

## Supplemental Data

### **A Recurrent De Novo Variant in *NACC1* Causes a Syndrome Characterized by Infantile Epilepsy, Cataracts, and Profound Developmental Delay**

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## Supplementary Data

### Case Reports

Individual 1: A 20-month-old Caucasian male was evaluated through the Undiagnosed Diseases Network for infantile spasms, acquired microcephaly, lamellar cataracts, failure to thrive, and global developmental delays.

He was born after an uneventful pregnancy at 39 weeks to a 34-year-old G<sub>1</sub>P<sub>0</sub> mother and a 32-year-old father, with a birth weight of 3.473 kg (60<sup>th</sup> percentile), a birth length of 48.3 cm (20<sup>th</sup> percentile), and an OFC of 34 cm (30<sup>th</sup> percentile). The neonatal course was complicated by difficulty latching to the breast requiring formula supplementation. A consistent left lateral gaze was also noted.

Developmental concerns were first noted at 4 months of age, when he was not rolling over, had poor head control, did not visually track and did not babble. Developmental regression was noted at 9 months with onset of seizures. Currently he is profoundly delayed with poor head control and does not sit independently, intentionally grasp objects or consistently track.

He was clinically diagnosed with infantile spasms at 9 months, confirmed by EEG showing hypsarrhythmia at 11 months old, at which time a 2-week course of ACTH was instituted with poor response. Seizures have been managed with Clonazepam and Topamax. Ketogenic diet was initiated at 19 months old due to recurrence of infantile spasms, and he has been seizure free since two weeks after diet was begun.

He was diagnosed with bilateral lamellar cataracts, without prominent subcapsular or nuclear component, at 7 months of age and underwent removal of these at 10 months of age.

A brain MRI at 1 year of age showed mildly delayed myelination within the peripheral white matter, and brain volume was mildly decreased with slight enlargement of ventricles. Brainstem auditory evoked response (BAER) at 10 months old revealed adequate hearing for speech development. A gastrostomy tube was placed at 11 months due to failure to thrive. Since infancy he has been difficult to soothe.

On exam at 20 months, he was small for his age, with the height at 76cm (<1<sup>st</sup> percentile), weight at 9.8 kg (3<sup>rd</sup> percentile) and OFC at 42 cm (<0.01<sup>th</sup> percentile). He had deep set eyes, depressed nasal bridge, slightly upturned nose, midface hypoplasia, and thick eyebrows. He had bilateral fifth finger clinodactyly, single palmar creases, small fifth toenails and dysplastic right toenail. He had a repetitive movement involving his upper extremities, in which he pulled the middle top part of his shirt downward with both hands.

Chromosome microarray was normal. A comprehensive epilepsy panel revealed a novel, heterozygous and maternally inherited *SCN2A* variant of unknown significance. Clinical WES (GeneDx) identified a novel heterozygous and maternally inherited *CRYGD* variant of unknown significance associated with autosomal dominant cataracts. The individual's maternal grandmother was also found to have both of these variants, and both the mother and maternal grandmother are neurologically normal with no history of cataracts. Family history was noncontributory.

Individual 2: A 12-year-old female presented to neurology clinic with epilepsy, microcephaly, cataracts, failure to thrive and severe developmental delay with regression. Her medical care had previously been managed in Saudi Arabia, so access to her full records was limited.

She was born at 36 weeks following induction of labor, but the reasons for this are unclear. By parental report she was small for gestational age and oligohydramnios was noted in the third trimester. She was admitted to the NICU for 7 days due to low weight and weakness.

The parents report normal development (she sat with support, rolled over) until 7-10 months of age when she began to lose motor skills, developed seizures, and was noted to be microcephalic.

She began having difficulty feeding, lost weight and was irritable with increased crying.

Currently all areas of development are profoundly impaired. She does not sit unsupported or speak. She does not have purposeful hand movement. She cries when she's hungry, and eats and drinks by mouth although there is concern with swallowing liquids.

She began having abnormal movements at 7 months of age which worsened by 10 months, described by parents as looking like seizures. At 11 months she was started on 3 AEDs during admission at a Saudi hospital, one of which was Clonazepam, but these records were unattainable. Her seizures have been managed solely with Clonazepam since 9 years old (duration of 3 years), although she continues to have seizures when doses of medication are missed. Seizures are described as paroxysmal quick jerk of arm upwards and head tilting to same side one time and then gradual relaxation. Seizures never last more than a few seconds, but can happen 100 times per day when off medications. She has never had a grand mal seizure. EEG at 12 years old showed only intermittent bifrontal slowing with no epileptiform discharges.

Her first cataract was diagnosed at 10 years of age, and the second at 11 years old. She has malformed globes.

A brain MRI at 17 months reportedly showed brain atrophy and fluid accumulation. Repeat imaging at 12 years old showed slight prominence of the extra-axial CSF spaces and the supratentorial ventricular system, arachnoid cyst in the left middle cranial fossa and left posterior parasagittal region, and developmental venous anomaly in the left centrum semiovale, in addition to the malformed globes. Spectroscopy revealed a slight decrease in NAA in the basal ganglia.

She has chronic anemia and also has had a reaction to sedation in the past that required CPR, intubation, and 1 week of ventilatory support.

On exam at 12 years of age, she was small with weight at 27.5 kg (<5 percentile), OFC at 46.5 cm (<2<sup>nd</sup> percentile). She was mildly dysmorphic, had bilateral cataracts and was hypertonic when awake. She did not react to visual stimuli although was responsive to touch and sound. She had Rett's-like hand automatisms.

Prior diagnostic workup included metabolic screening [serum amino acids (slightly elevated alanine), acylcarnitine profile, ammonia, lactate/pyruvate, lysosomal studies, very long chain fatty acids], CSF studies [neurotransmitters (slightly low 5-methyltetrahydrofolate), CSF amino acids and CSF lactate/pyruvate]. She also has a heterozygous pathogenic, maternally inherited variant in *FII* that was thought to explain her anemia, and a maternally inherited variant of uncertain significance in *G6PD* identified on WES. She has two healthy siblings who are neurologically normal.

Individual 3: An 18-1/2-year-old Caucasian male was evaluated for bilateral congenital cataracts, seizures, hypotonia since infancy, microcephaly, failure to thrive and profound developmental delay.

He was born at term via vaginal delivery to a 41-year-old G<sub>3</sub>P<sub>2</sub> mother and a 37-year-old father, both of Norwegian ancestry. His mother noted less movement during the pregnancy as compared to previous pregnancies. Birth weight was 4100 grams and birth length was 52 cm. Apgar score was 9. Hypotonia was noted at birth.

Developmental concerns were first noted at 8 weeks of age. He has had intensive psycho-motoric training, Domain patterning therapy from 14 months to 3 years of age, and Bobat neuro-developmental training. Although he was able to stand with support during early childhood, he has never achieved ambulation. From age 14 he was unable to sit without support due to spasticity. He has no language but does make sounds and can express his likes and dislikes. He recognizes his parents and siblings but has had long-standing tactile aversion leading to strong dislike of being touched or held. This issue has gradually improved in adolescence.

First seizure activity was noted at 6 months and he has had a long-standing history of epilepsy. Since 2 years of age he had tonic seizures. EEG at 5 ½ years of age showed increased activity, especially in the frontal lobe, related to his tonic-clonic seizures. Lamotrigine was initiated, which had no effect on the seizures and was discontinued after one year. Valproate therapy was effective, but EEG at 10 years of age still showed localized focal epileptic activity.

Bilateral congenital cataracts were diagnosed at 6 weeks of age, and he underwent surgery on the right eye at 1 year of age, and on the left eye at 2 years of age. He developed post-surgery cataracts and underwent laser surgery on the right lens at 2 years of age and left lens at 6 years of age.

MRI at 1 year of age showed reduced white matter and incomplete subcortical myelination of brain stem, thalamus and corpus callosum. MRI at 2 years of age showed increased myelination

as compared to the first imaging although still somewhat reduced related to age, reduced white matter and a hypoplastic corpus callosum with temporal lobes most affected. MRI at 11 years of age showed increased subarachnoid fluid over the frontal cerebral convexities, over both lateral fossae and along the interhemispheric fissures, as well as hyperintense foci in both hippocampi consistent with hippocampal sulcus remnant and myelinated but thin corpus callosum. The findings indicated generalized atrophy and mildly decreased brain volume. MR spectroscopy was notable for lower NAA levels left central hemisphere.

Constipation and gastroesophageal reflux noted at 5 years of age. Due to low weight, reduced growth and feeding difficulties, percutaneous endoscopic gastrostomy was performed at 6 ½ years of age; however at 18 years of age he continues to eat oral meals every day. Due to spasticity he has developed contractures of the feet, knees and hips, which have been treated with orthopedic interventions including triple arthrodesis, bilateral adductor tendonectomy, and right side femur/pelvic osteotomy to correct hip subluxation and stabilize the hips. His knee contractures have been managed with repeated Botulinum injections. He has developed thoracolumbar scoliosis.

Since early infancy he has had bouts of inconsolability and screaming for days at a time. Melatonin has been used for sleep disturbances. He has had Rett-like stereotypic hand movements since early childhood.

On exam at 6 ½ years of age he was microcephalic with OFC 49 cm (3 cm below 2.5 percentile). He had hyperreflexic patellar tendons, normal Achilles tendon reflexes and downturned plantar reflexes. He had increased muscle tone upper extremities. At 18 ½ years of age he had profound intellectual disability and stereotypic hand movements.

Previous testing included chromosomal microarray and Sanger sequencing for all known and available Rett/Angelman-like genes. Family history is noncontributory; he has two healthy siblings.

Individual 4: A 3-year, 7-month-old Caucasian male was evaluated for seizures, hypotonia, microcephaly, bilateral cataracts and glaucoma, bilateral sensorineural hearing loss (SNHL) and severe developmental delay. He was diagnosed with Stickler syndrome at 3 years of age by WES upon identification of a pathogenic *COL11A1* variant. His mother, who also has congenital SNHL and glaucoma, was found to have the same variant. However, this diagnosis did not explain his complete presentation.

He was born to a 30-year-old G<sub>1</sub>P<sub>0</sub> mother at 39 weeks gestation and weighed 7 pounds, 6 ounces. The pregnancy was complicated by gestational diabetes at 28 weeks, requiring insulin at 36 weeks. Labor was induced at 39 weeks gestation for maternal hypertension. The delivery was uncomplicated.

He began exhibiting developmental delays and feeding difficulties within the first month of life. Currently is able to roll over but cannot sit unassisted. He does not reach for or transfer toys.

Seizure activity was first observed at 4 months old; infantile spasms were diagnosed by EEG at 6 months and responded well to a 6 week course of ACTH. Levetiracetam was then initiated. He has been seizure free without AEDs since 2 years old (duration 17 months), and EEGs have been normal. He continues to have frequent sleep myoclonus.



Bilateral cataracts were diagnosed at birth and removed at 4 months of age. He has undergone bilateral goniotomy multiple times.

Brain MRI at 4 months showed mild delay in myelination and at 7 months showed significant interval cerebral volume loss. Brain imaging at 2 years, 2 months showed microcephaly and slight delay in myelination with considerable progression compared to previous imaging.

He has tracheomalacia, gastroesophageal reflux disease and feeding intolerance and disinterest which resulted in failure to thrive. Oral feeding improved during his ACTH treatment, however decreased significantly since completing therapy. Gastrostomy tube was placed at 9 months.

Mild hearing loss with normal tympanic membranes was found on ABR at 16 months.

He has periods of being irritable which last hours to days at a time where he has inconsolable crying. These are sometimes associated with constipation. He has sleep difficulties. He has a history of breath-holding spells which occurred several times a day prior to ACTH therapy; they seemed unrelated to seizures and cardiology evaluation was normal. The frequency of breath-holding spells has decreased significantly, and they now occur only when he is upset.

On exam at 3 years, 7 months he had diffuse hypotonia. Previous testing included chromosome microarray and CDG. His mother also has congenital hearing impairment and left sided glaucoma secondary to an eye injury; remaining family history was noncontributory.

Individual 5: A 9-year-old Caucasian female had been followed since age 8 months by medical genetics for epilepsy, microcephaly, bilateral cataracts, failure to thrive and severe developmental delay. Prior to WES she had a diagnosis of mitochondrial disease based on muscle biopsy that showed a reduction in several respiratory chain complexes.

She was born via full-term normal spontaneous vaginal delivery to a 30-year-old G<sub>1</sub>P<sub>0</sub> mother and a 33-year-old father, with a birth weight of 7 pounds 10 ounces. The neonatal course was uncomplicated.

Early developmental milestones were normal (good head control, rolled at 2 months of age, pushed to a prone position at 2 to 3 months of age). She had a social smile and tracked visually at approximately 2 months, although she never made eye contact. Currently she is profoundly impaired with no speech, no visual tracking and limited purposeful movement.

Myoclonic seizures began at 5 months of age consisting of myotonic jerking of her upper extremities, and a diagnosis of infantile spasms was tentatively made. An EEG showed hypsarhythmia during sleep and overall was consistent with diffuse epileptic encephalopathy. She was put on Zonisamide for presumed infantile spasms but continued to have clustering of seizures consisting of myotonic jerks as well as dozens of tonic jerks per day. At 10 months the Zonisamide was discontinued and her seizures doubled in frequency. Levetiracetam was started but discontinued after 2 weeks due to extreme irritability. Valproic acid was then initiated until an EEG at 11 months confirmed infantile spasms. ACTH was started at a low dose but spasms increased in intensity. Multiple AEDs were then attempted but did not result in improvement or had undesirable side effects, including Felbamate, Vigabatrin, Topamax, Lamotrigine and the ketogenic diet. Banzel (Rufinamide) was initiated at 2-½ years old, resulting in the first major improvement in her seizures. Seizures are currently well managed with Banzel, although there have been signs of breakthrough seizures with onset of puberty.

Nuclear cataracts were first noted in the right eye at 11 months and left eye at 12 months. She has had both cataracts as well as secondary cataracts removed during a total of four procedures.

A brain MRI at 5 months showed demyelination of the deep white matter. MR spectroscopy was normal.

She had feeding difficulties and formula intolerance requiring gastrostomy tube placement at 2-½ years of age, which was replaced 5 weeks later with a gastro-jejunal tube due to chronic vomiting. She has constipation and urine retention which has resulted in several UTIs. She has signs of precocious puberty since 5 years of age, and at 8 years of age hormonal changes were seen. She has had a single episode of difficulty waking up from anesthesia (following muscle biopsy at 2-½ years old).

She was a colicky baby and has had bouts of extreme irritability during childhood. At 2-½ years of age she began having choreic movements and increased sleep disturbance. Despite trials of Zolpidem, Lorazepam, Diazepam, Trazadone, Lortab, Gabapentin and Methadone, sleep patterns could not be stabilized. Around 5 years of age, an improvement was seen when calorie intake was lowered with a change to PurAmino formula, although she continues to have shorter bouts of extreme irritability. She has had breath-holding spells.

Previous diagnostic tests included a chromosome microarray, sequencing of the *MECP2*, *CDKL5* and *ARX* genes, enzyme testing for Neuronal Ceroid Lipofuscinosis types 1 and 2, metabolic screens [plasma amino acids (very mild elevation of alanine), homocysteine, acylcarnitine profile,

lactate, urine and plasma creatine studies, urine polyol studies (tenfold elevated ribitol), urine purine studies, urine organic acids (elevated lactate)]. CSF neurotransmitters and amino acids were normal. She had a muscle biopsy that showed a reduction in several respiratory chain complexes including complex 1 and complex 4, fulfilling minor criteria of the modified Walker criteria. Mitochondrial copy number was normal in the muscle sample and sequencing of the mitochondrial genome was likewise normal. She is the only child to her parents. Family history includes a paternal first cousin with seizures.

Individual 6: A 13-month-old male of Mexican ancestry was evaluated for developmental delay, hypotonia, infantile spasms, and bilateral sensory hearing loss.

He was born at 37 weeks gestation to a 31-year-old mother through induced vaginal birth after uneventful pregnancy and a 34-year-old father. The birth weight was 3.203 kg (30<sup>th</sup> percentile) and length was 48 cm (20<sup>th</sup> percentile). Slow heart rate was noted during birth.

Developmental concerns started at 4 months of age, when parents noticed that he would not laugh or look at them. At 6 months of age he failed a hearing and vision screen, and further evaluation identified bilateral sensorineural hearing loss and low vision, poor visual response, lack of tracking with both eyes, delayed visual maturation and bilateral hyperopia with astigmatism. At 9 months of age global developmental delay and hypotonia were noted. He began smiling and rolling over at this age. At 10 months old, breath holding spells with body stiffening was reported, followed by weakness. He had difficulty swallowing, and choked on thin liquids. He was not able to sit on his own, did not crawl, and had poor head control.

At 11 months old the first tonic seizure was observed. Video electroencephalogram (vEEG) indicated generalized hypsarrhythmia, frequent multifocal independent spike-wave discharges along with frequent biposterior or biparietal and occasionally generalized PFA. He completed a course of prednisolone which resolved his spasms. Zonisamide and clonazepam were initiated to control tonic seizures. The age of onset of seizures could not be determined, as they may have been present from birth but unrecognized. An MRI was performed at 12 months which was reported as unremarkable, with no gross structural anomaly and no acute intracranial findings; upon close review by an outside neuroradiologist volume loss was noted.

On physical examination at 12 months of age, he was noted to have central hypotonia, with weight of 7.95kg (4<sup>th</sup> percentile), height of 73cm (12<sup>th</sup> percentile), and a head circumference of 45.5cm (33<sup>rd</sup> percentile). He had a high arched palate, protuberant ears, hyperpigmented nevus, and bilateral, overlapping first, second, and third toes. He continues to have poor head control.

Chromosomal microarray, creatine phosphokinase (CK), thyroid stimulating hormone (TSH) and acylcarnitine profile were normal. Family history includes a 33-year-old maternal aunt who has a seizure disorder with onset at age 15.

Individual 7: A 12-year-old male was evaluated for developmental delay, seizure disorder, and cortical dysplasia, with prior medical care managed in Columbia.

He was born at 40 weeks gestation via C-section for a cephalopelvic disproportion from a 29-year-old primigravida mother and a 32-year –old-father.

Developmental concerns were noted shortly after birth with poor suck. He had prolonged crying episodes with right arm posturing similar to dystonic posture. At three months persistent startle and lack of interaction were noted. At 6 months he was unable to roll over. At 12 months he was diagnosed with cerebral palsy. He passed vision and hearing tests at 16 months. First words were spoken at age 2, and by age 10 his vocabulary totaled 20-40 words. Currently he has severe speech delay but is beginning to put two words together. He continues to have extensive drooling. He has autistic behaviors including hand flapping. He is able to walk but has an abnormal gait and requires leg braces for hyperextensibility. He startles easily, and episodes of startle lead to him falling over. He continues to make developmental progress.

At two years of age he had a single febrile seizure possibly due to influenza that included prolonged body stiffening. At 10 years of age he was noted to have multiple night time seizures with cyanotic, whole body convulsions. EEG confirmed electrical status epilepticus of sleep (ESES), which was well controlled with diazepam and most recent vEEG at age 10 showed no evidence of ESES. Currently he is not taking any medications. The timing of seizures suggested that additional episodes may have been missed over his childhood due to their night time occurrence.

MRI at 10 months of age showed an arachnoid cyst but no other abnormalities. MRI at age 2 was negative for intracranial injuries but showed enlarged adenoid tissue. The most recent MRI, at age 10 years, showed scattered areas of subcortical white matter T2/FLAIR hyperintensity involving the left superior frontal gyrus, posterior aspect of the left cingulate gyrus, left inferior frontal gyrus, and left supramarginal gyrus.

At eight months he was diagnosed with gastrointestinal reflux disease (GERD). He has a history of obstructive sleep apnea. Concerns for his vision at age ten resulted in the prescription for hyperopic correction lenses, although no strabismus was observed.

On exam at 12 years of age, weight was 53kg (61<sup>st</sup> percentile), height was 152cm (66<sup>th</sup> percentile), and his head circumference was 53cm (52<sup>nd</sup> percentile). He had myopathic face, midface hypoplasia, flat nasal bridge, high arched palate, protuberant ears, and low anterior hairline. He had a cafe au lait spot on right neck and multiple nevi. He continued to hold his mouth open and drooled. He continues to have problems with balance and falls frequently.

Previous metabolic testing included urine organic acids, plasma amino acids and urine mucopolysaccharides. Chromosomal microarray results were normal and genetic testing for TSC1/2, Fragile X, and X-linked Mental retardation (XLMR) panel were negative. The parents are of Colombian ancestry and consanguinity was denied. The individual has a younger brother with no medical condition. Paternal aunt had a history of seizures.

Table S1 Summary of exome sequencing platforms and coverage in seven unrelated individuals reported here with de novo *NACCI* p.Arg298Trp variant

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
<b>Sequencing laboratory</b>	GeneDx	GeneDx	Baylor Hopkins Centers for Mendelian Genomics	Baylor Genetics	Baylor Genetics	UCLA	UCLA
<b>Sequencing type</b>	Trio exome sequencing	Trio exome sequencing	Proband exome sequencing (Sanger sequencing of <i>NACCI</i> in proband and parents)	Proband exome sequencing (Sanger sequencing of <i>NACCI</i> in proband and parents)	Proband exome sequencing (Sanger sequencing of <i>NACCI</i> in proband and parents)	Trio exome sequencing	Trio exome sequencing
<b>Capture and library construction</b>	Agilent Clinical Research Exome Kit	Agilent Clinical Research Exome Kit	6plex using VCRome + PKv2	Biotin-labeled VCRome 2.1 in-solution Exome probes	Biotin-labeled VCRome 2.1 in-solution Exome probes	Agilent Clinical Research Exome Kit	Agilent Clinical Research Exome Kit
<b>Sequencing platform</b>	Illumina HiSeq2000	Illumina HiSeq2000	Illumina HiSeq2000	Illumina HiSeq2500	Illumina HiSeq2500	Illumina HiSeq2500	Illumina HiSeq2500
<b>Average depth of targeted bases</b>	73x	167x	84X	135x	235x	94x	78x
<b>Percentage of bases covered &gt;10x</b>	95.1%	97.5%	98.0%	98.3%	99.0%	96.50%	96.20%



Table S2 Additional variants detected by exome sequencing in seven individuals with a *NACCI* *de novo* p.Arg298Trp variant.

	Gene	Disease	Inheritance pattern	HGVS	Classification	Inherited from
<b>Individual 1</b>	<i>CRYGD</i>	Cataract 4, multiple types (MIM: 115700)	AD	c.118A>T (p.S40C) heterozygous	VUS	Mother
<b>Individual 2</b>	<i>F11</i>	Factor XI deficiency (MIM: 612416)	AD/AR	c.403G>T (p.E135X) heterozygous	pathogenic	Mother
	<i>G6PD</i>	Favism (MIM: 134700); Hemolytic anemia due to G6PD deficiency (MIM: 300908)	XL	c.1003G>A (p.A335T) heterozygous	VUS	Mother
<b>Individual 3</b>	<i>ABCB6</i>	Microphthalmia, isolated, with coloboma 7 (MIM: 614497)	AD	c.1A>G (p.M1?) heterozygous	Likely pathogenic	Unknown
<b>Individual 4</b>	<i>COL11A1</i>	Fibrochondrogenesis (MIM: 228520); Marshall syndrome (MIM: 154780); Stickerler syndrome, type II (MIM: 604841)	AD/AR	c.4084C>T (p.R1362X) heterozygous	Pathogenic	Mother
	<i>MYH9</i>	Macrothrombocytopenia and progressive sensorineural deafness [MIM:600208]; May-Hegglin anomaly [MIM:155100]; Sebastian syndrome [MIM:605249]	AD	c.5338C>T (p.R1780W) heterozygous	VUS	Mother
	<i>DYRK1A</i>	Mental retardation, autosomal dominant 7 [MIM:614104]	AD	c.1789G>A (p.A597T) heterozygous	VUS	Father
<b>Individual</b>	<i>ANKRD</i>	KBG syndrome	AD	c.5230C>	VUS	Father

<b>5</b>	<i>11</i>	(MIM: 148050)		G (p.H1744 D) heterozygo us		
	<i>ANKRD 11</i>	KBG syndrome (MIM: 148050)	AD	c.3019C> G (p.R1007G ) heterozygo us	VUS	Father
	<i>UPF3B</i>	Mental retardation, X-linked, syndromic 14 (MIM: 300676)	XL	c.1061G> A (p.R354Q) heterozygo us	VUS	Mother
<b>Individual 6</b>	No additional variant reported					
<b>Individual 7</b>	No additional variant reported					

Only heterozygous variants in known disease genes with autosomal dominant (AD) or X-linked (XL) inheritance and biallelic variants in known autosomal recessive (AR) disease genes are included here, and only for diseases where there is phenotypic overlap with the individuals' phenotypes.

Table S3 Summary of cases with *de novo* *NACCI* variants or deletions involving the *NACCI* from literature reports and DECIPHER database.

<b>Patient</b>	<b>Affected position</b>	<b>Sex</b>	<b>Size of deleted interval</b>	<b>Inheritance</b>	<b>Phenotype</b>	<b>Reference</b>
<b>Trio78</b>	<i>NACCI</i> c.1402C>T p.R468C	Female	1 nt	<i>de novo</i>	delayed developmental development, IQ level of about 45, autistic features and schizo-affective disorder	PMID 24896178, 23033978
<b>Pt13060</b>	<i>NACCI</i> c.946+2T>C	Male	1 nt	<i>de novo</i>	autism spectrum disorder	PMID 25363768
<b>Dolan et al, 2010, patient 1</b>	deletion, chr19:12498 237– 13126508	Female	680 kb	<i>de novo</i>	Developmental delay, IQ49, overgrowth, macrocephaly, hypotonia, esotropia, nystagmus, poor fixation	PMID 20613546
<b>Dolan et al, 2010, patient 1</b>	deletion, chr19:12536 641– 13794080	Female	1318 kb	<i>de novo</i>	Developmental and speech delay, overgrowth, macrocephaly, Optic nerve hypoplasia, exotropia	PMID 20613546
<b>Dolan et al, 2010, patient 2</b>	deletion, chr19:12793 474– 13104643	Male	327 kb	<i>de novo</i>	Severe speech delay, overgrowth, macrocephaly, seizures, hypotonia, optic atrophy, exotropia	PMID 20613546
<b>Dolan et al, 2010, patient 3</b>	deletion, chr19:12411 017– 13120904	Female	715 kb	<i>de novo</i>	Developmental and speech delay, overgrowth, macrocephaly, seizures, hypotonia, Chiari I malformation with syrinx, Optic nerve hypoplasia, exotropia, nystagmus	PMID 20613546
<b>Auvin et al, 2009</b>	deletion, chr19:12615 927– 13280259	Male	665 kb	not known	Global developmental delay, overgrowth with advanced bone age, macrocephaly, focal onset seizures on EEG, hypotonia, normal ophthalmologic exam.	PMID 19874387
<b>Lysy et al, 2010</b>	deletion, chr19:10246 651–	Female	3033 kb	<i>de novo</i>	Non-verbal, global developmental delay, hypotonia, ventriculo-	PMID 19842200

	13280203				megaly, strabismus, proptosis	
<b>Nimmakayalu et al., 2012, patient 1</b>	deletion, chr19:13016005–13415043	Female	399 kb	<i>de novo</i>	Significant developmental delay, moderate intellectual disability, generalized hypotonia, epilepsy, progressive unsteady gait. Tall stature and macrocephaly. MRI showed thinning of corpus callosum	PMID 23495138
<b>Nimmakayalu et al., 2012, patient 2</b>	deletion, chr19:13016005–13415043	Female	399 kb	<i>de novo</i>	Intellectual disability, macrocephaly, strabismus	PMID 23495138
<b>Bonaglia et al, patient 3</b>	deletion, chr19:12875220–14480616	Male	1605 kb	Maternal	intellectual disability, ADHD, seizures, hypotonia, normal eye exam	PMID 20648052
<b>Jorge et al., 2015</b>	deletion, chr19:12756718–13388309	Female	631 kb	not known	Intellectual disability, overgrowth with advanced bone age, hypotonia, ataxia, ventriculo-megaly, strabismus, nystagmus	PMID 26338046
<b>294330</b>	duplication, chr19:12344536-14642947	Male	2.30 Mb	Unknown	Global developmental delay	DECIPHER
<b>2539</b>	deletion, chr19:13161358-13265508	Male	104.15 kb	<i>de novo</i> constitutive		DECIPHER
<b>3820</b>	deletion, chr19:13081794-13265508	unknown	183.72 kb	Unknown	Intellectual disability, Strabismus, Tall stature	DECIPHER
<b>250047</b>	deletion, chr19:12754927-13419259	Male	664.33 kb	<i>de novo</i> constitutive		DECIPHER
<b>251206</b>	deletion, chr19:12819000-13609000	Female	790.00 kb	<i>de novo</i> constitutive	Ataxia, Intellectual disability, Tall stature, Visual impairment	DECIPHER
<b>253429</b>	deletion, chr19:13076503-	Female	1.60 Mb	<i>de novo</i> constit		DECIPHER

	14674918			utive		
<b>253780</b>	deletion, chr19:13068 773- 13528878	Fem ale	460. 11 kb	Unkno wn		DECIPHER
<b>256712</b>	deletion, chr19:13046 572- 14958141	Fem ale	1.91 Mb	<i>de novo constit utive</i>		DECIPHER
<b>269163</b>	duplication, chr19:11101 053- 13435131	Male	2.33 Mb	<i>de novo constit utive</i>	Abnormality of the central nervous system, Abnormality of the cornea, Atopic dermatitis, Delayed skeletal maturation, Intellectual disability, Microcephaly, Recurrent infections, Short stature, Wide mouth	DECIPHER
<b>2359</b>	duplication, chr19:12242 867- 14862610	Fem ale	2.62 Mb	Unkno wn	Clinodactyly of the 5th finger, Hypertelorism, Intellectual disability, Macrodonia, Microcephaly, Spastic diplegia	DECIPHER
<b>262646</b>	deletion, chr19:11892 746- 13611147	Male	1.72 Mb	<i>de novo constit utive</i>	Hydrocephalus, Intellectual disability, Patent ductus arteriosus	DECIPHER
<b>264317</b>	deletion, chr19:13182 191- 13842254	Male	660. 06 kb	<i>de novo constit utive</i>	Intellectual disability, Macrocephaly	DECIPHER
<b>265328</b>	deletion, chr19:12592 419- 13270578	Male	678. 16 kb	Unkno wn	Hearing impairment, Intellectual disability, Macrocephaly, Pectus carinatum, Recurrent fractures, Scoliosis, Tall stature, Visual impairment	DECIPHER
<b>292057</b>	duplication, chr19:13182 420- 13394529	Male	212. 11 kb	Unkno wn		DECIPHER
<b>267428</b>	deletion, chr19:12745 203- 13376358	Male	631. 16 kb	<i>de novo constit utive</i>	Deep palmar crease, Intellectual disability, moderate, Large for gestational age, Macrocephaly at birth,	DECIPHER

					Overgrowth, Prominent forehead, Sparse scalp hair, Spasticity	
<b>257523</b>	duplication, chr19:12841373-15929820	Male	3.09 Mb	<i>de novo</i> constitutive	Intellectual disability, Proportionate short stature	DECIPHER
<b>275609</b>	duplication, chr19:11574852-15028946	Male	3.45 Mb	Unknown	Growth hormone deficiency, Microcephaly, Moderate global developmental delay	DECIPHER
<b>265393</b>	duplication, chr19:10950425-14847355	Male	3.90 Mb	<i>de novo</i> constitutive		DECIPHER
<b>279720</b>	deletion, chr19:13243584-15633140	Female	2.39 Mb	Unknown	Hypoplasia of the corpus callosum	DECIPHER
<b>280489</b>	deletion, chr19:13081594-13270748	Male	189.16 kb	<i>de novo</i> constitutive	Global developmental delay	DECIPHER
<b>301615</b>	duplication, chr19:12997640-13476228	Female	478.59 kb	Unknown	Intrauterine growth retardation, Lymphedema	DECIPHER
<b>285391</b>	deletion, chr19:11338618-13327698	Male	1.99 Mb	<i>de novo</i> constitutive	Abnormality of the helix, Aplasia/hypoplasia of the corpus callosum, Dental crowding, High forehead, Hypoplasia of the optic tract, Intellectual disability, profound, Pectus excavatum, Pointed chin, Prominent ears, Scoliosis, Short nasal bridge, Ureteropelvic junction obstruction, Wide mouth	DECIPHER
<b>275388</b>	duplication, chr19:266117-59092570	Male	58.83 Mb	Unknown		DECIPHER
<b>292233</b>	deletion, chr19:13224132-13307044	Female	82.91 kb	Unknown		DECIPHER

<b>258888</b>	duplication, chr19:12997 640- 13611006	Fem ale	613. 37 kb	<i>de novo constit utive</i>		DECIPHER
<b>299787</b>	deletion, chr19:12433 780- 13419259	unkn own	985. 48 kb	<i>de novo constit utive</i>	Delayed speech and language development, Generalized neonatal hypotonia, Global developmental delay, Intellectual disability, mild, Nystagmus, Pectus excavatum of inferior sternum, Plagiocephaly, Triangular face	DECIPHER
<b>305287</b>	duplication, chr19:12880 402- 13497160	Fem ale	616. 76 kb	<i>de novo constit utive</i>	Epicanthus, High palate, Intrauterine growth retardation, Iron deficiency anemia, Postnatal microcephaly, Postnatal microcephaly, Short stature, Thin upper lip vermilion	DECIPHER
<b>302023</b>	deletion, chr19:12527 157- 13476228	Fem ale	949. 07 kb	<i>de novo constit utive</i>		DECIPHER
<b>284902</b>	duplication, chr19:12744 531- 13538042	Fem ale	793. 51 kb	<i>de novo constit utive</i>	Hyperactivity, Intellectual disability	DECIPHER
<b>331245</b>	deletion, chr19:12441 723- 13662245	Male	1.22 Mb	<i>de novo constit utive</i>	Aqueductal stenosis, Developmental stagnation, Generalized hypotonia, Global developmental delay, Hydrocephalus	DECIPHER