Appendix 2 (as submitted by the authors): Clinical summaries of individuals with a molecular diagnosis

Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit

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Retrospective cases

Individual 9:

Individual 9 was born to a healthy 36-year-old G3P2. There were no teratogenic exposures and no maternal illness but the pregnancy was complicated by poor growth detected at 32 weeks and 2 days. The parents were first cousins. The patient was induced for IUGR at 38 weeks and 2 days. Apgars were 9 and 9 at 1 and 5 minutes. She had feeding difficulties starting on her first day of life and would often become cyanotic during feeds. The feeding difficulties, including frequent regurgitation and significant nasal congestion, led to poor weight gain. A genetic assessment noted periorbital fullness, full cheeks, a long philtrum, a thin upper lip and a wide mouth. An echocardiogram showed a patent ductus arteriosus and patent foramen ovale as well as hypertrabeculation of the left ventricular apex consistent with left ventricular noncompaction or spongiform cardiomyopathy. Left-sided hip dysplasia was also diagnosed during her NICU

admission. On several occasions, she was hypertensive with no known cause. An MRI showed a thin corpus callosum, delayed myelination, and a mildly enlarged, bilobulated pituitary gland. She was discharged at 34 days of life. At her out-patient follow-up at 17 months of age in Genetics, she had global developmental delay, short stature, cortical vision impairment, and continued to have significant feeding issues secondary to gastroesophageal reflux and dysphagia with choking. She was enrolled in the research study at this time and NGS showed a novel homozygous missense mutation in *FTO* [NM_001080432.2): c.956C>T; p.(Ser319Phe)] (Table 2)[1]. This variant has not been seen before though a single, homozygous pathogenic mutation in the same domain had been reported previously in a single family with growth and developmental delay, dysmorphic facies, and morbidity in early childhood (OMIM: 612938).[2] The genetic evidence for a causative mutation was compelling for this case (the rarity of the mutation, the phenotype correlation, *in-silico* prediction programs).

Individual 11:

Individual 11 was born to healthy 29 year old G3P1. There were no teratogenic exposures or maternal illness though the pregnancy was complicated by massive ascites diagnosed at 28 weeks of gestation. A fetal echocardiogram was normal. The individual was born at 38 weeks and 3 days by caesarian section. His birthweight was 4.8kg (97-99%) and Apgars were 2, 5 and 6 at 1, 5, and 10 minutes, respectively. He required positive pressure ventilation and subsequent intubation and was transferred to the NICU. An abdominal ultrasound showed massive ascites with associated cysts in upper right and left quadrants. A dysmorphic examination after birth was limited given the significant edema. During his NICU admission he was also found to have bilateral hip dysplasia and required a Pavlik harness. He was discharged from the NICU at day

32 of life. On follow-up assessment it was noted his limbs showed rhizomelic shortening and he had brachydactyly with single palmar creases. A pelvic X-ray showed small iliac wings and sciatic notches and horizontal inferior iliac bones. The X-rays were suggestive of a short-rib thoracic dysplasia (ciliary chondrodysplasia). Genetic testing for mutations in the more common and available genes (*ITF80* and *DYNC2H1*) was normal. Given his past admission to the NICU he was enrolled as a retrospective case for the targeted NGS panel testing at age 3. NGS showed compound heterozygous mutations in *WDR19* (NM_025132.3) (Table 2). The mutations were a novel nonsense mutation [c.1600G>T; p.(Glu534*)] and a known pathogenic missense mutation [c.2129T>C; p.(Leu710Ser)].[3] This was in keeping with a diagnosis of Sensenbrenner syndrome/cranioectodermal dysplasia (OMIM 614378) that is a type of ciliary chondrodysplasia.

Individual 15:

Individual 15 is a boy born to a healthy G6P4 woman after an uncomplicated pregnancy with no teratogenic exposures at 37 weeks and 5 days. Parents were first degree cousins. Birthweight was 2.8 kg (50-85%). During the first day of life he was noted to be difficult to rouse, have poor feeds and was therefore admitted to the NICU. A genetic assessment noted severe ptosis, epicanthus inversus, short nose with long philtrum, high arched palate and down-turned corners of his mouth as well as hypotonia. Distal flexion contractures to distal upper and lower extremities were present that slowly resolved. He was given a diagnosis of arthrogryposis multiplex congenita. Oral feeds were difficult and he underwent a G-tube placement at 35 days of life. He was enrolled into the NICU Study, retrospectively, at 5 years of age. In the interim he required surgery for his ptosis. He also was diagnosed with a supraventricular tachycardia at 10 months of life. Results of an EMG were in keeping with myasthenic syndrome or a possible

congenital myopathy. Genetic testing of *MTM1* and a microarray were normal. He further underwent clinical NGS panel testing for the congenital myasthic syndrome genes. Both the NICU study and the clinical panel identified a novel homozygous change in *CHRND* [NM_000751.1: c.932+5G>A] (Table 2). This change is predicted to result in abnormal splicing by several prediction programs (Alamut, Mutation taster).

Individual 18:

Individual 18 was born to a 30 year old G4P2 at term. During the pregnancy there were no teratogenic exposures or maternal illness. The pregnancy was complicated by intrauterine growth restriction (IUGR) and an apparent enlarged cisterna magna though a prenatal diagnosis of Dandy-Walker malformation was also considered. At birth the patient required positive pressure ventilation and APGARS were 6 and 8 at 5 and 10 minutes. Birthweight was 2.3 kg (15^{th} percentile). On examination he had a sacral dimple, increased tone to his extremities and bilateral contractures of third and fifth digits. An echocardiogram showed a patent ductus arteriosus and an MRI was consistent with an enlarged cisterna magna with no other central nervous system malformations. He was enrolled in the NICU study at 13 months. At that time of enrollment he also had evidence of global developmental delays and feeding difficulties. His facial features were dysmorphic. He had post-natal microcephaly (<3%). He had deep-set and down-slanted palpebral fissures with a hooded appearance. There was a prominent nasal root and ears were low-set and mildly dysplastic. His mouth was downturned. NGS revealed a *de novo* mutation in *DYRK1A* (NM_001396.3) (Table 2). The c.951+4_951+7delAGTA mutation is predicted to affect splicing. The history of IUGR, developmental delays and feeding difficulties and his facial gestalt were in keeping with *DYRK1A*-related intellectual disability (OMIM 614104).[4,5]

The Prospective cases

Individual 2:

This individual has been presented in detail as a clinical report.[6] The patient was born prematurely to a healthy 30 year old G4P0A3 at 27 weeks and 3 days. At birth the neonate was edematous, hypotonic, and cyanotic with poor respiratory effort. He was also hypotensive soon after birth and became anuric for four days, despite extensive medical treatment. Laboratory markers were consistent with acute renal failure. Treatment with vasopressin on Day 8 of life showed an excellent response and he was able to maintain his blood pressure. However, after discharge, his blood pressure progressively declined and potassium levels slowly increased (5.7mmol/L). Renal function also progressively deteriorated and reached Chronic Kidney Disease (CKD) stage 3 at the time the result of research panel testing became available at 12 months of age. NGS with the targeted panel showed compound heterozygous frameshift deletions in *ACE* (NM_000789) (Table 2). The mutations were [c.820_821delAG; p.(Arg274Glyfs*117)] and [c.3521delG; p.(Gly1174Alafs*12)]. The knowledge of the bi-allelic mutations in the *ACE* gene provided a diagnosis of renal tubular dysgenesis (OMIM 267430) and prompted further laboratory investigations. A renin level (76800 ng/L, normal range; 6.3-149ng/L) and an aldosterone (<103pmol/L) were performed. ACE level was 2 U/L (normal range 14-108U/L). Given these findings, the patient was immediately started on fludrocortisone and renin levels decreased dramatically to 7200ng/L and potassium levels normalized. His estimated GFR (Schwartz) was 69ml/min/1.73m^2, which placed him to CKD stage 2 that was an improvement from his pre-diagnosis status.

Individual 6:

Individual 6 was born to a 33-year-old G1P0 by spontaneous vaginal delivery at 40+5 weeks' gestational age. He was noted to have extremely large, firm inguinal masses that were not able to transilluminated. He was transferred to the NICU and scheduled for bilateral inguinal hernia repair on day 2 of life. He had flexion contractures of upper and lower limbs with low truncal tone. The surgery was uncomplicated and resulted in a full reduction of hernias.

Bilateral hip dysplasia was confirmed by hip ultrasound. His skin was felt to be lax though this improved over time. On day 18 of life he was noted to have abnormal movements and an EEG showed evidence of epileptiform activity. The epileptiform spikes arose independently over both the left and right central areas. NGS showed a *de novo* mutation in *SCN1A* (NM_001202435.1) [c.620T>G; p.(Val207Gly)] in the patient (Table 2). This was not present in the available databases and was predicted to be damaging by *in-silico* prediction programs (SIFT, Polyphen2, and Mutation taster). The seizures responded well to clobazam and phenobarbital.

While the *de novo SCN1A* mutation explained the early-onset seizures, the explanation for the inguinal hernias, hip dysplasia and lax skin at presentation has yet has to be determined. Significant motor delays are still present at 11 months of age. He is able to turn his head although he has limited head control and he cannot sit or roll independently. Social and language skills are appropriate for age.

Individual 8:

Individual 8 was born to a healthy 26 year old G1P0. At birth, he showed poor respiratory effort and low tone. Positive pressure ventilation and chest compressions were required and he was subsequently transitioned to CPAP and then intubated. The newborn demonstrated significant

neurological impairment including limited limb movement, marked axial hypotonia with hypertonia of extremities, a weak grasp and suck, and no gag reflex. The child was enrolled in the NICU research project at this time. Upon further questioning there was noted a family history of a maternal uncle who had passed away in early childhood. Medical records were obtained and showed the maternal uncle had died of a myotubular myopathy. As a result, targeted Sanger sequencing of *MTM1* was requested for the patient. The child was shown to have a mutation in *MTM1* (NM_000252.2) [c.594C>A; p.(Tyr198*)], by both next-generation sequencing (Table 2) and Sanger sequencing. During his admission the child's breathing became increasingly irregular, he required frequent suctioning and he also experienced cardiac arrest in the context of aspiration pneumonia. He had evidence of a major ischemic event on MRI and, taken with his molecular diagnosis, the overall prognosis was considered poor. Supportive measures and comfort care were the focus of his management plan and the child passed away at Day 33 of life.

Individual 19:

Individual 19 of the study was born to a healthy 37 year old G3P2 woman. There were no exposures to teratogenic or history of maternal illness. Prenatal ultrasound noted a congenital diaphragmatic hernia (CDH) but no other congenital malformations. There was no family history of CDH and parents were non-consanguineous. The child was born at 38+2 weeks gestation. She was 3.5 kg (50-85%). She was non-dysmorphic. She had surgery at Day 2 of life for her CDH. On day 13 of life it was noted she had proteinuria that proceeded to frank nephrotic syndrome. She requires daily dialysis and is awaiting a kidney transplant. A *de novo* mutation in *WT1* was identified (NM_024426.4) [c.1406A>C; p.(His469Pro)]. The nucleotide alteration has

not been reported previously but the same amino acid residue at position 469 has been substituted from a Histidine to a Tyrosine in Denys-Drash syndrome[7] that is in keeping with the patient phenotype. She continues to receive daily dialysis at 215 days of life and is still admitted in the hospital.

References:

1. Daoud H, Zhang D, McMurray F, et al. Identification of a pathogenic FTO mutation by next-generation sequencing in a newborn with growth retardation and developmental delay. *J Med Genet.* 2015.
2. Boissel S, Reish O, Proulx K, et al. Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. *Am J Hum Genet.* 2009;85(1):106-111.
3. Bredrup C, Saunier S, Oud MM, et al. Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene WDR19. *Am J Hum Genet.* 2011;89(5):634-643.
4. Ruaud L, Mignot C, Guët A, et al. DYRK1A mutations in two unrelated patients. *Eur J Med Genet.* 2015;58(3):168-174.
5. van Bon BW, Coe BP, Bernier R, et al. Disruptive de novo mutations of DYRK1A lead to a syndromic form of autism and ID. *Mol Psychiatry.* 2015.
6. Richer J, Daoud H, Geier P, et al. Resolution of refractory hypotension and anuria in a premature newborn with loss-of-function of ACE. *Am J Med Genet A.* 2015.
7. Baird PN, Cowell JK. A novel zinc finger mutation in a patient with Denys-Drash syndrome. *Hum Mol Genet.* 1993;2(12):2193-2194.