

# A novel *KMT2D* mutation resulting in Kabuki syndrome: A case report

JUN LU<sup>1</sup>, GUILING MO<sup>2</sup>, YAOJUN LING<sup>1</sup> and LIJUAN JI<sup>1</sup>

<sup>1</sup>Pediatric Department, Central South University Xiangya School of Medicine Affiliated Haikou Hospital, Haikou, Hainan 570208; <sup>2</sup>Department of Molecular Genetics, Guangzhou Kingmed Center for Clinical Laboratory Co., Ltd., Guangzhou, Guangdong 510330, P.R. China

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**Abstract.** Kabuki syndrome (KS) is a rare genetic syndrome characterized by multiple congenital anomalies and varying degrees of mental retardation. Patients with KS often present with facial, skeletal, visceral and dermatoglyphic abnormalities, cardiac anomalies and immunological defects. Mutation of the lysine methyltransferase 2D (*KMT2D*) gene (formerly known as *MLL2*) is the primary cause of KS. The present study reported the case of a 4-year-old Chinese girl who presented with atypical KS, including atypical facial features, unclear speech and suspected mental retardation. A diagnosis of KS was confirmed by genetic testing, which revealed a nonsense mutation in exon 16 of *KMT2D* (c.4485C>A, Tyr1495Ter). To the best of our knowledge, this is a novel mutation that has not been reported previously. The present case underscores the importance of genetic testing in KS diagnosis.

## Introduction

Kabuki syndrome (KS), first reported by Japanese researchers Kuroki *et al* (1) and Niikawa *et al* (2), is a rare genetic disorder characterized by intellectual disability and multiple congenital anomalies (3). The incidence of KS is officially 1 in 32,000 individuals (4). The cardinal diagnostic manifestations of KS include characteristic facial features, mild-to-moderate mental retardation, dermatoglyphic abnormalities, skeletal anomalies, and postnatal growth deficiencies (5). In addition, speech and language deficits are commonly present in patients with KS (6). Etiologically, KS was attributed originally to loss-of-function mutations in the gene encoding lysine methyltransferase 2D (*KMT2D*), namely *KMT2D* (formerly *MLL2*), with an autosomal dominant inheritance pattern (7).

More recently, the KS phenotype was identified in boys with a loss-of-function mutation in *KDMA6* on the X chromosome, the product of which is important for *KMT2D* function (8). Currently, no effective treatments for KS are available (9). Treatments are required that are beneficial for KS patients with developmental disability or cognitive limitations; group therapy may be an option (10). The present study reports the case of a young Chinese girl with unclear speech (nasal and muffled pronunciation) and possible mental retardation, who was diagnosed with KS based on DNA sequencing analysis of *KMT2D*.

## Case report

Written informed consent was obtained from the patient's family. A 4-year-old Chinese girl was admitted to the Central South University Xiangya School of Medicine Affiliated Haikou Hospital (Haikou, China) on March 8th, 2015 with unclear speech (nasal and muffled pronunciation), which her parents indicated had been present for >2 years, and possible mental retardation. Mental retardation was suspected due to the weak expressive language ability of the patient compared with healthy peers.

The patient was a first-born child with no siblings, born full-term via uncomplicated vaginal delivery when her mother was 24 years old. There was no history of consanguineous marriage in the family, and no family history of genetic disorders. The patient's father was employed as a road construction engineer and her mother had previously worked with cosmetics. During the first trimester, prior to awareness of the pregnancy, the patient's mother reportedly had a febrile illness and received oral cold medication at a local clinic (no records available). The patient's mother did not consume alcohol, smoke, or take any other drugs or medications during the pregnancy.

At 1 year of age, the patient was diagnosed with right congenital hip dysplasia, and was treated successfully with external bracing. The patient's speech was delayed; she was only able to say one word by 1 year of age. Upon admission to the hospital at 4 years of age, the patient was able to speak in 5- and 6-word sentences. Her speech was unclear, with muffled pronunciation and a nasal tone. No seizures, meningitis, or other severe illnesses were reported. In addition, no problems

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**Correspondence to:** Dr Jun Lu, Pediatric Department, Central South University Xiangya School of Medicine Affiliated Haikou Hospital, 43 Renmin Road, Haikou, Hainan 570208, P.R. China  
E-mail: lu139762@163.com

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with hearing or difficulties with understanding and following instructions were observed. The patient was able to recognize simple colors and the numbers 1-5, and could color with a crayon. However, she was unable to read, write, or perform simple addition. Her motor and social skills were observed to be normal.

A physical examination revealed that the patient was of normal height (~100 cm) and weight (~16 kg). However, she presented with abnormal facial features, including slanted eyes and sparse eyebrows (Fig. 1A), a broad nose and depressed nasal tip (Fig. 1B), and eversion of the lateral parts of the lower eyelids (Fig. 1C). In addition, she had a high palate, but without a cleft. Her fifth digits were short with clinodactyly (Fig. 1D), but she had normal palmar creases. The results of heart and respiratory system examinations were normal, and there was no hepatosplenomegaly. Neurologically, the patient exhibited normal physiological reflexes and did not exhibit any pathological reflexes. Her muscle strength appeared to be normal.

Blood analysis revealed normal complete blood count, electrolyte measurements, blood lipid levels and renal and liver functional markers, but high blood lactic acid levels (2.8 mmol/l, August 2013; 3.4 mmol/l, January 2015; normal range, 0.7-2.1 mmol/l). In addition, the patient's blood ammonia levels were normal and a urine mucopolysaccharide test was weakly positive [5  $\mu$ l (+), 15  $\mu$ l (+++) and 25  $\mu$ l (+++)]. Gas chromatography-mass spectrometry analysis of the patient's urine and blood revealed no evidence of any amino acid or aliphatic acid metabolic disorders. Furthermore, G-banding karyotyping demonstrated that the patient had macroscopically normal chromosomes (46, XX). Chest X-rays, retinal examinations, and brain magnetic resonance imaging results were also normal. Notably, X-rays of the fingers revealed the presence of three phalanges in the short fifth digits of the two hands.

Due to the clinical manifestations and urine mucopolysaccharide test results, it was suspected initially that the patient may be suffering from mucopolysaccharidosis type III (MPS III). MPS III and KS are characterized by atypical facial features together with developmental and/or speech delays. Specifically, MPS III is characterized by an early-onset developmental and/or speech delay subsequent to an initial period of normal development (11). The developmental delays evolve into a progressive cognitive decline, behavioral abnormalities and severe hyperactivity that does not respond to treatment with stimulants (11). The somatic features of MPS III, however, are relatively mild, including a dolichocephalic skull shape with a short forehead, prominent eyebrows, an everted and thick lower lip, and an upturned upper lip with a protruding philtrum (11). The patient in the present study exhibited abnormal facial features and delayed speech, but displayed no overt behavioral abnormalities, hyperactivity, or MPS III-characteristic facial features, and her urine MPS test was only slightly abnormal. Therefore, a diagnosis of MPS III was excluded. Autism was also considered due to the patient's delayed speech development. Autism is a neurodevelopmental disorder that is characterized by abnormalities in reciprocal social and communicative behaviors, and an inflexible adherence to routinized patterns of thought and behavior (12). The patient did not display any difficulties in understanding or following instructions given by her parents or preschool teacher. In addition, the patient gave reliable responses and maintained eye contact when spoken to.

Furthermore, sensory processing difficulties are ubiquitous, albeit highly variable, among people with autism. The patient of the present study presented no evidence of sensory hyper- or hypo-sensitivity, or any motor coordination delays, further indicating that she did not have autism.

Following the exclusion of MPS III and autism, KS was considered as a possible diagnosis. Various clinical characteristics of the patient supported a diagnosis of KS, including abnormal facial features (Fig. 1A-C), short fifth digits with clinodactyly (Fig. 1D), skeletal anomalies, delayed language development and unclear speech. DNA sequence analysis of *KMT2D*, the gene primarily associated with KS, was performed. A peripheral blood sample was first obtained from the sample, and DNA was extracted from blood with a QIAamp Blood DNA Mini kit (catalog no. 51106; Qiagen GmbH, Hilden, Germany). High-throughput sequencing of *KMT2D* was performed using the TruSight One Sequencing Panel (Illumina, San Diego, CA, USA). The sequence was analyzed in ANNOVAR software ([annovar.openbioinformatics.org/en/latest/](http://annovar.openbioinformatics.org/en/latest/)) (13), and various databases, including 1,000 genomes ([www.1000genomes.org/](http://www.1000genomes.org/)), Exome Sequencing Project 6500 ([evs.gs.washington.edu/EVS/](http://evs.gs.washington.edu/EVS/)), Single Nucleotide Polymorphisms ([www.ncbi.nlm.nih.gov/SNP/](http://www.ncbi.nlm.nih.gov/SNP/)) and Human Gene Mutation Data ([www.hgmd.cf.ac.uk/ac/index.php](http://www.hgmd.cf.ac.uk/ac/index.php)), were used for screening and annotation of gene variants in accordance with the American College of Medical Genetics and Genomics guidelines (14). The DNA sequence analysis revealed a mutation in *KMT2D* (Fig. 2), which confirmed the diagnosis of KS.

Subsequently, the patient's parents underwent polymerase chain reaction (PCR) genetic testing together with their daughter with the following *KMT2D*-Exon 16 primers, designed using Primer Premier software version 5.0 (Premier Biosoft International, Palo Alto, CA, USA): Forward, 5'-TATGATGTT GAGTATGTGACAGAGG-3' and reverse, 5'-AATCCTAGC AGTGAAGAGACCAT-3'. The primers were used to amplify the *KMT2D* gene using an ABI 9700 PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Exon 16 was amplified in a reaction solution containing 1  $\mu$ l 10X PCR buffer, 0.35  $\mu$ l dNTPs (10 mmol/l), 0.07  $\mu$ l FastStart Taq DNA Polymerase (5 U/ $\mu$ l; Roche Diagnostics, Basel, Switzerland), 1  $\mu$ l genomic DNA (100 ng/ $\mu$ l), 1  $\mu$ l each primer (3.2  $\mu$ mol/l) and 5.6  $\mu$ l ddH<sub>2</sub>O. The cycling conditions were as follows: 94°C for 12 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, and a final elongation step at 72°C for 10 min, as previously described (5,15). An ABI 3500 Sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.) was then used to sequence the PCR products by the Sanger method, as previously described (15), and the resultant sequences were compared with known sequences of KS-associated genes (NCBI sequences, NM\_003482.3 and NG\_027827.1). The patient was found to harbor a nonsense mutation in exon 16 of the *KMT2D* gene (c.4485C>A, Tyr1495Ter; Fig. 3A); however, neither of her parents carried this mutation (Fig. 3B and C). Based on the clinical manifestations of the patient and the identification of a *de novo* nonsense mutation of *KMT2D*, a diagnosis of KS was established.

There is currently no cure for KS, and the only treatments available are supportive and psychological therapies (9). However, early diagnosis of the syndrome is essential for optimal management. There are several features that should be

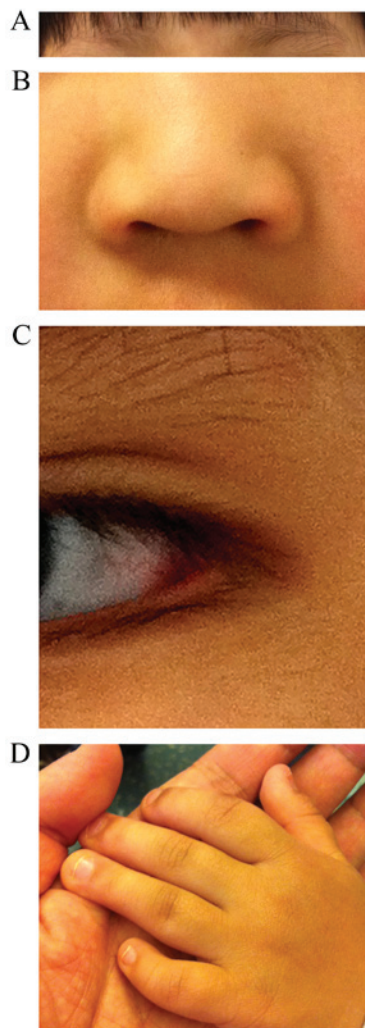


Figure 1. Photographs of the patient's physical features demonstrating the presence of (A) sparse eyebrows, (B) a broad nose with a depressed nasal tip, (C) eversion of the lateral part of the lower eyelid, and (D) short fifth digits with clinodactyly.

monitored, including the patient's height, weight, head circumference, vision and hearing (9). The patient described in the present report was referred immediately for speech therapy with the aim of improving her speech quality.

The patient's prognosis is unclear; therefore, a long-term follow-up plan was implemented with the intention of providing potentially beneficial supportive and psychological therapies as required. At a follow-up in May 2015, following 10 months of speech training, the patient's speech remained unclear. Following her diagnosis in our hospital, she has not been subjected to any further tests, has not exhibited any new manifestations of pathology and has not been administered any other treatments beyond speech therapy. The current case underscores the importance of genetic testing in enabling a clear differentiated diagnosis of KS.

## Discussion

The present study reports a new case of the rare genetic syndrome KS, which is characterized primarily by intellectual deficiencies, multiple congenital malformations, and

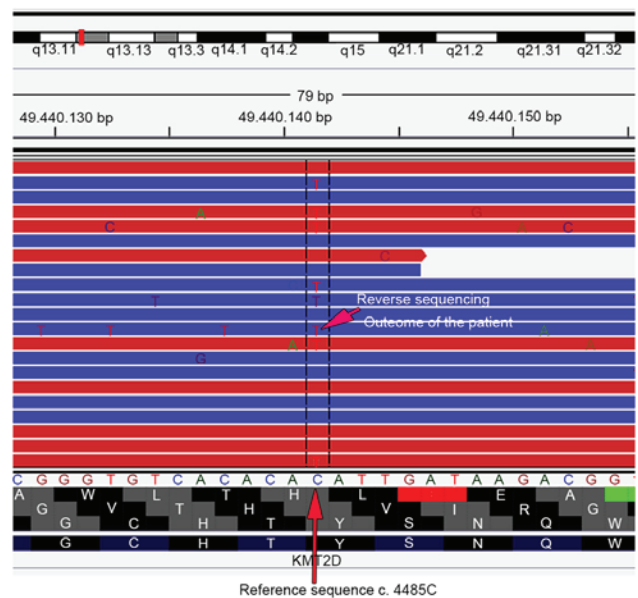


Figure 2. High-throughput reverse DNA sequencing results for the patient demonstrate the presence of a mutation in *KMT2D* (c.4485T; center red arrow) compared with the reference sequence (c.4485C; lower red arrow). *KMT2D*, lysine methyltransferase 2D.

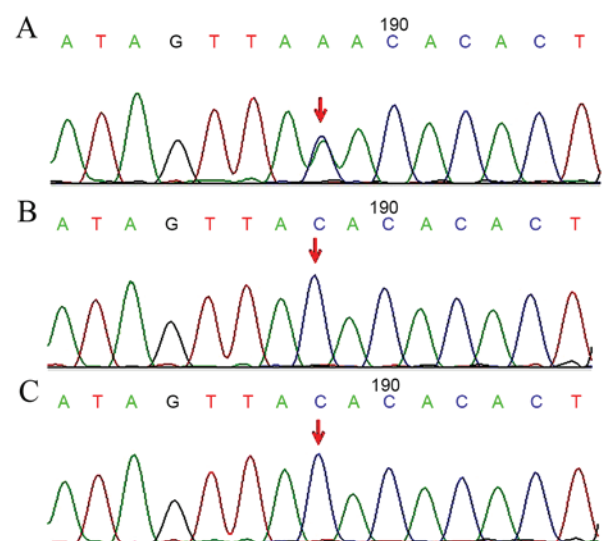


Figure 3. Sanger sequencing results for (A) the patient, and the patient's (B) mother and (C) father. The analysis demonstrated the presence of a nonsense *KMT2D* mutation (c.4485C>A, Tyr1495Ter; red arrow) in the patient, and the absence of a mutation at c.4485C (red arrows) in her parents. *KMT2D*, lysine methyltransferase 2D.

specific craniofacial abnormalities (16). More specifically, KS is characterized by the following five, mainly external, structural features: i) A dysmorphic face, including eversion of the lower lateral eyelids and arched eyebrows with sparse lateral eyebrows; ii) dermatoglyphic abnormalities, with increased digital ulnar loops and hypotenar loop patterns, absence of digital triradius, and the presence of fingertip pads in 93% of patients; iii) skeletal anomalies, with brachydactyly and spinal deformities with or without sagittal cleft vertebrae in 92% of patients; iv) mild-to-moderate mental retardation in



92% of patients; and v) postnatal growth deficiencies in 83% of patients (17). Patients with KS frequently exhibit internal malformations of the heart, kidneys, gastrointestinal system, skeletal system and/or eyes (18). Certain patients present with immunological defects and increased susceptibility to respiratory infections. Speech and language deficits are common in KS, with the most prominent deficit being dysarthria, characterized by imprecise pronunciation of consonants, a harsh vocal quality, hypernasality, reduced speech rate and stress, and a distorted pitch (6).

KS has an incidence rate of 1 in 32,000 individuals; however, this figure is likely to be underestimated due to missed diagnosis and misdiagnosis (4). The vast majority of reported KS cases are sporadic (18). Diagnosis of KS can be challenging due to the spectrum of clinical, radiological and biological factors associated with KS, various complications, and incomplete penetrance in certain cases (16). Additionally, numerous other conditions present with features similar to those observed in KS, including global developmental delays, fetal alcohol syndrome, autism spectrum disorder and Down syndrome (19). The majority of patients diagnosed with KS are diagnosed based primarily on the presentation of archetypical features. Currently, although genetic testing of families is important for genetic counseling, confirmatory genetic testing is performed only in a minority of cases.

Mutations in *KMT2D* are present in 55-80% of KS-diagnosed individuals subjected to genetic testing (9); *KMD6A* mutations have been observed in the remaining KS cases in which genetic testing was performed, although there may be other as yet unidentified genetic mutations that lead to KS (20). A number of different *KMT2D* mutations have been reported to date, including missense mutations, nonsense mutations, frameshift mutations, splice site mutations, and indel mutations (21). The majority of these result in a truncated protein product. In the present study, a novel nonsense mutation in exon 16 of *KMT2D* (c.4485C>A, Tyr1495Ter) was identified. This mutation is a loss-of-function mutation in which codon 1495 is replaced with a termination codon, resulting in truncation of the encoded KMT2D protein product. KMT2D is a histone methyltransferase, comprised of 5,537 amino acid residues, that serves an important role in regulating gene transcription. KMT2D methylates lysine 4 of histone 3; this methylation serves as a marker of gene activation (22).

Numerous *de novo* *KMT2D* mutations have been identified in sporadic KS cases (20). Among KS cases that are not sporadic, autosomal dominant inheritance of KS due to parent-to-child transmission of *KMT2D* mutations has been observed (23). In the present study, the patient's parents were not found to carry *KMT2D* mutations, which indicates that the patient harbored a *de novo* genetic mutation.

Although the molecular mechanisms underlying the KS phenotype development remain to be elucidated, the clinical manifestations of KS are generally presumed to be due to the loss of KMT2D functionality. This view is supported by the fact that the other gene in which mutations have been demonstrated to cause KS, *KDM6A*, encodes a histone demethylase that interacts with KMT2D (24). The *KMT2D* mutation found in the present case (c.4485C>A) appears to be novel in that it was not listed among the hundreds of previously identified KS-causing mutations (5) represented in the Leiden Open

Variation Database ([www.lovd.nl/](http://www.lovd.nl/)), the PubMed database of the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)), the European Molecular Biology Laboratory ([www.embl.org/](http://www.embl.org/)), or the Human Gene Mutation Database ([www.hgmd.org/](http://www.hgmd.org/)).

In conclusion, clinicians should be aware that there are a variety of clinical manifestations of KS. In the present study, the patient had abnormal facial features and unclear speech; however, her overall presentation was not clearly indicative of KS. Genetic testing should be conducted to confirm KS diagnosis.

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