Infrequent Manifestations of Kabuki Syndrome in a Patient with Novel *MLL2* Mutation

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Key Words

Diaphragmatic hernia · Hypoglycemia · Kabuki syndrome · *MLL2*

Abstract

We present a case of a 9-month-old Hispanic female with Kabuki syndrome with some infrequent manifestations including a single umbilical artery, butterfly vertebrae, a small larynx, a preauricular pit, microtia with internal ear abnormalities, abnormal calcium metabolism, premature thelarche, neonatal/persistent hypoglycemia and eventration of the diaphragm. She was found to have a previously unreported nonsense *MLL2* mutation. This is the first case that includes all such findings occurring simultaneously that was genotyped.

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Kabuki syndrome (KS) is a rare multiple congenital anomaly disorder characterized by a combination of 5 main criteria that include mild-to-moderate intellectual disability, postnatal short stature, dermatoglyphic abnormalities, craniofacial anomalies with characteristic dysmorphic features, and skeletal findings [Niikawa et al., 1981; Geneviève et al., 2004]. Other less common mani-

festations include early breast development in infant girls (25%), neonatal hypoglycemia (7–8%), persistent hypoglycemia (1%), and diaphragmatic defects (4–8%) [Niikawa et al., 1981; Geneviève et al., 2004; Adam and Hudgins, 2005; Armstrong et al., 2005]. Recently, mutations in the *MLL2* gene were identified in KS patients. This gene has been found to be mutated in approximately 70% of patients tested [Ng et al., 2010; Hannibal et al., 2011; Micale et al., 2011; Paulussen et al., 2011; Banka et al., 2012]. Here we present a case with all of the above mentioned infrequent manifestations, an unreported *MLL2* mutation and a variant of unknown clinical significance.

Patient and Methods

Genomic DNA was isolated from lymphocytes using standard procedures. All 54 coding exons of the *MLL2* gene were amplified by polymerase chain reaction (PCR). PCR products were visualized on a 2% agarose gel and then purified using Qiaquick® PCR Purification Spin Columns (Qiagen, Valencia, Calif., USA). The products were then sequenced by a standard protocol using the Big Dye® Version 2 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA). Both strands were sequenced to confirm alterations. The reference mRNA sequence used is NM_003482.3, with codon 1 corresponding to the ATG start codon. Primer sequences are available upon request.

Fig. 1. Left: patient at 9 months of age with typical dysmorphic features. Right, top: right-sided microtia. Right, middle: hypoplastic 5th fingernail. Right, bottom: fingertip pads.

Clinical Report

The female patient was born at 36 weeks' gestation by cesarean section to non-consanguineous Hispanic parents. Her mother was 40 years old, but no amniocentesis was performed. Prenatal and family histories were unremarkable. Birth weight was 3,145 g (75–90th centile), length 46 cm (25–50th centile), and head circumference 32 cm (25–50th centile). During the neonatal period, she was noted to have right-sided microtia, hypoplastic 5th toenails, a single umbilical artery, and butterfly vertebrae. There was a history of brief thrombocytopenia that improved without intervention. The patient had dysphagia and slow growth requiring a temporary gastrostomy tube placement. Other studies and procedures that were done in the 1st few weeks of life include a flexible laryngoscopy that documented an unusually small larynx, an echocardiogram with a patent ductus arteriosus and a patent foramen ovale, a temporal bone CT that showed findings of right aural atresia with likely fused right malleus and incus, a renal ultrasound that showed an unusually small left kidney, and a normal brain MRI, including pituitary gland. At the age of 9 months she had global developmental delay. Her weight was 6.95 kg (<3rd centile), length 64.7 cm (<3rd centile) and head circumference 42 cm (3rd–10th centile). Dysmorphic facial features included long palpebral fissures with long eyelashes, arched eyebrows that were sparse laterally, left-sided ptosis, a hemangioma on her upper eyelids, right-sided microtia with atresia of the ear canal, left-sided preauricular pit, thin upper lip with micrognathia, and a high and narrow palate (fig. 1). She also had gynecomastia, toe overlap, 5th digits with hypoplastic nails, brachydactyly, and distal joint hypermobility with a fleshy texture of her skin. The fingertip pads were not particularly prominent, but her neurological examination was significant for generalized hypotonia.

On day of life 3, chest X-rays showed a density at the base of the right thorax, followed by an ultrasound of the chest that confirmed the finding and suggested a protrusion of a portion of the right lobe of the liver into the lower chest. At surgery, the diaphragm

Fig. 2. Sequencing analysis of the *MLL2* gene. The sequencing analysis detected 2 previously unreported changes. The first alteration, c.9931C>T (p.Q3311X), is shown in **A**. The second change, shown in **B**, is a c.10101G>T (p.L3367F). Neither change was detected in the parents of the proband, indicating that both alterations are de novo. Asterisks: mutant and normal nucleotide in the proband and the parents, respectively.

and the liver were found to be adhered in the underside and with a membrane covered defect at the fascial level, consistent with an eventration rather than a hernia. The defect was repaired without any difficulties. The patient also had neonatal hypoglycemia and needed high intravenous glucose concentrations to maintain euglycemia. Her calcium levels were low during the 1st few days, and she needed several boluses of intravenous calcium before stabilizing. Blood growth hormone, vitamin D, thyroid hormone and cortisol levels were within normal results. However, insulin levels were inappropriately elevated, and she was started on diazoxide on day of life 7 that she continues to receive to the present day.

Results

High-resolution chromosome analysis and a whole-genome array CGH using Affymetrix Genome wide SNP 6.0® array (Calif., USA) platform did not reveal any

Fig. 3. Allele-specific PCR and sequencing analysis of the *MLL2* gene. A forward PCR primer was designed to be specific for the c.9931C>T (p.Q331X) mutation in the *MLL2* gene. PCR utilizing this allele-specific primer amplified only the patient's DNA (upper panel: lane 1, 100-bp ladder; lane 2, DNA from a normal control; lane 3, DNA from the proband; and lane 4, no DNA control). Sequence analysis of the proband's allele-specific PCR product detected the c.10101G>T (p.L3367F) variant (asterisk), indicating that both alterations are in cis (lower panel).

abnormalities. *MLL2* sequencing revealed 2 previously unreported changes. The first is a C>T transition at nucleotide 9,931 (c.9931C>T) which results in the substitution of a stop codon for a glutamine at amino acid 3,311 (p.Q331X) (fig. 2A). This mutation occurs in a glutamine-rich region of exon 34. Although this nonsense mutation has not been previously reported, it is expected to result in a truncated protein. The second alteration is a c.10101G>T which results in a missense change (p.L3367F) (fig. 2B). This specific nucleotide change is not listed in the Human Gene Mutation Database, dbSNP, the Exome Variant Server or the 1000 Genome Browser; however, a different nucleotide change (c.10101G>C; rs148143899) which results in the same amino acid substitution is listed in both dbSNP and the 1000 Genome Browser occurring on a total of 2 alleles (<http://www.hgmd.cf.ac.uk/ac/index.php>; <http://www.ncbi.nlm.nih.gov/snp/>; <http://evs.gs.washington.edu/EVS/>; <http://browser.1000genomes.org>). It should also be noted that the p.L3367F alteration is predicted by both PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>) and SIFT (<http://sift.jcvi.org/>) algorithms to be a benign variant. Neither of the changes was identified in the proband's

parents. To determine whether the alterations were in cis or trans in the patient, allele-specific PCR was performed and followed by sequence analysis. The results indicated that the alterations are present on the same allele (fig. 3).

Discussion

The patient presented here had many of the common findings and medical complications that can be seen in KS. These include the typical facial appearance, postnatal growth deficiency with dysphagia, developmental delay, cardiac defects, hypotonia, palate abnormalities, joint hypermobility, and skeletal anomalies. Less frequent clinical features also seen in this patient include a single umbilical artery, butterfly vertebrae, premature thelarche, and neonatal hypocalcemia. Furthermore, our patient had neonatal hypoglycemia that persisted several months due to hyperinsulinism and a diaphragmatic eventration. These last 2 findings have been reported at a low frequency in patients with KS [Philip et al., 1992; Ewart-Toland et al., 1998; Wilson, 1998; Donadio et al., 2000; van Haelst et al., 2000; Bereket et al., 2001; McGaughan et al., 2001; Geneviève et al., 2004; Adam and Hudgins, 2005; Armstrong et al., 2005; Ma et al., 2005; Vaux et al., 2005; Sethi and Faridi, 2006; Abdel-Salam et al., 2008].

In addition to hyperinsulinism, the only other previously reported cause of hypoglycemia is isolated ACTH deficiency, but in many other cases of either neonatal or persistent hypoglycemia, the cause remains unknown [Philip et al., 1992; Ewart-Toland et al., 1998; Wilson, 1998; Bereket et al., 2001; Geneviève et al., 2004; Armstrong et al., 2005; Ma et al., 2005]. Diaphragmatic defects have also been rarely reported in KS and include congenital diaphragmatic hernia and unilateral or bilateral eventration of the diaphragm [Lynch et al., 1995; van Haelst et al., 2000; McGaughan et al., 2001; Adam and Hudgins, 2005; Vaux et al., 2005; Abdel-Salam et al., 2008]. The frequency has been estimated to be low (4–8%) but more common than originally suspected, particularly in non-Asian female patients, like the present case [Donadio et al., 2000; Adam and Hudgins, 2005].

MLL2 regulates the expression of several genes that are implicated in cell adhesion, cytoskeleton organization, growth impairment and cell motility and have an important role during early embryogenesis and organogenesis [Issaeva et al., 2007; Glaser et al., 2009]. Among those regulated genes, there are different members of the *HOX*

complex family, an important group of mesenchymally derived master regulatory genes that control organ-specific axial patterning of emerging embryonic tissues from the 3 germ layers and have been implicated in a variety of embryological developmental abnormalities, including congenital diaphragmatic hernias [Volpe et al., 2003; Glaser et al., 2006; Martínez et al., 2007; Mallo et al., 2010]. Furthermore, *MLL2* is also known to interact with estrogen receptor- α , one of the mediators of the biological effects of estrogen in estrogen responsive tissues, and transcriptionally regulates *HOXC6*, a critical player in mammary gland development. This provides a potential biological mechanism for the premature thelarche seen in our patient and occasionally in KS [Mo et al., 2006; Ansari et al., 2011].

MLL2 haploinsufficiency seems to be the more likely mechanism responsible for KS [Ng et al., 2010]. Most variants in the *MLL2* gene have been de novo, as is in our case [Ng et al., 2010; Hannibal et al., 2011; Micale et al., 2011; Paulussen et al., 2011; Banka et al., 2012]. In our patient, 2 separate previously unreported changes were identified. In line with the previous studies which showed that nonsense mutations are the most frequent type of variants, we found a nonsense change, c.9931C>T (p.Q3311X), predicted to be pathogenic. A missense change in exon 34 of the *MLL2* gene, c.10101G>T (p.L3367F), was also identified in the present case in cis configuration with the nonsense mutation. Although likely benign, this specific nucleotide change has not

previously been reported. Even if the alteration was able to affect protein function in some regard, the fact that the nonsense mutation occurs earlier in the gene would likely negate any potential impact by this missense change.

Initial attempts for a genotype-phenotype correlation showed that patients with KS and a *MLL2* mutation were more likely to have characteristic facial features, dysphagia, urogenital anomalies, early breast buds, joint dislocations, cleft palate, and growth retardation compared to those clinically diagnosed who did not have any mutation [Hannibal et al., 2011; Paulussen et al., 2011; Banka et al., 2012]. A larger group of patients with *MLL2* testing and phenotype description should be done to establish potential correlations.

In summary, we report a case of a patient with many infrequent findings that do not particularly lead the clinician to make the diagnosis of KS based on the phenotype alone such as neonatal hypoglycemia and hypocalcemia, persistent hypoglycemia, premature thelarche, diaphragmatic eventration, severe microtia with aural atresia and middle ear abnormality. The presence of some of these unusual clinical findings in patients with KS can be particularly helpful in the early recognition of this syndrome before the more recognizable facial features or medical findings develop. This case also illustrates the importance of molecular confirmation in cases with atypical presentation.

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