Report of the First Clinical Case of a Moroccan Kabuki Patient with a Novel *MLL2* Mutation

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Key Words

Kabuki syndrome · *MLL2* · Splicing mutation

Abstract

Kabuki syndrome (also known as Niikawa-Kuroki syndrome) is a rare autosomal disorder, characterized by an unusual face, short stature, skeletal, visceral and dermatoglyphic abnormalities, cardiac anomalies, mental retardation, and immunological defects. Point mutations and large intragenic deletions and duplications of the mixed lineage leukemia 2 (*MLL2*) and exons deletions of lysine demethylase 6A (*KDM6A*) genes have been identified as its underlying causes. We report on the first description of a Moroccan Kabuki syndrome patient with typical facial features, developmental delay, finger pads, and other anomalies carrying a novel splice site mutation in the *MLL2* gene that produces a truncated and likely pathogenetic form of *MLL2* protein.

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Kabuki syndrome (KS, OMIM 147920), described by two independent groups of Japanese scientists [Kuroki et al., 1981; Niikawa et al., 1981], is a rare autosomal domi-

nant disorder. KS is characterized by facial features, including long palpebral fissures with eversion of the lateral third of the lower eyelids, short columella with a broad and depressed nasal tip, prominent ears, and a cleft or high-arched palate, postnatal short stature, skeletal anomalies, moderate mental retardation, and dermatoglyphic anomalies [Adam and Hudgins, 2005]. Other clinical findings, including cleft lip and/or cleft palate, anorectal anomalies, precocious puberty, lower lip pits, hearing loss, inner ear abnormalities, craniosynostosis, and central nervous system anomalies have also been observed [Niikawa et al., 1988; Matsumura et al., 1992; Franceschini et al., 1993; Kokitsu-Nakata et al., 1999; Igawa et al., 2000; Petzold et al., 2003; Abdel-Salam et al., 2008; Lodi et al., 2010; Martínez-Lage et al., 2010; Yoshioka et al., 2011]. KS is mainly due to loss-of-function mutations of the mixed lineage leukemia 2 (*MLL2*) gene at 12q12q13.12, identified by a whole exome sequencing approach [Ng et al., 2010]. *MLL2* encodes a large protein that belongs to the SET1 family of human SET-domain protein methyltransferase superfamily. Specifically *MLL2* methylates the lysine 4 of histone H3 (H3K4), an epigenetic marker correlated with transcriptional active chromatin [Issaeva et al., 2007], regulating a set of genes in-

Fig. 1. Patient with dysmorphic features.

involved in embryogenesis and development [Ansari and Mandal, 2010]. Recently, de novo partial or complete deletions of the Xp11.23 located gene, lysine demethylase 6A (*KDM6A*), have been identified as additional causes of KS, confirming the genetic heterogeneity of the disease [Lederer et al., 2012], although these genetic events appear extremely rare in the pathogenesis of KS [Priolo et al., 2012]. *KDM6A* demethylates di- and trimethyl-lysine 27 on histone H3 maintaining embryonic stem cell pluripotency and plasticity during embryonic development and X chromosome inactivation [Hong et al., 2007]. *KDM6A* and *MLL2* act together in the epigenetic control of transcriptionally active chromatin by counteracting polycomb-group (PcG) proteins that are involved in the tri- and dimethylation of H3K27 [Schuettengruber et al., 2007].

Here we describe the first Moroccan KS patient with typical facial features characteristic for the syndrome, developmental delay and fetal fingertip pad. This patient carries a novel mutation in the *MLL2* gene that results in abolishment of a splice donor site, leading to aberrant RNA isoform with consequent production of a truncated *MLL2* protein.

Case Report

A 2-year-old boy was referred for medical genetics consultation because of the association of a dysmorphic facies and soft cleft palate. He is the firstborn of a healthy consanguineous couple (second degree), a 33-year-old mother and 38-year-old father, with no rel-

Table 1. Clinical signs of the patient

Craniofacial abnormalities

Eye abnormalities

- Arched eyebrows
- Long palpebral fissures
- Lower palpebral eversion
- Long eyelashes
- Epicanthus

Nasal abnormalities

- Depressed nasal tip
- Short nasal septum

Ear abnormalities

- Prominent ears
- Detached ears

Oral abnormalities

- Soft cleft palate
- Micrognathia

Microcephaly

Postnatally reduced weight and growth

Neurologic abnormalities

- Hypotonia
- Myopathic facies
- Psychomotor and developmental delay

Skeletal abnormalities

- Brachydactyly of hands
- Short fifth middle phalanges
- Short fifth fingers
- Short metacarpals
- Brachydactyly of feet
- Short metatarsals

Fingertip pads

Genitourinary abnormalities

- Micropenis

Susceptibility to infections

- Recurrent upper respiratory infections

evant familial history. The pregnancy was not medically followed, but it was reported without complications. At birth, he weighed 3,500 g; he had a length of 52 cm and 36 cm for head circumference. He presented neonatal hypotonia. His parents reported psychomotor and developmental delay and an increased susceptibility to infections with recurring upper respiratory tract infections during his 2 first years of life.

Clinical examination at 2 years of age showed a delay in growth development with a weight of 12 kg (25th centile), height of 81.5 cm (3rd–10th centile) and an OFC of 47.8 cm (25th centile), respectively. He presented with myopathic facies, long palpebral fissures, lower palpebral eversion, arched eyebrows, epicanthus, long eyelashes, depressed nasal tip, short nasal septum, micrognathia, and prominent and detached ears (fig. 1). He also had soft cleft palate scheduled for a surgical treatment, brachydactyly of hands and feet, short fifth fingers and finger pads. The urogenital examination showed a micropenis with a penile length of 3 cm (10th centile). He presented with developmental delay; he was still walking with support, and his speech was delayed. The clinical signs are summarized in table 1. X-rays of the skull, cardiac and abdomino-

Fig. 2. Scheme explaining the effect of the c.4693+1G>A mutation identified in the reported patient. Schematic representation of the *MLL2* gene region between exons 16 and 19, including the c.4693+1G>A splice site mutation. Numbers indicate exons; larger boxes indicate coding region; horizontal lines indicate introns. A scheme showing the structure of wild-type (**A**) and aberrant (**B**) open reading frame. Wild-type and mutant *MLL2* protein domain structures are shown. The relevant donor and acceptor splice sites are indicated for the wild-type and mutated sequences. Arrows

indicate the region of the primers used in the RT-PCR experiments. The electropherograms show the wild-type sequence in the normal individuals and the c.4693+1G>A heterozygous mutation in the patient. PHD = Plant homeodomain finger; HMG-box = high mobility group; CC = coiled coil; FYRN = FY-rich domain, N-terminal region; FYRC = FY-rich domain, C-terminal region; SET = Su(var)3-9, Enhancer-of-zeste, Trithorax domain; post SET = post SET domain.

genito-urinary ultrasonographies were normal (data not shown). The results of laboratory examinations were within normal limits, and a chromosomal investigation revealed a normal male karyotype, 46,XY. On clinical examination, both the parents and their second sibling of 9 months were normal.

Informed consent was obtained from the proband's parents prior to implementation of the molecular assay. Peripheral blood was collected from the affected child and his parents. Molecular genetic testing for suspected KS was performed by complete DNA sequence analysis of the entire coding region and flanking introns

of the *MLL2* gene, as reported previously [Micale et al., 2011]. This led to the identification of the de novo heterozygous donor splice site variant c.4693+1G>A in intron 17 (fig. 2). The variant was not found in the 1,000 genome database (<http://www.1000genomes.org/>) nor in the Exome Variant Server, suggesting that it is a rare event with potential pathological effect. The c.4693+1G>A splice site variant was evaluated for putative alteration of regulatory process at the transcriptional or splicing level with NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2>) and NNSPLICE (http://www.fruitfly.org/seq_tools/splice.html). This variant was predict-

ed to alter the splicing process by abolishing the donor splice site in the intron 17. RT-PCR and direct sequencing of amplified fragments from RNA prepared from the proband showed that the mutation activates a cryptic donor splice site in the exon 17, leading to a frameshift transcript with the generation of a truncated MLL2 protein (fig. 2).

Discussion

KS is characterized by a typical dysmorphic appearance together with cognitive and growth delay, in addition to a variety of anomalies [Niikawa et al., 1981].

To date, approximately 300 distinct *MLL2* mutations have been characterized in patients with KS. The majority of *MLL2* mutation-positive KS patients carry truncating mutations, including nonsense mutations, deletions, insertion of a single base, and small duplications. Splice-site, frameshift and missense mutations have also been described. The majority of them was de novo and predicted to cause pathology on the basis of haploinsufficiency or nonfunctional MLL2 protein. In accordance with its biological role, the lack of functional MLL2 protein impairs the histone methylation pathway affecting proper activation of certain genes in many organs and tissues that could result in some of the abnormalities of postnatal development of KS.

In this study, we reported the molecular characterization of an intronic c.4693+1G>A *MLL2* variant in a 2-year-old Moroccan boy with KS; to the best of our knowledge, it had never been documented before. This mutation occurred de novo because it was not revealed in hematologic cells of the parents. However, a gonadal mosaicism could not be excluded. The risk for an individual family's germ cell mosaicism depends on the percentage of affected gametes in the germ cells of the parent with mosaicism; therefore, gonadal mosaicism may have important implications in genetic counseling, though famil-

ial KS recurrence by germinal mosaicism has never been reported till date.

Computational prediction confirmed by experimental evidences showed that the *MLL2* identified variant leads to a premature codon stop in exon 18. It was well documented that genetic variants in disease-responsible genes that disrupt the splicing code have a key role in human hereditary disorders [Cartegni et al., 2002; Wang and Cooper, 2007]. In fact, it has been estimated that more than 60% of sequence variations are associated with splicing aberrations that should be considered as a primary mechanism of gene inactivation [López-Bigas et al., 2005].

The predicted protein lacks the methyltransferase activity conferred by SET domain of the MLL2 protein, strongly suggesting that this variant affects the physiological function of MLL2 altering the epigenetic and transcriptional regulatory properties of the protein. This notion supports that the c.4693+1G>A splice site mutation is likely the cause of the phenotype observed in our patient. Nevertheless, leukocytes are the main source of RNA; caution should be taken when interpreting these results, since differential tissue-specific alternative splicing events could mask the real splicing outcome of a DNA variant.

Our report of the first Moroccan case with KS emphasizes that *MLL2* mutations are a major cause of KS.

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