**HGGA, Volume *3***

## Supplemental information

# Variants in *PHF8* cause a spectrum of X-linked neurodevelopmental disorders and facial dysmorphology

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**Individual 2: Clinical report**

Individual 2 was born G0P0→1 to healthy non-consanguineous parents by emergency Caesarean section after a failed Ventouse at 40 weeks + 10 days. He had a birth weight of 3.92 kg, head circumference of 37.3 cm, and a length of 54 cm. Apgar scores were 6 at 1 minute, and 8 at 5 minutes. He was intubated and ventilated for a few minutes, and initially exhibited poor feeding which soon resolved. At birth, he displayed a hoarse cry, and he had sufficient facial dysmorphology to be noted at birth. In the neonatal period he had jaundice which resolved with standard phototherapy. There was no family history of similar issues in either the paternal or maternal lineages,

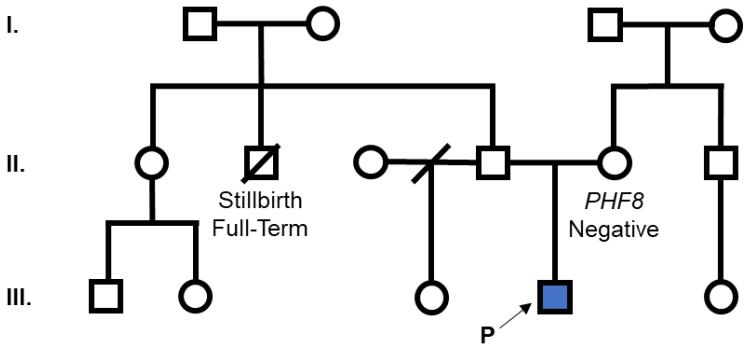
Over the next two decades he was followed for developmental delay and intellectual disability. At age 9 months he first was able to sit, he began to crawl at 14 months, and walk at 16 months. He was admitted to hospital at age 16 months with an *E. Coli* urinary tract infection. He was successfully treated with intravenous antibiotic. While he was admitted, developmental delay was noted and documented. At 21 months he had not attained speech or normal babble, he was hypotonic and exhibited hand flapping and hand wringing reminiscent of Rett syndrome. During this visit his weight was 13.8 kg, his head circumference was 50.6 cm, and his length 85.6 cm. He was noted to have coarse facial features, hypertelorism, short, upslanting palpebral fissures, and low set ears with deficient lobes. His behavior included mouthing (+++) and he exhibited continuous moaning noises. Spatulate digits especially thumbs and first toes were observed. He had broad first toes and thumbs with soft skin and lax joints.

By age 3 years and 9 months he still had not attained any speech. He had persistent cough and intermittent fevers with frequent hospital admissions. Motor development was delayed as he exhibited very poor balance and coordination and he was diagnosed with autism. MRI showed no abnormalities. At age 8 years 10 months his weight was 28.9 kg, his height approximately 130 cm, and his head circumference was 55.3 cm. Global developmental delay with severe learning difficulties was evident and he still had not attained speech or babble although he was able to accomplish some basic signing. Non-nutritive oral behaviors became more pronounced, and he exhibited drooling with extensive licking and chewing behavior with hand biting when anxious or frustrated. He had poor sleep patterns and was treated with melatonin. Motor

development improved, and gait was no longer ataxic. A high palate was observed, but no clefting. The most recent visit with Individual 2 occurred when he was 17 years of age after he completed puberty. His IQ was measured at 38, and he is able to speak two words: Mum and yes. He had swollen proximal interphalangeal joints and he continued showing stereotypic hand movements and hand biting. Mild scoliosis began at age 16 years and facial features have become increasingly coarse with age.

**Individual 2: Molecular characterization**

Previous genetic investigations included normal karyotype 46;XY, and normal cytogenomic microarray analysis using an Agilent custom array-CGH (genomic plus 5 probes per exon) and an analysis pipeline version: clinical-filter 0.0.27; convex 4.0.0; DDG2P version: 14-11-01. At age 17, exome sequencing using an Agilent SureSelect Exome Plus; HiSeq sequencing and analysis pipeline version: clinical-filter 0.0.27; dng-pipeline 0.10.9; hgi-vcf-generation 1.0.0; HGI Sequencing Data Improvement. (WES) 2.0.0; DDG2P version: 14-11-01 showed a mutation in *PHF8* c.704+1G>A, (X:54,043,027-54,043,027 PHF8 ENST00000357988.5: c.704+1G>A) which is a splice site variant that abolishes the canonical splice donor site for intron 7. This corresponds to c. c.596+1G>A on the NM_015107.2 transcript. The unaffected mother does not harbor the mutation, and no other family members are similarly affected. No other female family members have been tested (Pedigree – Individual 02).




Pedigree – Individual 2

**Individual 5: Clinical report**

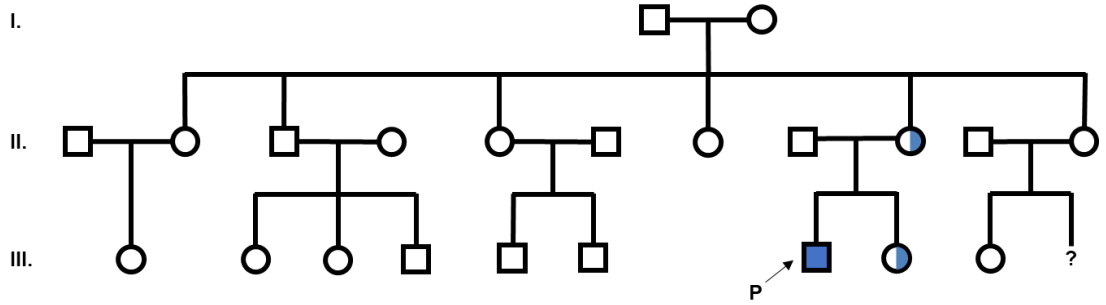
Individual 5 is an Afro-Caribbean 11-year-old boy born to a G0P0→1 mother who had no known prenatal exposures. He was born in the United States without any prenatal or perinatal complications. However, he moved to the resource-limited country of his family origin as a small child, which delayed an appropriate genetics evaluation. He presented with a diagnosis of autism, moderate developmental delay, hyperactivity, asthma, kidney stones, and mild dysmorphic features. His facial features include a long face with a prominent chin, bilateral ptosis, short palpebral fissures with a mild upslant, hypertelorism, bilateral epicanthus, sparse eyebrows that are thin medially, a flat broad nasal bridge, columella below the nares, full lips, bilateral underdeveloped ear helices, and mild facial asymmetry with possible left hypoplasia. He has a high arched palate. Joint laxity was noted when he was 8 years old. Head circumference was 52.6 cm (50$^{th}$%) and his interpupillary distance was 5.75 cm (75$^{th}$-97$^{th}$%).

At age nine he was able to write simple sentences and perform simple addition and subtraction, consistent with moderate developmental delay. He has never had a period of regression and continues to learn new skills. His family history is noncontributory. He has a full sister without similar issues. His father is alive and well, and he has no family history of intellectual disability. His mother is healthy, as are her parents, four sisters, and one brother. Both parents are of Afro-Caribbean ancestry. There is no history of consanguinity, birth defects, developmental delays, or recurrent miscarriage.

**Individual 5: Molecular characterization**

Previously, at the age of seven, Individual 5 obtained cytogenomic microarray from Progenity using an Affymetrix CytoScan containing approximately 2,696,500 probes with an overall resolution of 1.15 kb. No clinically significant copy number changes were detected, and he was reported as normal arr(1-22x2(XY)x1. Fragile X syndrome testing (Progenity) was also obtained, where he was shown to have one *FMR1* allele with 30 CGG repeats (normal range). Proband-only exome sequencing identified a hemizygous likely pathogenic variant in PHD Finger Protein 8 (*PHF8*), NM_015107.2: c.1996delG; p.(Glu666Argfs*163). The variant was verified by Sanger sequencing. His unaffected

mother and sister also carried this variant, but in a heterozygous state. Nobody else in the family is similarly affected, and other female family members were unavailable for carrier testing.




Pedigree – Individual 5

**Individuals 6, 7, and 8: Clinical report**

Individual 6 is a 9-year-old boy, born from non-consanguineous parents of European ancestry. During pregnancy, bilateral clubfoot was detected by ultrasound imaging. An amniocentesis was performed and fetal karyotype was normal. He was born at term with normal growth parameters. At neonatal exam, the clubfoot malformation was confirmed. A ventricular septal defect was also detected, which resolved spontaneously. He underwent surgical correction of the feet malformation at 3 and 7 years. At the age of 2.5 years, he also had surgical correction of a bilateral vesico-ureteral reflux (grade IV and V).

Motor development was normal, with acquisition of independent walking at the age of 18 months. He had speech delay, with first complete sentences pronounced at the age of 5 years. Now, at the age of 9, he has fine motor dyspraxia and behavioral troubles (tantrums, emotional lability). He has astigmatism and hypermetropia. He has learning difficulties due to attention deficiency and dyslexia, but no intellectual deficiency (total IQ 80). At clinical exam at 9 years, his growth parameters were in the normal range (height 136 cm, P30; weight 34.4 kg, P65; head circumference 55 cm, P80). He had hypertelorism, puffy upper eyelids, mild synophris, large palpebral fissures, and a high nasal bridge. Cardiovascular and neurological exams were normal.

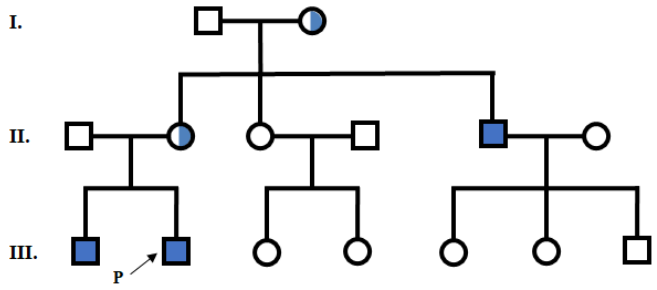
Individual 7 is the affected older brother of individual 6. He was born at term, after an uneventful pregnancy, with normal growth parameters and without any

congenital malformation. His psychomotor development was normal. At the age of 11 years, he had no intellectual impairment but had learning difficulties due to dyspraxia, dyscalculia and attention deficit. Craniofacial characteristics were very similar to his brother.

Individual 8 is the 46-year-old affected maternal uncle of individuals 6 and 7. He had normal psychomotor development but learning difficulties due to dyspraxia, attention deficit, behavioral troubles and borderline intellectual disability (total IQ 69, verbal comprehension index 81, fluid reasoning index 78, working memory index 68, processing speed index 69). At clinical exam, he had high and large forehead, high nasal bridge, short nasal alae, long columella and long philtrum.

**Individuals 6, 7, and 8: Molecular characterization**

In individual 6, molecular karyotype and Fragile X screening were negative. Due to familial history suggestive for an X-linked disease, a complete sequencing of the X chromosome was performed with SureSelect Agilent capture and NextSeq Illumina technology. This analysis identified a hemizygous variant in PHD Finger Protein 8 (*PHF8*), NM_001184896.1: c.1135C>T; p.(Gln379*). This corresponds to c.1030C>T; p.(Gln343*) on the *PHF8* NM_015107.2 transcript. The variant was verified by Sanger sequencing. Familial segregation analysis confirmed the presence of this variant in the hemizygous state in the affected individuals 7 (brother) and 8 (maternal uncle), and at heterozygous state in the mother and the maternal grandmother of individuals 6 and 7. This variant was not found in the population database (GnomAD) and was predicted to be a loss of function allele.



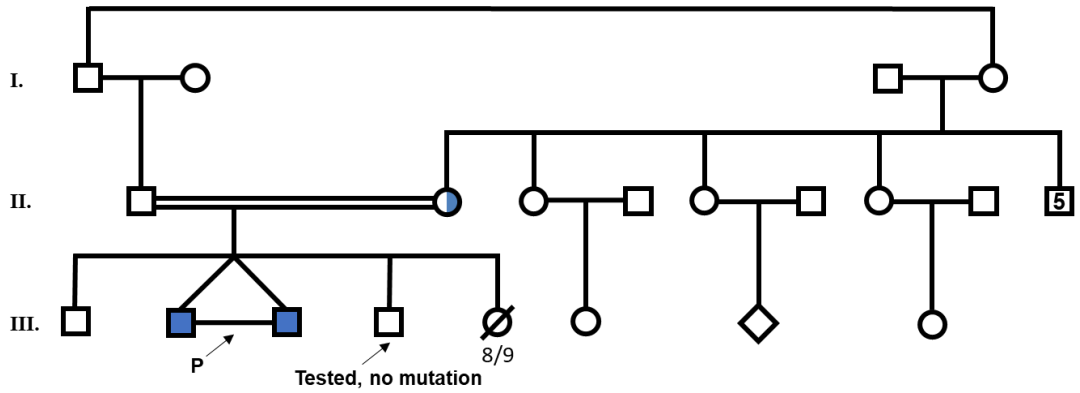

Pedigree – Individuals 6, 7, and 8

**Individuals 9 and 10: Clinical report**

Individuals 9 and 10 were identical twins born to consanguineous first cousin parents who are of North African (Morocco) ancestry and currently living in Europe. The mother had insulin dependent diabetes but was well regulated in pregnancy. These twins were first seen at the age of 2, where they were observed to have the same clinical presentation. They had microcephaly (<p2), no cleft lip, nor cleft palate. Both had severe intellectual disability, autism spectrum disorder, and developmental delay with almost no speech. They had an extrapyramidal movement disorder. Brain MRI was done at age 11 which showed bilateral polymicrogyria in both twins. These patients also had dextrocardia, which was diagnosed by autoradiography. Dysmorphology features included mild hypertelorism with a downslanting, broad nasal bridge, broad eyebrows with medial flaring, long eye lashes, prominent philtrum, tapering fingers, and single palmar creases.

**Individuals 9 and 10: Molecular characterization**

Trio exome sequencing revealed a *PHF8* g.54014379T>C; c.1839-2A>G (NM_001184896.1) variant in individual 10. This corresponds to c.1731-2A>G on the *PHF8* NM_015107.2 transcript. Sanger sequencing showed that his similarly affected twin had the same variant. This variant is at -2 bp of exon 17 and is predicted to affect splicing. The mother who is not affected, was a heterozygous carrier for this variant. A healthy brother was tested, he did not have the variant. No other family members were available for testing. Previous genetic investigations included testing for fragile X syndrome, microarray, and standard karyotype.



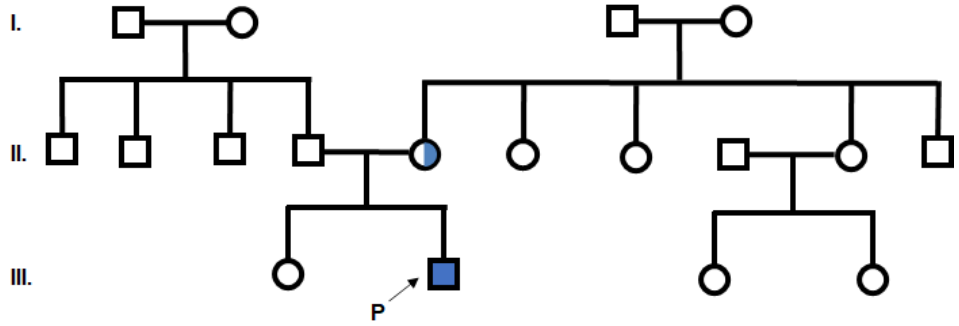

Pedigree – Individuals 9 and 10

**Individual 11: Clinical report**

Individual 11 was a 4-year-old boy (now deceased) of North African (Morocco) ancestry whose family is currently living in Europe. Consanguinity was denied.

**Individual 11: Molecular characterization**

Trio exome sequencing showed a g.54014379T>C: NM_001184896.1: c.1839-2A>G variant in the *PHF8* gene with the mother being an unaffected heterozygous carrier. This is the same variant that is found in Individuals 9 and 10, but the family relationship was not understood. Previous genetic testing for Individual 11 included a normal microarray (Affymetrix Cytoscan HD Array). No other family members were tested.




**Individual 11: Pedigree**

**Individual 15: Clinical report**

Individual 15 was born to healthy non-consanguineous parents at 38 weeks and 5 days. He had a birth weight of 2.92 kg, a birth length of 48.5 cm and a head circumference of 33 cm. Cleft lip and palate was noticed during the second trimester prenatal ultrasound. There was no gestational diabetes, no known exposure to toxins, and no infectious events during the pregnancy. He received a velopharyngoplasty, and a cartilage graft at the level of the nasal pyramid. There was no family history of similar issues in either the paternal or maternal lineages.

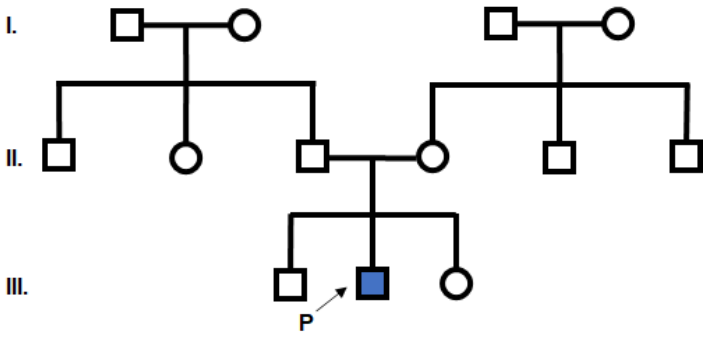
He was diagnosed with hearing loss (transmission) at the age of 2 and was quickly fitted with a hearing aid. A malformation was not detected with a CT scan focusing on the inner ear. He had language delay thought to be related to phonation disorders that were managed by a speech therapist. When younger, he had mild learning difficulties without intellectual disability. Currently he has no other

developmental delay and he has no academic difficulties. He plays rugby as activity. He has not had any other notable health problems.

On his last exam at age 14 he had a + 1 SD for height, + 2 SD for weight and - 1 SD for head circumference. He was noted to have a rather large nose at the root and at the tip. He had slight hypertelorism, a short columella, and a maxillary retrofusion. He had an ogival palate with a short soft palate. No anomalies of the extremity or other malformation were found.

**Individual 15: Molecular characterization**

Previous genetic investigations included apparently normal antenatal karyotype 46,XY and a normal microarray-CGH. Analysis by next-generation sequencing of a panel of genes implicated in syndromic facial clefts identified a novel hemizygous *PHF8* (NM_015107.2) pathogenic c.1965_1966dup; p.Glu656Valfs*174 variant. The variant was presumed to be *de novo*, as it was not found in the unaffected mother.




**Individual 15: Pedigree**

**Individual 16: Clinical report**

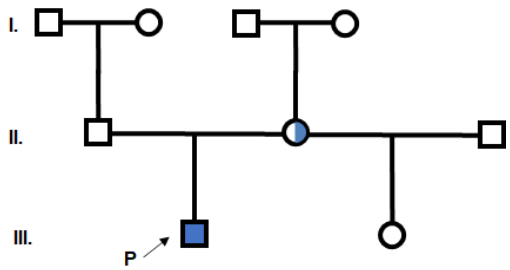
Individual 16 was born to healthy non-consanguineous parents after cesarean section for breech presentation at 36 weeks and 4 days. He had a birth weight of 2.95 kg, a birth length of 47 cm and a head circumference of 36 cm. He was hospitalized for cleft lip and palate, hypertonia of legs, and jaundice. The bilateral cleft lip and palate was noticed prenatally in the first trimester by ultrasound. There was no gestational diabetes, no known intake of toxins, and no infectious events during the pregnancy. There was no family history of similar presentation in either the paternal or maternal lineages.

His neonatal history was significant for craniosynostosis and apneas. A brain MRI showed tied cranio-occipital malformations without the typical Chiari malformation but with a hypoplasia posterior fossa, and a thin corpus callosum. Cardiac and abdominal ultrasound was normal. Facial dysmorphology included bilateral cleft lip palate which was surgically corrected soon after birth. He also had macroglossia with bifid tongue, sacral dimple, and macrocephaly.

He was followed for developmental delay and intellectual disability. At age 9 months he was able to perform eye tracking and he could sit unaided at 14 months. We walked at 3 years. He received physiotherapy and speech therapy and he was able to pronounce few words when 4 years old. Psychological therapy has improved some of his behavior disorders. At 6 years of age he was +3 SD for height, +1.5 SD for weight and +3 SD for head circumference.

**Individual 16: Molecular characterization**

An antenatal karyotype was apparently normal (46,XY) and microarray-CGH was also normal (NGS-MiSeq Illumina). No pathogenic variants were found with an intellectual deficiency panel. A next-generation sequencing-based panel of genes implicated in syndromic facial clefts identified a novel *PHF8* (NM_015107.2) hemizygous pathogenic variant c.294-1820_597-603del. The absence of coverage of exon 5 and 6 was confirmed by long-range PCR followed by Sanger sequencing of the intronic breakpoint. The deletion of two in frame exons predicts the synthesis of a protein lacking about 100 amino acid residues including part of the JmjC domain. This pathogenic variant was identified in the unaffected mother by Sanger sequencing. No other family members were tested

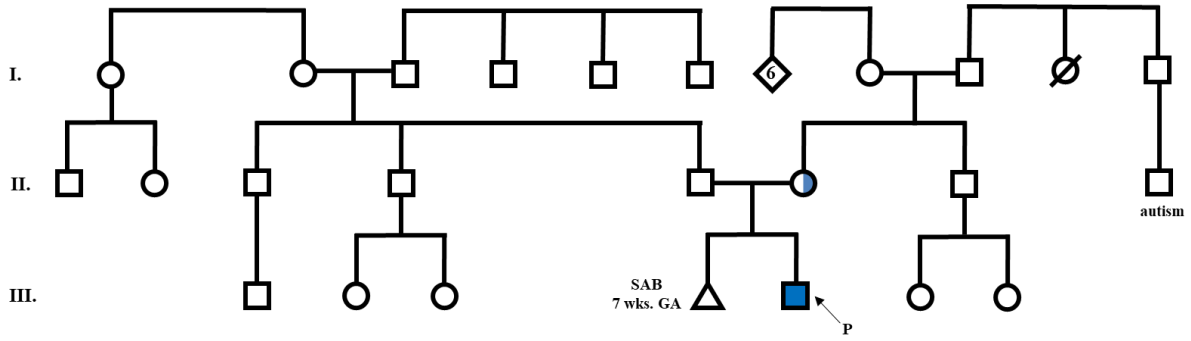



Individual 16: Pedigree

**Individual 17 (VUS): Clinical report**

Individual 17 was a 38-week gestation male infant, born after a pregnancy remarkable for preterm labor at 27 weeks treated with bedrest. His birthweight was 2.495 kg. There was deceleration of fetal growth for the last 4 weeks and thus labor was induced. Delivery was notable for fetal heart deceleration, vacuum-assist vaginal delivery and an atypical appearing placenta. Neonatal course was notable for jaundice treated with phototherapy. His birth length was 48.26 cm. He had poor latch but took a bottle well. He had reflux requiring formula change. Head circumference at 6-1/2 months of age was at the 25$^{th}$ percentile. Early developmental milestones were mildly delayed with walking at 16 months. Initial developmental concern was at 18 months of age when he was not speaking. He had severe impairments across receptive, expressive and pragmatic language domains. He had episodes of developing language and then stopping and becoming nonverbal again. He did not learn pointing, signing or picture exchange despite efforts to teach these skills. He had poor retention of learned skills. With intensive attention, at 7-8 years of age he acquired a larger vocabulary in two languages and can speak in 3-5-word sentences. Some of his language is scripted. He has improved social skills. He can perform simple chores at home. He toilet trained at 7 years. There are concerns for attentional deficits.

Medical history is complicated by a tight Chiari I malformation that required decompression at 2.5 years of age. The presenting signs included gagging, balance and gait issues, irritability, headaches and torticollis. He also has history of deformational plagiocephaly, eustachian tube dysfunction and cryptorchidism,
At 8 years he was at the 88$^{th}$ centile for height, 76$^{th}$ centile for weight and 54$^{th}$ centile for BMI. Head circumference was at the 21$^{st}$ centile at 3.5 years of age. His exam notes posteriorly rotated and prominent ears, normally spaced somewhat short palpebral fissures, mildly asymmetric nares, intact palate, mild retrognathia, and flat feet. Trio exome sequencing showed a maternally inherited hemizygous missense variant of uncertain significance in *PHF8* c.143A>G causing a p.(Tyr48Cys) substitution in the encoded protein.




Pedigree of Individual 17.

**Individual 18 (VUS): Clinical report**

Individual 18 was born at term after an unremarkable pregnancy and delivery. He had a birth weight of 4.05 kg and a length 54.6 cm. Global developmental delays were detected at his 18-month well-child visit with notation of walking at 15 months and no words. He was referred to an early development treatment program. His first words emerged at 24 months. He was diagnosed with severe speech apraxia. He was referred for formal developmental assessment at 31 months that diagnosed significant cognitive deficits, significant delay in expressive language and articulation, moderate delay in receptive language, decreased attention, decreased strength and coordination, difficulty crossing the midline, decreased vestibular and proprioceptive sensory processing. He received an autism spectrum disorder diagnosis. He has difficulty initiating and maintaining sleep, and has night terrors. He had global developmental regression after Chiari decompression but was able to regain these skills.

Medical health includes colic, gastroesophageal reflux, inguinal hernia, recurrent otitis, adenotonsillectomy, single febrile seizure and Chiari I malformation decompression. He has macrocephaly (z-score 1.65-2.27), tall stature (z-score 1.07-1.38) and elevated weight (z-score 1.79-2.41) and body mass index (z-score 1.50-2.59). Physical exam noted mildly cupped ears, round face, flat nasal bridge, upturned nasal tip, malar flatness, somewhat long philtrum and low normal muscle tone. Trio exome sequencing showed a hemizygous maternally inherited VUS in exon 4 *PHF8*: c.257C>T; p.(Thr86Met)

Previous genetic testing included a chromosomal microarray that showed an 88 kb deletion at 7q35 (146498890-146587308) that was not maternally inherited. This

deletion is within the *CNTNAP2* gene that has been linked to autism susceptibility. Autosomal recessive inheritance of *CNTNAP2* mutation results in a specific syndrome but sequencing of the *CNTNAP2* gene in this individual was normal. His family history included a sister who briefly needed speech therapy for articulation, a maternal half-sister with speech delay, a maternal half-sister with dyslexia and a maternal half-brother (individual 19 in this study) who had failure to thrive, hypotonia, and global developmental delay.

**Individual 19 (VUS): Clinical report**

Individual 19 was the maternal half-brother to Individual 18. He was born at 38-3/7 weeks weighing 2.72 kg after a pregnancy complicated by maternal hospitalization for UTI and pneumonia at 32 weeks. Delivery was induced without complication. The neonatal course was complicated by poor latch and emesis. He was hospitalized at 3 weeks for continued GE reflux and emesis with poor weight gain that prompted a switch to low allergen formula.

At 6 months he had ongoing growth failure, scrotal bruising, unexplained rib fractures, hypotonia and global developmental delay. He developed head control at 9 months, walked at 18 months, and had first words at 18 months. At the time of this report, he has only been seen by a medical geneticist through virtual visit and thus the exam details are more limited. His weight at 22 months was at the 25$^{th}$ centile. A visual inspection during the telemedicine consultation noted a high forehead, posteriorly rotated and mildly prominent ears due to outward positioning of superior helix, anteverted nares, broad nasal tip, somewhat long philtrum, mild retrognathia, and supernumerary nipples. His mother had a relatively small head, prominent ears and smooth philtrum.

Targeted analysis showed a maternally inherited hemizygous c.257C>T; p.(Thr86Met) VUS in *PHF8*. This is the same variant that was found in his maternal half-brother. Testing done due to unexplained rib fractures found a VUS in *COL1A1*, c.1691G>A; p.(Arg564His). Chromosomal microarray showed a 585 kb duplication 17p13.3 (hg19:2252823-2840218) considered to be a variant of uncertain significance.

**Individual 20: Clinical report**

This individual was a 17-year-old boy who is of mixed European and African (Kenya) ancestry. He was born at 37 weeks' gestation weighing 5 pounds 8 ounces and measuring 21 inches to a 44-year-old woman and her 24-year-old partner. An amniocentesis was performed because of advanced maternal age and revealed an apparently normal, 46,XY karyotype. Delivery was via cesarean section due to concerns for pregnancy-induced hypertension, He had a brief stay in the Neonatal Intensive Care Unit after birth and was discharged home with his mother 2 days after birth.

Concerns with his development arose in early childhood. He sat at 8 months, walked at about 10 months, uttered his first words at 14 months of age, and began stringing words together after 3 years. He has been provided with developmental and educational support since infancy. At 14 years of age he was enrolled in a self-contained classroom for most of the school day. He was able to do simple reading and writing. He was able to add, subtract and multiply but not divide. He has social difficulties. He does not recognize social cues and tends to be overly literal in his thinking. His ongoing hyperactivity and impulsivity appear to alienate him from others.

His most recent neuropsychological testing was performed at 14 years of age and his cognitive skills were estimated to be in the low average to average range (18$^{th}$ centile). His perceptual reasoning skills were noted to be in the average range (30$^{th}$ centile) and his verbal comprehension skills were noted to be in the low average range (14$^{th}$ centile). His full-scale IQ was noted to be 86. He was noted to be gaining skills relative to his peers over time when comparing these results with his pervious neuropsychological testing findings at 6 years of age when he was noted to have mild to moderate intellectual disability.

At 13 ½ years of age, his height was at the 83$^{rd}$ centile, his weight at the 62$^{nd}$ centile and his head circumference at the 75$^{th}$ - 98$^{th}$ centile. His had deep set appearing eyes, a broad nasal tip, short philtrum and a maxillary overbite, a mildly shortened appearing neck and pigmentary differences including several hyperpigmented macules of which only 1 measured greater that 1.5 cm and a hypopigmented macule. He has a history of mild mitral valve prolapse that has been stable in appearance over a 7-year period.

**Individual 20: Molecular characterization**

This individual's genetic work-up included a normal SNP chromosome microarray analysis and normal Fragile X test for the CGG expansion in *FMR1*. A autism/intellectual disability gene panel revealed a hemizygous variant of uncertain significance in *PHF8*, c.808 C>T; p.(Arg270Cys) with his mother being an unaffected carrier.