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증 례

# 분자유전학적으로 진단된 가부키 증후군 1례

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# A Case of Kabuki Syndrome Confirmed by Genetic Analysis: A Novel Frameshift Mutation in the *KMT2D* Gene

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Kabuki syndrome is a rare congenital disorder that causes multiple birth defects and mental retardation. Mutation of the lysine methyltransferase 2D (*KMT2D*) gene is the primary cause of Kabuki syndrome. We report a 4-year-old Korean girl diagnosed with Kabuki syndrome based on distinctive facial features (eversion of the lower lateral eyelid, arched eyebrows, depressed nasal tip, prominent ears), skeletal anomalies, short stature, and molecular analysis, which revealed a novel frameshift mutation in the *KMT2D* gene. A 4-year-old patient had a past history of congenital cardiac malformations (coarctation of the aorta, ventricular septal defect, atrial septal defect, patent ductus arteriosus), subclinical hypothyroidism and dysmorphic features at birth including webbed neck, short fingers, high arched palate, micrognathia and horseshoe kidney. She showed unique facial features such as a long palpebral fissure, long eyelashes, arched eyebrows with sparseness of the lateral third, broad nasal root, anteverted ears, and small mouth. Her facial features suggested Kabuki syndrome, and genetic analysis discovered a novel heterozygous frameshift mutation (c.4379dup, p.Leu1461Thrfs*30) in exon 15 of the *KMT2D* gene. The diagnosis of our 4-year-old patient was made through thorough physical examination and history taking, and genetic testing. It is challenging to diagnose patients with Kabuki syndrome at birth, since the characteristic facial features are expressed gradually during growth. Clinical suspicion aroused by regular follow-ups may lead to earlier diagnosis and interventions.

**Key words:** Kabuki syndrome, *KMT2D* mutation

## Introduction

Kabuki syndrome is a rare genetic disorder characterized by multiple congenital anomalies, variable degree of mental retardation, short stature, skeletal and visceral abnormalities (deformed spinal column with or without sagittal cleft vertebrae and brachydactyly), and distinct facial features that resemble the make-up of actors in Kabuki, the traditional Japanese theater[1)]. The

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characteristic facial features of Kabuki syndrome include long palpebral fissures and eversion of the lower lateral eyelid, arched eyebrows with the lateral one-third dispersed or sparse, a depressed nasal tip, and prominent ears[2]. It was first introduced by a Japanese geneticist in 1981, with an incidence of 1/32,000 in Japan[3]. Although Kabuki syndrome is reportedly rare in Korea, considering the many reports of other populations, the frequency of Kabuki syndrome in the Korean population presumably approximates that of the Japanese population[4]. Two genes have shown to be mutated in patients with Kabuki syndrome: *KMT2D* at 12q13.12 and *KDM6A* at Xp11.3. Kabuki syndrome is most often caused by a mutation in the *KMT2D*[5,6], a gene that encodes a histone methyltransferase, a protein important in the epigenetic control of active chromatin states[7]. Subsequent mutation screenings in Kabuki syndrome cohorts found a *KMT2D* nutation in 55.8% to 80% of patients[8]. Heterozygous deletions in a second gene, *KDM6A*, are another cause of Kabuki syndrome[9].

Here, we report a 4-year-old female patient with a complex phenotype diagnosed with Kabuki syndrome by molecular testing, which revealed a novel frameshift mutation in *KMT2D*, at chromosome 12q13.

## Case report

Our patient was born at a gestational age of 35 weeks and 6 days by cesarean section. The patient was of normal height (45 cm, 25-50$^{th}$ percentile) and weight (2.39 kg, 25-50$^{th}$ percentile) at birth. Cesarean section was chosen due to prenatally known congenital heart disease (coarctation of the aorta, ventricular septal defect (VSD), atrial septal defect (ASD), large patent ductus arteriosus (PDA) in the fetus. The patient was the second child of a 38-year-old mother, and there was no family history of any congenital anomalies. She underwent coarctation repair surgery, patch closure of VSD, and primary closure of ASD at the 23$^{rd}$ day after birth. She was discharged from the hospital after 2 months of post-operative care. During 2 months of hospitalization, she was diagnosed with subclinical hypothyroidism (TSH level 17.75 IU/mL, free T4 1.09 μg/dL at 8$^{th}$ day of birth) and started taking synthyroxine medication. She had experienced two hypoglycemic episodes, glucose level 47 mg/dL, 30 mg/dL in each episodes (at 5$^{th}$, 7$^{th}$ day of birth). Insulin level was not checked at that time, but transient hyperinsulinism due to perinatal stress was suspected as the possible cause of hypoglycemia. The physical examination at birth showed webbed neck, short fingers, high arched palate, and microgenia. Abdominal computed tomography (CT) for further evaluation of congenital malformations revealed the horseshoe kidney. During follow-up, we conducted regular thyroid function tests and she continued taking 16.5 mcg of synthyroxine medication. Chromosomal studies for rule out of Turner syndrome, fluorescent in-situ hybridization study for 22q11.2 microdeletion syndrome was performed, and the results were negative at that time.

By the time the patient was 4 years old, she showed unique facial features: long palpebral fissures, long eyelashes, arched eyebrows with sparseness of the lateral third, broad nasal root, anteverted ears, and a small mouth. Moreover, she had brachydactyly and fetal finger pads. Growth assessment revealed short stature (90 cm, Height-SDS=-2.61) and compared to previous growth assessments suggested postnatal growth delay. Evaluation of her motor development revealed that she was able to control her neck when she was 6 months old, sit without support when

she was 17 months old, and walk with support when she was 3 years old. She spoke her first words at the age of two and first two-word sentences at the age of four. She knew a limited number of words and could construct limited sentences.

Considering the patient's known multiple organ abnormalities and facial features, skeletal features, postnatal short stature, we conducted a genetic analysis of the *KMT2D* gene first. Written informed consent for genetic testing was obtained. Genomic DNA was isolated from peripheral blood leukocytes by the QIAamp DNA Mini Kit (Qiagen, Hamburg, Germany). PCR was carried out using gene-specific primers for the *KMT2D* gene. A novel heterozygous frameshift mutation (c.4379dup, p.Leu1461Thrfs*30) in exon 15 of the *KMT2D* gene was identified (Fig. 1).

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, occupational therapy, and activities daily living training. At a follow-up in November 2017, following 14 months of speech training, the patient was not fluent in speech, but showed slight improvement in speech fluency. Although current treatments available for Kabuki syndrome are only supportive and psychological therapies[10], early diagnosis of the syndrome is important for optimal management. Frequent monitoring of height, weight, and head circumference, developmental milestones, vision and hearing is recommended for individuals with Kabuki syndrome.

## Discussion

Kabuki syndrome is a multisystem genetic disorder with characteristic facial features, skeletal anomalies, mental retardation, and postnatal growth delay[2,3,11]. The most distinctive feature of Kabuki syndrome is facial dysmorphism, including long palpebral fissures with eversion of the lower eyelid, long, dense eyelashes and arched eyebrows, prominent ears, depressed nose, a thin upper and full lower lip, and corners of the mouth that slant downwards.

Kabuki syndrome patients are typically born with normal body measures, but feeding difficulties caused by poor sucking and swallowing lead to failure to thrive and postnatal short stature (58 %)[7,8,12]. Varying degrees of intellectual disability are also common (90%) in patients with Kabuki

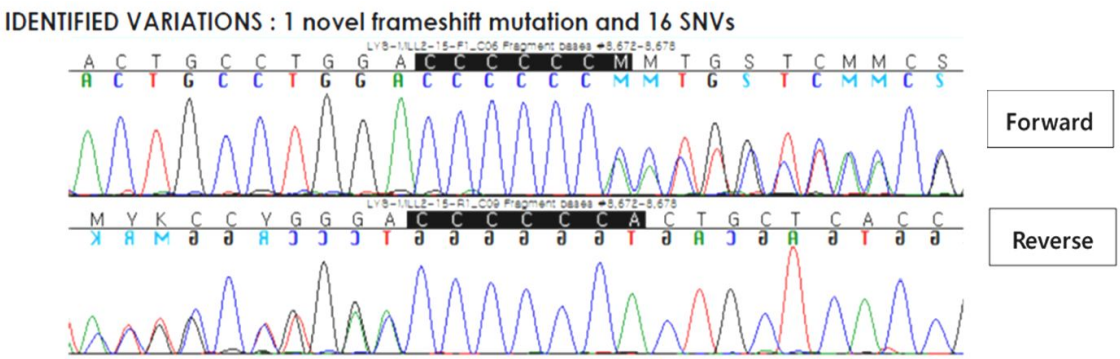


**Fig. 1.** A novel heterozygous frameshift mutation detected in exon 15 of the *KMT2D* gene (c.4379dup, p.Leu1461Thrfs*30).

syndrome[7,12]. Congenital heart defects are common with the most frequent malformations being atrial septal defects, ventricular septal defects, and aortic coarctation. Endocrinologic complications of Kabuki syndrome include growth hormone deficiency, premature thelarche[3,13]. Rare endocrinologic findings in Kabuki syndrome include hypothyroidism, hyperinsulinism hypoglycemia, diabetes insipidus, primary ovary dysfunction, and abnormal pituitary findings on magnetic resonance images[1,14]. Urinary tract anomalies such as hydronephrosis and renal position abnormalities are found in approximately 30-40% of patients[7,8]. Skeletal abnormalities include rib and vertebral malformations, scoliosis, brachydactyly, and/or clinodactyly of the fifth digit. Presence of persistent fetal finger pads is a distinct skin and soft tissue abnormality of Kabuki syndrome patients[15].

Mutation of *KMT2D*, the gene that encodes a Trithorax-group histone methyltransferase, a protein important in the epigenetic control of active chromatin states, is the primary cause of Kabuki syndrome[5,6,16]. Mutations in *KMT2D* are present in 55-80% of Kabuki syndrome patients subjected to genetic testing[10]. Numerous different *KMT2D* mutations have been identified in diagnosed Kabuki syndrome patients, and most of these mutations result in a truncated protein product. In 2015, Cheon et al. had described 12 Korean patients with suspected Kabuki syndrome in whom direct Sanger sequencing or exome sequencing was performed, and 11 out of 12 patients expressed *KMT2D* mutations, and 1 patient expressed KDM6A mutation. The 11 *KMT2D* mutations included five small insertions or deletions causing frameshifts, four nonsense mutations, and two missense mutations. The mutations identified were relatively evenly distributed across exons 14 to 46 of the *KMT2D* gene[4]. In the present study, a novel heterozygous frameshift mutation (c.4379dup, p.Leu1461Thrfs*30) in exon 15 of the *KMT2D* gene was identified. *KMT2D* is a histone methyltransferase that plays an important role in regulating gene transcription. *KMT2D* methylates histone H3 lysine 4, whose methylations serve as a gene activation mark[17].

Since the characteristic facial features of Kabuki syndrome are expressed gradually during the growth period, it is quite challenging to diagnose the syndrome at birth or during infancy. Dentici et al. suggested that although the facial dysmorphism is less prominent in infancy, some facial characteristics, such as large prominent ears and elongated palpebral fissures and particularly those associated with hypotonia, feeding difficulties and malformations, may prompt clinical suspicion of Kabuki syndrome during the first year of life[18].

An early diagnosis of Kabuki syndrome is necessary for systemic evaluation and early therapeutic intervention to address possible growth delays, intellectual disability, conductive hearing loss, and endocrinologic dysfunction. The diagnosis of our 4-year-old patient was made by thorough physical examination and history taking, and eventually revealed a novel frameshift mutation in the *KMT2D* gene. Thus, it is important to consider Kabuki syndrome for patients with facial dysmorphisms and multisystem congenital malformations. Mutation analysis of *KMT2D* may be a useful method for early diagnosis and counseling of Kabuki syndrome. The molecular mechanisms underlying the Kabuki syndrome phenotype development is not clearly demonstrated. More functionally-oriented studies are needed and furthermore, other genes associated with Kabuki syndrome need to be discovered.

## 요 약

가부키 증후군(Kabuki syndrome)은 특징적인 얼굴(아치형의 넓은 눈썹, 낮은 코끝, 큰 귓바퀴, 아래 외측 안검 외전), 골격계 이상, 출생 후 성장 지연, 그리고 경도의 정신 지체를 특징으로 나타내는 선천성 이상 질환이다. 또한, 선천성 심장기형, 비뇨생식기 기형, 구개구순열을 포함한 위장관 기형, 안검 하수 등의 증상이 동반되기도 한다. 감염과 면역질환, 경련, 내분비 문제, 청력 손실이 나타날 수 있다.

가부키 증후군의 대부분은 12번 염색체의 장완에 위치한 *KMT2D* 유전자의 돌연변이에 의해 발병된다. *KMT2D* 유전자의 돌연변이로 생성된 비정상적인 *KMT2D* 단백질로 인해 신체의 여러 장기와 조직의 특정 유전자의 활성이 정상적으로 이뤄지지 못하여, 결과적으로 가부키 증후군의 특징적인 외형과 기능장애가 나타난다.

출생시 선천성 심장 기형, 갑상선 기능 저하 및 일과성 저혈당 과거력이 있던 환아가 만 4세경 성장 지연, 아치형 눈썹, 낮은 코끝, 아래 외측 안검 외전, 큰 귓바퀴 등의 얼굴 생김새를 보여 시행한 분자유전학적 검사에서 *KMT2D*의 exon 15에서 새로운 frameshift mutation이 발견되어 증례를 보고하는 바이다.

가부키 증후군은 성장 지연 뿐 아니라, 발달 장애 및 행동 장애, 사춘기 여아의 경우 조기 가슴 발육을 포함한 내분비적 문제, 사시, 안검하수 등의 안과적 문제, 만성 중이염, 청력 손실, 감염, 경련 등 다양한 증상을 나타낼 수 있기 때문에 조기에 질환을 진단하고, 임상증상에 따라 적절한 중재를 할 수 있도록, 정기적으로 신장, 몸무게, 머리둘레, 발달상태, 청각과 시각상태를 확인하는 것이 중요하다. 따라서 여러 장기의 선천 기형을 보이는 환아의 경우, 임상의는 출생 당시에는 두드러지지 않을 수 있는 특징적인 얼굴 생김새, 성장 지연, 정신 지체 등의 이상에 대해 유의하여야 한다. 분자유전학적 검사는 임상적으로 가부키 증후군이 의심되는 환아를 조기에 진단할 수 유용한 방법이며, 앞으로도 가부키 증후군과 연관된 유전자 변이에 대한 연구가 더욱 필요할 것으로 사료된다.

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