

Whole Exome Sequencing Reveals De Novo Pathogenic Variants in *KAT6A* as a Cause of a Neurodevelopmental Disorder

Francisca Millan,^{1*} Megan T. Cho,¹ Kyle Retterer,¹ Kristin G. Monaghan,¹ Renkui Bai,¹ Patrik Vitazka,¹ David B. Everman,² Brooke Smith,² Brad Angle,³ Victoria Roberts,³ LaDonna Immken,⁴ Honey Nagakura,⁴ Marc DiFazio,⁵ Elliott Sherr,⁶ Eden Haverfield,¹ Bethany Friedman,¹ Aida Telegrafi,¹ Jane Juusola,¹ Wendy K. Chung,⁷ and Sherri Bale¹

¹GeneDx, Gaithersburg, Maryland

²Greenwood Genetic Center, Greenville, South Carolina

³Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois

⁴Specialty For Children Genetics, Austin, Texas

⁵Children's Outpatient Center of Montgomery County, Rockville, Maryland

⁶Institute of Human Genetics, University of California, San Francisco, California

⁷Departments of Pediatrics and Medicine, Columbia University Medical Center, New York, New York

Manuscript Received: 5 March 2015; Manuscript Accepted: 6 April 2016

Neurodevelopmental disorders (NDD) are common, with 1–3% of general population being affected, but the etiology is unknown in most individuals. Clinical whole-exome sequencing (WES) has proven to be a powerful tool for the identification of pathogenic variants leading to Mendelian disorders, among which NDD represent a significant percentage. Performing WES with a trio-approach has proven to be extremely effective in identifying de novo pathogenic variants as a common cause of NDD. Here we report six unrelated individuals with a common phenotype consisting of NDD with severe speech delay, hypotonia, and facial dysmorphism. These patients underwent WES with a trio approach and de novo heterozygous predicted pathogenic novel variants in the *KAT6A* gene were identified. The *KAT6A* gene encodes a histone acetyltransferase protein and it has long been known for its structural involvement in acute myeloid leukemia; however, it has not previously been associated with any congenital disorder. In animal models the *KAT6A* ortholog is involved in transcriptional regulation during development. Given the similar findings in animal models and our patient's phenotypes, we hypothesize that *KAT6A* could play a role in development of the brain, face, and heart in humans. © 2016 Wiley Periodicals, Inc.

Key words: neurodevelopmental disorder; intellectual disability; whole exome sequencing

INTRODUCTION

Neurodevelopmental disorders, affecting intelligence, behavior, and/or motor functioning, are common, with 1–3% of the general

How to Cite this Article:

Millan F, Cho MT, Retterer K, Monaghan KG, Bai R, Vitazka P, Everman DB, Smith B, Angle B, Roberts V, Immken L, Nagakura H, DiFazio M, Sherr E, Haverfield E, Friedman B, Telegrafi A, Juusola J, Chung WK, Bale S. 2016. Whole exome sequencing reveals de novo pathogenic variants in *KAT6A* as a cause of a neurodevelopmental disorder. Am J Med Genet Part A 170A:1791–1798.

population being affected by global developmental delay (DD) or intellectual disability (ID) [Roeleveld et al., 1997; Leonard and Wen, 2002; Michelson et al., 2011]. According to the American Association on Intellectual and Developmental Disabilities, ID can

Conflict of interest: Francisca Millan, Megan Cho, Kyle Retterer, Kristin Monaghan, Renkui Bai, Patrik Vitazka, Eden Haverfield, Bethany Friedman, Aida Telegrafi, Jane Juusola, and Sherri Bale are employees of GeneDx. Wendy Chung is a consultant for BioReference Laboratories. The other authors have no conflicts to declare.

*Correspondence to:

Francisca Millan, GeneDx, 207 Perry Parkway, Gaithersburg, Maryland, MD 20877.

E-mail: fmillanzamora@genedx.com

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 2 May 2016

DOI 10.1002/ajmg.a.37670

be stratified by measuring the intelligence quotient (IQ), and can also be classified into syndromic intellectual disability (SID) and non-syndromic intellectual disability (NS-ID). In SID, individuals have additional clinical features or co-morbidities, whereas in NS-ID the presence of ID is the sole clinical feature [Kaufman et al., 2010]. The cause of ID is hypothesized to be monogenetic in 25–50% of individuals, and this percentage increases with the severity of ID [Kaufman et al., 2010]. A genetic etiology is more frequently identified by karyotype, chromosome microarray analysis, and subtelomeric FISH in individuals with SID relative to NS-ID [Michelson et al., 2011]. However, before the advent of next generation sequencing technologies, the underlying cause remained unexplained in at least 50–60% of individuals with ID [Kaufman et al., 2010; Willemsen and Kleefstra, 2014] when evaluated only by molecular cytogenetics, Fragile X, and metabolic testing.

Whole-exome sequencing (WES) has proven to be a powerful tool in identifying causative pathogenic variants in known genes associated with Mendelian disorders [Yang et al., 2013] as well as for the discovery of novel disease genes [Gonzaga-Jauregui et al., 2012]. De novo pathogenic variants have been shown to be a major cause of sporadic human genetic disease associated with decreased reproductive fitness [Veltman and Brunner, 2012]. Performing WES in trios with a proband and both parents has proven to be an extremely effective approach in identifying de novo pathogenic variants as a common cause of neurodevelopmental disorders [Vissers et al., 2010; de Ligt et al., 2012; Ku et al., 2013]. Because of the genetic heterogeneity and the incomplete knowledge of all the genes causing ID and the effectiveness of trio WES analysis to identify de novo variants, many patients referred for WES have a neurological disorder [Yang et al., 2013, 2014].

We report six unrelated individuals with a neurodevelopmental disorder with severe speech delay who underwent WES with a trio approach, in whom de novo heterozygous predicted pathogenic novel variants in the *KAT6A* gene were identified.

PATIENTS AND METHODS

Patients

Patients were clinically evaluated at their respective institutions as part of routine clinical care. Clinical informed consent was obtained by the provider from the parents of each patient. Clinical information from the patients is described below and in Table I. Facial features can be seen in Figure 1.

Patient 1 is a 2-year-old female who presented in the neonatal period with an atrial septal defect and mild mitral valve prolapse that required surgical correction. She was noted to be microcephalic a few months after birth. The patient had failure to thrive and was noted to have early developmental delays and rolled over at 11 months, sat unsupported at 18 months, and is non-ambulatory and non-verbal at age 27 months. Her physical exam is significant for facial dysmorphisms including coarse facial features, loose skin on the face, short nose with a broad base, and small dysplastic ears with a right-sided preauricular sinus. Additionally, she has a supernumerary left nipple, hypotonia, and brisk reflexes.

Patient 2 is a 9-year-old male with a history of hypotonia, global developmental delay, and microcephaly. He is currently non-verbal but communicates well using sign language. He performs academically at a kindergarten level, and the results of his psychological assessments have been variable, with most measures showing function in the below average to mildly deficient range. He had right-sided cryptorchidism requiring orchiopexy, an umbilical hernia, and recurrent ear infections with an eardrum perforation. His early history was also notable for feeding difficulties, gastroesophageal reflux, and chronic cough, and he has experienced significant constipation without proven celiac disease but with a good response to a gluten-free diet. Physical exam was notable for mild microcephaly with head circumference at the 3rd to 10th centile, and facial dysmorphism including a prominent bridge with a prominent and downturned nasal tip, a thin upper lip, a narrow anterior palate, and relatively large and posteriorly rotated ears. He also has an asymmetric pectus carinatum, finger and elbow laxity, and 2–3 toe syndactyly. He has mitral valve prolapse with regurgitation. His extensive previous work-up was only remarkable for a maternally inherited 550 kilobase tandem micro-duplication on chromosome 14q23.3 (hg18: 65,357,663–66,160,407).

Patient 3 is an 11-year-old female who was first evaluated at age 3 months for microcephaly, significant feeding difficulties and global delay in developmental milestones. Between 12 and 24 months old, she was admitted to the hospital twice due to episodes of acute febrile illness with poor feeding, associated with developmental regression. She was diagnosed with a presumed short chain fatty acid dehydrogenase deficiency disorder. However, at age 2 years, she had no additional episodes of metabolic decompensation or developmental regression, and this diagnosis was questioned. Her history was also notable for severe gastroesophageal reflux, vomiting, constipation, and failure to thrive that improved when nutritional supplementation was started at age 6 years. She walked at age 24 months and said her first word at age 6 years. Currently, she has few words, but has more receptive language. She recognizes letters and some words and does simple math. She has minor facial dysmorphism including hypotelorism. Her previous work up is notable for a brain MRI at age 2 years that was remarkable for microcephaly, without additional anatomical abnormalities.

Patient 4 is a 5-year-old female with a history of developmental delay and hypotonia. She had a patent foramen ovale and patent ductus arteriosus. She had a history of laryngomalacia and gastroesophageal reflux which resolved. Her physical exam is remarkable for normocephaly and facial dysmorphisms including bilateral ptosis, epicanthal folds, bulbous nasal tip, and micrognathia. She sat around 7–8 months and began walking at 19 months. At age 3 years she was still nonverbal, though she used at least one sign to communicate and was capable of following multi-step commands. At 5 years of age she communicates using 3 words, approximately 50 signs, and augments her communication using the augmentative and alternative communication application Proloquo2Go on her iPad. Her extensive previous workup included a brain MRI showing hyperintense signal in the posterior periventricular white matter in the T2/FLAIR sequences, low total and free carnitine with a normal acylcarnitine profile,

TABLE I. Clinical Characteristics and Sequence Variants Found in Reported Patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Novel variant	c.3462delA, p.Gly1155Alafs*21	c.3287delG, p.Cys1096Phefs*27	c.3230delA, p.Asn1077Metfs*46	c.3070C>T, p.Arg1024Ter	c.1928 A>G, p.Asn643Ser	c.3040-1_3040delGA
Inheritance	De novo	De novo	De novo	De novo	De novo	De novo
Gender	Female	Male	Female	Female	Female	Male
Age	2 years	9 years	11 years	5 years	6 years	29 years
Family history	Two unaffected siblings	Two unaffected siblings	Unremarkable	Unaffected younger brother	Unaffected younger maternal half-brother	24 year old sister with ID and autism
Global Developmental Delay/ Intellectual Disability	+	+	+	+	+	+
Motor Development	Sat unsupported at 18 months, non-ambulatory at 2 years	Walked at 24 months	Walked at 24 months	Walked at 19 months	Walked at 19 months	Walked at 22 months
Speech	Non-verbal	Non-verbal	4–5 words at age 7 years	Three words at 5 years	Three words at 3 years	Non-verbal
Hypotonia	+	+	–	+	+	+
Failure to Thrive	+	–	+	–	+	+
Height	5–10th percentile	10–25th percentile	25–50th percentile	>75th percentile	10–25th percentile	10–25th percentile
Weight	25–50th percentile	75th percentile	10–25th percentile	>75th percentile	25–50th percentile	50th percentile
OFC	<3rd percentile	<10th percentile	25th percentile	25–70th percentile	25–50th percentile	50–75th percentile
Microcephaly	+ (A)	Mild microcephaly	–	–	–	–
Facial dysmorphism	+	+	+	+	+	+
Congenital heart disease	ASD	–	–	–	–	–
GI	Vomiting, dysphagia	Constipation	Severe GERD Constipation	Resolved GERD	–	Severe GERD in infancy
Brain CT/MRI	N	N	abN ^a	abN ^b	N	abN ^c

DD, Developmental Delay; ID, Intellectual Disability; OFC, Occipito-Frontal Circumference; A, Acquired; C, congenital; ASD, Atrial Septal Defect; MRI, Magnetic Resonance Imaging; N, Normal; abN, abnormal.

^aMicrocephaly, no parenchymal abnormalities.

^bHyperintense signal in posterior periventricular white matter in the T2/FLAIR sequences.

^cMissing olfactory bulb.



FIG. 1. Facial features of probands. Patient 1 (2-year old) with microcephaly, coarse facial features, loose skin on the face, short nose with a broad base, small and dysplastic ears with a right-sided preauricular sinus. Patient 2 (9-year old) with prominent nasal bridge with downturned tip, a thin upper lip, a narrow anterior palate, and relatively large and posteriorly-rotated ears. Patient 5 (6-year old) with a flattened midface, downslanting palpebral fissures, widely spaced eyes, a broad nose with bulbous tip, a long columella that extends below nares, a thin upper lip, protruding tongue, widely spaced teeth and small ears with thickened helices. Patient 6 (29-year old) with a bulbous nose. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>.]

homoplasmic rare variants m.1342C>T, m.7440T>C, m.8578C>T in mtDNA present in her clinically unaffected mother, and a DLD gene splicing variant detected by a mitochondrial-disorder associated nuclear genes panel, without a second DLD variant identified.

Patient 5 is a 6-year-old female with developmental delay, intellectual disability, hypotonia, and stereotypical behaviors. Her physical exam is notable for dysmorphic facial features such as a flattened midface, recessed hairline, downslanting palpebral fissures, widely spaced eyes, a broad nose with bulbous tip, a long columella that extends below the nares, a thin upper lip, protruding tongue, widely spaced teeth and small ears with thickened helices. She sat at 5 months and walked at 19 months of age.

Patient 6 is a 29-year-old male with Autism Spectrum Disorder, intellectual disability, epilepsy, a bulbous nose, and obsessive-compulsive behaviors. He has a history of severe gastroesophageal reflux as an infant and subsequent failure to thrive, which resolved. Developmentally he was delayed, achieving independent walking at 22 months, and is presently non-verbal. He developed epilepsy at age 9 years with complex partial seizures, and secondary generalization. He is currently on antiepileptic medication and has been seizure-free for 2 years. His brain MRI was remarkable for absence of an olfactory bulb.

METHODS

One thousand twenty-eight patients with a suspected monogenic cause for their DD/ID and both parents, when available, had their whole blood or isolated DNA sent to GeneDx, Inc. in Gaithersburg, Maryland for clinical WES. Informed consent for clinical WES was obtained from the parents by the clinical provider. The study was approved by the Institutional Review Board of Columbia University. Genomic DNA was extracted from submitted whole blood and exon targets were isolated by capture using the Agilent SureSelect Human All Exon V4(50 Mb) kit (Agilent Technologies, Santa Clara, CA). One microgram of DNA was sheared into 350–400 bp fragments, which were then repaired, ligated to adaptors, and purified for subsequent PCR amplification. Amplified products were then captured by biotinylated RNA library baits in solution following the manufacturer's instructions. Bound DNA was isolated with streptavidin-coated beads and re-amplified. The final isolated products were sequenced using the Illumina HiSeq 2000 or 2500 sequencing system with 100-bp paired-end reads (Illumina, San Diego, CA). The sequence data were aligned to the published human genome build UCSC hg19/GRCh37 reference sequence using BWA with the latest internally validated version at the time of sequencing, progressing from BWA v0.5.8 through BWA-Mem v0.7.8 [Li and Durbin, 2009]. Targeted coding exons and splice junctions of known protein-coding RefSeq genes were assessed for average depth of coverage with a minimum depth of 10× required for inclusion in downstream analysis. Local realignment around insertion-deletion sites and regions with poor mapping quality was performed using the Genome Analysis Toolkit Indel Realigner v1.6. [Van der Auwera et al., 2013]. Variant calls were generated simultaneously on all sequenced family members using SAMtools v0.1.18 [Li et al., 2009]. All coding exons and surrounding intron/exon boundaries up to 13 bp 5' and 6 bp 3' of

the splice junction were analyzed. Automated filtering removed common sequence changes (defined as $\geq 10\%$ minor allele frequency in 1000 Genomes database) [1000 Genomes Project Consortium, 2012]. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. Whole-exome sequencing data for all sequenced family members was analyzed using GeneDx's XomeAnalyzer (a variant annotation, filtering, and viewing interface for WES data), which includes nucleotide and amino acid annotations, population frequencies (NHLBI Exome Variant Server and 1000 Genomes databases), in silico prediction tools, amino acid conservation scores, and mutation references. Variants were filtered based on inheritance patterns, variant type, population frequencies, and gene lists of interest in relation to the patient's major phenotypic features, as appropriate. Resources including the Human Gene Mutation Database (HGMD), 1000 Genomes database, NHLBI Exome Variant Server (ESP), OMIM, PubMed, and ClinVar were used to evaluate genes and detected sequence changes of interest. Interrogation of the 1028 exome profiles of individuals and parents was undertaken to identify de novo variants occurring in the same gene among patients with overlapping clinical features. In genes in which multiple de novo variants were observed the probability of such an observation occurring by chance was evaluated using a false discovery rate adjusted *P*-value calculated using the Transmission and de novo Association (TADA) algorithm [He et al., 2013]. Identified sequence changes of interest were confirmed and segregation within the family was determined in all family members by conventional di-deoxy DNA sequence analysis using an ABI3730 (Life Technologies, Carlsbad, CA) and standard protocols on a new DNA preparation. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (<http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/>) and guidelines from the American College Of Medical Genetics and Genomics and the Association for Molecular Pathology are followed for this purposes [Richards et al., 2015].

RESULTS

Interrogation of 1,028 individuals and parental exomes revealed 10 independent trios in which de novo protein altering variants were identified in the *KAT6A* gene. The pathogenic variants in *KAT6A* and associated phenotypes have been previously reported for four of these families [Arboleda et al., 2015; Tham et al., 2015]. Here, we present the clinical phenotypes of the six additional cases and the observed de novo mutations (Table I, Figure 2). Exome sequencing of the six patients in this report produced an average of ~ 14 GB of sequence per sample. Mean coverage of captured regions was $\sim 170\times$ per sample, with $>96\%$ having a depth of coverage of at least $10\times$. All cases met the Illumina quality control criteria for variant calling. Filtering of common sequence changes resulted in $\sim 5,000$ variants per proband sample. In most of the patients, only one gene, *KAT6A*, showed de novo rare and predicted damaging sequence variations that were thought to be the cause of the patients' phenotype.

In addition to the *KAT6A* variant, patient 2 was identified to be hemizygous for a novel missense in *SLC6A8*, c.820 G>A

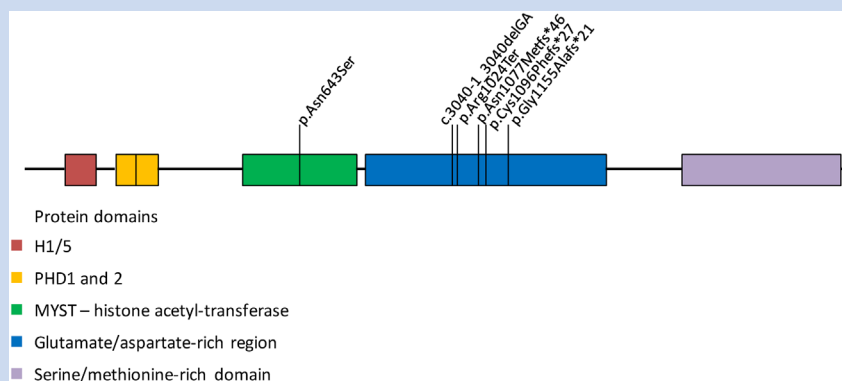


FIG. 2. Schematic representation of *KAT6A* protein and its domains. Vertical lines represent the position at which the sequence variations found in the *KAT6A* gene in the six patients reported here would affect the encoded protein. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>.]

(p.Val274Met). Pathogenic variants in *SLC6A8* account for the X-linked cerebral creatine deficiency syndrome, characterized by intellectual disability, seizures, autism, and behavioral disorders in males. This novel missense variant produces an amino acid substitution of similar structural and chemical properties, and is thus not expected to lead to major alterations in protein structure. In silico analysis with SIFT, PolyPhen, and MutTaster predicted this to be a damaging change. However, the patient's phenotype is not consistent with this disorder, since he has never had a seizure and his brain MRI showed normal myelination and no structural abnormalities. Of note, the patient's urine creatine and guanidinoacetate levels were normal on two separate occasions, thus suggesting that the variant is functionally benign. Patient 2 was also found to be heterozygous for the paternally inherited c.838C>T (p.Arg280Trp) missense variant in *CBL*. Pathogenic variants in *CBL* cause several congenital anomalies that overlap with neurofibromatosis type 1, Noonan syndrome, Costello syndrome, Cardiofaciocutaneous syndrome, and Legius syndrome [Niemeyer et al., 2010]. Although reduced penetrance for variants in this gene has been reported [Martinelli et al., 2010], the patient's phenotype is not consistent with this group of disorders. Thus, the c.838C>T missense variant in *CBL* was deemed to be of uncertain significance.

The false discovery rate adjusted *P* value of observing nine truncating de novo variants and one missense de novo variant in this population is 2.4×10^{-12} using the TADA algorithm [He et al., 2013]. The novel variants identified in *KAT6A* were confirmed by Sanger sequencing in all six patients and were absent in their respective parents. *KAT6A* has a RVIS score of -3.09 and a percentile of 0.48% indicating that it is intolerant to sequence variation [Petrovski et al., 2013]. The majority of the alleles found in this group of affected individuals is predicted to truncate the protein. None of the variants are present in the NHBLI Exome Variant Server, the Database of Single Nucleotide Polymorphisms (dbSNP), the 1000 Genomes databases or in a local database. Furthermore, predicted truncating sequence variations in *KAT6A* are absent among the healthy individuals in our internal

database of 7,757 exomes, and there is only one frameshift variant in this gene listed in the NHBLI Exome Variant Server that we have seen internally, which has repeatedly failed to confirm with Sanger sequencing and is therefore likely an artifact. To our knowledge, there are no reports linking structural variants or copy number variations in *KAT6A* to any congenital clinical disorder in humans. This gene, however, has long been known for its structural involvement in acute myeloid leukemia [Borrow et al., 1996].

The de novo missense variant in Patient 5 (p.Asn643Ser) occurs in a highly conserved region of the *KAT6A* gene, involving the catalytic MYST-type histone acetyltransferase domain [Voss and Thomas, 2009]. Interestingly, this substitution occurs two residues upstream from the first acetyl-coenzyme A binding region of the catalytic domain and is also included in the region that mediates interaction with the Brpf1 protein, a multidomain complex that is required for Hox gene expression and segmental identity [Laue et al., 2008]. In leukemia, this substitution is located in the region that regulates transcription through its interaction with the RUNX1 protein, a DNA-binding transcription factor [Perez-Campo et al., 2013]. In silico analysis with SIFT, PolyPhen and MutTaster produce consistently damaging scores of 1, 0.998, and 1, respectively, supporting its pathogenic classification. The Raw CADD score for this sequence variation is 4.58, and it has a PHRED value of 25.5 indicating that this variant is likely damaging.

Patient 6 has a de novo splice site variant (c.3040-1_3040delGA) that is expected to disrupt the acceptor site by two predictors (Berkeley Drosophila Genome Project and NetGene2).

DISCUSSION

In all of six cases we describe, there is a common phenotype of moderate to severe neurodevelopmental delay with most individuals having absent or minimal verbal communication skills. Many also have hypotonia and facial dysmorphism, two of them have microcephaly and one has mild congenital heart disease. All of them harbor unique and novel de novo sequence variations in *KAT6A*, most of which are predicted to truncate the encoded

protein. Besides the *KAT6A* variants, we did not identify other molecular events that would account for these patients' phenotypes. Although the neurodevelopmental findings are relatively non-specific, the significant speech impairment, associated microcephaly, and the overall severity of the ID suggest a shared phenotype across the patients. Therefore, the de novo pathogenic variants observed in *KAT6A* are the most likely etiology for the ID in these individuals.

KAT6A is located on 8p11.21, consists of 18 exons, and encodes the 2004 amino acid *KAT6A* (MOZ/MYST3) histone acetyl-transferase protein [Reifsnyder et al., 1996; Champagne et al., 2001]. *KAT6A* was first identified at recurrent translocations causing a particularly aggressive form of acute myeloid leukemia [Borrow et al., 1996] and further characterized to be widely expressed [Voss and Thomas, 2009]. It is a member of the MYST family of histone acetyl-transferases (HATs), which also includes the *KAT8*, *HTATIP*, *KAT7*, and *KAT6B* genes [Voss and Thomas, 2009]. Histone acetylation/deacetylation modulates transcription through epigenetic changes. Histone acetylation is catalyzed by histone acetyl-transferases, which transfer acetyl groups to lysines in the tail of core histones leading to the "unraveling" of the nucleosomes, exposing chromatin and thus promoting transcription. In contrast, deacetylation of histones leads in general to transcriptional silencing [Kouzarides, 2007].

Mammalian development requires a very precise temporal and spatial expression of a large number of genes. One key mechanism regulating gene expression is chromatin structure, and histone modifications play a pivotal role in this modulation. Many members of the HATs protein families have been involved in different disorders such as *CREBBP* and *EP300* in Rubinstein-Taybi syndrome [Petrij et al., 1995; Roelfsema et al., 2005], and *TAF1* in X-linked Parkinson-Dystonia [Makino et al., 2007]. Among the MYST family, *KAT6B* has been identified as the cause for Say-Barber-Biesecker-Young-Simpson syndrome [Clayton-Smith et al., 2001] and Genitopatellar syndrome [Simpson et al., 2012]. Additionally, pathogenic variants in *ANKRD11* and *KANSL1*, which regulate HATs, have been identified as causing KBG syndrome [Sirmaci et al., 2011] and 17q21.31 microdeletion syndrome [Koolen et al., 2012] respectively. De novo changes in genes involved in histone regulation through either methylation or ubiquitination have also been associated with congenital heart disease [Zaidi et al., 2013].

KAT6A has been studied in a number of animal models and suggest a possible role in human development. In zebrafish, a truncating mutation of the *KAT6A* ortholog leads to a loss of expression of the *hoxa2b* and *hoxb2a* genes in cranial neural crest cells of the branchial arches, resulting in erroneous homeotic differentiation [Miller et al., 2004]. Conversely, overexpression of *kat6a* is able to rescue the phenotype of zebrafish embryos treated with 1-(2-[trifluoromethyl] phenyl) imidazole, a compound known to abrogate first pharyngeal arch development [Kong et al., 2014].

In mouse models, depending on the knockout allele and genetic background, *Kat6a* homozygous mutants may show different features. One type of *Kat6a* homozygous mutant dies between E14.5 and E18.5, and lacks hematopoietic stem cells [Katsumoto et al., 2006; Thomas et al., 2006]. In a different model, homozygous

Kat6a mutants show anterior homeotic transformations of both the axial skeleton and neural tube, namely supernumerary vertebrae, an absent pair of ribs, and an additional cervical segment in the nervous system. *Kat6a* histone acetylation activity is decreased, leading to reduced transcription of Hox gene loci [Voss et al., 2009]. These latter mice also display craniofacial and cardiac defects similar to the human DiGeorge syndrome. The molecular mechanism underlying these defects is reduced *Tbx1* and *Tbx5* expression, further implicating the *Kat6a* gene in cardiac, pharyngeal apparatus, and facial development [Voss et al., 2012]. Interestingly, all of the individuals reported here have facial dysmorphisms and one of them has mild structural cardiac involvement. Given the similar findings in animal models and our patient's phenotypes, we hypothesize that *KAT6A* could play a role in development of the brain, face, and heart in humans.

All of the patients included in the present report had an extensive workup before reaching a diagnosis with WES. Of note, *KAT6A* de novo predicted damaging variants were observed in 10 out of 1028 individuals referred for WES at GeneDx with a neurodevelopmental disability, representing ~1% of the cases. Although not a major cause of ID, *KAT6A*-related disorders are likely still under-diagnosed because the clinical features are non-specific.

Although the majority of the pathogenic variants reported in this case series produce a premature termination of the protein, all of them occur either in the last exon or close to last exon-exon junction and thus are not predicted to lead to nonsense-mediated mRNA decay (NMD). The splicing variant present in patient 6 is predicted to damage, but not completely destroy the acceptor site for exon 18, the last one of this sequence. All these changes in the protein structure take place downstream from the histone acetyltransferase domain, encoded by exons 10 through 15. However, the variants are predicted to compromise the serine-methionine rich region of the protein, encoded solely by exon 18. The serine-methionine rich region interacts with RUNX1 transcriptional activation protein and has been demonstrated to lead to enhanced transcription in gene reporter assays [Champagne et al., 2001]. Additional research will be needed to better delineate the spectrum of phenotypic variability associated with *KAT6A* pathogenic variants, and functional studies will be necessary for a better understanding of the mechanisms underlying this disorder.

ACKNOWLEDGMENTS

We gratefully acknowledge all the patients and their families.

REFERENCES

- 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* 491:56–65.
- Arboleda VA, Lee H, Dorrani N, Zadeh N, Willis M, Macmurdo CF, Manning MA, Kwan A, Hudgins L, Barthelmy F, Miceli MC, Quintero-Rivera F, Kantarci S, Strom SP, Deignan JL, UCLA Clinical Genomics Center, Grody WW, Vilain E, Nelson SF. De novo nonsense mutations in *KAT6A*, a lysine acetyl-transferase gene, cause a syndrome including

- microcephaly and global developmental delay. *Am J Hum Genet* 96:498–506.
- Borrow J, Stanton VP, Jr, Andresen JM, Becher R, Behm FG, Chaganti RS, Civin CI, Distche C, Dubé I, Frischauf AM, Horsman D, Mitelman F, Volinia S, Watmore AE, Housman DE. 1996. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREBB-binding protein. *Nat Genet* 14:33–41.
- Champagne N, Pelletier N, Yang XJ. 2001. The monocytic leukemia zinc finger protein MOZ is a histone acetyltransferase. *Oncogene* 20:404–409.
- Clayton-Smith J, O'Sullivan J, Daly S, Bhaskar S, Day R, Anderson B, Voss AK, Thomas T, Biesecker LG, Smith P, Fryer A, Chandler KE, Kerr B, Tassabehji M, Lynch SA, Krajewska-Walasek M, McKee S, Smith J, Sweeney E, Mansour S, Mohammed S, Donnai D, Black G. 2001. Whole-exome-sequencing identifies mutations in histone acetyltransferase gene KAT6B in individuals with the Say-Barber-Biesecker variant of Ohdo syndrome. *Am J Hum Genet* 89:675–681.
- de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. 2012. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* 367:1921–1929.
- Gonzaga-Jauregui C, Lupski JR, Gibbs RA. 2012. Human genome sequencing in health and disease. *Annu Rev Med* 63:35–61.
- He X, Sanders SJ, Liu L, De Rubeis S, Lim ET, Sutcliffe JS, Schellenberg GD, Gibbs RA, Daly MJ, Buxbaum JD, State MW, Devlin B, Roeder K. 2013. Integrated model of de novo and inherited genetic variants yields greater power to identify risk genes. *PLoS Genet* 9:e1003671.
- Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T, Kitabayashi I. 2006. MOZ is essential for maintenance of hematopoietic stem cells. *Genes Dev* 20:1321–1330.
- Kaufman L, Ayub M, Vincent JB. 2010. The genetic basis of non-syndromic intellectual disability: A review. *J Neurodev Disord* 2:182–209.
- Kong Y, Grimaldi M, Curtin E, Dougherty M, Kaufman C, White RM, Zon LI, Liao EC. 2014. Neural crest development and craniofacial morphogenesis is coordinated by nitric oxide and histone acetylation. *Chem Biol* 21:488–501.
- Koolen DA, Kramer JM, Neveling K, Nillesen WM, Moore-Barton HL, Elmslie FV, Toutain A, Amiel J, Malan V, Tsai AC, Cheung SW, Gilissen C, Verwiel ET, Martens S, Feuth T, Bongers EM, de Vries P, Scheffer H, Vissers LE, de Brouwer AP, Brunner HG, Veltman JA, Schenck A, Yntema HG, de Vries BB. 2012. Mutations in the chromatin modifier gene KANSL1 cause the 17q21.31 microdeletion syndrome. *Nat Genet* 44:639–641.
- Kouzarides T. 2007. Chromatin modifications and their function. *Cell* 128:693–705.
- Ku CS, Polychronakos C, Tan EK, Naidoo N, Pawitan Y, Roukos DH, Mort M, Cooper DN. 2013. A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. *Mol Psychiatry* 18:141–153.
- Laue K, Daujat S, Crump JG, Plaster N, Roehl HH, Tübingen 2000 Screen Consortium, Kimmel CB, Schneider R, Hammerschmidt M. 2000. The multidomain protein Brpf1 binds histones and is required for Hox gene expression and segmental identity. *Development* 135:1935–1946.
- Leonard H, Wen X. 2002. The epidemiology of mental retardation: Challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev* 8:117–134.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25:1754–1760.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079.
- Makino S, Kaji R, Ando S, Tomizawa M, Yasuno K, Goto S, Matsumoto S, Tabuena MD, Maranon E, Dantes M, Lee LV, Ogasawara K, Tooyama I, Akatsu H, Nishimura M, Tamiya G. 2007. Reduced neuron-specific expression of the TAF1 gene is associated with the X-linked dystonia-parkinsonism. *Am J Hum Genet* 80:393–406.
- Martinelli S, De Luca A, Stellacci E, Rossi C, Checquolo S, Lepri F, Caputo V, Silvano M, Buscherini F, Consoli F, Ferrara G, Digilio MC, Cavaliere ML, van Hagen JM, Zampino G, van der Burgt I, Ferrero GB, Mazzanti L, Screpanti I, Yntema HG, Nillesen WM, Savarirayan R, Zenker M, Dallapiccola B, Gelb BD, Tartaglia M. 2010. Heterozygous germline mutations in the *CBL* tumor-suppressor gene cause a Noonan syndrome-like phenotype. *Am J Hum Genet* 87:250–257.
- Michelson DJ, Shevell MI, Sherr EH, Moeschler JB, Gropman AL, Ashwal S. 2011. Evidence report: Genetic and metabolic testing on children with global developmental delay: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 77:1629–1635.
- Miller CT, Maves L, Kimmel CB. 2004. *Moz* regulates Hox expression and pharyngeal segmental identity in zebrafish. *Development* 131:2443–2461.
- Niemeyer CM, Kang MW, Shin DH, Furlan I, Erlacher M, Bunin NJ, Bunda S, Finkelstein JZ, Sakamoto KM, Gorr TA, Mehta P, Schmid I, Kropshofer G, Corbacioglu S, Lang PJ, Klein C, Schlegel PG, Heinzmann A, Schneider M. 2010. Germline *CBL* mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nat Genet* 42:794–800.
- Perez-Campo FM, Costa G, Lie-a-Ling M, Kouskoff V, Lacaud G. 2013. The MYSTTerious MOZ, a histone acetyltransferase with a key role in hematopoiesis. *Immunology* 139:161–165.
- Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ. 1995. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* 376:348–351.
- Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. 2013. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet* 9:e1003709.
- Reifsnnyder C, Lowell J, Clarke A, Pillus L. 1996. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat Genet* 14:42–49.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL, ACMG Laboratory Quality Assurance Committee. 2015. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 17:405–424.
- Roeleveld N, Zielhuis GA, Gabreëls F. 1997. The prevalence of mental retardation: A critical review of recent literature. *Dev Med Child Neurol* 39:125–132.
- Roelfsema JH, White SJ, Ariyürek Y, Bartholdi D, Niedrist D, Papadia F, Bacino CA, den Dunnen JT, van Ommen GJ, Breuning MH, Hennekam RC, Peters DJ. 2005. Genetic heterogeneity in Rubinstein-Taybi syndrome: Mutations in the CBP and EP300 genes cause disease. *Am J Hum Genet* 76:572–580.
- Simpson MA, Deshpande C, Dafou D, Vissers LE, Woollard WJ, Holder SE, Gillessen-Kaesbach G, Derks R, White SM, Cohen-Snuijff R, Kant SG, Hoefloot LH, Reardon W, Brunner HG, Bongers EM, Trembath RC. 2012. De novo mutations of the gene encoding the histone acetyltransferase KAT6B cause genitopatellar syndrome. *Am J Hum Genet* 90:290–294.

- Sirmaci A, Spiliopoulos M, Brancati F, Powell E, Duman D, Abrams A, Bademci G, Agolini E, Guo S, Konuk B, Kavaz A, Blanton S, Digilio MC, Dallapiccola B, Young J, Zuchner S, Tekin M. 2011. Mutations in ANKRD11 cause KBG syndrome, characterized intellectual disability, skeletal malformations, and macrodontia. *Am J Hum Gene* 89:289–294.
- Tham E, Lindstrand A, Santani A, Malmgren H, Nesbitt A, Dubbs HA, Zackai EH, Parker MJ, Millan F, Rosenbaum K, Wilson GN, Nordgren A. 2015. Dominant mutations in *KAT6A* cause intellectual disability with recognizable syndromic features. *Am J Hum Genet* 96:507–513.
- Thomas T, Corcoran LM, Gugasyan R, Dixon MP, Brodnicki T, Nutt SL, Metcalf D, Voss AK. 2006. Monocytic leukemia zonc finger protein is essential for the development of long-term reconstituting hematopoietic stem cells. *Genes Dev* 20:1175–1186.
- Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thilbaut J, Banks E, Garimella KV, Altshuler D, Gabriel S, DePristo MA. 2013. From FastQ data to high confidence variant calls: The Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* 11:11.10.1–11.10.33.
- Veltman JA, Brunner HG. 2012. De novo mutations in human genetic disease. *Nat Rev Genet* 13:565–575.
- Vissers LE, de Ligt J, Gilissen C, Janssens I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, de Vries BB, Brunner HG, Veltman JA. 2010. A de novo paradigm for mental retardation. *Nat Genet* 42:1109–1112.
- Voss AK, Thomas T. 2009. MYST family histone acetyltransferases take center stage in stem cells and development. *Bioessays* 31:1050–1061.
- Voss AK, Collin C, Dixon MP, Thomas T. 2009. Moz and retinoic acid coordinately regulate H3K9 acetylation, Hox gene expression, and segment identity. *Dev Cell* 17:674–686.
- Voss AK, Vanyai HK, Collin C, Dixon MP, McLennan TJ, Sheik BN, Scambler P, Thomas T. 2012. MOZ regulates the *Tbx1* locus, and Moz mutation partially phenocopies DiGeorge syndrome. *Dev Cell* 23:652–663.
- Willemsen MH, Kleefstra T. 2014. Making headway with genetic diagnostics of intellectual disabilities. *Clin Genet* 85:101–110.
- Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA, Eng CM. 2013. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 369:1502–1511.
- Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A, Landsverk M, Craigen WJ, Bekheirnia MR, Stray-Pedersen A, Liu P, Wen S, Alcaraz W, Cui H, Walkiewicz M, Reid J, Bainbridge M, Patel A, Boerwinkle E, Beaudet AL, Lupski JR, Plon SE, Gibbs RA, Eng CM. 2014. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA* 312:1870–1879.
- Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, Romano-Adesman A, Bjornson RD, Breitbart RE, Brown KK, Carriero NJ, Cheung YH, Deanfield J, DePalma S, Fakhro KA, Glessner J, Hakonarson H, Italia MJ, Kaltman JR, Kaski J, Kim R, Kline JK, Lee T, Leipzig J, Lopez A, Mane SM, Mitchell LE, Newburger JW, Parfenov M, Pe'er I, Porter G, Roberts AE, Sachidanandam R, Sanders SJ, Seiden HS, State MW, Subramanian S, Tikhonova IR, Wang W, Waburton D, White PS, Williams IA, Zhao H, Seidman JG, Brueckner M, Chung WK, Gelb BD, Goldmuntz E, Seidman CE, Lifton RP. 2013. De novo mutations in histone-modifying genes in congenital heart disease. *Nature* 498:220–223.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.