Novel Heterozygous Variants in *KMT2D* Associated with Holoprosencephaly

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Abstract

Lysine methyltransferase 2D (*KMT2D*; OMIM 602113) encodes a histone methyltransferase involved in transcriptional regulation of the beta-globin and estrogen receptor as part of a large protein complex known as activating signal cointegrator-2 -containing complex (ASCOM). Heterozygous germline mutations in the *KMT2D* gene are known to cause Kabuki syndrome (OMIM 147920), a developmental multisystem disorder. Neither holoprosencephaly nor other defects in human forebrain development have been previously associated with Kabuki syndrome. Here we report two patients diagnosed with alobar holoprosencephaly in their antenatal period with *de novo* monoallelic *KMT2D* variants identified by trio-based exome sequencing. The first patient was found to have a stop-gain variant c.12565G>T (p.Gly4189*), while the second patient had a missense variant c.5A>G (p.Asp2Gly). Phenotyping of each patient did not reveal any age-related feature of Kabuki syndrome. These two cases represent the first report on association between *KMT2D* and holoprosencephaly.

Graphical Abstract
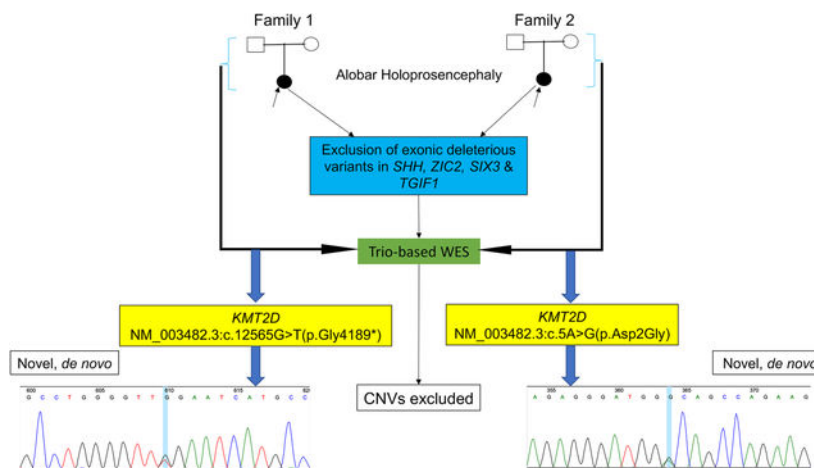
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1. INTRODUCTION

Holoprosencephaly (HPE) is a structural brain defect characterized by incomplete cleavage of the developing forebrain during embryogenesis.[1] It is considered as one of the most common developmental abnormalities in humans, with an estimated prevalence of approximately 1 in 250 conceptuses and 1 in 10,000 live births.[2] Classically, HPE can occur in a non-syndromic form or as part of recognizable monogenic conditions and chromosomal anomalies. To date, non-syndromic HPE has been associated with pathogenic variants in nearly 17 genes, while syndromic HPE features in at least 25 monogenic conditions.[3,4] The gene *KMT2D* has not been shown to play a major role in the regulation of human forebrain development. *KMT2D* encodes a histone methyltransferase responsible for the trimethylation of the fourth lysine residue of histone H3 protein, which is an epigenetic mark associated with transcriptionally active genes.[5] The encoded protein is part of a large protein complex known as activating signal cointegrator-2-containing complex (ASCOM) that is involved in transcriptional regulation of the beta-globin and estrogen receptor genes.[6] Germline pathogenic variants in *KMT2D* are known to cause Kabuki syndrome (KS), a condition characterized by specific craniofacial features, infantile hypotonia, mild-to-moderate intellectual disability, skeletal defects and dermatoglyfic abnormalities.[7,8] Various structural brain anomalies have been occasionally described in individuals affected with KS; [9] however, no association with HPE has ever been reported, regardless of the gene/variant involved. Likewise, HPE has not been previously associated with *KMT2D*. In this study, we report on two patients with HPE and alterations in *KMT2D* identified using exome sequencing.

2. MATERIALS AND METHODS

All research procedures were conducted in accordance with the Declaration of Helsinki. This study was approved by the National Human Genome Research Institute (NHGRI) Institutional Review Board. Written informed consent was obtained from each family, including consent to publish photographs. The two patients with HPE included in this study were part of our NHGRI cohort of 277 individuals with HPE (135 trios and 142 singletons). Patients were evaluated by brain imaging (prenatal ultrasound) and postnatal clinical examination. The two patients were initially screened for exonic variants in the four most common genes known to cause HPE, namely *SHH* (OMIM 600725), *ZIC2* (OMIM 603073), *SIX3* (OMIM 603714) and *TGIF1* (OMIM 602630) using Sanger sequencing as previously recommended.[10] Trio-based exome paired-end sequencing was performed at the NIH Intramural Sequencing Center (NISC) following the HiSeq2000 Sequencer protocol (Illumina, San Diego, CA) as previously described.[11] The mean read depth for each sample was 79.8x. Novel variants were filtered using stringent criteria, including ExAC database allele frequency of zero, gene constraint based on a probability of being loss-of-function intolerant (pLI) score higher than 0.95 or missense z-score higher than 3.09, and *de novo*

inheritance. Sequence variants identified were validated by Sanger sequencing on an ABI 3730xl Sequencer (Life Technologies, Grand Island, NY) according to the manufacturer's protocol. Copy number variation (CNV) prediction from exome data was done using the XHMM (eXome-Hidden Markov Model) caller. We used GATK to generate the depth of coverage statistics required for XHMM from the BAM files of our HPE cohort and a control set. GATK output was then run through the XHMM pipeline, generating a VCF file containing each predicted CNV. We then annotated each CNV for genes contained and cytogenetic region using Annovar. A custom Perl script was used to evaluate for any de-novo CNVs in the HPE probands. This work utilized the computational resources of the NIH HPC Biowulf cluster (http://hpc.nih.gov).

3. RESULTS

3.1 Clinical report

3.1.1 Case 1—The patient was a female fetus (Figure 1: A), first of three pregnancies of a non-consanguineous union. Her two living siblings were healthy and there was no relevant family history. Her healthy mother and father were respectively aged 27 and 23 at the time of the pregnancy, and there was no reported history of teratogen exposure. The pregnancy with her was uneventful until 19 weeks' gestation when a diagnosis of alobar HPE was made on ultrasound examination. Screening for common fetal aneuploidies by means of amniocentesis and G-banding karyotype was negative for trisomies 13, 18, 21 and sex chromosomes aneuploidies (46,XX). No other prenatal genetic investigation was performed. The pregnancy ended at 22 weeks of gestation following preterm labor and delivery of a nonviable fetus. No post-mortem examination was performed. The clinical features of the patient are summarized in Table 1.

3.1.2 Case 2—The patient is a 5-day-old female (Figure 1: B1& B2), fifth of six siblings from a non-consanguineous union between her 39-year-old mother and her 40-year-old father. The mother had two first trimester miscarriages and there is a family history of autism spectrum disorder, with a brother, a sister and a maternal half-sister affected. The pregnancy with her was uneventful until 18 weeks when she was diagnosed with alobar HPE by means of ultrasound examination. No history of teratogen exposure was recorded. Screening for common fetal aneuploidies using cell-free DNA testing, revealed diploid chromosomes 13, 18 and 21 with two X chromosomes. No other prenatal genetic investigation was performed. She was born at 38 weeks 5 days by normal vaginal delivery with Apgar scores of two and seven at 1 and 5 minutes respectively. Her birth weight was 2490 g (5th percentile), Length of 44 cm (<5th percentile) and head circumference 29.5 cm (<5th percentile). The patient was resuscitated soon after birth for absent spontaneous breathing or chest movement and was admitted afterward in the neonatal intensive care unit for respiratory failure. Postnatal imaging performed were limited to head ultrasound, repeated echocardiographies and chest X-rays. She died on postnatal day 5 in the context of body temperature instability, recurrent generalized tonic-clonic seizures, myocardial dysfunction and respiratory failure. The family opted not to have post-mortem examination. The patient's prenatal and postnatal clinical features identified are summarized in Table 1.

3.2 Molecular analysis

Using trio-based exome sequencing, a novel *KMT2D* heterozygous variant was identified in each patient (Table 1). Both variants were confirmed by Sanger sequencing. The variant occurred *de novo* in each family, as none of the parents was found to be a carrier. Case 1 was found to have a deleterious stop-gain variant, NM_003482.3:c.12565G>T(p.Gly4189*) on exon 39 (CADD scaled score: 47; pLI score: 1). This variant is predicted to result in a truncated protein missing amino acids 4190–5537. The missing region contains all functional domains (N- and C-terminal FYR, SET and Post-SET), LXXLL motifs 3 to 6, the last two zinc fingers, and several predicted post-translational modification residues. The predicted S-adenosyl-L-methionine (SAM) binding site at position 5474–5475 which is essential for the methyltransferase activity is also lost. Case 1 had one other *de novo* variant, a missense variant of unknown significance: *FMC1* (NM_001244584.2):c.8>T (p.Ala3Val). There is no known human disease associated with *FMC1*. Case 2 had a deleterious missense variant, NM_003482.3:c.5A>G(p.Asp2Gly) on exon 1 (CADD scaled score: 12.9; missense z-score of 3.73). In addition to ExAC database, this missense variant was not found in other population databases including 1000 Genomes Project and dbSNP. This position does not involve any known or predicted functional domain, but it represents a non-conservative amino acid change expected to alter the conformation of the protein. The variants were respectively classified as pathogenic (c.12565G>T; p.Gly4189*) and likely-pathogenic (c. 5A>G; p.Asp2Gly) based on the 2015 American College of Medical Genetics and Genomics-Association for Molecular Pathology criteria.[12] One additional *de novo* variant was found in Case 2, *ADAM11* (NM_002390.6), c.570G>A:p.Arg190Arg, a synonymous change not considered significant. No pathogenic CNV was identified in both patients. In the Supplementary Materials, variants filtered in Cases 1 and 2 are listed (Table S1 & Table S2). In addition to the two *de novo* variants that are discussed above, no other variants that may explain the HPE phenotype in both patients were found.

4. DISCUSSION

The etiology of HPE is complex and heterogeneous, involving genetic and environmental determinants. HPE is known as a feature of several developmental disorders, including numeric and structural chromosomal abnormalities, CNVs, as well as at least 25 monogenic conditions.[13,14] HPE also occurs in a nonsyndromic form with various modes of inheritance reported, including autosomal dominant with reduced penetrance and variable expressivity, autosomal recessive and a growing evidence of oligogenic inheritance.[4,15] The two patients reported in this study were diagnosed with alobar HPE in utero by ultrasound examination. Alobar HPE, which is the most severe subtype in the HPE spectrum, is classically characterized by agenesis of the corpus callosum and olfactory bulbs, fused thalami, a midline monoventricle and complete absence of the interhemispheric fissure with or without associated craniofacial defects.[16] Development of alobar HPE is thought to be promoted by defective signals from the prechordal plate that are essential for the patterning and morphogenesis of the forebrain.[17] Both patients presented with craniofacial features known to be part of the clinical spectrum of alobar HPE, which could be classified as type IV (case 1) and type III (case 2) of DeMyer's categories of facial phenotypes in severe forms of HPE. [18] The clinical diagnosis of KS is hardly achievable in fetuses or newborns since

recognizable physical features of the condition evolve over time.[8] One can argue that in both patients, the severity of craniofacial defects associated with HPE could have concealed any craniofacial characteristic suggestive of KS. However, age-related extra-cephalic features of KS that could be assessed in each patient, such as prominent fingertip pads, were absent. Cleft palate and/or occasionally cleft lip and congenital heart defects, most commonly left-sided obstructive defects, are among the structural defects associated with KS.[8,19] While the cardiovascular phenotype of Case 2 includes hypertrophic cardiomyopathy which has been sporadically reported in KS, the characteristic HPE-associated median cleft lip and cleft palate present in Case 1 are rather not typical to KS.[20] Considering the age of the two patients, it would be cumbersome to attempt a syndromic classification of both HPE cases. Genes involved in the pathogenesis of HPE are expected to be expressed in the prosencephalic neural folds that give rise to the forebrain during primary neurulation.[17] The two patients reported here were indentified with novel deleterious variants in *KMT2D*, a gene not previously associated with defects in human forebrain development. Investigations of the mutational spectrum of *KMT2D* have shown that truncating mutations including nonsense and frameshift are the most common mutations types, followed by missense mutations. Exons 10, 31, 34, 39 and 48 are known as hotspots, with the majority of trucating mutations involving exon 39 and 48.[21,22] These data support our finding in Case 2 who was identified with a nonsense variant (c.12565G>T (p.Gly4189*)) in exon 39. Most *KMT2D* missense variants are confined in regions encoding C-terminal domains.[21,7] Though, some of these variants do not involve any known functional domain of the protein. The missense variant (c.5A>G (p.Asp2Gly)) identified in Case 1, although located in the first exon of the gene and not involving any known domain of the protein, is predicted *in sillico* to be damaging. The role of *KMT2D* in several developmental processes remains to be clarified. *KMT2D* morpholino knockdown in zebrafish results in reduced overall brain size and defect in neural progenitor cells' ability to differentiate in the forebrain and midbrain.[23] Similarly, Lee and collaborators did not report features of HPE in *Kmt2d* knockout mice embryos, which died at embryonic day 9.5.[24] The contrast between the brain phenotype of our two patients and the results of the above animal studies may be explained by their respective study method which was not designed to comprehensively explore the development of the forebrain. Therefore, there is a need for further investigation of the role of *KMT2D* in HPE pathogenesis. Based on previous evidences, many individuals affected with HPE carry a heterozygous mutation in an HPE-associated gene, which either occurs *de novo* or is transmitted following an autosomal dominant mode of inheritance.[14] Consistently, the two patients reported in this study were found to carry a *de novo* heterozygous variant in *KMT2D*, predicted to be highly deleterious.

This report presents for the first time an association between HPE and heterozygous mutations in *KMT2D*, a gene known to cause KS. Further studies will attempt to clarify the role of *KMT2D* in the regulation of forebrain development and pathogenesis of HPE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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Figure1:
Clinical photographs of the two patients with alobar HPE **A**: postmortem image of case 1 showing an apparently well-developed fetus with craniofacial dysmorphism, including closely spaced eyes, very depressed nasal bridge with flat nose, and cleft of the upper lip and palate. **B1** and **B2**: case 2 facial images showing a metopic ridge and features characteristic of cebocephaly. Publication of these photographs is supported by consents from the families.

Table 1:

Summary of clinical and molecular data of the two patients with HPE

	Case 1	Case 2
Clinical features*KMT2D* variant	NM_003482.3:c.12565G>T (p.Gly4189*)	NM_003482.3:c.5A>G (p.Asp2Gly)
Gender	Female	Female
Ethnicity	Caucasian	Caucasian
Antenatal		
Gestational age at diagnosis of HPE (weeks)	18	19
Termination of pregnancy	+[†]	−[‡]
Intrauterine Growth Restriction	−	+
Polyhydramnios	−	+
Brain abnormalities		
Completely absent interhemispheric fissure	+	+
Single ventricle	+	+
Fused thalami	+	+
Agenesis of the corpus callosum	+	+
Craniofacial features		
Microcephaly	+	+
Hypotelorism	+	+
Midface hypoplasia	+	+
Depressed nasal bridge	+	−
Flat nose	+	−
Proboscis-like nose	−	+
Median cleft lip	+	−
Cleft palate	+	−
Cardiovascular features		
Right ventricular dilation and hypertrophy with decreased systolic function	−	+
Other extra-cephalic features	−	−

[†]:
+ indicates presence of the feature;

[‡]:
- indicates absence of the feature