Case Report

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Fetal Phenotype and Prenatal Diagnosis of Kabuki Syndrome

Yan Pan, Hong Yao, Gongli Chen, Qiong Tan, Qing Chang, Yongyi Ma*, Zhiqing Liang*

Abstract

Kabuki syndrome (MIM 147920) is an autosomal dominant rare disease featured with multiple malformations and mental retardation. The main clinical manifestations of Kabuki syndrome are characteristic facial features, skeletal abnormalities, dermatoglyphic abnormalities, postpartum growth retardation, mild to moderate mental retardation, as well as other structural and functional abnormalities that may involve multiple systems. The establishment of diagnosis needs to be combined with clinical phenotype and the discovery of pathogenic mutation. Compared with the abundant descriptions and records of genotype-phenotype of postpartum patients, few prenatal diagnosis cases of Kabuki syndrome had been reported, which partially result from lacking the knowledge of its phenotype in fetuses that might suggest the diagnosis. This report performed comprehensive prenatal examinations to identify a fetus's etiology with multiple structural anomalies characterized by ascites, thickening of local skin, and cardiac abnormalities. We ruled out intrauterine infection, thalassemia, and chromosome abnormality by corresponding tests. Finally, trio whole-exome sequencing revealed a de novo heterozygous variation c.15641g > A (p.r5214h) in exon 48 of the *KMT2D* gene was the fetus's genetic pathogeny causing Kabuki syndrome. This result suggests that Kabuki syndrome should be in the suspected etiology list for prenatal hydrops/ascites. Our study confirmed that prenatal whole-exome sequencing is an efficient tool for diagnosing fetal abnormalities, and a multidisciplinary team is necessary for providing pregnancy guidance to patients.

Keywords: Prenatal diagnosis; Ultrasonography abnormality; Kabuki syndrome; *KMT2D* gene; Whole exome sequencing

Introduction

Kabuki syndrome (KS) is a multiple malformations and mental retardation syndrome characterized by distinctive facial features, skeletal abnormalities, dermatoglyphic abnormalities, postpartum growth retardation, mild to moderate mental retardation, as well as other structural and functional abnormalities that may involve multiple systems.[1,2] According to the causative gene and inheritance pattern, KS can be divided into two types: Type I (OMIM # 147920) caused by pathogenic variants in *KMT2D*, in autosomal dominant inheritance[3] and Type II (OMIM # 300867) caused by pathogenic variants in *KDM6A*, in X-linked dominant inheritance.[4] It has been reported that *KMT2D* mutation accounts for about 70% of clinically diagnosed patients.[5,6]

The diagnosis of KS is based on typical clinical manifestations and molecular genetics findings. To date, most of KS patients are diagnosed after birth.[7] As most KS cases are born to unaffected parents and caused by de novo mutations, identifying the genotype-phenotype of affected fetus is important to discover and prenatal diagnosis of this syndrome before birth. In this paper, we diagnosed a case of KS fetus with multiple ultrasound abnormalities by comprehensive prenatal examination, and the prenatal phenotype of a specific mutation was established. Moreover, we also reviewed the fetal phenotype of KS cases reported in previous literature, in order to provide reference for prenatal diagnosis and consultation regarding this genetics syndrome.

Case presentation

Patients

The pregnant woman aged 37 years, with a spouse aged 46 years. The couple were previously married and both had healthy children with their former spouses. Due to delayed liquefaction of semen, in vitro fertilization via intracytoplasmic sperm injection was performed in an assisted reproduction center. After a blastocyst transfer, the female conceived successfully. The couple attended at our prenatal diagnosis clinic for consultation because of fetal ultrasound abnormalities found at 26th weeks of gestation. Both the couple had no obvious disease phenotypes and denied their families had genetic disease histories. The study protocol was approved by our institutional ethics committee, batch number for KY201940t. Patient's consent was obtained for publication of the clinical histories and pictures.

Methods

Sample collection

The fetal cord blood sample was collected by ultrasound-guided percutaneous umbilical sampling at the 26th week

Department of Gynecology & Obstetrics, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400030, China.

**Corresponding authors: Zhiqing Liang, and Yongyi Ma, Department of Gynecology & Obstetrics, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400030, China. E-mail: zhi_lzliang@163.com (Liang Z); mayongyi@aliyun.com (Ma Y).*

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of pregnancy. Meanwhile, peripheral blood samples of the couple were also collected for the subsequent tests.

Consanguinity identification and maternal contamination assessment

Genomic DNA was extracted from fetal cord blood and peripheral blood lymphocytes of the parents, using a TIANamp Genomic DNA Kit (TIANGEN, Beijing). Biological kinship identification of family samples and assessment of matern-al cell contamination in prenatal sample were conducted by using PowerPlex16 HS System (Promega, Madison, WI).

Intrauterine infection test

Toxoplasma, rubella, cytomegalovirus and herpes simplex virus (TORCH) immunoglobulin G (IgG) and immunoglobulin M (IgM) detection for maternal and fetal plasma were conducted with an enzyme-linked immunosorbent assay kit (Autobio, Shanghai, China), while B19 and cytomegalovirus in fetal cord blood were detected by polymerase chain reaction (PCR) (Easy Diagnosis Biomedicine, Wuhan, China).

Karyotype analysis and genome microarray analysis

Cord blood cells were cultured routinely and G-banding karyotype analysis was performed. The karyotype was described according to "ISCN 2013 Standard for International System of Cytogenetics". Genome microarray analysis was conducted by using Infinium Global Screening Array-24 v1.0 BeadChip (Illumina, San Diego, CA) according to the manufacturer's protocol. Interpretation of the copy number variation detected was carried out according to the American College of Medical Genetics and Genomics (ACMG) latest guidelines.[8]

Thalassemia gene test

Three types of α-thalassemia deletions including Southeast Asia, 3.7 and 4.2 were detected by one tube multiplex Gap-PCR. Known pathogenic variations causing thalassemia (http://globin.cse.psu.edu/globin/hbvar/) in hemoglobin-alpha locus and hemoglobin-beta locus gene were screened by high-throughput custom capture sequencing.

Prenatal whole-exome sequencing (WES)

Trio WES was performed. The WES mainly included library preparation, hybridization capture, and high-throughput sequencing. For library preparations, the DNA was broken into 200–300 bp fragments by ultrasonic waves and the libraries were prepared by using the KAPA LTP Library Preparation Kit Illumina® Platforms (KAPA Biosystems, Cape Town, South South Africa) with reference to its specifications. The hybridization capture involved the capture of target molecules and removal of non-target molecules in the mixed libraries and this was conducted by IDT xGen Exome Research Panel (Integrated DNA Technologies, Inc., Coralville, Iowa, USA) and customized with xGen Lockdown® Probes. The captured libraries were sequenced on HiSeq X10 (Illumina) with paired end run to obtain 2× 150bp reads with at least 100X depth of coverage. Then the quality of the original sequencing data was evaluated and saved for downstream analysis.

Biological information analysis

High-quality paired-end reads were aligned to the human reference genome sequence from University of California Santa Cruz Genomics Institute database (build 37.1 version hg19, http://genome.ucsc.edu/) using BurrowsWheeler Alignment tool. All variants were filtered first against 1000 genomes project database, for a minor allele frequency ≥1% and ExAC hom AC ≥3. Obtained variants were further selected according to co-segregation, genetic model, and with a minor allele frequency <1% in three databases (1000 genomes project_EAS, ExAC, gnomA-D_EAS). Single nucleotide polymorphisms and indels occurring in exons and canonical splice sites were further analyzed. Interpretation and classification of mutations found were conducted according to ACMG classification standards and guidelines for genetic mutations.[9]

Results

Clinical features

The pregnant performed first trimester screening and serial ultrasound exams at her local hospital. The nuchal translucency value was below 2.5 mm and non-invasive prenatal testing showed a low risk of trisomies 21, 18, and 13. Serial ultrasound exams at 25th week gestation showed single umbilical artery, fetal ascites, and isolated left renal collecting system. Color doppler ultrasound examination in our hospital at 26th weeks showed fetal ascites with a larger spacing of dark area of liquid about 18 mm, slight pericardial effusion with depth 2.7 mm, tricuspid regurgitation with systolic regurgitant volume about 4.5 × 3.6 mm^2, separation of left renal pelvis with a spacing about 8.5mm, single umbilical artery, thickening nuchal fold (NF) with the thickness about 7.4 mm and slight thickening of anterior nasal skin (5.7 mm). The amniotic fluid was less than normal with the largest amniotic fluid pool being 34 mm and placenta dimidiata was observed (Fig. 1). The blood type of the pregnant is A RhD positive, irregular antibodies I, II, and III were also negative.

Prenatal examination

TORCH examination

The TORCH test for maternal serum showed IgG positive of anti-herpes simplex virus and anti-cytomegalovirus, and negative pathogen IgM in all ranges. TORCH test for fetal cord blood showed negative IgG and IgM of related pathogens. PCR test results showed negative parvovirus B19 and cytomegalovirus as well.

Chromosome analysis

Cord blood karyotype was normal 46, XY. In chromosome microarray analysis, copy number mutations above 100K or other mutations related to known micro-deletion and micro-repeat syndrome were not detected.

Gene test

Short tandem repeat analysis confirmed the biological kinship between the fetus and the parents. No pathogenic changes were detected in thalassemia gene test. WES revealed a de novo heterozygous variant, c.15641G>A (p.

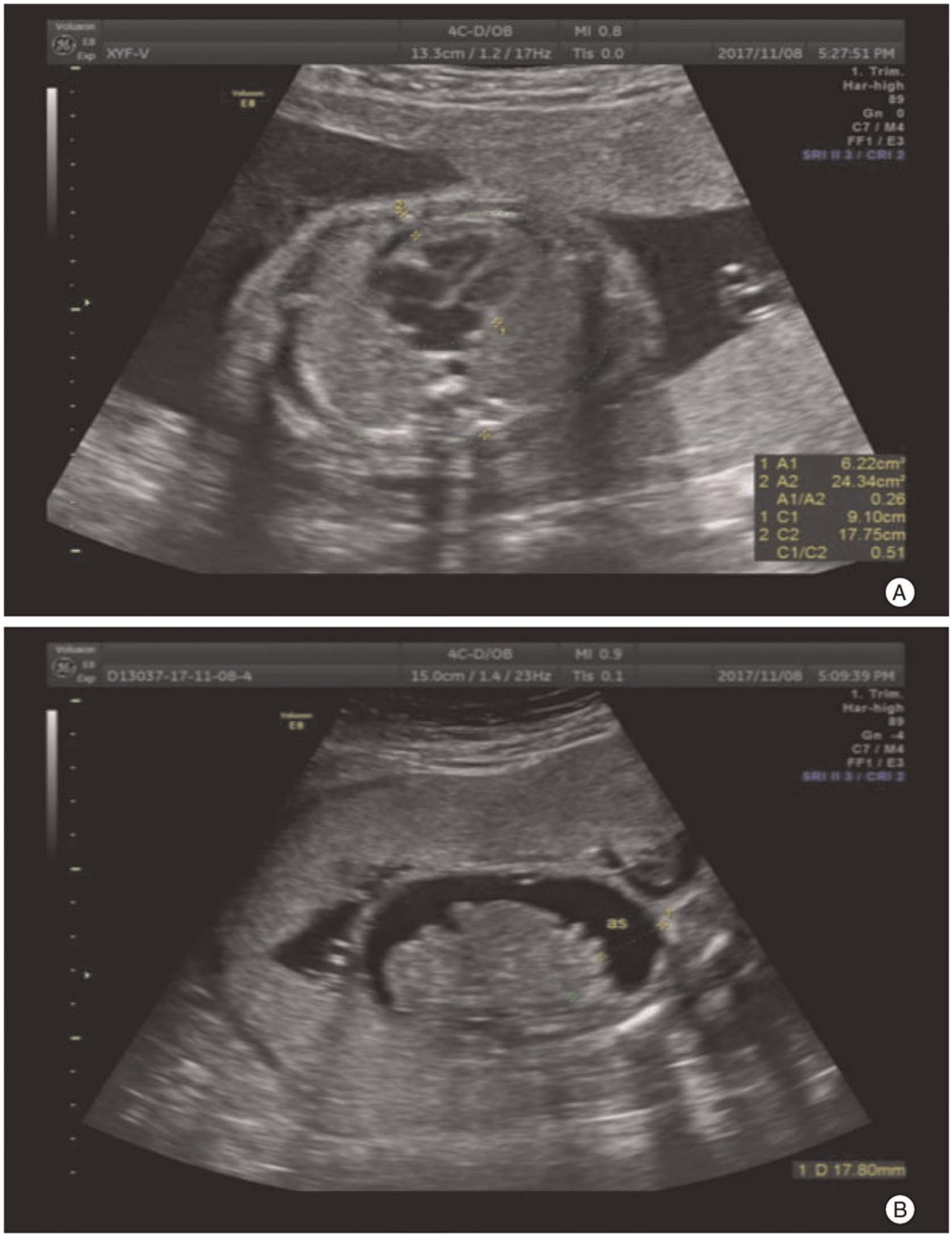

Figure 1. The fetal ultrasonography. A The slight fetal pericardial effusion (yellow circles indicate cardio-thoracic proportion, a small spacing of dark area of liquid besides the cardiac cavity indicates pericardial effusion). B The fetal ascites (yellow line indicates fetal ascites).

R5214H), in exome 48 of the *KMT2D* (NM_003482) gene, which was a missense variation resulting in the conversion of arginine to histidine at amino acid position 5214 in the FYRC domain of C-terminus. This variant was not found in databases of the normal population (1000G/ExAC/gnomAD), and the site was highly conserved between different species. Several bioinformatics software predicted that this mutation was detrimental to the protein function. Previous literates had reported that the *KMT2D* p.R5214H was found in three non-consanguineous KS patients which diagnosed after birth, and was defined as variation unknown significance or likely pathogenic in these studies.[3,10,11] The ClinVar database included this mutation (variation ID: 523665) and annotated it as pathogenic. Based on evidences and referring to ACMG guideline, this mutation was classified as pathogenic (PS1 + PM1 + PM2 + PP3 + PP5). As *KMT2D* mutation related KS is autosomal dominant inheritance, the diagnosis of KS affected was established.

Genetic consultation and follow-up

Prenatal genetic consultation was provided at 30 weeks of gestation. The couple knew clearly the fetal KS involvement. The clinical phenotype of KS and recurrence risks were informed of as well, in order that they could make their own pregnancy decision. However, the patient did not return to our clinic for consultation since then. In two telephone follow-up visits at half a year and 1 year, the pregnant woman told us that the Doppler ultrasound examination at the 34th week of pregnancy in another hospital showed that the fetal ascites regressed and no other obvious structural abnormality were found. The obstetrician at the hospital refused to perform odinopoeia for her because they thought the fetus was healthy and had no indication for termination of pregnancy. A baby boy was delivered by cesarean section at the 38th week of pregnancy. The baby's KS facial features became increasingly obvious 6 months after birth. He had feeding difficulty, hypotonia, and delay of growth and development. Complex congenital heart disease was found by ultrasound examination. The baby was diagnosed clinically as KS by a pediatrician.

Discussion

Approximately 3% of pregnancies will show a fetal structural anomaly in a sonogram, which can range from a single minor defect to severe multisystem anomalies. Determination of the causes of these abnormalities through prenatal diagnosis is critically important on consultation, intervention, and pregnancy decisions.

In this case, the fetus showed multiple ultrasound abnormalities including the presence of ascites, thickening of local skin, and cardiac abnormalities, among which hydrops fetalis was the most notable. Numerous etiologies may lead to nonimmune hydrops fetalis, including genetic defects, intrauterine infection, metabolic abnormalities and structural malformations, and so on. Different cause may lead to different pregnancy outcome. Its diagnosis depends on comprehensive prenatal examination and analysis.[12,13] We carried out examinations for intrauterine infection, thalassemia, karyotype, and chromosome microarray analysis on fetal samples, and the negative results showed that the possibility of fetal edema and other abnormalities caused by the above reasons could be almost completely ruled out. By WES, the de novo heterozygous mutation, c.15641G>A (p. R5214H) of *KMT2D* gene, was detected in the fetus. This mutation was classified as pathogenic, therefore the fetus was diagnosed as KS involvement. The clinical diagnosis results obtained from the follow-up after birth also confirmed our conclusions.

KS is a rare multiple congenital anomaly syndrome caused by epigenetic dysregulation, which has wide range of prenatal and postnatal phenotypes.[14] Mutations in *KMT2D* and *KDM6A* are responsible for most of these patients.[15] Postnatal KS patients have typical clinical features, including distinctive facial features (long palpebral fissures with eversion of the lateral third of the lower eyelid, arched and broad eyebrows, short columella with depressed nasal tip, prominent and/or cupped ears), minor skeletal malformations, dermatoglyphic abnormalities, growth retardation, mild to moderate intellectual disability. Other structural or functional abnormalities may also be found (eg, blue sclera, hearing disorders, urinary system malformations, cardiovascular abnormalities, and immune hypo-function).[1,2] Typical dysmorphic features are key clues for preliminary clinical recognition of KS. However, due to the variable expressivity, broad spectrum phenotypes, and overlapping with other hereditary syndromes (eg, Charge syndrome), the establishment of KS diagnosis needs to combined the clinical phenotype and pathogenic mutations discovery.

There are abundant descriptions and records of genotype-phenotype of postnatal diagnosed KS patients. However, due to vary reports of prenatal diagnosis cases, the knowledge of the prenatal phenotype of KS is still very insufficient. Considering that the majority of KS is caused by de novo mutation, and the parents of the patients are normal childbearing population, it is important to collect more phenotype-genotype data of KS fetuses for supporting prenatal diagnosis, clinical consultation, and pregnancy choice.

Retrospective analysis of the prenatal ultrasound data is an effective approach to obtaining the phenotype-genotype of KS fetuses. Chen *et al.* reviewed the natural history of a series of postnatal diagnosed KS, and found that 69% could be found at least 1 prenatal ultrasound abnormality. The most common types of abnormalities were polyhydramnios (41%), single umbilical artery (31%), congenital heart defects (22%), renal abnormalities (19%) and the rest were lymphocystis, NF thickening, intrauterine growth restriction, edema, oligohydramnios, and short humeral. However, genotypic data are not provided in this article.[14] Long *et al.*[16] reviewed the prenatal ultrasound of 2 KS children caused by *KMT2D* mutations, one of which showed hypoplastic left heart syndrome, hydrothorax, and ascites, the other presented intrauterine growth retardation, single umbilical artery, enhancement of an intestinal echo, dilatation of unilateral ureter, small mandible, NF thickening, and thickening of anterior nasal skin as well as ascites in the middle and late pregnancy periods. In addition, KS may also manifest as cleft lip and palate[17,18] and congenital hydrocephalus[19] during the fetal period. The above literature shows that a large number of KS fetuses have recognizable ultrasound abnormal phenotypes, and these phenotypes are highly heterogeneous.

Recently, prenatal diagnosis of KS cases has been reported. Normand *et al.*[20] detected 146 fetuses with ultrasound abnormalities, of which four cases can be definitely diagnosed as KS involvement, and these were presented mainly as complex cardiac defects (4/4) and renal structural abnormalities (3/4). Lord *et al.*[21] detected 610 fetuses with ultrasound abnormalities, and confirmed three cases of KS. The ultrasound abnormalities were edema, multisystem abnormalities, and cardiac defects respectively. There was still another suspected KS, with ultrasound manifestation of NF thickening. Petrovski *et al.*[22] detected 234 fetuses with ultrasonography abnormalities, and confirmed one case of KS with ultrasonography manifestation of unilateral renal dysplasia, intrauterine growth retardation, lumbar hemiverte-bra, and complex cardiac defects. The above KS cases were all caused by *KMT2D* de novo mutations, but showed a wide phenotype spectrum. The current strategy of clinical genetic diagnosis adopting genomic sequencing is phenotype-driven analysis, with the application of WES in prenatal diagnosis of fetal ultrasound abnormalities, the accumulation of more prenatal phenotypic-genotypic data will contribute to the analysis and interpretation of the results.

It is worth noting that the symptom of our case was relatively severe between late second trimester and early third trimester, but greatly relieved or even disappeared in late third trimester (after the 34th week). The remission of ultrasonic manifestations results in the obstetricians and patient neglecting or doubting the results of genetic diagnosis, which eventually leads to the unexpected KS birth. This outcome suggests that not only comprehensive detection strategy should be adopted for prenatal diagnosis of ultrasound abnormalities, but also multidisciplinary cooperation and communication are necessary for providing explicit pregnancy guidance to patients.

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Conflicts of Interest

None.

Data Availability

Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

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