HGG Advances

ARTICLE

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

Andrew K. Sobering,[1,2,3,31,*] Laura M. Bryant,[4,31] Dong Li,[4] Julie McGaughran,[5] Isabelle Maystadt,[6] Stephanie Moortgat,[6] John M. Graham, Jr.,[7] Arie van Haeringen,[8] Claudia Ruivenkamp,[8] Roos Cuperus,[9] Julie Vogt,[10] Jenny Morton,[11] Charlotte Brasch-Andersen,[12,13] Maria Steenhof,[12] Lars Kjærsgaard Hansen,[14] Élodie Adler,[15] Stanislas Lyonnet,[15] Veronique Pingault,[15] Marlin Sandrine,[16] Alban Ziegler,[16] Tyhiesia Donald,[17] Beverly Nelson,[17] Brandon Holt,[18] Oleksandra Petryna,[19] Helen Firth,[20] Kirsty McWalter,[21] Jacob Zyskind,[21] Aida Telegrafi,[21] Jane Juusola,[21] Richard Person,[21] Michael J. Bamshad,[22,23,24] Dawn Earl,[22] University of Washington Center for Mendelian Genomics, Anne Chun-Hui Tsai,[25] Katherine R. Yearwood,[26] Elysa Marco,[27] Catherine Nowak,[28] Jessica Douglas,[28] Hakon Hakonarson,[4,29,30] and Elizabeth J. Bhoj[4,29,30,*]

Summary

Loss-of-function variants in *PHD Finger Protein 8* (*PHF8*) cause Siderius X-linked intellectual disability (ID) syndrome, hereafter called PHF8-XLID. PHF8 is a histone demethylase that is important for epigenetic regulation of gene expression. PHF8-XLID is an under-characterized disorder with only five previous reports describing different *PHF8* predicted loss-of-function variants in eight individuals. Features of PHF8-XLID include ID and craniofacial dysmorphology. In this report we present 16 additional individuals with PHF8-XLID from 11 different families of diverse ancestry. We also present five individuals from four different families who have ID and a variant of unknown significance in *PHF8* with no other explanatory variant in another gene. All affected individuals exhibited developmental delay and all but two had borderline to severe ID. Of the two who did not have ID, one had dyscalculia and the other had mild learning difficulties. Craniofacial findings such as hypertelorism, microcephaly, elongated face, ptosis, and mild facial asymmetry were found in some affected individuals. Orofacial clefting was seen in three individuals from our cohort, suggesting that this feature is less common than previously reported. Autism spectrum disorder and attention deficit hyperactivity disorder, which were not previously emphasized in PHF8-XLID, were frequently observed in affected individuals. This series expands the clinical phenotype of this rare ID syndrome caused by loss of *PHF8* function.

Introduction

Control of gene expression occurs through the interaction of a repertoire of regulatory factors. Gene regulation is complex, and is affected by a number of systems, including DNA binding proteins, enzymes that modify DNA bases, microRNA, and a large family of histone modification enzymes.[1] The interaction between DNA and histones may be regulated by methylation of arginine[2,3] and lysine amino acids[4] as well as a diverse array of other histone modifications.[5–7] Histone methylation status is controlled by enzymes that may methylate or demethylate and the methylation status affects binding of other proteins to specific regions of the histone tails. Loss-of-function variants

[1]AU/UGA Medical Partnership, Department of Basic Sciences, University of Georgia Health Sciences Campus, Athens, GA 30602, USA; [2]St. George's University, Department of Biochemistry, St. George's, Grenada, West Indies; [3]Windward Islands Research and Education Foundation, True Blue, St. George's, Grenada, West Indies; [4]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; [5]Genetic Health Queensland, RBWH, Brisbane and The University of Queensland School of Medicine, Brisbane, QLD 4029, Australia; [6]Centre de Génétique Humaine, Institut de Pathologie et de Génétique, 6041 Gosselies, Belgium; [7]Medical Genetics, Department of Pediatrics, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA 90048, USA; [8]Leiden University Medical Center, 9600, 2300 RC Leiden, the Netherlands; [9]Juliana Children's Hospital, HAGA Medical Center, The Hague, the Netherlands; [10]Birmingham Women's and Children's NHS Foundation Trust, Birmingham Women's Hospital, Birmingham B15 2TG, UK; [11]West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's Hospital NHS Foundation Trust, Birmingham B15 2TG, UK; [12]Department of Clinical Genetics, Odense University Hospital, Odense 5000, Denmark; [13]Human Genetics, Department of Clinical Research, University of Southern Denmark, Odense 5000, Denmark; [14]Department of Paediatrics, Odense University Hospital, Odense 5000, Denmark; [15]Fédération de Médecine Génomique and *Imagine* Institute, Université de Paris, Hôpital Necker-Enfants Malades, APHP, 75015 Paris, France; [16]Reference Center for Genetic Deafness, Fédération de Médecine Génomique and *Imagine* Institute, Université de Paris, Hôpital Necker-Enfants Malades, APHP, 75015 Paris, France; [17]Clinical Teaching Unit, St. George's University School of Medicine, St. George's, Grenada, West Indies; [18]Department of Anatomical Sciences, St. George's University, Grenada, West Indies; [19]Hackensack University Ocean Medical Center, Department of Psychiatry, Hackensack, NJ 08724, USA; [20]Department of Clinical Genetics, Cambridge University Hospitals, Box 134, Cambridge CB2 0QQ, UK; [21]Clinical Genomics, GeneDx, Gaithersburg, MD 20877, USA; [22]Seattle Children's Hospital, Seattle, WA 98105, USA; [23]Departments of Pediatrics and Genome Sciences, University of Washington, Seattle, WA 98195, USA; [24]Brotman-Baty Institute, Seattle, WA 98195, USA; [25]University of Oklahoma, Section of Genetics, 800 Stanton L Young Boulevard, Oklahoma City, OK 73117, USA; [26]University Health Services, St. George's University, Grenada, West Indies; [27]Cortica Healthcare, Marin Center, 4000 Civic Center Dr, Ste 100, San Rafael, CA 94903, USA; [28]Boston Children's Hospital, Division of Genetics and Genomics, 60 Temple Place, 2nd Floor, Boston, MA 02111, USA; [29]Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA; [30]Department of Pediatrics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[31]These authors contributed equally
*Correspondence: andrew.sobering@uga.edu (A.K.S.), bhoje@chop.edu (E.J.B.)
https://doi.org/10.1016/j.xhgg.2022.100102.
© 2022 The Author(s). This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

of *PHF8* (MIM: 300560), along with a number of other genes involved with regulation of histone methylation, have been associated with intellectual disability (ID) and developmental disorders in humans.[8,9]

PHF8 belongs to a sequence-related group of nuclear proteins that function in epigenetic gene regulation.[10] PHF8 contains 1,024 amino acids and has multiple domains including a zinc-finger plant homeodomain (PHD), a Jumanji (JmjC) domain, several nuclear localization signal domains, and a serine-rich region.[11–13] The PHD domain recognizes methylated histones, and the JmjC domain has catalytic histone demethylation activity.[14] PHF8 has dual functions. The PHD domain preferentially recognizes trimethylated H3K4 (H3K4me3),[15] an epigenetic marker typically associated with active transcription and the catalytic JmjC domain is responsible for preferential demethylation of H3K9me2.[14,16,17] In this way, an epigenetic marker associated with transcriptional repression is removed. By recognizing a mark of transcriptional activation and removing an adjacent modification associated with transcriptional repression, PHF8 plays a role in switching genes from a repressed state to an active state. PHF8 has also been shown to demethylate H3K9me1.

PHF8 orthologs have been studied in various model systems that show that it plays diverse roles in gene regulation, growth, and development. Examples include regulation via RNA polymerase II,[15] regulation of ribosomal gene transcription,[16] development of colorectal cancer,[18] and migration of endothelial cells.[19] Loss of *Phf8* in mouse embryonic stem cells (mESCs), neural progenitor cells, and mouse embryonic fibroblasts causes a severe reduction in proliferation of these cell types.[20] Loss of *Phf8* also causes changes in stem cell differentiation. *Phf8* knockout in mESCs shifts differentiation toward cardiac and mesodermal cell fate.[21]

Various neuronal functions for *PHF8* have been elucidated. *JMJD-1.2*, the *Caenorhabditis elegans* ortholog for human *PFH8,* is required for correct axon guidance.[22] In addition, *Phf8* null mice were shown to have impaired learning and memory.[23] Interestingly, a previous study found that mice deficient in *Phf8* had a resilience to anxiety and depression but no difference in learning and memory, possibly due to using a different background strain.[20] Other studies demonstrate that *Phf8* plays a role in astrocyte differentiation,[24] and also the regulation of neuronal differentiation through the retinoic acid receptor.[25]

In humans, *PHF8* is X-linked and its loss of function leads to developmental delay and dysmorphology. The first report of PHF8-XLID was enabled by linkage analysis, which implicated genes in the Xp11.3-q21.3 region in midline development, orofacial clefting, and ID in three related individuals.[26] This initial discovery led to the eponym of Siderius X-linked mental retardation syndrome (MIM: 300263), which is hereafter called PHF8-X-linked ID (PHF8-XLID). Six years later, the three individuals described in the initial report by Siderius et al., were found to have a 12-base pair deletion affecting the splice donor site for intron 8 of *PHF8,* and causing a frameshift of the encoded protein. In addition, an unrelated individual with a stop-gain variant in exon 7 of *PHF8* was described.[11] In 2007, one additional individual was described.[27] To our knowledge, there are only two additional reports describing individuals with pathogenic *PHF8* variants, but photographic documentation of the phenotype was not included.[12,13]

Here, we describe 11 additional variants and 16 individuals affected with predicted *PHF8* loss of function. We also describe five additional individuals with four different *PHF8* variants of unknown significance (VUS). Identification of families from clinicians and researchers worldwide was enabled by MatchMaker Exchange (MME),[28] GeneMatcher,[29] MyGene2,[30] Deciphering Developmental Disorders,[31] and Decipher.[32]

Materials and methods

We evaluated the facial dysmorphology, development, and behavioral characteristics among a cohort of 16 individuals who were identified to have predicted loss-of-function variants in *PHF8* after either exome, X-exome, or gene panel sequencing. The five individuals with a *PHF8* VUS had next-generation sequencing-based testing. Three of the VUS were identified by trio exome sequencing, and one by analysis of an ID/autism spectrum disorder (ASD) gene panel. The parents of all affected individuals gave written permission to publish clinical information. Written permission to publish photographs was obtained from the parents of all affected individuals except for individuals 13, 14, and 21, who are not included in the image panels. All investigators who enrolled participants in this study had ethical oversight from their respective institutional review boards.

Results

We identified 16 male individuals of various ancestries who harbor predicted loss-of-function variants in *PHF8*. Of the individuals in this cohort, nine were of Western European ancestry, three were Moroccan, two were Asian-Indian, and one was Afro-Caribbean. Photographs of the affected individuals show the wide range of facial dysmorphology caused by disruption of *PHF8* (Figure 1). In some individuals, photographs showing the evolution of the facial dysmorphology with age were available. Two of these individuals have an elongated face that tended to become more apparent with age (Figure 2).

Features of affected individuals

Our cohort consisted of 16 individuals with predicted loss-of-function variants in *PHF8*. From measurements obtained at the most recent evaluation, six of these individuals had microcephaly, two had macrocephaly, and the remainder had head circumference within the normal range. Hypertelorism was observed in 11 individuals and hypotelorism was seen in one. Five individuals had low-set ears and, of these, four had posterior angulation of

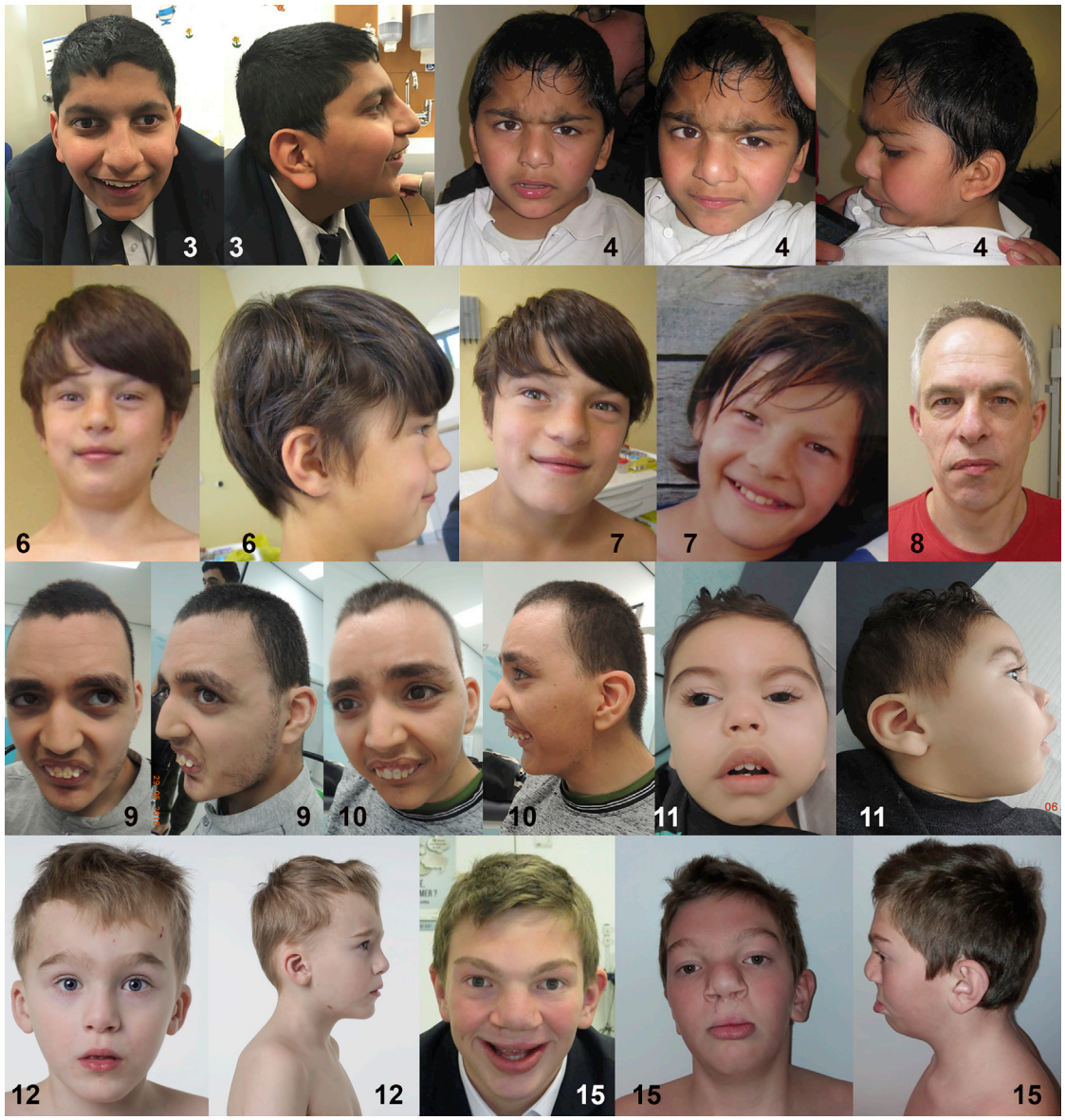


Figure 1. Facial features of individuals with predicted loss-of-function variants leading to PHF8-XLID
Photographs of individuals 3, 4, 6, 7, 8, 9, 10, 11, 12, and 15.

the ear. An elongated face was seen in eight individuals, and a borderline long face was seen in an additional three. When photographic evidence was available, the facial elongation appeared to become more pronounced with age. Retrognathia was seen in 10 individuals with a borderline presentation in one more. A broad nasal tip was observed in five individuals. Cleft lip/palate was seen in three individuals, two of which also had a high-arched palate. A high-arched palate in the absence of frank orofacial clefting was seen in an additional three others.

All affected individuals exhibited developmental delay (DD) and speech delay. All except two individuals had some degree of ID. There was a wide range of ID: borderline mild in two, mild in three, moderate in four, and severe in five. One individual had language delay thought to be due to hearing loss, but otherwise he did not have ID. The other individual who was reported to not have ID had dyscalculia. Psychomotor DD was seen in 12 affected individuals. Gross motor delay was seen in 12, and fine motor delay was seen in 14. Five individuals were reported to have recurrent seizures.

Ophthalmological and auditory abnormalities were also seen among individuals in our cohort, albeit never in the same individual. Six individuals had notable ophthalmologic features, which included unexplained unilateral squint, myopia, astigmatism, and hypermetropia. Auditory abnormalities were observed less frequently than vision problems, and included one individual who required ventilation tubes, one who was hearing impaired, one with unilateral sensorineural hearing loss, and one with progressive bilateral conductive hearing loss. The

Figure 2. Evolution of the facial features of individuals affected with PHF8-XLID
Photographs of individuals 1, 2, 5, and 16.

reported age at walking for 13 of the 16 individuals was delayed with a mean of 20 months and standard deviation of 7 months. Two individuals were not able to walk, and had to use a wheelchair. Birth length was available from seven individuals and the mean was 48.25 cm (50th centile); birth weight was 2.68 kg (10th centile), and birth head circumference from six individuals had a mean of 33.4 cm (25th centile).

Seven of the individuals in our cohort were diagnosed with ASD. Seven individuals carried a diagnosis of attention deficit hyperactivity disorder (ADHD), while only two individuals were diagnosed with both ASD and ADHD. The Autism Diagnostic Observation Schedule was used to diagnose ASD in two individuals, and clinical impressions or unspecified testing was used for the others. Some individuals were not tested for ADHD, three because of severe ID. Several were not tested for ASD because it was not clinically indicated. Aggressive behavior was documented in five individuals, and two individuals were noted to have anxiety. The distribution of the craniofacial dysmorphology features, developmental findings, and behavioral aspects of the affected individuals is shown in Figure 3.

Developmental regression, or loss of previously attained milestones, was not seen in any individual within the cohort of individuals who have a predicted *PHF8* loss-of-function variant. Feeding issues at infancy were observed in 10 of the 16 individuals and included inability to breast feed, requirement for percutaneous endoscopic gastrostomy, failure to thrive, typical problems associated with cleft lip/palate, and excessive drooling. In early childhood one individual developed gastroesophageal reflux disease, another was noted to have poor chewing ability and to prefer soft foods.

Brain MRI was obtained for six different individuals and significant findings were observed in five. Individual 2 showed normal brain MRI findings at age 3 years. Individual 3 had a mildly increased intensity of the subarachnoid space and high signal in the white matter around the posterior horns of the lateral ventricles. Individuals 9 and 10, who are monozygotic twins with consanguineous parents, had polymicrogyria and cortical dysplasia upon MRI at age

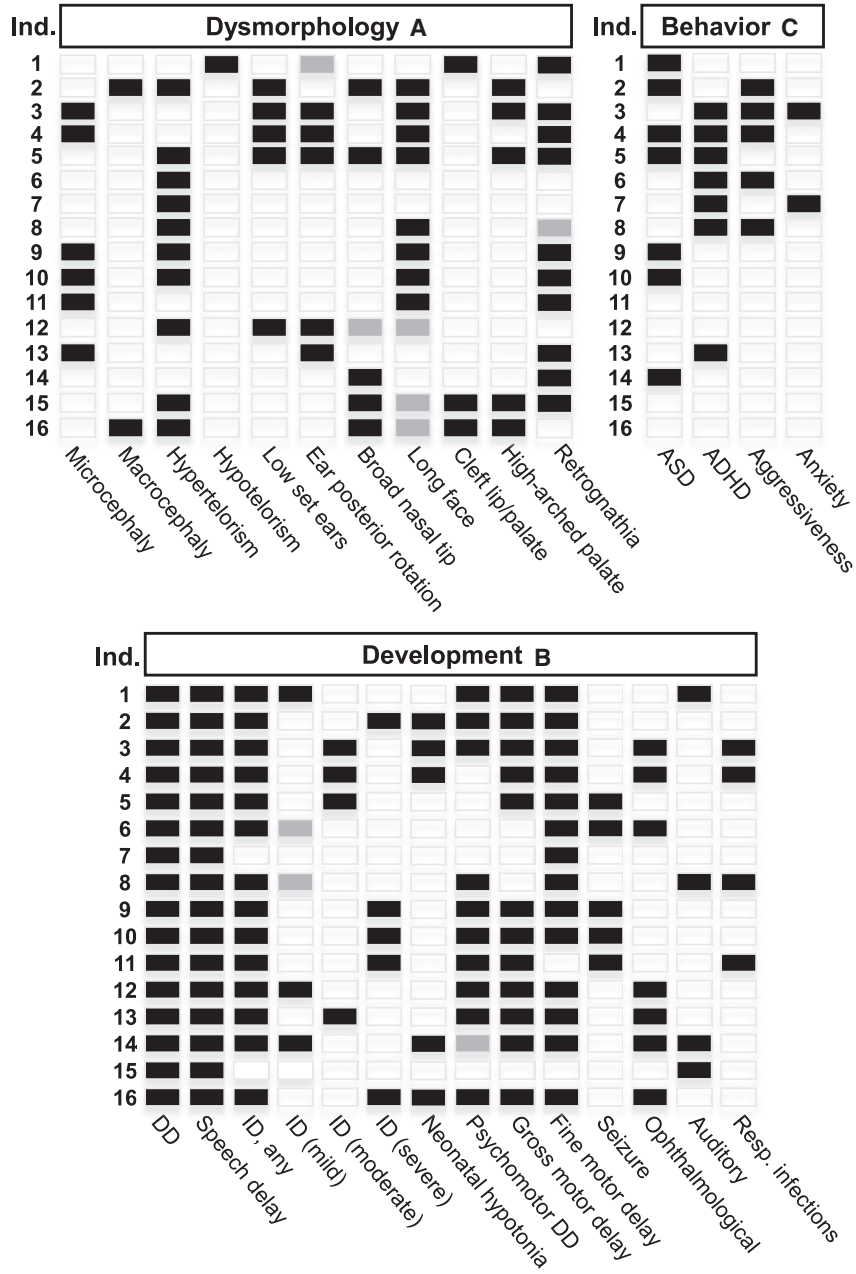


Figure 3. Features of individuals with PHF8-XLID

(A) Characteristic dysmorphology features.

(B) Delay and neurological features.

(C) Behavioral characteristics. Partially filled in boxes (gray) indicate borderline feature.

11 years. Individual 11 had an abnormal signal intensity in the striatum, normal cerebrum, normal gyri, and abnormal signal intensity in the caudate nucleus and globus pallidus, but the thalamus was normal. Individual 16 had tied cranio-occipital malformations without the typical Chiari malformation, but with a hypoplasia posterior fossa and a thin corpus callosum. A compilation of the collected clinical data for each individual in this cohort is in Table S1. Clinical descriptions for a subset of these individuals may be found in the supplemental material.

PHF8 VUS

We also present five male individuals (17–21 years) who were identified with a hemizygous VUS in *PHF8* following either exome sequencing or gene panel analysis. No other explanatory variant was identified for these individuals. All had mild to severe ID, and two exhibited regression of previously attained milestones. As with the individuals in the pathogenic/likely pathogenic cohort, the facial features of the individuals with *PHF8* VUS are heterogeneous. Orofacial clefting was not observed in any of these individuals (Figure 4). Overall, the clinical phenotype of the individuals with VUS appears to show a broad range of phenotypic variability similar to that seen in patients with predicted loss-of-function variants.

Genetic investigations

All *PHF8* variants in our cohort were identified by next-generation sequencing (NGS). One individual was diagnosed by NGS of a custom panel of genes known to be associated with ID, two via a panel designed to detect genes associated with cleft lip/palate, and one individual was identified by X-exome sequencing. The remainder were identified via exome sequencing. In three families, additional affected individuals were identified following diagnosis in a sibling or a nephew. Of the 12 mothers who had children affected with PHF8-XLID, 10 were shown to be unaffected carriers and 2 did not have a *PHF8* variant suggesting *de novo* inheritance. Skewed X-inactivation was tested in 3 of the 10 healthy carrier mothers. In two of the mothers, completely skewed X-inactivation was observed and, in the third, the test was uninformative. A schematic of PHF8 with the placement of previously reported variants and the variants reported here is shown in Figure 5. Of the variants we describe here, four were previously reported in large-scale sequencing studies.[9,33] In addition, several other predicted loss-of-function *PHF8* variants were described in other next-generation sequencing-based studies,[9,34,35] but detailed clinical information was not included. Previously reported *PHF8* loss-of-function variants,[9,11–13,26,27,33–35]

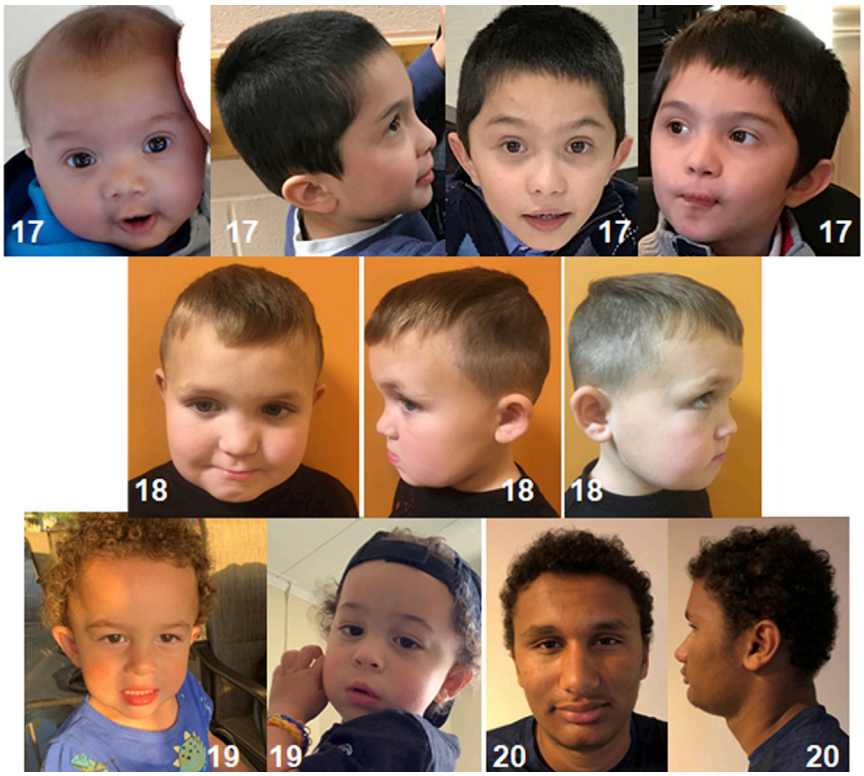


Figure 4. Facial features of individuals who have intellectual disability and VUS in *PHF8*
Photographs of individuals 17, 18, 19, and 20.

the loss-of-function variants described in this study, and the VUS we report are listed in Table 1.

Most affected individuals had previous genetic testing. Microarray was obtained for 13, fragile-X syndrome testing was obtained for 10, and karyotype was obtained for 8 individuals. Angelman syndrome was suspected and tested for in three individuals, and one was tested for myotonic dystrophy. One individual underwent testing via a gene panel for ID and DD which was uninformative. Two individuals underwent metabolic testing, which included analysis of amino acids, organic acids, glycosaminoglycans, oligosaccharides, acylcarnitines, and very long chain fatty acids. The previous genetic and biochemical testing obtained for the individuals in this cohort is in Table S2.

Of the individuals with *PHF8* VUS, three had trio exome sequencing, one had targeted analysis of *PHF8*, because of the known *PHF8* variant in a maternal half-brother, and one had a trio ID/ASD gene panel. In all cases, the *PHF8* VUS was inherited from an unaffected carrier mother. None of the mothers were tested for skewed X-inactivation.

Discussion

The five previously published reports describing PHF8-XLID include photographic documentation of six individuals, and written descriptions of two more. All six of the individuals who were described with photographs were either adult or in late adolescence, so descriptions of PHF8-XLID in young children has previously not been available. Six of the eight previously reported individuals exhibited facial clefting and this was viewed as a defining characteristic feature of PHF8-XLID. It is likely that this is because cleft lip/palate was a screening criterion combined with ID in the first four reports.[11,12,26,27] In the Ibarluzea 2020 report, orofacial clefting was not used as one of the screening criteria,[13] so a predicted *PHF8* loss-of-function variant was detected from a panel of genes known to cause X-linked ID. In our cohort, only 3 of the 16 patients with predicted loss-of-function variants had a cleft lip/palate and none of the individuals with VUS had a cleft lip/palate, consistent with a less severe phenotype. While cleft lip/palate can be caused by pathogenic variants in *PHF8*, it does not appear to be a consistent diagnostic feature of PHF8-XLID.

Unbiased screening by either exome, X-exome, or ID gene panel sequencing reveals the broad range of phenotypic heterogeneity and variable expressivity displayed by individuals who harbor *PHF8* loss-of-function variants. The only clinical feature common to all individuals was DD and speech delay. ID was seen in all except for two individuals, and the severity ranged from borderline mild to severe. Within our cohort of individuals who had predicted *PHF8* loss-of-function variants, there were three sets of siblings. The clinical features within these sibling pairs was relatively consistent compared with the overall cohort. For instance, individuals 6 and 7 had either mild or no ID, individuals 3 and 4 had moderate ID, and individuals 9 and 10, who are consanguineous identical twins, had severe ID. In addition many other clinical features were similar within these sibling pairs.

All except one variant described in the previous reports of PHF8-XLID were predicted to cause truncation of *PHF8* and disruption of the catalytic JmjC domain. The one exception was a missense variant leading to a Phe279Ser substitution in the encoded protein.[27] *In vitro* functional studies demonstrated that the Phe279Ser variant abolished the lysine demethylase activity of the encoded protein,[36] suggesting that aberrant epigenetic regulation of gene expression leads to the disorder. Five of the loss-of-function variants we describe (in seven individuals) are located outside of the JmjC domain. All of these variants either lead to a frameshift or are predicted to affect splicing. Notably, the individuals who we describe with predicted loss-of-function variants outside of the JmjC domain do

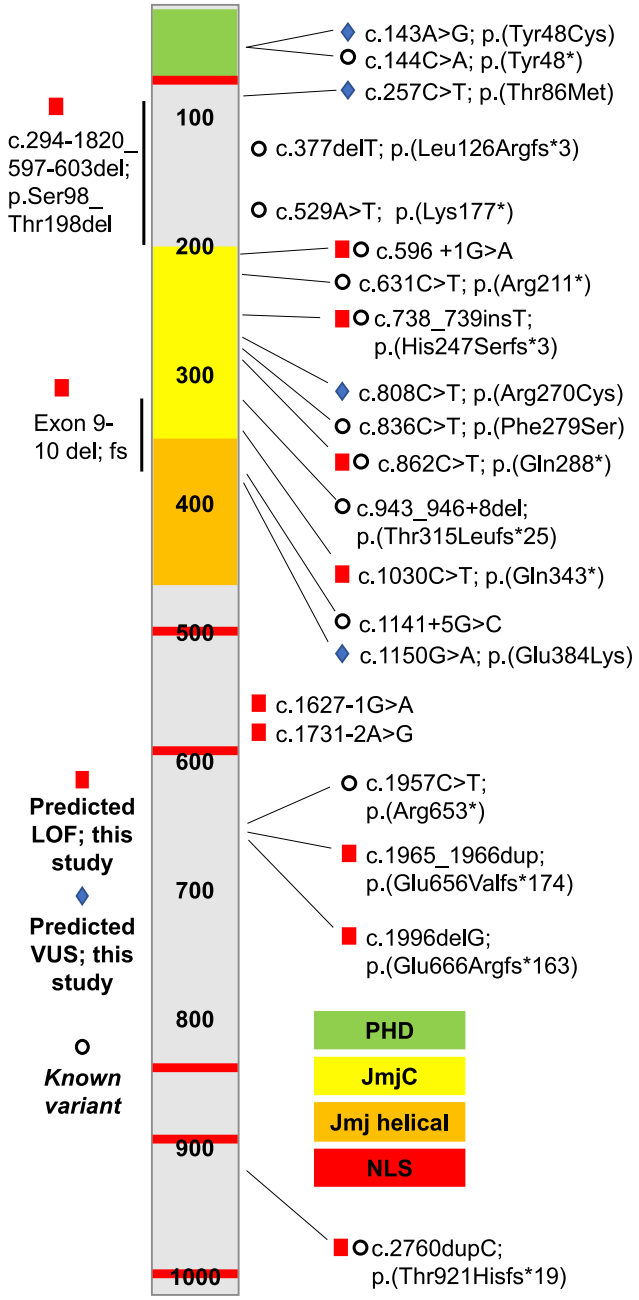


Figure 5. Schematic of PHF8 protein structure annotated with variants

Known *PHF8* variants are indicated with open circles; predicted loss-of-function *PHF8* variants are indicated with red squares, and predicted *PHF8* VUS are indicated with blue diamonds. Protein domains of PHF8 are color coded. PHD, plant homology domain; JmjC, jumonji C domain; NLS, nuclear localization signal.

not have different clinical features or phenotypic severity than those with variants within the JmjC domain. Notably, one of the individuals with a loss-of-function variant outside of the JmjC domain is one of the three patients in our cohort with a cleft lip/palate. Four of the variants we identified were found by either an ID gene panel, a cleft lip/palate panel, or X-exome sequencing. Since exome sequencing was not used for these individuals, it is possible that they may have additional but unknown variants that contribute to their phenotype.

A variant predicted to cause an in-frame deletion of a serine amino acid at position 969 of PHF8 was reported in two individuals and a carrier mother.[37] However, we did not include Ser969del as a deleterious variant in this study for three reasons. First, it is abundantly found in gnomAD in both heterozygous males and homozygous females.[38] The second reason is that it is annotated as benign or likely benign in ClinVar.[39] Finally, functional studies were not done to characterize this variant.

Our cohort includes five individuals with four previously unreported missense variants. The functional implication is currently unknown and future study of these VUS could allow us to better understand the importance of other PHF8 domains. Functional studies to assess PHF8 activity would be useful to help characterize these missense variants. Three reports of microdeletion or unspecified mutation within the Xp11.22 region describe individuals with phenotypes similar to PHF8-XLID. Although the deletions in these patients spanned multiple genes, their phenotypic features are similar to that seen in individuals with predicted loss of function of only *PHF8*.[40–42]

The methylcytosine epigenetic signatures of various genes involved in histone methylation have been established.[43] For example, the epi-signature of Claes-Jensen syndrome due to pathogenic loss of function of the X-linked gene *KDM5C* is significantly different between unaffected individuals, carrier mothers, and affected males.[44] However, a recognizable epi-signature was not observed from an analysis of nine males affected with PHF8-XLID. It is possible that a larger sample size is needed, or that DNA samples from a tissue other than blood might be needed to uncover a potentially altered methylation pattern for this disorder.[45]

Overall, PHF8-XLID appears to be characterized by DD, ID, and craniofacial anomalies. Cleft lip or palate is found in a much smaller percentage of affected individuals who were found through unbiased sequencing. The phenotypic variability does not appear to be linked to the variant location in individuals who harbor a null allele. Future studies aimed at evaluating the cause of the variability would be helpful to predict disease severity and developmental progression as well as the interpretation of VUS in *PHF8*.

Ethical oversight

This study was performed in accordance with the ethical standards detailed in the Declaration of Helsinki. The parents or guardians of all participants provided written informed consent for inclusion in this study.

Web resources

OMIM, http://www.omim.org/

Table 1. Known variants in *PHF8*

Ind.	var #	*PHF8* NM_015107.2 Variant	Protein consequence	Variant effect	Inh.	Variant first identified by	Source
This study (predicted loss of function)							
1	1	del exons 9-10	p.Gly316_Arg380del	Intragenic del with frameshift	Maternal	Gene panel (ID)	*This study*
2	2	c.596+1G>A	Unknown	Splice site	*de novo*	Trio exome, DDD	
3, 4	3	c.862C>T	p.(Gln288*)	Stop gain	Maternal	Quad exome, DDD	
5	4	c.1996delG	p.(Glu666Argfs*163)	Frameshift	Maternal	Proband exome	
6,7,8	5	c.1030C>T	p.(Gln343*)	Stop gain	Maternal	X-exome	
9,10,11	6	c.1731-2A>G	Unknown	Splice site	Maternal	Trio exome	
12	7	c.1627-1G>A	Unknown	Splice site	*de novo*	Trio exome	
13	8	c.738_739insT	p.(His247Serfs*3)	Frameshift	Maternal	Trio exome, DDD	
14	9	c.2760dupC	p.(Thr921Hisfs*19)	Frameshift	Maternal	Trio exome	
15	10	c.1965_1966dup	p.(Glu656Valfs*174)	Frameshift	*de novo*	Gene panel (CL/P)	
16	11	c.294-1820_597-603del	p.Ser98_Thr198del	Deletion	Maternal	Gene panel (CL/P)	
This study (variants of unkwown significance)							
17	12	c.143A>G	p.(Tyr48Cys)	Missense	Maternal	Trio exome	*This study*
18,19	13	c.257C>T	p.(Thr86Met)	Missense	Maternal	Trio exome	
20	14	c.808C>T	p.(Arg270Cys)	Missense	Maternal	Trio ID gene panel	
21	15	c.1150G>A	p.(Glu384Lys)	Missense	Maternal	Trio exome	
PHF8 variants in previously described indviduals							
23		c.144C>A	p.(Tyr48*)	Stop gain	Maternal	XLID panel (NGS)	Ibarluzea et al.[13]
25,26		c.836C>T	p.(Phe279Ser)	Missense	Maternal	Sanger seq	Koivisto et al.[27]
27		c.529A>T	p.(Lys177*)	Stop gain	Not noted	Sanger seq	Abidi et al.[12]
28		c.631C>T	p.(Arg211*)	Stop gain	Maternal	Sanger seq	Laumonnier et al.[11]
29,30,31		c.943_946+8del	p.(Thr315Leufs*25)	Frameshift	Maternal	Linkage analysis	Siderius et al.[26]

(*Continued on next page*)

Table 1. *Continued*

Ind.	var #	*PHF8* NM_015107.2 Variant	Protein consequence	Variant effect	Inh.	Variant first identified by	Source
PHF8 variants described in large-scale exome sequencing projects							
na		c.596+1G>A	Unknown	Splice site	Not noted	Exome	Faundes et al.[9]
na		c.738_739insT	p.His247Serfs*3	Frameshift	Not noted	Exome	
na		c.862C>T	p.(Gln288*)	Stop gain	Not noted	Exome	
na		c.1957C>T	p.(Arg653*)	Stop gain	Not noted	Exome	
na		c.377delT	p.(Leu126Argfs*3)	Frameshift	Not noted	Exome	Posey et al.[34]
na		c.2760dupC	p.(Thr921Hisfs*19)	Frameshift	Not noted	Exome	Retterer et al.[33]
na		c.1141+5G>C	Unknown	Splice site	Not noted	Exome	Redin et al.[35]

The table includes the variants described in this study, variants described in previously published reports, and variants described in large-scale sequencing projects. All variants were coded according to the NM_015107.2 *PHF8* transcript.

gnomAD, https://gnomad.broadinstitute.org/
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/variation/

Data and code availability

The published article includes a supplemental table with the full clinical dataset generated and analyzed during the study. All exome sequencing data are not publicly available due to privacy or ethical restrictions.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2022.100102.

Acknowledgments

Sequencing was provided by the University of Washington Center for Mendelian Genomics (UW-CMG) and was funded by NHGRI and NHLBI grants UM1 HG006493 and U24 HG008956, by the Office of the Director, NIH under Award Number S10OD021553. We thank the patients and their families for participating in this study. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]. This study makes use of DECIPHER (http://decipher.sanger.ac.uk), which is funded by Wellcome. See Nature PMID: 25533962 or www.ddduk.org/access.html for full acknowledgement.

Declaration of interest

J.J., J.Z., A.T., R.P., and K.M. are employees of GeneDx, Inc. All other authors have no conflict of interest to declare.

Received: December 14, 2021
Accepted: March 18, 2022

References

1. Ahsendorf, T., Müller, F.J., Topkar, V., Gunawardena, J., and Eils, R. (2017). Transcription factors, coregulators, and epigenetic marks are linearly correlated and highly redundant. PLoS One *12*, 1–25. https://doi.org/10.1371/journal.pone.0186324.
2. Di Lorenzo, A., and Bedford, M.T. (2011). Histone arginine methylation. FEBS Lett. *585*, 2024–2031. https://doi.org/10.1016/j.febslet.2010.11.010.
3. Zhang, J., Jing, L., Li, M., He, L., and Guo, Z. (2019). Regulation of histone arginine methylation/demethylation by methylase and demethylase (Review). Mol. Med. Rep. *19*, 3963–3971. https://doi.org/10.3892/mmr.2019.10111.
4. Husmann, D., and Gozani, O. (2019). Histone lysine methyltransferases in biology and disease. Nat. Struct. Mol. Biol. *26*, 880–889. https://doi.org/10.1038/s41594-019-0298-7.
5. Taylor, B.C., and Young, N.L. (2021). Combinations of histone post-translational modifications. Biochem. J. *478*, 511–532. https://doi.org/10.1042/BCJ20200170.
6. Ryu, H.Y., and Hochstrasser, M. (2021). Histone sumoylation and chromatin dynamics. Nucleic Acids Res. *49*, 6043–6052. https://doi.org/10.1093/nar/gkab280.

7. Villaseñor, R., and Baubec, T. (2021). Regulatory mechanisms governing chromatin organization and function. Curr. Opin. Cell Biol. *70*, 10–17. https://doi.org/10.1016/j.ceb.2020.10.015.
8. Vallianatos, C.N., and Iwase, S. (2015). Disrupted intricacy of histone H3K4 methylation in neurodevelopmental disorders. Epigenomics *7*, 503–519. https://doi.org/10.2217/epi.15.1.
9. Faundes, V., Newman, W.G., Bernardini, L., et al. (2018). Histone lysine methylases and demethylases in the landscape of human developmental disorders. Am. J. Hum. Genet. *102*, 175–187. https://doi.org/10.1016/j.ajhg.2017.11.013.
10. Piazza, R., Magistroni, V., Redaelli, S., et al. (2018). SETBP1 induces transcription of a network of development genes by acting as an epigenetic hub. Nat. Commun. *9*, 2192. https://doi.org/10.1038/s41467-018-04462-8.
11. Laumonnier, F., Holbert, S., Ronce, N., et al. (2005). Mutations in PHF8 are associated with X linked mental retardation and cleft lip/cleft palate. J. Med. Genet. *42*, 780–786. https://doi.org/10.1136/jmg.2004.029439.
12. Abidi, F.E., Miano, M.G., Murray, J.C., and Schwartz, C.E. (2007). A novel mutation in the PHF8 gene is associated with X-linked mental retardation with cleft lip/cleft palate. Clin. Genet. *72*, 19–22. https://doi.org/10.1088/1367-2630/15/1/015008.
13. Ibarluzea, N., de la Hoz, A.B., Villate, O., et al. (2020). Targeted next-generation sequencing in patients with suggestive X-linked intellectual disability. Genes (Basel) *11*, 1–22. https://doi.org/10.3390/genes11010051.
14. Horton, J.R., Upadhyay, A.K., Qi, H.H., Zhang, X., Shi, Y., and Cheng, X. (2010). Enzymatic and structural insights for substrate specificity of a family of jumonji histone lysine demethylases. Nat. Struct. Mol. Biol. *17*, 38–43. https://doi.org/10.1038/nsmb.1753.
15. Fortschegger, K., de Graaf, P., Outchkourov, N.S., van Schaik, F.M.A., Timmers, H.T.M., and Shiekhattar, R. (2010). PHF8 targets histone methylation and RNA polymerase II to activate transcription. Mol. Cell Biol. *30*, 3286–3298. https://doi.org/10.1128/MCB.01520-09.
16. Feng, W., Yonezawa, M., Ye, J., Jenuwein, T., and Grummt, I. (2010). PHF8 activates transcription of rRNA genes through H3K4me3 binding and H3K9me1/2 demethylation. Nat. Struct. Mol. Biol. *17*, 445–451. https://doi.org/10.1038/nsmb.1778.
17. Yu, L., Wang, Y., Huang, S., et al. (2010). Structural insights into a novel histone demethylase PHF8. Cell Res. *20*, 166–173. https://doi.org/10.1038/cr.2010.8.
18. Lv, Y., Shi, Y., Han, Q., and Dai, G. (2017). Histone demethylase PHF8 accelerates the progression of colorectal cancer and can be regulated by miR-488 in vitro. Mol. Med. Rep. *16*, 4437–4444. https://doi.org/10.3892/mmr.2017.7130.
19. Gu, L., Hitzel, J., Moll, F., et al. (2016). The histone demethylase PHF8 is essential for endothelial cell migration. PLoS One *11*, 1–15. https://doi.org/10.1371/journal.pone.0146645.
20. Walsh, R.M., Shen, E.Y., Bagot, R.C., et al. (2017). Phf8 loss confers resistance to depression-like and anxiety-like behaviors in mice. Nat. Commun. *8*, 1–11. https://doi.org/10.1038/ncomms15142.
21. Tang, Y., Hong, Y.Z., Bai, H.J., et al. (2016). Plant homeo domain finger protein 8 regulates mesodermal and cardiac differentiation of embryonic stem cells through mediating the histone demethylation of pmaip1. Stem Cell *34*, 1527–1540. https://doi.org/10.1002/stem.2333.
22. Riveiro, A.R., Mariani, L., Malmberg, E., et al. (2017). JMJD-1.2/PHF8 controls axon guidance by regulating Hedgehog-like signaling. Development *144*, 856–865. https://doi.org/10.1242/dev.142695.
23. Chen, X., Wang, S., Zhou, Y., et al. (2018). Phf8 histone demethylase deficiency causes cognitive impairments through the mTOR pathway. Nat. Commun. *9*, 114. https://doi.org/10.1038/s41467-017-02531-y.
24. Iacobucci, S., Padilla, N., Gabrielli, M., et al. (2021). The histone demethylase PHF8 regulates astrocyte differentiation and function. Development *148*, 1–14. https://doi.org/10.1242/dev.194951.
25. Qiu, J., Shi, G., Jia, Y., et al. (2010). The X-linked mental retardation gene PHF8 is a histone demethylase involved in neuronal differentiation. Cell Res. *20*, 908–918. https://doi.org/10.1038/cr.2010.81.
26. Siderius, L.E., Hamel, B.C.J., van Bokhoven, H., et al. (1999). X-linked mental retardation associated with cleft lip/palate maps to Xp11.3-q21.3. Am. J. Med. Genet. *85*, 216–220. https://doi.org/10.1002/(SICI)1096-8628(19990730)85:3<216.
27. Koivisto, A.M., Ala-Mello, S., Lemmelä, S., Komu, H.A., Rautio, J., and Järvelä, I. (2007). Screening of mutations in the PHF8 gene and identification of a novel mutation in a Finnish family with XLMR and cleft lip/cleft palate. Clin. Genet. *72*, 145–149. https://doi.org/10.1111/j.1399-0004.2007.00836.x.
28. Philippakis, A.A., Azzariti, D.R., Beltran, S., et al. (2015). The matchmaker Exchange: a platform for rare disease gene discovery. Hum. Mutat. *36*, 915–921. https://doi.org/10.1002/humu.22858.
29. Sobreira, N., Schiettecatte, F., Valle, D., and Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum. Mutat. *36*, 928–930. https://doi.org/10.1002/humu.22769.
30. Sobreira, N., Arachchi, H., Buske, O.J., et al. (2017). Matchmaker exchange. Curr. Protoc. Hum. Genet. *95*, 9.31.1–9.31.15. https://doi.org/10.1002/cphg.50.
31. Fitzgerald, T.W., Gerety, S.S., Jones, W.D., et al. (2015). Large-scale discovery of novel genetic causes of developmental disorders. Nature *519*, 223–228. https://doi.org/10.1038/nature14135.
32. Firth, H.V., Richards, S.M., Bevan, A.P., et al. (2009). DECIPHER: database of chromosomal imbalance and phenotype in humans using ensembl resources. Am. J. Hum. Genet. *84*, 524–533. https://doi.org/10.1016/j.ajhg.2009.03.010.
33. Retterer, K., Juusola, J., Cho, M.T., et al. (2016). Clinical application of whole-exome sequencing across clinical indications. Genet. Med. *18*, 696–704. https://doi.org/10.1038/gim.2015.148.
34. Posey, J.E., Harel, T., Liu, P., et al. (2017). Resolution of disease phenotypes resulting from multilocus genomic variation. N. Engl. J. Med. *376*, 21–31. https://doi.org/10.1056/nejmoa1516767.
35. Redin, C., Gérard, B., Lauer, J., et al. (2014). Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. J. Med. Genet. *51*, 724–736. https://doi.org/10.1136/jmedgenet-2014-102554.
36. Loenarz, C., Ge, W., Coleman, M.L., et al. (2010). PHF8, a gene associated with cleft lip/palate and mental retardation, encodes for an Nε-dimethyl lysine demethylase. Hum. Mol. Genet. *19*, 217–222. https://doi.org/10.1093/hmg/ddp480.

37. Nava, C., Lamari, F., Héron, D., et al. (2012). Analysis of the chromosome X exome in patients with autism spectrum disorders identified novel candidate genes, including TMLHE. Transl. Psychiatry *2*, 1–12. https://doi.org/10.1038/tp.2012.102.
38. (2012). gnomAD. https://gnomad.broadinstitute.org/variant/X-53966800-TGAG-T?dataset=gnomad_r2_1.
39. (2012). ClinVar. https://www.ncbi.nlm.nih.gov/clinvar/variation/445714/.
40. Qiao, Y., Liu, X., Harvard, C., et al. (2008). Autism-associated familial microdeletion of Xp11.22. Clin. Genet. *74*, 134–144. https://doi.org/10.1111/j.1399-0004.2008.01028.x.
41. De Wolf, V., Crepel, A., Schuit, F., et al. (2014). A complex Xp11.22 deletion in a patient with syndromic autism: exploration of FAM120C as a positional candidate gene for autism. Am. J. Med. Genet. Part. A. *164* (12), 3035–3041. https://doi.org/10.1002/ajmg.a.36752.
42. Raynaud, M., Gendrot, C., Dessay, B., et al. (1996). X-linked mental retardation with neonatal hypotonia in a French family (MRX15): gene assignment to Xp11.22-Xp21.1. Am. J. Med. Genet. *64*, 97–106. https://doi.org/10.1002/(SICI)1096-8628(19960712)64:1<97.
43. Aref-Eshghi, E., Rodenhiser, D.I., Schenkel, L.C., et al. (2018). Genomic DNA methylation signatures enable concurrent diagnosis and clinical genetic variant classification in neurodevelopmental syndromes. Am. J. Hum. Genet. *102*, 156–174. https://doi.org/10.1016/j.ajhg.2017.12.008.
44. Schenkel, L.C., Aref-Eshghi, E., Skinner, C., et al. (2018). Peripheral blood epi-signature of Claes-Jensen syndrome enables sensitive and specific identification of patients and healthy carriers with pathogenic mutations in KDM5C. Clin. Epigenet. *10*, 1–11. https://doi.org/10.1186/s13148-018-0453-8.
45. Aref-Eshghi, E., Kerkhof, J., Pedro, V.P., et al. (2020). Evaluation of DNA methylation episignatures for diagnosis and phenotype correlations in 42 mendelian neurodevelopmental disorders. Am. J. Hum. Genet. *106*, 356–370. https://doi.org/10.1016/j.ajhg.2020.01.019.

HGGA, Volume *3*

Supplemental information

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

Andrew K. Sobering, Laura M. Bryant, Dong Li, Julie McGaughran, Isabelle Maystadt, Stephanie Moortgat, John M. Graham Jr., Arie van Haeringen, Claudia Ruivenkamp, Roos Cuperus, Julie Vogt, Jenny Morton, Charlotte Brasch-Andersen, Maria Steenhof, Lars Kjærsgaard Hansen, Élodie Adler, Stanislas Lyonnet, Veronique Pingault, Marlin Sandrine, Alban Ziegler, Tyhiesia Donald, Beverly Nelson, Brandon Holt, Oleksandra Petryna, Helen Firth, Kirsty McWalter, Jacob Zyskind, Aida Telegrafi, Jane Juusola, Richard Person, Michael J. Bamshad, Dawn Earl, University of Washington Center for Mendelian Genomics, Anne Chun-Hui Tsai, Katherine R. Yearwood, Elysa Marco, Catherine Nowak, Jessica Douglas, Hakon Hakonarson, and Elizabeth J. Bhoj

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,

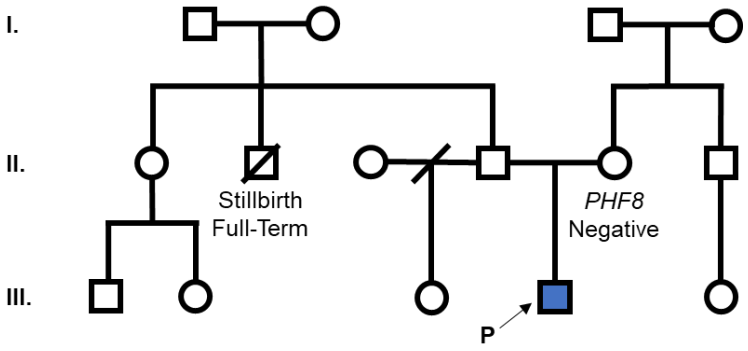
Over the next two decades he was followed for developmental delay and intellectual disability. At age 9 months he first was able to sit, he began to crawl at 14 months, and walk at 16 months. He was admitted to hospital at age 16 months with an *E. Coli* urinary tract infection. He was successfully treated 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

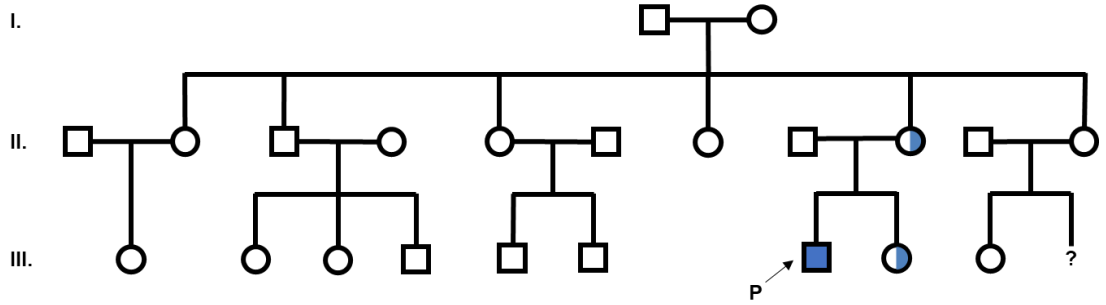
Individual 5 is an Afro-Caribbean 11-year-old boy born to a G0P0→1 mother who had no known prenatal exposures. He was born in the United States without any prenatal or perinatal complications. However, he moved to the resource-limited country of his family origin as a small child, which delayed an appropriate 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 (50th%) and his interpupillary distance was 5.75 cm (75th-97th%).

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.

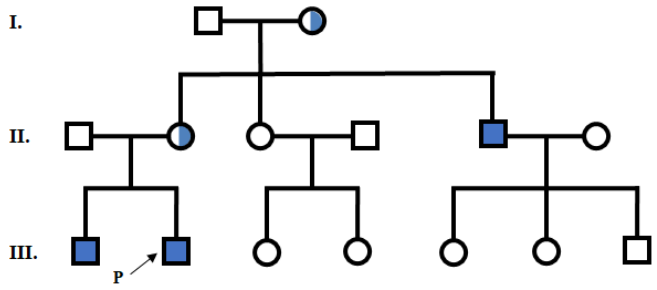
Individual 7 is the affected older brother of individual 6. He was born at term, after an uneventful pregnancy, with normal growth parameters and without any

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.



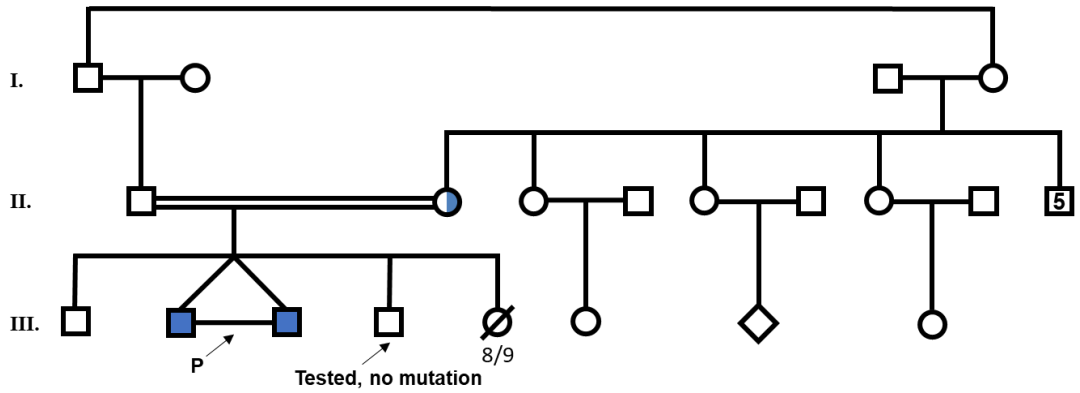
Pedigree – Individuals 6, 7, and 8

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.



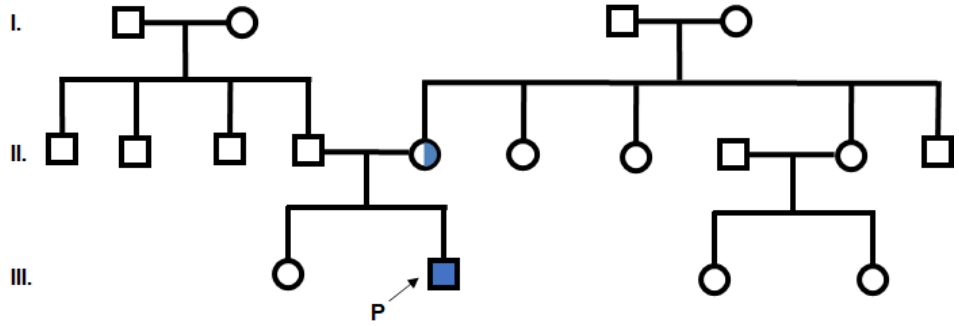
Pedigree – Individuals 9 and 10

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.

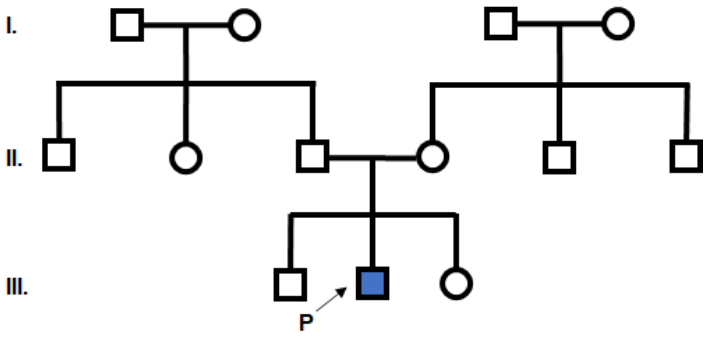
He was diagnosed with hearing loss (transmission) at the age of 2 and was quickly fitted with a hearing aid. A malformation was not detected with a CT scan focusing on the inner ear. He had language delay thought to be related to phonation disorders that were managed by a speech therapist. When younger, 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

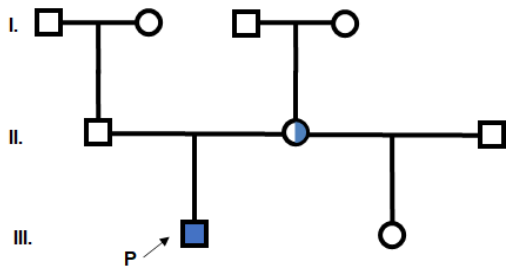
Individual 16 was born to healthy non-consanguineous parents after cesarean section for breech presentation at 36 weeks and 4 days. He had a birth weight of 2.95 kg, a birth length of 47 cm and a head circumference of 36 cm. He was hospitalized for cleft lip and palate, hypertonia of legs, and jaundice. 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

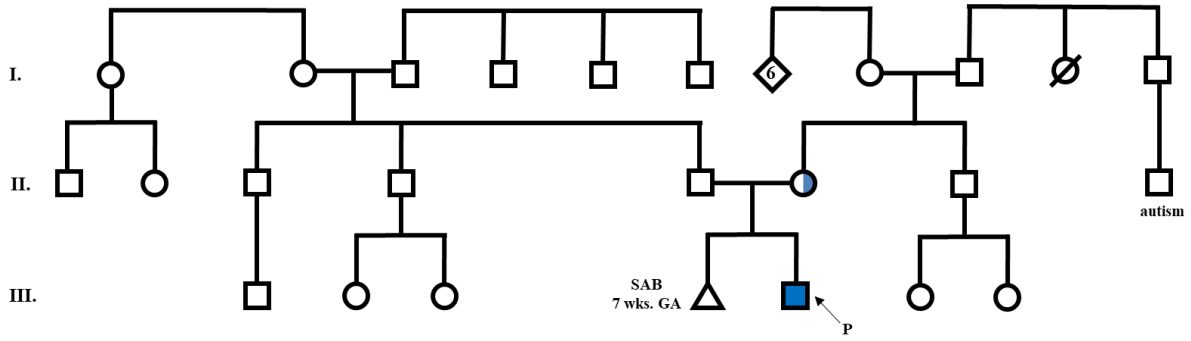


Individual 16: Pedigree

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 25th 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 88th centile for height, 76th centile for weight and 54th centile for BMI. Head circumference was at the 21st 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 25th 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 (18th centile). His perceptual reasoning skills were noted to be in the average range (30th centile) and his verbal comprehension skills were noted to be in the low average range (14th 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 83rd centile, his weight at the 62nd centile and his head circumference at the 75th - 98th 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.