Supplemental Information

CASE VIGNETTE 1: CHANGE IN TESTING STRATEGY FROM EXOME TO SINGLE GENE BASED ON EVALUATION

A 25-month-old girl was referred for ES because of hypotonia, abdominal distension, feeding difficulties, and failure to thrive (FTT) in the setting of global developmental delays. On physical examination, she demonstrated a behavioral pattern of finger twirling, rocking, and irregular breathing, findings that together with postnatal-onset microcephaly and global developmental delays suggested the diagnosis of Rett syndrome. The testing strategy was changed to tiered testing. Tier 1 comprised targeted testing with *MECP2* and *FOXG1* gene sequencing and exon-level copy number variant testing; tier 2 was ES if necessary. Tier 1 testing revealed a de novo p.Arg270* in *MECP2*, a well-described mutation in Rett syndrome.

CASE VIGNETTE 2: CANDIDATE GENE RESULT RETURN

A 4-day-old girl with severe congenital dilated cardiomyopathy (DCM) and severe biventricular dysfunction was referred for rapid ES and mtDNA analysis in the setting of negative biochemical screening test results for systemic disease. Her family history was notable for parental consanguinity, with her parents being half-second cousins but neither having any cardiac findings. mtDNA analysis revealed negative results but ES reported the following:

- *LMOD2* gene: homozygous for p.Trp398* (biparental inheritance confirmed); candidate gene result; and
- *OBSCN* gene: heterozygous for p.Gly4287Val, pat -VUS.

Leiomodins, encoded by *LMOD1, LMOD2,* and *LMOD3,* represent a subgroup of tropomodulin protein family and are regulators of the thin filament actin length in muscles. At the time, only *LMOD3* had a known disease association: autosomal recessive nemaline myopathy. Biallelic loss of function mutation in *LMOD2* represents a plausible pathogenic mechanism for the patient's congenital DCM on the basis of the mouse knock-out data and basic science research regarding protein function and tissue expression pattern (predominant isoform in the cardiac muscle). Other investigations for muscle, liver, and mitochondrial disease all being negative further supported the proposed pathogenicity of *LMOD2* mutation for this patient.

The paternally inherited p.Gly4287Val missense variant in *OBSCN* was not felt to be causative of congenital DCM, and its role in cardiomyopathy continues to be investigated.

On the basis of the ES results, the patient was cleared for cardiac transplant because clinical and genetic data did not reveal neurometabolic multisystemic diagnosis. The patient was listed for a transplant at 2 weeks of age and received it at 10 months. She sustained a middle cerebral artery stroke with transient seizure activities while waiting for the transplant because of her critical cardiac status, which has impacted her development. This information was also used for family planning for a subsequent pregnancy with the birth of an unaffected healthy sibling.

CASE VIGNETTE 3: DUAL DIAGNOSES

A 34-month-old boy was referred for ES because of congenital heart defects (truncus arteriosus and pulmonary artery stenosis), thymic aplasia, bilateral colobomas, failure to thrive (FTT), severe gastroesophageal reflux, and dysphagia necessitating gastrostomy tube feedings, abnormal brain MRI with mild abnormalities in the brainstem and pons, dysmorphic posterior fossa and narrowing of the craniocervical junction, central hypotonia, and global developmental delays. Previous genetic testing had included normal SNP-based chromosomal microarray analysis, MLPA for 22q11.2 deletion, and *CHD7* gene sequencing. Physical examination revealed several dysmorphic features, hypotonia, and delays that all supported the need for an ES test that identified dual diagnoses:

- *CHD4* gene: heterozygous p.Met1192Arg, de novo; diagnostic of Sifrim-Hitz-Weiss syndrome; and
- *CFTR* gene: p.Phe508del (mat) and p.Phe1052Val (pat) diagnostic of a mild form of cystic fibrosis.

Sifrim-Hitz-Weiss syndrome provided an explanation for most of his multisystemic involvement. The

biallelic *CFTR* mutations are not expected to contribute to his FTT phenotype, but the mild cystic fibrosis diagnosis uncovered by exome (despite a negative newborn screen result) is critical for his ongoing medical management, particularly with regard to respiratory infections.

CASE VIGNETTE 4: POSITIVE FOR ACMG SECONDARY FINDINGS

A 40-year-old woman was referred for ES because of findings of developmental delays, a learning disability with IQ of 98, seizures, microcephaly, bipolar disorder, autism spectrum disorder evolving spasticity and gait ataxia mild scoliosis, unilateral cataract, and a history of FTT and feeding difficulties with resulting short stature. Mild dysmorphic features were identified on examination, and she was referred for simultaneous chromosomal microarray analysis and ES testing. Chromosomal microarray analysis revealed a de novo and novel 1.66 Mb duplication of 19p13.3 that was subsequently felt to be likely pathogenic, especially in light of a nondiagnostic exome. ACMG secondary finding genes were analyzed and were positive for *BRCA1* c.68_69delAG mutation that was paternally inherited. This is a known founder mutation among the Ashkenazi Jewish population. Her father had been recently diagnosed with prostate cancer, a known cancer risk association for men with *BRCA1* gene mutations. Other at-risk family members were referred to consider *BRCA1* testing.

CASE VIGNETTE 5: EXOME DIAGNOSIS WITH IMMEDIATE MEDICAL MANAGEMENT CHANGE

An 11-year-old girl presented for a follow-up evaluation because of progressive bilateral sensorineural hearing loss requiring cochlear implantation, a more recent diagnoses of bilateral optic atrophy due to a 2-year history of worsening vision, and emerging gait difficulties. Her initial genetic testing (at 8 years of age for sensorineural hearing loss) had included a chromosomal microarray analysis and a hearing loss gene panel that included 66 nuclear genes and targeted mtDNA testing. The results revealed homozygosity for *GJB2* gene p.Gly4Asp variant interpreted as VUS and presence of the m.963T>C variant in the mtDNA. Because the *GJB2* p.Gly4Asp variant was reinterpreted as likely benign, the mtDNA variant also could not explain her evolving phenotype; her sample was sent for ES and full mtDNA analysis. The results revealed the following:

- *SLC52A2* gene: p.Tyr50* (mat) and p.Ala420Thr (pat) diagnostic of Brown-Vialetto–Van Laere syndrome, type 2 (also known as riboflavin transporter deficiency neuronopathy type 2). This gene was not on the initial hearing loss gene panel; and
- mtDNA: m.8976T>C (p.Leu149Pro) at 38% heteroplasmy, de novo (likely benign variant).

Brown-Vialetto–Van Laere syndrome represents a progressive neuronopathy with early-onset sensorineural deafness, nystagmus, progressive optic atrophy, vision loss, progressive pontobulbar palsy, severe diffuse muscle weakness and wasting resulting in respiratory insufficiency, and loss of independent ambulation. It is also a treatable diagnosis with high-dose riboflavin, particularly when identified earlier in the clinical course. At 15 years of age, she has had some improvement in her vision and gait without progression of her findings, including stable electromyography and nerve conduction study results. Her younger brother, tested presymptomatically, was also found to have the biallelic *SLC52A2* mutation and started on riboflavin therapy at 4 years of age. At 7 years of age, he is clinically unaffected but has electrophysiologic evidence of sensory axonal neuropathy on EMG and NCV testing that has improved with treatment.