstructive sleep apnoea and had adenotonsillectomy but symptoms recurred. On examination her OFC was 50.4 cm (+1 SD) with height and weight on the 75th centile. She had rough wiry hair out of keeping with the rest of the family. She had a tall forehead, a broad nasal tip, a high arched palate, a shallow philtrum and a wide mouth. She had bilateral 5th finger clinodactyly. When reviewed at 6 years her mother raised concerns that her appearance had coarsened with time and that her voice sounded hoarse. She still was not toilet trained. She was putting 3 or 4 words together and had obtained a place at a specialised additional needs school. At this time her height and weight were on the 98th centile. Bone age was normal. Abdominal ultrasound detected mild dilatation of the right renal pelvis but no masses or other abnormalities were detected. Thyroid function was normal and urinary metabolic screen was negative. Trios WES as part of the DDD Study revealed a *de novo* variant in *CLTC* (NM_004859.3: c.4663C>T: p.Gln1555*).

281177; *CLTC* (c.4667G>A: p.Trp1556*): Individual 281177 is an 11 year old male who was born after an uneventful pregnancy by planned caesarean section at 40 weeks gestation weighing 3.5Kg [-.02 SD]. There were no perinatal problems, there was some delay in attaining head control but no other concerns were noted in the first year. He sat unaided at 6 months and walked unaided at 12 months. He was generally healthy throughout infancy. The first significant cognitive concern was related to his delay in acquisition of both receptive and expressive language. He did not have clear words until he was over three years old. He was first seen by clinical genetics services aged 7 years 8 months for investigation of learning disability. At that age his height was 127.3 cm [.26 SD], weight 25.7 kg [.26 SD] and his head circumference was 54.3 cm [.34 SD]. He had no major dysmorphisms or malformations. There were no focal neurological signs. He was generally a pleasant and cooperative boy but his parents reported significant behavioral problems at home, in particular he would become in appropriately angry over minor issues. He has been formally assessed for autistic spectrum disorder but he did not fulfill the diagnostic criteria. Following the clinical genetics appointment DNA was taken for array CGH which was normal. He was recruited to the DDD Study in 2013. On trio-based

exome sequencing a *de novo* nonsense mutation was identified in *CLTC* (NM_004859.3:c.4667G>A: p.Trp1556*).

4) Individuals with *de novo* variants in *DHDDS* (NM_024887.3):

indvSG; *DHDDS* (c.110G>A: p.Arg37His): This is a globally delayed, nonverbal girl who recently started walking independently at 4 years of age. She has dozens of seizures per day that started at 18 months of age which are exquisitely photosensitive- even going outside in the sunlight sets her off. Her seizures consist of eyelid fluttering lasting a few seconds often with throwing her head back, suggesting absence seizures with eyelid myoclonia. There is no post-ictal state. No medication has been successful at treating her seizures (valproic acid, lamotrigine, levetiracetam, ethosuxamide); however, parents feel valproic acid (divalproex) had made the greatest difference but she still has dozens of seizures daily. Brain MRI was normal at the age of 12 months. Other investigations found negative include clinical array CGH and comprehensive epilepsy panel sequencing from Transgenomics. Clinical WES at BCM-Miraca diagnostic laboratory did not reveal any likely pathogenic variant, however, it identified a missense in *DHDDS* (NM_024887.3; c.110G>A:p.Arg37His) which was subsequently confirmed to be *de novo* by Sanger sequencing in the parents and the child.

HSJ0762; *DHDDS* (c.110G>A: p.Arg37His): This is a 6 years old male with normal antenatal and postnatal history born to non-consanguineous parents. Low tone was evident since birth and always showed some tremor when performing an action. He sat at 9 months and walked at 21 months. He said his first words at the age of 2 and at 5 years of age he was able to communicate with sentences. At 12 months of age he started having myoclonic seizures requiring hospitalization; brain MRI and lumbar puncture (LP) including amino acids were normal. EEG showed generalized epileptiform activity. He started on high dose levetiracetam and became somnolent therefore dose reduced. At 22 months of age he experienced "staring spells" 2-3 seconds during times of illness and a few episodes of sudden falling to the ground. At the age of 25 months the EEG showed abnormal background with no gradient and generalized discharges. He experienced ongoing myoclonic seizures only at time of fevers and atypical absence seizures also increased with fever. Levetiracetam was increased to 70mg/kg/day and valproate was added resulting in a better seizure control. At the age of 36 months he showed a wide based gait, difficulty running, tremor, 4 point crawl up stairs. At age 5 years he was still having atypical absences with atonic semiology a couple of times a week (was seizure free for 1 year 2015-2016). No further myoclonic events. VPA increased in 2016 and no seizures since. Other Investigations done and found negative: aCGH, epilepsy panel (GeneDx), MELAS/MERFF, Carnitine, acylcarnitine, amino acids, organic acids, ammonia, 2nd LP normal CSF/serum glucose, neurotransmitters. Trio WGS sequencing identified a *de novo* missense in *DHDDS* (NM_024887.3: c.110G>A:p.Arg37His).

indvEF; *DHDDS* (c.632G>A: p.Arg211Gln): This individual is a six-year-old female with a history of global developmental delay, hypotonia, tremor, ataxia, and seizures. Hypotonia (axial and appendicular) was first noted around seven months of age. Around the same time, head tremors, eventually progressing to involving her arms and legs, were also noted. In retrospect, her parents feel she had tremors in early infancy. The tremor is mild, present at rest but worsens with activity. It is absent during sleep. Her early development was normal, with rolling over and sitting up at six months,

pulling to stand at ten months, and walking by 14.5 months. As she began to walk, she was noted to be ataxic and has continued to have an unsteady gait. Otherwise, her development was relatively normal up until age two years. Evidence of global developmental delay manifested after age two years. She has global developmental delay, although she has not had regression and is making progress. She has difficulty processing information and her level of understanding is unclear. Her seizures began around four years of age and were initially described as staring blankly and eye fluttering, followed by a postictal period of weakness and confusion. An EEG in the past was abnormal with epileptiform activity. Levetiracetam was started but discontinued because she would not tolerate it. Seizures occurred every two to three months. Seizures are now well controlled with lamotrigine. Brain imaging was essentially normal and did not reveal a cause for her neurologic symptoms (a Chiari I malformation was identified). She was born full term at 8 lbs 12 ounces and 21.5 inches long. Past medical history is significant for laryngomalacia and torticollis in infancy. She has had a substantial weight gain in the setting of insatiable appetite and is undergoing endocrinological workup for Cushing's syndrome and thyroid disorders. Family history is unremarkable. Her father had seizures in childhood, but not now. Her mother is in good health. Neither parent has intellectual disability, tremors, or ataxia. She has a sister and a brother who are both growing and developing normally. No one else in the family has health problems like indvEF. Physical exam at age 5 years revealed thick, dark hair, hypotonia and unsteady gait, but she was able to walk on her own. Speech was relatively easy to understand but she displayed some articulation errors. Genetic work up (chromosome microarray and deletion testing for spinal muscular atrophy) was initially normal. Trio WES (GeneDx) revealed a *de novo* missense in DHDDS (NM_024887.3; c.632G>A: p.Arg211Gln).

MDB31882; *DHDDS* (c.632G>A: p.Arg211Gln): This 35 year-old male was born after an uneventful pregnancy and normal delivery from nonconsanguineous healthy parents. After an unremarkable neonatal period, global developmental delay became evident. He acquired trunk control at 18 months, and walked unsupported at 30 months. Impaired social skills, repetitive behaviors, sensory-perceptual abnormalities, and language development delay suggested the diagnosis of autistic spectrum disorder, at the age of 4 years. From early infancy, he showed fluctuations in mood and activity levels with periods of marked anxiety and restlessness alternated with periods of apathy and hypokinesia. At the age of 2 years, distal upper limb tremor was noticed; which became associated with postural, action and stimulus-sensitive multifocal non-epileptic cortical myoclonus (involving trunk and upper limbs), from the age of 6, which turned out to be generalized during the following two years. From the age of 9, paroxysmal eyelid myoclonias and staring were associated with generalized polyspike-waves on the EEG recording. Epileptic seizures were successfully treated with sodium valproate, which did not affect movement disorders and clinical fluctuation. From late adolescence forward, this individual experienced a progressive switch to an hypokinetic rigid syndrome associated with generalized tremor. Clinical status continued to fluctuate between status of increased tremulousness and multifocal myoclonus (lasting 1 week/month) and akinesia and catatonia (lasting 1 week/month). Several pharmacological attempts (piracetam, benzodiazepines, dopaminergic and anti-dopaminergic drugs) resulted ineffective in improving neuromotor disorders and/or preventing clinical fluctuations. On the last examination at the age of 35 years, he showed generalized tremor, facial myokimia, poly-mini-myoclonus of the fingers, bradykinesia, hypomimia, rigidity, freezing and impaired postural reactions. Neuropsychological and behavioral phenotype was characterized by severe intellectual disability and

frontal lobe impairment features with verbal perseveration, disinhibition and unsuitable joviality. Structural and functional evaluation of brain (MRI, 1H-MRS, EMG, Flash and pattern visual-, motor- and somatosensorial-evoked potentials) were normal. An extensive neurogenetic and neurometabolic work-up, including IEF profiling of transferrins, failed in identifying any diagnostic cue. WES identified a heterozygous predicted-damaging missense in DHDDS (NM_024887.3: c.632G>A:p.Arg211Gln) that was subsequently confirmed by Sanger sequencing to be *de novo*.

indvNCJ; *DHDDS* (c.632G>A: p.Arg211Gln): This individual is a 7 year old female with a moderate-severe intellectual disability and a movement disorder. She is the second child of healthy parents and was born after an uncomplicated pregnancy. She had a secondary caesarian section because of abnormal CTG. Her APGAR scores were 6 after 1 minute and 8 after 5 minutes. pH umbilical artery 7.21 (Base excess -2.0), pH umbilical vein 7.25 (Base excess -1.8). Her birth weight was 4035 gram. At the age of 3 months she had an airway infection due to RS virus for which ventilation and tube feeding was needed for several days. Around the age of 1 year parents noticed she developed different than their older child. At the age of 1 year and 10 month she was formally tested and global developmental delay was identified. She functioned at the level of a 1 year old (Bayley Scales of Infant Development (BSID)-II-NL non-verbal, rough score 61, development index 55, Dutch non-speech test receptive and perceptive language below first percentile). When she started walking at the age of 2,5 years, parents noticed a movement disorder. She said her first words at the age of 3,5 year, and speaks full sentences at the age of 7 years. The family history revealed neither movement disorders nor intellectual disability nor seizures. At the age of 1 year and 4 months parents thought she might have Rett syndrome. Clinical and molecular investigation (MECP2) showed no evidence for this diagnosis. Also array CGH (Agilent 180 K custom HD-DGH microarray; (AMADID-nr 27730)) was done around that period and showed a normal female pattern. At the age of 4.5 years, physical exam revealed no dysmorphisms except for a high palate, missing tooth element and a hyperpigmentation at the right side of the thorax. An intention tremor was seen and ataxia was suspected. Genetic analysis of the FMR1-gene showed no CGG-expansion and whole exome sequencing filtered for genes causing developmental delay no potential pathogenic variants were identified (Radboud MC, Netherlands). At the age of 5.5 years eye examination including examination of the fundi showed no abnormalities. The pediatric neurologist noted that the child displayed abnormal movement with especially dystonia, but also random movements of the face, eyebrows and mouth. No intention tremor was seen. Metabolic pediatrician clinically saw no evidence for a disorder of metabolism. Analysis panel movement disorders and open exome analysis initially revealed no potential pathogenic variants (Radboud MC, Netherlands). Lumbar puncture, MRI and MR spectrometry revealed no abnormalities. At the age of 7 years reanalysis of exome data revealed a *de novo* missense in *DHDDS* (c.632G>A: p.Arg211Gln). Standard metabolic investigation in blood and urine has been performed including sialo-transferines, but no abnormalities were identified. She recently had her first seizures. They started with jerky movements from her right arm and in lesser extent her right leg. She remained conscious during this period. On video the movement are classified as cortical myoclonus. After half an hour her whole body cramped for one minute. She had several periods of these insults afterwards. Her movement disorder is described as jerky movements especially in action, partly related to ataxia, partly related to myoclonus. A recent EEG was of limited assessability, but no clear epileptiform activity is seen.

5) Individuals with *de novo* variants in *NUS1* (NM_138459.4):

indvKW; *NUS1* (c.743delA: p.Asp248Alafs*4): This is a male (8years, 9 months) whose had global developmental delay and currently shows moderate ID. He has language delay and is difficult to understand. Seizures started at the age of 12 months as generalized myoclonic epilepsy versus convulsive epilepsy, nocturnal jerks. EEG testing showed bifrontal epileptiform activity. The seizures are controlled with Levetiracetam. History of ataxia post doses of Levetiracetam was initially noted and was later resolved but incoordination persists. Following periods of increased seizure activity he has regression of language. Brain MRI (2y3mo) and array CGH were normal. Clinical exome sequencing (GeneDx) revealed a de novo frameshift mutation in NUS1 (NM_138459.4: c.743delA: p.Asp248Alafs*4).

HSJ0623; *NUS1* (c.128_141dup: p.Val48Profs*7): Individual HSJ0623 is a 15 years old boy and is the first child of a non-consanguineous French-Canadian couple. The pregnancy was unremarkable except for preterm contractions for which the mother was placed on bed at 7 months of gestation. The individual was born at term after an unremarkable delivery. He presented with low birth weight (2 489g, 3rd percentile) but without signs of perinatal distress (APGAR 9-10-10). He presented at 10 months of age with seizures and mild motor delay. His initial seizures were described as myoclonic absences with behavioural arrest, facial and palpebral myoclonus, lasting 5-10 seconds and occurring 5 times per day. The seizures responded to valproic acid. He had one febrile tonic-clonic seizure at 18 months of age. He presented with atonic seizures (drops) at 2 years of age, sometimes with a vague sensory aura, occurring up to 100 times per day. These seizures were quite refractory to treatment (various combinations of valproic acid, lamotrigine, levetiracetam, ethosuximide, clonazepam, carbamazepine and stiripentol), but responded to a combination of valproic acid and clobazam at age 7.5 years old. He has had only 2 seizures since 10 years of age on this combination. EEGs revealed diffuse background slowing with rhythmic bifrontal high amplitude rhythmic theta discharges. A video-EEG monitoring at 7.5 years revealed diffuse background slowing and epileptic discharges with bi 2082 frontal small amplitude spikes with secondary generalization manifesting as absence seizures or head drops. The walked at 16 months of age. However, he can now run, climb stairs, play sports. He is described as clumsy on fine motor tasks (he can eat and dress independently but needs help with buttons and zip and uses adapted pencils to write). He has moderate intellectual deficiency and was diagnosed with autism spectrum disorder (ADI and ADOS) at 7 years of age. He spoke his first words at one year of age and now communicates with short sentences. He can engage in brief conversations. He is known for inattention but has not tolerated the side effects of psychostimulants (Adderal and Ritalin). He receives special education in a TEACH classroom. On examination at 10 years of age, his head circumference was 56 cm (98th percentile), his height was 154 cm (>97th percentile). He has no dysmorphic traits and his neurological exam is entirely normal apart from mild postural and kinetic tremor without other signs of cerebellar impairment (no ataxia, dyskinesia, dysarthria, etc). On investigation, a brain CT scan at 18 months and a brain MRI at 8 years of age were unremarkable. The karyotype, CGH and Fragile X screens were normal. Serum lactate, ammonia, amino acid screen were normal, as was a urinary organic acid, purines/pyrimidines, creatine and GUAC profiles. *SCN1A* sequencing was negative. Whole genome sequencing revealed a *de novo* variant in *NUS1* (NM_138459.4:c.128_141dup: p.Val48Profs*7).

HSJ0627; *NUS1*: 1.3 kb del exon2: Individual HSJ0627 is a 29 years old woman and is the first child of Caucasian non-consanguineous parents of European descent. She was born at 8 months of pregnancy by C-section due to suspected fetal distress. The delivery was uneventful and the baby did not present apparent signs of a perinatal hypoxic-ischemic event. She presented at 2.5 years of age with febrile myoclonic status epilepticus. She than developed myoclonic absences with behavioural arrest and eyelid flutters as well as limb myoclonus, lasting less than 1 minute. She also developed sudden drop attacks, often with a premonitory feeling (malaise?), occurring 1-2 times per week. She received various combinations of valproic acid, levetiracetam, clobazam, felbamate, lamotrigine and clonazepam. At her last evaluation, her seizures were relatively well controlled with a combination of valproic acid, lamotrigine and clonazepam. Her EEGs revealed generalized spike-wave and poly-spike wave activity. This individual presented with a mild motor delay. She walked at 17 months of age. She can run, jump and swim but is unable to ride a bicycle. She is autonomous on fine motor skills: she can eat, dress and wash independently. Her writing is imprecise and clumsy. Her language skills seemed unaffected as she started talking around one year of age, has a fluent spontaneous speech with full sentences. She can read short sentences and functions at an equivalent of 1st grade. On examination at 29 year, the head circumference was at the 25th percentile. Eye pursuits were saccadic but saccades were normal. She had mild dysarthria, mild postural and kinetic tremors but no ataxia. Her reflexes were brisk. Brain MRIs were repeated 3 times and were unremarkable. An extensive metabolic screen was normal (including serum ammonia, lactate, amino acid screen, urinary organic acid screen and creatine/GUAC dosages). An array CGH was normal. Genome sequencing revealed a *de novo* intragenic deletion of ~1.3 kb in exon 2 of the *NUS1* (NM_138459.4).

6) Individuals with *de novo* variants in *RAB11A* (NM_004663.4):

HK055; *RAB11A* (c.71A>G: p.Lys24Arg): This male individual was born normally at term with normal birth weight 3710g, length 51 cm and occipitofrontal circumference (OFC) 35 cm (-0.5 SD); Apgar score 9/9. After the birth moderate muscular hypotonia was noticed and spinal muscular atrophy was excluded by molecular testing. Soon after the birth he showed good weight gain. At 5 months of age his length was 68 cm (0 SD), weight was 10.9 kg (+3 SD) and OFC 42.7 cm (-1 SD). His main clinical problem was muscular hypotonia. Brain MRI showed dilated lateral ventricles. At 13 months of age developmental delay became more evident. He turned and crawled, but did not sit, stand or walk. He said few words. He had 3 episodes of unconsciousness with perioral cyanosis in early infancy, but EEG showed normal background activity in awake and sleep state. Cardiac pathology was excluded by normal results in electrocardiography and ultrasound investigation. At 2 years and 1 month of age his height was 87 cm (0 SD) and weight 13.6 kg (0 SD). Acquired microcephaly was noticed – OFC 46 cm (-2.5 SD). He had muscular hypotonia, walked with the aid and had axial ataxia. He said only few words. Passive understanding of simple speech was evident. He showed aggressive behavior. He has rough and curly hair, hypertelorism, epicanthal folds, astigmatism, single palmar crease in the left side, abnormal fatty skin folds and inverted nipples. EEG and ENMG were normal. Griffith scale at 2years and 1 month showed delay in development corresponding to the age of 12 months. He is presently 5.5-year-old boy with moderate ID and microcephaly – his OFC was 49 cm (-2.5 SD). His height is 112 cm

(0 SD) and weight 30 kg (+4 SD), KMI=24 (obesity). He has a clumsy walk. Griffith scale-III at 5years and 2 months showed delay in development. His cognitive skills correspond to that of an age of 2 years and his social skill to that of an age of 2 years and 5 months. EEG showed abnormal background activity, but no epileptic charges. Brain MRI at 3 years of age showed bilateral widening of third and lateral ventricles, which indicate central brain atrophy and bilateral periventricular white matter damage; corpus callosum is relatively thin. Chromosomal microarray analysis showed no abnormal chromosomal copy number variations. Extensive metabolic investigations were done, which were in normal range (amino and organic acids in serum and urine, acylcarnitines and transferrine isoelectric focusing in serum, creatine and guanidinoacetate in urine and neurotransmitters in cerebrospinal fluid). Trio exome sequencing identified a novel de novo missense in *RAB11A* (NM_004663.4: c.71A>G: p.Lys24Arg).

HSJ0637; *RAB11A* (c.244C>T: p.Arg82Cys): Individual HSJ0637 is a 9.5 years old girl and the only child of a French-Canadian non-consanguineous couple. The prenatal ultrasounds revealed mild dilatation of the lateral ventricles (14-15 mm at 19 weeks of gestation), which stabilized to 13 mm at 21 weeks of gestation. A prenatal fetal MRI at 25 weeks of gestation was unremarkable, as was a prenatal fetal echocardiogram. The virology screens were negative but hematological investigations revealed maternal anti PLA1 antibodies, which were treated prenatally. The child was born by C-section at 37 weeks of gestation and did not display signs of perinatal distress (APGAR 8-8-9, birth weight and length at the 10th and 25th percentile, HC 33 cm (10th percentile). She presented a mild physiological jaundice, which resolved after a few days, as well as anemia and neutropenia that resolved spontaneously at a few weeks of age. She was referred for progressive post-natal microcephaly at 6 months of age (stabilized at the 2nd percentile since 10 month of age). The child presented seizures at 4 months of age consisting of erratic limb myoclonus followed by flexion spasms of the limbs and trunk. The initial EEG revealed abundant multifocal epileptic activity with sustained spike-wave discharges over both occipital areas. An EEG monitoring at 6 months revealed hypsarrhythmia and electrodecremential responses during spasms confirming the diagnosis of West syndrome. The epileptic spasms were refractory to therapy, including various combinations of nitrazepam, clobazam, vigabatrine (initiated at 6 months) and topiramate. The epileptic spasms subsided around 2 years of age. However, at 8 months of age, the child had developed focal seizures with behavioral arrest, horizontal nystagmus (towards the right), chewing automatisms and version of the head and trunk towards the right, lasting less than a minute. She was treated with combinations of topiramate, clobazam and valproic acid with partial responses. The seizures were controlled with the addition of levetiracetam at one year of age. Similar episodes of behavioral arrests with head deviation were noted after weaning the levetiracetam at 3 years of age, but an EEG monitoring during the episodes did not reveal concomitant epileptic activity and the episodes were considered non epileptic. Since the age of 3, this individual has had rare episodes of behavioral arrest and head deviation, lasting less than 2 minutes, and that seem to respond at least partially to stimulation. The parents have declined medication since that time. Of note, repeated EEGs (including one at 9.5 years of age) revealed diffuse slowing of the background rhythms with persistent multifocal epileptic activity with low amplitude spikes followed by large slow waves occurring in short bouts of 3-4 seconds at 2 Hz, in the frontal, temporal, occipital areas bilaterally and independently. Nonetheless, interictal EEGs at 2 and 5 years

old were unremarkable apart from diffuse background slowing. She had developmental regression at the onset of seizures around 4 months of age with reduced interest and hypotonia. She eventually learned to turn from back to front at 18 months of age, could sit with support at 2.5 years and sat independently at 3.5 years old. She has moderate to severe intellectual deficiency with autistic traits. She is non-verbal but uses a few communication signs. Her visual contact is limited. She likes musical games and action-reaction games but has relatively restricted interested otherwise. She brings objects to her mouth and can hold her bottle to drink. She has trouble swallowing and drools easily. She needs help for most daily tasks. On formal examination at 5 years of age, she had microcephaly (47 cm, 2^{nd} percentile) and a height of 109 cm (25^{th} percentile). There were no other dysmorphic traits. She has moderate axial hypotonia and tends to lean forward but can sit up if prompted. There were no signs of spasticity, the limb tone and reflexes were normal. She could lift her limbs against gravity and perform simple directed tasks. On investigation, a brain ultrasound at 3 months of age revealed increase subarachnoid spaces. A brain MRI at 6 months of age revealed progressive diffuse brain atrophy with enhanced lateral ventricles, partial agenesis of the corpus callosum (rostrum and splenium) and myelination delay. The spectroscopy revealed a decreased NAA signal. The karyotype and array CGH were normal as was an extensive metabolic screen (serum lactate, ammonia, CK, amino acid screen, acylcarnitine profile, glycosylation screen, and urinary purines and pyrimidines profile). The urinary organic acid screen revealed non-specific increases in oxalic and glyceric acids. A heart ultrasound and bone XR were normal. Sequencing of the MECP2, FOXG1 and CDKL5 genes were negative. An ophthalmology evaluation at 1 year of age revealed visual inattention (with absent P100 wave on visual evoked potentials). The auditory screen was unremarkable. Whole genome sequencing revealed a de novo missense in *RAB11A* (NM_004663.4:c.244C>T: p.Arg82Cys).

24631; *RAB11A* (c.461C>T: p.Ser154Leu): This male individual was born at term by caesarean section as breech presentation, following a normal pregnancy apart from borderline gestational diabetes. His birth weight and OFC were 4.3kg and 36.5cm, respectively. He had a urinary tract infection at 7 months, found to have a dilated left calix and ureter. He had numerous hearing tests due to concerns about speech delay, but all were normal. He has language delay; at age 4 he had some 2 word phrases and an array of single words. He rolled at 12 months and walked from 31 months. He was diagnosed with moderate developmental delay. He has limited attention span and is highly distractible. He did not have hypotonia or seizures. He was found to have a mildly raised phenylalanine level. Genetic analysis identified 2 variants in PAH, c.117C>G & c.805A>C, but these were not judged to be contributing to his delay. On examination aged 3 years & 11 months, height was 104.4cm (60th centile) and OFC was 50cm (18th centile). He shows bilateral frontal cowlicks, thin upper lip and single palmar crease on the left. Standard karyotype, Fish for 22q11, CGH array, all normal. MRI aged 6 showed partial agenesis of the corpus callosum, but was otherwise normal. Trio exome sequencing as part of the DDD study revealed a *de novo* missense variant in *RAB11A* (NM_004663.4:c.461C>T: p.Ser154Leu).

84049; *RAB11A* (c.461C>T: p.Ser154Leu): This female individual was born to non-consanguineous family. Possible poor fetal movements were noted but she was delivered at term by Caesarean (birth weight 3.43kg, OFC 36cm). She sat at 8 months but never crawled. She pulled to stand at 20 months

and walked at the age of 3 years. Her language skills are delayed. She had moderate GDD. At the age of 9 years, 11 months she was coping at mainstream school with one to one support. She had obesity from early on despite the fact that her parents were very strict as to intake and that she was active and not food-seeking. She has no history of seizures. Brain MRI was not done. aCGH, PWS methylation, UPD14 studies were all normal. Trio exome sequencing as part of the DDD study revealed a *de novo* missense variant in *RAB11A* (NM_004663.4:c.461C>T:p.Ser154Leu).

7) Individuals with *de novo* variants *GABBR2* (NM_005458.7) or *SNAP25* (NM_130811.3):

HSJ0048; *GABBR2* (c.2077G>T: p.Gly693Trp): Individual HSJ0048 is a 14 years old boy and the second child of a non-consanguineous French-Canadian couple. He was born at term after an uneventful pregnancy. There were no perinatal complications, the APGAR score was 8-9-9 and the birth head circumference was 34.5 cm (P50). He presented at 11 months of age with severe global developmental delay and seizures. The initial seizures were brief focal seizures with impaired awareness characterized by behavioral arrest and perioral cyanosis, which sometimes progressed to become bilateral tonic-clonic seizures, lasting 20-60 seconds. He was initially treated with carbamazepine but within a month he had clusters of epileptic spasms with bouts of flexion spasms of the trunk and limbs. The EEG at 1 year of age revealed modified hypsarrhythmia. Carbamazepine was therefore discontinued and vigabatrin was initiated, with complete resolution of the epileptic spasms. The seizures recurred around 4.5 years of age, with focal seizures with impaired awareness presenting as behavioral arrest, visual fixation, forced laughter, sometimes with a myoclonic jerk of the axial musculature, lasting less than 10 seconds. These seizures were refractory to valproic acid, but responded to topiramate. The seizures recurred after two years and were now longer (30 seconds to 1 minute), occurring 1-2 times per day, together with primary generalized tonic-clonic seizures lasting 1-3.5 minutes and occurring up to 8 times per day. The seizures did not respond to serial combinations of topiramate, carbamazepine, clobazam, phenytoin, levetiracetam and lacosamide. However, partial seizure control was achieved with the addition of lamotrigene at 13 years of age. At last follow-up, the child was receiving a combination of lamotrigene, lacosamide, clobazam and phenytoin and was still presenting brief generalized tonic-clonic seizures (<30 seconds) once per week, mostly during sleep. He presented a severe global developmental delay. He had axial hypotonia from the first few months of age. He started rolling from back to belly at 17 months of age and could sit with support around 2 years of age. He cannot sit on his own, does not crawl or stand. He has profound intellectual deficiency, remains non-verbal, has no communication skills, poor visual contact, grunts but does not point. He presents very limited interests for objects and people in his environment and does not reach for objects placed in front of him. He presents frequent bouts of aggressiveness and self-inflicted injuries despite treatment with benzodiazepines, antipsychotic medications (risperidal and clozapine) and clonidine. On examination at 12 years of age, the head circumference was 56 cm (90^{th} percentile). There were no dysmorphic traits but he presented occipital plagiocephaly. He had no eye contact, was drooling and had a tendency to keep his mouth open. He presented major axial hypotonia, necessitating truncal support to sit up, and thoracic scoliosis. He also had moderate limb hypotonia with hyporeflexia, but could lift his limbs against gravity. On investigation, a brain CT scan and a brain MRI at 13 months of age revealed increased sub-arachnoid spaces, mostly bi-frontal, as well as *ex vacuo* dilatation of the

lateral ventricles. The karyotype, sub-telomeres, Fragile X and 15q13 FISh studies were negative. A metabolic screen, including serum lactate, ammonia, amino acid VLCFA and transferrin glycosylation screens as well as the urinary organic acid screen were negative. An electromyogram was performed and was non-contributory, but a muscle biopsy revealed signs of underuse myopathy. Whole genome sequencing revealed a *de novo* variant in *GABBR2* (NM_005458.7: c.2077G>T: p.Gly693Trp).

HSJ0745; *SNAP25* (c.496G>T: p.Asp166Tyr): Individual HSJ0745 is a 23 years old male. He was born at term after an uneventful pregnancy from a non-consanguineous French-Canadian couple. He presented mild respiratory distress at birth, requiring nasopharygeal aspirations of meconium-tainted amniotic fluid, which resolved quickly (APGAR score 5-6-9). He was hospitalized in the first few months of life for recurrent apneas with cyanosis and bradycardia. The investigation, including EEG, eosophageal pH monitoring, ECG and Holter monitorings, were normal. The apneas resolved spontaneously at 6 months of age. He also presented with severe constipation in the first months of life, for which an extensive gastroenterological investigation was conducted and was found to be normal (including a rectal manometry, rectal biopsy and contrast imaging of the intestines). The child was followed in neurology since the first year of life for global developmental delay which evolved towards a moderate ID. He started crawling at 14 months of age and took his first steps at 2 years of age. He is now active and autonomous: he can run, climb, plays hockey and basketball and drives a bicycle. He has no major issues with fine motor skills: he eats, dresses and writes independently. He had a moderate language delay (first words at 2 years) but he now speaks fluently with full sentences. His speech is sometimes imprecise with some degree of speech dyspraxia. His receptive abilities are intact. He received special education and now continues training in a special school for adults with cognitive impairment. He recognizes written words but cannot read sentences. He can write his name and most letters but cannot write sentences. He can count but cannot do arithmetical calculations (no additions or subtractions). He developed epilepsy at 18 months of age with nocturnal tonic-clonic seizures and a few brief episodes of arrest of activity with confusion during daytime, suggestive of focal seizures with impaired awareness. The seizures were controlled with valproic acid but recurred upon weaning. He eventually required a combination of valproic acid and clobazam to control seizures, and the clobazam was weaned at the age of 18 years. He remains on valproic acid and has been seizure-free for the last 2 years. His initial EEGs revealed generalized spike-wave discharges that became almost constant during sleep (continuous spike-wave seizures during slow-wave sleep). The last available EEG at age 15 years revealed intermittent generalized discharges during drowsiness. On examination, he presents a few minor dysmorphic traits with upslanted short palpebral fissures, telecantus and short thumbs. He has speech dyspraxia. The neurological exam is otherwise unremarkable. A brain MRI at 20 years of age revealed mild diffuse cortical atrophy. The genetic investigation, including array CGH, Fragile X screen and *GRIN2A* gene sequencing was negative. His genome sequencing revealed a *de novo* missense in *SNAP25* (NM_130811.3:c.496G>T: p.Asp166Tyr).

Supplemental figures

Figure S1: % of the complete coding sequence (CCDS) and genome bases covered at ≥ 10x in the CENet DEE trios

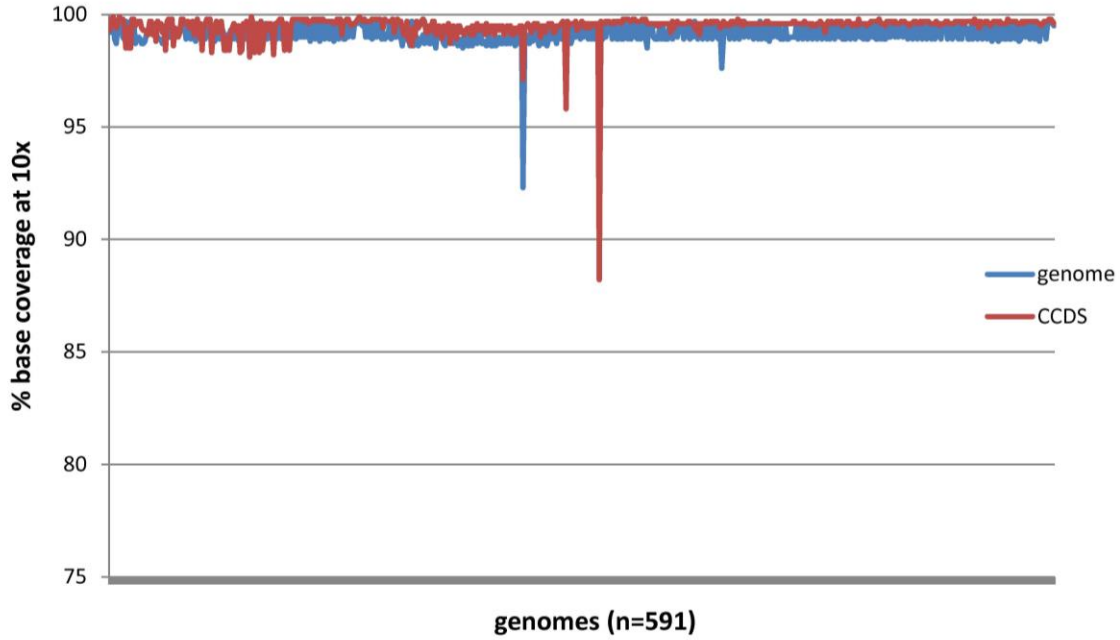
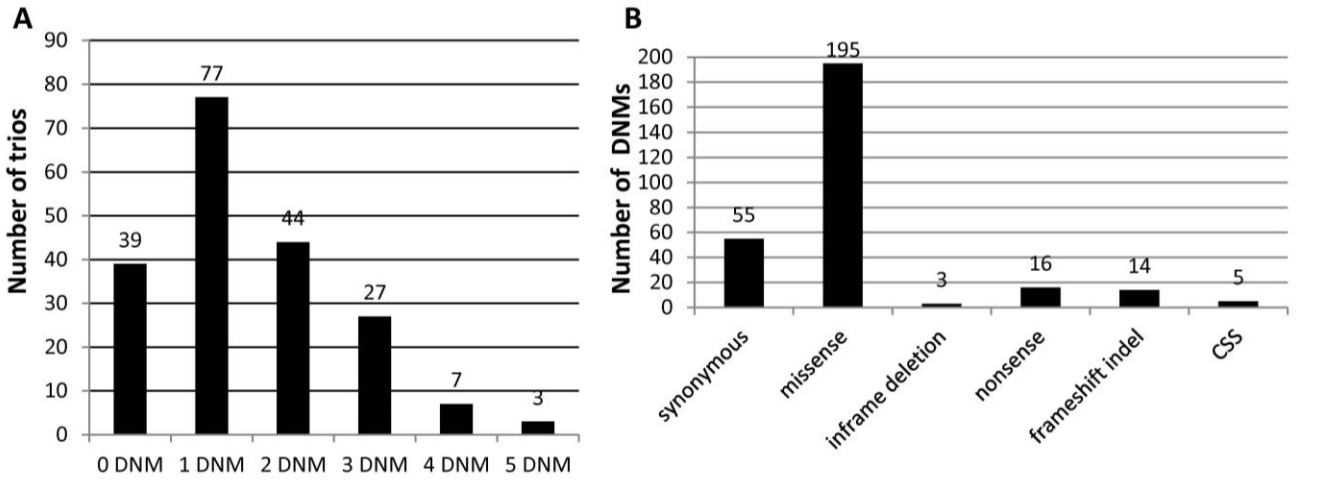


Figure S2. Number (A) and type (B) of Sanger-validated DNMs in the coding and canonical splice sites (CSS) per trio



Supplemental Tables

Table S1. Composition of the CENet DEE cohort used for WGS

DEE phenotype	**number of cases**
Unclassified developmental and epileptic encephalopathies (DEE)	84
Lennox-Gaustaut syndrome (LGS)	16
Infantile spasms (IS)	18
Early-onset epileptic encephalopathy (EOEE)	63
Myoclonic astatic epilepsy (MAE)	5
Childhood Absence Epilepsy	3
Dravet syndrome (DS)	3
Ohtahara syndrome (OS)	2
CSWS/Landau-Kleffner spectrum disorders	2
Early myoclonic epilepsy (EME)	1
Total	**197**

Table S2. Composition of the DEE cohort used for MIPs screening.

DEE phenotype	**number of cases**
Devastating epileptic encephalopathy in school-aged children (DESC)	1
Dravet syndrome (DS)	7
Epilepsy-aphasia syndromes (EAS)	36
Unclassified developmental and epileptic encephalopathy (DEE)	210
Epilepsy of infancy with migrating focal seizures (EIMFS)	1
Early myoclonic encephalopathy (EME)	2
Early-onset absence epilepsy (EOAE)	3
Early-onset EE (EOEE)	41
Electrical status epilepticus during slow-wave sleep (ESES)	1
Febrile infection-related epilepsy syndrome (FIRES)	10
Hemiconvulsion-hemiplegia epilepsy (HHE)	4
Infantile spasms (IS)	111
Lennox-Gaustaut syndrome (LGS)	57
Late-onset spasms (LOS)	2
Myoclonic astatic epilepsy (MAE)	100
Migrating partial seizures in infancy (MPSI)	3
Ohtahara syndrome (OS)	4
Progressive myoclonus epilepsy (PME)	2
Total	**595**

Table S3. DEE and ID cohorts used in the DNM meta-analyses

cohort	PMID	phenotype	number of trios	sequencing	total trios	
CENeT (current study)	-	DEE	197	WGS	624	5948
Halvardson et al. (2016)	27334371	DEE	39	WES		
Hino-Fukuyo et al. (2015)	25877686	DEE	14	WES		
Epi4K Consortium (2014)	25262651	DEE	356	WES		
Michaud et al. (2014)	24781210	DEE	18	WES		
DDD (2017)	28135719	ID	4293	WES	5324	
Lelieveld et al. (2016)	27479843	ID	820	WES		
Lopes et al. (2016)	26740508	ID	19	WES		
Gilissen et al. (2014)	24896178	ID	50	WES		
Hamdan et al. (2014)	25356899	ID	41	WGS		
de Ligt et al. (2012)	23033978	ID	50	WES		
Rauch et al. (2012)	23020937	ID	51	WES		

Table S4. Variants called per genome

variant type	average number of variants/affected individual (n=197)	average number of variants/parent (n=394)
SNVs	3790550	3789423
insertion	180005	179822
deletion	212653	212528
total genomic variants	**4183208**	**4181772**
stopgain_SNV	97	97
stoploss_SNV	43	42
frameshift_deletion	112	113
frameshift_insertion	163	163
missense_SNV	11005	11013
nonframeshift_deletion	135	135
nonframeshift_insertion	94	94
synonymous_SNV	11873	11885
total variants in coding regions	**23521**	**23542**