CLINICAL REPORTS

Subject 1

Subject 1 is a 4-year, 3-month-old Hispanic male who carries a *de novo* 1p36.23 deletion (minimum deletion chr1:8,509,888-8,803,072; maximum deletion chr1:8,497,191-8,813,784, hg19) which includes coding exons 1-10 of the *RERE* gene.

He was conceived naturally by non-consanguinous parents. His mother has mild cerebral palsy, speech problems and learning disabilities. Despite these issues, she went on to graduate from a technical college. His father and a paternal aunt have biopolar disorder, and a sister had some developmental delays but is now doing well in school. Pregnancy was complicated by gestational diabetes managed with diet. He was delivered at term via a scheduled repeat caesarian section. At birth, he weighed 3.26 kg (43rd centile) and was mildly jaundiced but did not require phototherapy.

He rolled over at 8 months of age, sat by himself at approximately 8 months of age, crawled at 10 months of age and walked at 14 months of age. He receives physical therapy for gross motor impairment, has an abnormal gait with toe-walking, and has been prescribed ankle braces. He could say "mom" and "dad" at 15 months of age but stopped talking abruptly until two years of age. At 3 years, 10 months of age, he could use two-word phrases and follow a 1-step command without gesture, but could not follow 2-step commands. He has been diagnosed with speech apraxia. Parents report that he covers his ears when exposed to loud noises. A hearing evaluation was normal. A brain MRI obtained at 3 years, 11 months of age was normal.

At 4 years, 3 months of age, he weighed 16.3 kg (35th centile) and his length was 100 cm (13th centile). On examination, he was found to have a triangular face, mild plagiocephaly, normal tone, and a right ankle contracture. Developmental testing using the Capute Scales put his

language skills around 29 months and his cognitive/adaptive skills around 31.5 months, ultimately bringing a diagnosis of mixed receptive-expressive language disorder.

Subject 2

Subject 2 is an 8-month-old Hispanic male who carries a *de novo* c.248dupA, p.Ser84Valfs*4 variant in *RERE*. A nuchal translucency scan at 9 weeks, 6 days of gestation revealed a cystic hygroma. At 14 weeks of gestation the nuchal translucency was normal at 2.2 mm. Chorionic villus sampling showed a normal male chromosomal compliment. An array-based copy number variant analysis and a Noonan syndrome panel—testing for pathogenic variants in *BRAF*, *CBL*, *HRAS*, *KRAS*, *MAP2K1*, *NRAS*, *PTPN11*, *RAF1*, *SCHOC2*, and *SOS1*—were performed prenatally and were also normal. Anatomy ultrasounds and fetal echocardiography did not identify any structural anomalies. He was born at 38 weeks gestation via vaginal delivery. Birth weight was 2.955 kg (20th centile).

In the first months of life he had multiple urinary tract infections. Radiographic studies showed no evidence of hydronephrosis or vesicoureteral reflux. He was noted to be hypertonic with the upper limbs being more affected that the lower limbs. His gross motor development was normal: he rolled over at 3 months and started sitting on his own at 7 months. At 8 months of age his length was at the 25th centile, his weight was at the 5th centile and his occipital frontal circumference (OFC) was at the 25th centile. On physical examination he was noted to have bifrontal narrowing, a low anterior hairline, mild hypertelorism, bilateral epicanthal folds, downslanting palpebral fissures, synophrys, mild hypoplastic helices, redundant nuchal skin and spasticity of all four extremities with upper limbs being more affected than lower limbs,.

Subject 3

Subject 3 is a 21-year-old male of European descent who carries a *de novo* c.4300T>C, p.Ser1434Pro variant in *RERE*. Pregnancy was complicated by twin gestation with loss of the second twin at 10 weeks of gestation. He was born via vaginal delivery. At birth he weighed 3.402 kg (54^{th} centile) and was 53.3 cm (97^{th} centile) in length. He rolled over at 6 months of age, sat at 9 months of age, crawled at 18 months of age and walked at 3 years of age. He said his first words at approximately 9 months of age and put two words together between 2 and 3 years of age.

As an infant he was noted to have choking episodes which eventually subsided. He was found to have an atrial septal defect for which he underwent a transcatheter closure. He is currently treated with atenolol for aortic root dilatation (Z score = 3.9).

At 7 years of age he started displaying recurrent episodes of cyanosis without respiratory difficulty. A muscle biopsy was conducted and showed that he had significant and reproducible reduction in succinate cytochrome c reductase with 19% activity compared to healthy controls. His NADH activity and NADH cytochrome c reductase activity were 40% compared to healthy controls. He was also noted to have mild hypotonia, ataxia, scoliosis, bilateral ptosis, esotropia, developmental delay and obsessive compulsive disorder.

At 8 years of age, he started displaying apneic spells consistent with sleep apnea. His memory improved, and despite speech delays he was more verbal. At 9 years of age he began complaining of throbbing headaches that were keeping him up at night. A brain MRI revealed a thin corpus callosum, flattening of the brain stem and a thin rim of sella. Hearing loss evaluations revealed chronic tympanic membrane perforation on the right and very mild sensorineural

hearing loss on the left. He ultimately finished high school in special education classes. He can read, tell the time and recognize community signs.

At 19 years, 7 months of age his height was 163.3 cm (3rd centile), and his weight was 59.9 kg (14th centile). He was noted to have repetitive hand movements, limited speech, an elongated and myopathic face with midfacial retraction, bilateral ptosis, deep-set eyes, a high arched palate, spatulated thumbs and hyperconvexity of his fingernails and toenails. He currently speaks in up to 4 to 5 word sentences and also uses sign language to communicate.

Subject 4

Subject 4 is a 13-year-old female of European descent who carries a *de novo* c.4303C>T, p.His1435Tyr missense variant in *RERE*. Pregnancy was uneventful and she was born at term at 39 weeks by cesarean section for breech presentation. At birth she weighed 2.980 kg (29nd centile), was 46 cm (5th centile) in length, and had an OFC of 35 cm (83rd centile). Neonatal hypotonia was noted in the first days of life without feeding difficulties. She held her head up at 6 months of age, sat unaided at 18 months of age and walked at 2.5 years of age. A brain MRI obtained at 5 years of age showed hemispheric cerebellar dysplasia with abnormal lobule and fissure orientation in the inferior hemispheres. Onset of puberty was noted between 6 and 7 years of age. Treatment for precocious puberty was initiated at 8 years of age and continued until she was 13 years old.

At 13 years of age her height was 148 cm (10th centile), her weight was 47 kg (55th centile), and her OFC was 54 cm (62nd centile). She has severe intellectual disability and severe speech delay with a vocabulary of less than 20 words and an inability to use two-word phrases.

Hearing evaluation was normal. She often has temper tantrums. She is toilet trained and does not have seizures nor sleep disturbances.

Subject 5

Subject 5 is a 22-year-old female of Japanese and European descent who carries a *de novo* c.4304A>G, p.His1435Arg variant in *RERE*. She was born by induced delivery at 40 2/7 weeks of gestation following an uneventful pregnancy. At birth she weighed 3.6 kg (78^{th} centile), was 53 cm long (95^{th} centile) and had an OFC of 33 cm (23^{rd} centile). Her Apgar scores were 8 and 9. Shortly after birth, she was noted to be hypotonic and hypoxic. There was evidence of lung disease, central hypoventilation as well as poor respiratory effort. She was transferred to the neonatal intensive care unit where she stayed for two weeks. She received supplemental oxygen and was intubated and ventilated for a short period of time. During her hospitalization she was diagnosed with an atrial septal defect. A chest x-ray revealed 11 paired ribs. She was discharged home on supplemental oxygen and monitoring. Supplemental oxygen was discontinued at three months of age.

In her first years of life, she suffered from asthma, with one episode requiring hospitalization at age 1.5. A pulmonary evaluation discovered mild, asymptomatic tracheomalacia which has since resolved along with her asthma. During childhood she was noted to have strabismus and hyperopia and briefly wore glasses. Currently, her ocular acuity is considered normal, but she has been diagnosed with cortical visual impairment.

Global developmental delays were noted in infancy. At 1.5 years of age, acoustic emittance testing revealed normal (type A) tympanograms but behavioral audiometry was inconclusive due to cognitive immaturity. Subsequent high frequency auditory brainstem

response evaluation (ABR) was normal but middle and low frequencies were not tested. She walked and spoke her first word at age 5. At the age of 8 she used roughly 25 words. An MRI of the brain performed at age 2, showed mildly prominent CSF spaces. An EEG obtained at 2 years, 10 months of age was normal.

At 21 years, 1 month of age, her height was 143 cm (<1st centile, -3.08 SD), her weight was 63.6 kg (73rd centile), her body mass index (BMI) was 31.1 (99th centile), and her OFC was 55.5 cm (73rd centile). In addition to short stature and obesity, she was noted to have upslanted palpebral fissures, large ears with overfolded helices, a right preauricular pit, small hands and feet, brachydactyly, and hyperconvex toenails. She is able to walk without assistance, but remains incontinent and is dependent in all activities of daily living. In adolescence she experienced menometrorrhagia. Laboratory evaluations including a chromosome analysis, fragile X molecular testing, a subtelomeric probe study, array-based copy number variant analysis, Prader-Willi syndrome methylation testing, acylcarnitine profile, urine organic acids, plasma amino acids and a very long chain fatty acid study were non-diagnostic.

Subject 6

Subject 6 is an 8-year, 6-month-old male of Asian Indian descent who carries two *de novo* heterozygous variants in *RERE*: a c.4304A>T, p.His1435Leu variant and a c.3292C>G, p.Leu1098Val variant. The phase of these variants is unknown. He was also found to carry a *de novo* c.1147C>T, p.Arg383Trp variant in the protein phosphatase 2 regulatory subunit, alpha gene (*PPP2R2A*, MIM #604941, NM_002717.3). He was conceived naturally by non-consanguineous parents. He was born via cesarean section at 39 and 4/7 weeks of gestation following a pregnancy complicated only by a prenatal diagnosis of bilateral pelviectasis.

Parents became concerned about his development at 5 to 6 months of age when he did not develop a social smile and was not regarding faces. He sat by himself at approximately 1 year of age, could pull to a standing position at 2 years of age and walked independently at 3.5 years of age. He began clapping at 12 months of age but had lost that skill by 16 months of age. Over time he was noted to have intellectual disability, and he was diagnosed with autism spectrum disorder at 6 years of age. He has never developed a social smile, and he is not toilet trained. He uses monosyllabic sounds without purpose. He does not point, use picture icons or sign. He is currently enrolled in the second grade in special education classes and receives physical, occupational, and speech therapy.

At 6 years, 7 months of age an audiological evaluation revealed mild, bilateral sensorineural hearing loss. A brain MRI obtained at 2 years, 1 month of age was normal. An ophthalmologic exam revealed bilateral myopia and exotropia. Corrective surgery was performed at 7 years of age, and his bilateral exotropia was successfully reduced.

At 8 years, 6 months of age, his height was 116.4 cm (6^{th} centile), his weight was 22.2 kg (26^{th} centile) and his OFC was 50.5 cm (22^{nd} centile). On physical exam he was noted to have prominent, cupped ears, a triangular-shaped face, mild fifth finger clinodactyly, 2^{nd} toes that override his 1^{st} toes bilaterally, three single café au lait macules, and ankle valgus deformities of the feet resulting in pronation for which he wears braces.

Subject 7

Subject 7 was a male of European descent who died at 33 days of life. He carried a *de novo* c.4313_4318dupTCCACC, p.Leu1438_His1439dup variant in *RERE*. Pregnancy was complicated by polyhydramnios and gestational diabetes mellitus. He was born prematurely at

36 4/7 weeks of gestation via spontaneous vaginal delivery. His birth weight was 2.550 kg (24^{th} centile), his length was 47 cm (37^{th} centile) and his OFC was 33.5 cm (37^{th} centile). At birth he was flaccid and required positive pressure ventilation with 100% oxygen for apnea and cyanosis which persisted despite stimulation. At five minutes of life, heart rate decreased to 70 bpm despite positive pressure ventilation. However, prior to intubation, he cried and his heart rate increased. Apgar scores were 2, 4, 7 and 9. He was started on CPAP and transferred to a neonatal intensive care unit at a different institution. On arrival he was noted to have low tone and increased work of breathing for which he was intubated. Blood glucose was low at 1.7 mmol/l (31 mg/dl).

In an effort to pass various nasal tubes, it was discovered that there was a physical obstruction. Flexible nasopharyngoscopy on the first day of life revealed a posterior bony obstruction, and he was transferred to a tertiary care center for further investigation and management. A choanal CT revealed a bony narrowing with thickening of the vomer in posterior portion of both choanae and bilateral membranous choanal atresia. A bronchoscopy showed enlarged arytenoids (80%), small bilateral vocal cord granulomas, subglottic erythema and grade 1 subglottic stenosis. Extubation attempts were unsuccessful so he received a tracheostomy at 17 days of life and remained on full ventilator support throughout his life.

Physical exam findings included a flat nasal bridge, a large prominent forehead, bilateral ptosis, left-sided iris coloboma and corneal clouding, small, low-set ears, excessive nuchal skin, bilateral contractures of the 2^{nd} and 3^{rd} digits, widely spaced nipples, hypospadias, and axial hypotonia with normal deep tendon reflexes. An ophthalmological assessment revealed a left central corneal opacity, an inferonasal iris coloboma and anterior segment dysgenesis. No evidence for a chorioretinal coloboma was seen by ultrasound.

A head ultrasound performed on day of life 3 revealed diffuse white matter changes with increased echogenicity and concerns for simplified sulcation. A brain MRI performed on the seventh day of life confirmed a simplified gyral pattern with unusually large ventricles suggestive of delayed brain maturation. Multiple punctate periventricular ischemic lesions were also detected along with restricted diffusion in the splenium of the corpus callosum. Audiometry was not performed, but MRI and CT scans revealed normal semicircular canals.

An echocardiogram performed on day 5 revealed an atrial septal defect, a ventricular septal defect, a small patent foramen ovale and mildly dilated right ventricle. Over time, he developed heart failure with pulmonary edema and elevated right ventricle and diastolic pressures (>50% systemic) suggestive of persistent pulmonary hypertension. The parents and medical personnel decided to extubate and palliate the baby given his multiple medical issues and poor prognosis. He died at 33 days of age.

An autopsy confirmed the dysmorphic features and congenital anomalies described above. Analyses of the brain confirmed simplified gyration of cerebral cortex and also showed some atrophy of the frontal lobes, dysplasia of the inferior olivary and dentate nuclei, optic nerve hypoplasia, and mild to moderate ventriculomegaly. There was also evidence of multifocal neuroglial heterotopia around the base of the brain, close to the arachnoid space, particularly on the left side under the lenticular nucleus and hypoxic-ischemic damage predominantly in the posterior regions of the cerebral hemispheres with neuronal loss in the cerebellar cortex and extensive reactive gliosis. A postmortem skeletal survey revealed no significant abnormalities.

Subject 8

Subject 8 is an 8-year, 3-month-old female of European descent who carries a *de novo* c.4313_4318dupTCCACC, p.Leu1438_His1439dup variant in *RERE*. Pregnancy was complicated by advanced maternal age, polyhydramnios requiring amniotic fluid reduction, prenatal diagnosis of truncus arteriosus type I, intrauterine growth retardation and fetal hand posturing. There was no history of infections, hypertension, or diabetes during the pregnancy. Prenatal testing included an amniocentesis that revealed a normal chromosomal compliment and normal FISH studies for 22q11 microdeletion.

She was delivered at 39 1/7 weeks gestation by emergency cesarean section because of decreased fetal heart tones. Apgar scores were 1 and 6 at one and five minutes, respectively. She was initially intubated and suctioned below the vocal cords on two separate occasions. She was taken to the neonatal intensive care unit (NICU) for respiratory distress. Birth weight was 2.415 kg (3^{rd} centile), length was 47 cm (13^{th} centile) and OFC was 32 cm (6^{th} centile).

While in the NICU she was found to have bilateral choanal atresia. An ophthalmological evaluation showed right chorioretinal and iris colobomata and anisometropia. Truncus arteriosus was confirmed and was repaired at 1 month of age. Following surgery she had neonatal supraventricular tachycardia (SVT) which resolved by 1 year of age.

She has progressive sensorineural hearing loss and wears a hearing aid in the left ear and has a cochlear implant on the right side. Her temporal bone CT showed bilateral cochlear dysplasia. Other surgeries include pilonidal cyst incision, drainage and removal of a sacral cyst, myringotomy, gastrostomy tube placement, appendectomy, and a petrous apicectomy.

Her developmental history is significant for developmental delay and intellectual disability. She is currently in the first grade attending a mixture of regular and special education classes. She has no speech. She cannot stand or walk without assistance but can walk with

assistance for short distances and she uses a gait trainer. She is wheelchair-bound for longer distances. There has been no history of behavioral concerns, seizures, abnormal tone, or developmental regression.

At 7 years, 9 months of age she was evaluated for short stature. Thyroid function studies, LH/FSH/Estrogen, IGF-1 and IGFBp-3 were normal. Bone age was advanced showing an ossification pattern typical of a 10-year-old female. She has also been diagnosed with neuromuscular thoracolumbar scoliosis, bilateral hip dysplasia, and bilateral equinus contractures.

At 8 years, 3 months of age, her height was 114.3 cm (0.4^{th} centile), her weight was 20.4 kg (4^{th} centile) and her OFC was 47 cm ($<1^{st}$ centile). She had a flattened facial profile, hypertelorism, a right-sided iris coloboma, normally set ears with very small lobules and prominent antihelicies, a broad nasal bridge, a III/VI systolic murmur and bilateral hockey stick palmar creases.

Subject 9

Subject 9 is a 4-year-old female of European descent who carries a *de novo* c.4391A>G, p.His1464Arg variant in *RERE.* Pregnancy history is limited, but she has a twin brother who is healthy. She has an older brother who was diagnosed with dyslexia but is otherwise healthy and a paternal uncle who has Smith-Lemli-Opitz syndrome.

She was originally referred for expressive language delay, hypotonia and trouble completing gross motor activities. Upon further investigation, she was diagnosed with autism spectrum disorder, developmental delay, obsessive compulsive disorder and stereotypic

behaviors. Her parents report erratic behavior and extreme separation anxiety. She has difficulty falling asleep and maintaining sleep but has since improved with Guanfacine administration.

At her most recent physical examination, her height and weight were at the 95th centile, and her OFC was at the 50th centile. She was found to have hirsutism affecting the back and arms, synophrys, hypertelorism, an upturned nose and a wide mouth.

Table S1. Structural birth defects and sensorineural hearing loss among genotypic groups

Mutation Type	Loss of Function Mutations*						PM	Point Mutations in Atrophin-1 Domain										
	S1	S2	Fr S8	Fr S9	Fr S10	Fr S6	Fr S7	Fr S1	Fr S3	Fr S4	Fr S5	S3	S4	S5	S6	Fr S2	S7	S8	S9
Age/Sex	4y M	8m M	10y M	7y M	14 y F	6y F	11y M	3y M	2y M	9y F	12y M	21 y M	13y F	22y F	8y M	15m M	33d M	8y F	4y F
Structural brain anomalies on MRI	-	N/D	-	+	N/D	N/D	+	+	+	+	+	+	+	-	-	+	+	N/D	N/D
Structural eye anomalies	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	+	+	-
Congenital heart defects	-	-	-	+	-	-	-	+	+	-	-	+	-	+	-	+	+	+	-
Renal anomalies	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
Sensorineural hearing loss	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	N/D	+	-

F = Female, Fr = Fregeau et al., M = Male, PM = Point mutations not in the Atrophin-1 domain, S = Subject, - = Not reported, + = Reported, N/D = Not Done

* Partial deletions, stop-gain variants or frameshift variants