SUPPLEMENTAL TEXT- Complete Clinical Case History

The patient was the second child born to a 44-year-old mother and a 47-year-old father. The pregnancy was complicated by gestational hypertension and by premature cervical shortening but was otherwise unremarkable. The patient was born at term by urgent cesarean section due to fetal bradycardia. At birth, his heart rate was noted to be over 100 beats per minutes, and Apgar scores were 9 and 9. The patient was noted to have increased work of breathing and a fever of 102 degrees Fahrenheit shortly after birth. He was treated with a 48-hour course of antibiotics, but blood culture resulted as negative. The patient was noted to have hypoglycemia in the first day of life, for which he received IV glucose. He was also noted to have hyperbilirubinemia at 48 hours of life, for which he received photo therapy. He was discharged home with his mother on day of life three.

As an infant, the patient reportedly never cried and moved very little. His hands were continuously fisted with abducted thumbs, and his great toes were reported to be in constant hyperextension until 2 months of age. The patient was first seen by a neurologist at three months of age, at which time mild left-sided torticollis with right-sided restriction was noted. This improved with physical therapy.

The patient has severe developmental delay. He has made slow progress with intensive therapies and has not experienced any developmental regressions. He attained moderate head control and began rolling at 18 months of age. He was able to come to a sitting position independently at 36 months of age and to creep or crawl at 48 months of age. At five years of age, he was not yet able to pull himself to a standing position or to stand unsupported, although he did begin taking steps with a gait trainer. He began feeding himself finger foods at 24 months and using and holding a sippy cup at 26 months. At 6 years he was not yet using utensils to feed himself or drinking from a regular cup, and his parents reported fatigue with chewing. At 6 years he had a spontaneous vocabulary of approximately ten words, although these were not understandable to most people, was using a combination of verbal and sign. Decreased oral motor strength and planning difficulties were felt to be impacting his ability to produce new verbalizations and signs. He was not yet using two or more words together.

Neurological exam at 31 months noted marked difficulty in initiating saccades, with frequent turning of head to track objects. Tone was low axially, and bulk was mildly reduced. He was able to move all extremities at least antigravity. When held to bear weight on his lower extremities. Truncal titubation and an uncoordinated gait were noted but were felt to reflect weakness more than ataxia. He had difficulty with head control, with weakness and titubation, and he frequently held his neck in hyperextension. Truncal titubation was also noted. He had exhibited frequent, complex dyskinetic movements of the upper extremities as well as occasional jerking movements. There

was no cortical fisting, fasciculations, or myotonia. He was appropriately responsive to touch in all extremities. Deep tendon reflexes were 1+ and symmetric in the bilateral upper extremities and 2+ in the lower extremities with an upgoing toe on the right.

The patient's height had been in the normal to low range in infancy and early childhood (-1.17 SD at birth, -1.35 SD at 31 months), and his weight and head circumference have been consistently within normal range (-0.66 SD at birth, -0.42 SD at 3 years). His height has slowly dropped off with age, and he now has short stature (-2.36 SD at 6 years). By contrast, his older sister's height is at the 75th percentile. His mother and father's adult heights are 170.2 cm (5'7") and 172.2 cm (6'0"), respectively, predicting a mid-parental target adult height of approximately 177.5 cm (5'10").

The patient has some mild dysmorphic features (Figure 1). He has brachycephaly and a flattened occiput. His eyes are mildly deep-set. He has mild prognathism, widely spaced teeth, and torus palatinus. His ears are mildly low set, with thickened helices and underdeveloped tragus and antitragus bilaterally. When evaluated at 31 months of age he inverted nipples and a prominent suprapubic fat pad. His hands and feet were small (-2.04 and -3.3 SD, respectively), and his fingers were tapered. His overall coloring, most prominently his hair, was noted to be unusually fair for his family, and his parents reported that his hair grew very slowly. He also had eczema.

The patient has been generally healthy throughout his life. His hearing and vision are normal, although he does have intermittent strabismus. He has no history of seizures. He had some early issues with feeding and weight gain that were attributed to a cow's milk protein allergy. He had retractile testes and a right inguinal hernia, for which he underwent bilateral orchidopexy and hernia repair. He was noted to have persistent enlargement of a left inguinal lymph node at 4 years of age. An excisional biopsy showed no evidence of malignancy. At 4 years of age, he was noted to have an intermittent bulge in his anterior neck when straining. Imaging indicated that this likely represented extension of a normal thymus through the thoracic inlet.

Brain magnetic resonance imaging studies at 11 months at 3.5 years showed mild prominence of the ventricular system and extra-axial spaces but were otherwise unremarkable. Electroencephalograms at 9 months and 4 years of age showed mild to moderate background slowing indicative of mild diffuse cerebral dysfunction. Electromyography and nerve conduction velocity studies at 10 months of age were unremarkable, showing no evidence for neuropathy or myopathy.

Pathology examination of the patient's hair revealed scant pigment and focally complete absence of melanin. These findings are reportedly distinct from what would be seen in typical blond hair and were felt to be suggestive of Griscelli syndrome. Of note, the patient did not have known or suspected immune deficiency, and a review of his genome sequencing data did not identify candidate variants in *MYO5A*, *RAB27A*, or *MLPH*

A biopsy of the right vastus lateralis muscle at 31 months revealed mild myopathic features, with moderate excess variation in fiber size with smaller type I fibers than type II fibers. Prominent mitochondria were seen by enzymatic histochemical stains, but it was not clear if this represented technical artifact. Electron microscopy did not reveal any diagnostic abnormalities but did note clusters of mitochondria and occasional large mitochondria.

Extensive metabolic testing was negative. This included testing for congenital disorders of glycosylation and multiple studies of cerebrospinal fluid.

The patient also underwent extensive genetic testing. Karyotype, chromosomal microarray, and methylation studies for Prader-Willi syndrome and Angelman syndrome were normal, as were *SMN1* and *SMN2* copy numbers. Panel-based testing for congenital myasthenia and cutaneous albinism were also negative. Mitochondrial genome sequencing and deletion studies were reportedly negative. Clinical trio exome sequencing revealed no findings. Clinical quad exome sequencing, including the patient's unaffected older sister, did not reveal any findings; however, subsequent research-based analysis of the data highlighted a novel de novo missense variant (NM:001991.3:c.2033G>C (p.A678G)) in *EZH1*, a gene with no established disease association. This finding was Sanger confirmed in a CLIA lab. Two additional research-based findings of lesser interest were also Sanger confirmed but were ultimately not felt to be strong candidates. Upon retrospective review, the *EZH1* variant was present in data from this patient’s original exome sequencing.