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Analysis of *MLL2* gene in the first Brazilian family with Kabuki syndrome

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Abstract

Most cases with Kabuki syndrome (KS) have been reported sporadically, however familial cases of KS are described showing evidence that this syndrome is inherited as a dominant trait with variable expressivity. We report on two related individuals with facial findings characteristic of Kabuki syndrome. The proband had arched eyebrows, long and upward slanting palpebral fissures, cleft lip and palate, retromicrognathia, brachydactyly of hands and feet, stubby fingers, nail hypoplasia, and prominent finger pads. Her mother had, as main characteristics, eyebrows with dispersed lateral half, long and upward slanting palpebral fissures, retrognathia, dysplastic and posteriorly rotated ears, prominent finger pads, brachydactyly of feet, learning difficulties, and psychomotor development delay. DNA sequencing revealed a novel missense mutation in the *MLL2* gene of both the proband and her mother. The mutation (R5432Q) was found in the exon 51, within the SET domain of the gene, which confers methyltransferase activity on the protein. Therefore the epigenetic and transcriptional regulatory properties of this protein may be altered and may be the cause of phenotype observed in both the proband and her mother. These clinical signs and the molecular evidence in this family further support the evidence for KS having a dominant inheritance pattern with variable expressivity. To our knowledge this is the first report of a Brazilian family with recurrence of this syndrome.

Keywords

Kabuki syndrome; dominant inheritance; familial recurrence; *MLL2*

Introduction

Kabuki syndrome (KS; OMIM 147920) was recognized and proposed by Niikawa et al. [1981] and Kuroki et al. [1981] in Japan. This condition is characterized by cognitive and growth retardation with specific craniofacial malformations including arched eyebrows sparse in the lateral halves, long eyelashes, a long palpebral fissures with ectropion of the lateral third of the lower eyelids, a broad and/or depressed nasal tip.

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Other common signs are skeletal abnormalities, unusual dermatoglyphic patterns, mild to moderate mental retardation, hearing loss, and postnatal growth retardation [Halal et al., 1989]. Clinical findings such as cleft lip and/or cleft palate, anorectal anomalies, precocious puberty, lower lip pits, 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 GM et al., 2008; Martínez-Lage et al., 2010; Lodi et al., 2010; Yoshioka et al., 2011].

In 2010 Ng et al. published a whole exome sequencing approach to detect genes in which mutations might cause Kabuki syndrome and identified loss-of function mutations in the mixed-lineage leukemia 2 (*MLL2*) gene in nine out of ten cases studied in the discovery cohort. As a follow up, they screened *MLL2* in an additional 43 Kabuki cases to ultimately characterize 33 distinct mutations, and therefore confirming that this gene (*MLL2*) is a compelling candidate for Kabuki syndrome [Ng et al., 2010]. Since *MLL2* has been identified as a candidate gene for KS more than 150 distinct mutations have been characterized in patients with KS [Ng et al., 2010; Hannibal et al., 2011; Li et al., 2011; Micale et al., 2011; Paulussen et al., 2011].

MLL2 is a large gene that encodes a 5,262 amino acid protein that belongs to the SET protein family [Prasad et al., 1997]. *MLL2* is a major transcriptional and epigenetic regulator due to its histone methyltransferase activity that specifically methylates the Lysine-4 position of histone H3 [Issaeva et al., 2007]. The SET domain of *MLL2*, contained in coding regions 51-53, confers the methyltransferase activity of this protein [Issaeva et al., 2007]. Mutations in this region have already been characterized in cases of Kabuki Syndrome.

In this paper we describe the first Brazilian family with recurrence of typical facial of Kabuki syndrome that presents with mutation in *MLL2* on both affected individuals, further supporting the evidence that KS is inheritance as an autosomal dominant trait with variable expressivity.

Clinical Report

The proband (Fig. 1A-B) is a 5 months old Brazilian girl. She was born by Cesarean section at 37 weeks of gestation complicated by bleeding in the first trimester and urinary tract infection in the first and last trimester. The parents were nonconsanguineous 18-year-old, G1P1 mother and a 21-year-old father. Her birth weight was 2,050 g (<3rd centile), length was 44 cm (3rd-10th centile) and head circumference (OFC) of 31 cm (<2nd centile). Clinical examination, at age of 5 months and 8 days showed weight, height and OFC as 6,250 g (25th centile), 59.8 cm (3rd-10th centile), and 40 cm (2nd-50th centile), respectively. She presented with arched eyebrows with dispersed lateral halves, long and upward slanting palpebral fissures, epicanthus, long eyelashes, broad and depressed nasal tip, short nasal septum, cleft lip and palate, retromicrognathia, brachydactyly of hands and feet, stubby fingers, nail hypoplasia, and prominent finger pads. The cleft lip was repaired at age of 5 months and 18 days and cleft palate was repaired at age of 13 months. Reevaluation at 13 months (Fig. 2A-D) showed weight of 8,600 g (3rd-10th centile), stature of 73 cm (3rd centile), and head circumference of 44.5 cm (2nd-50th centile). She has global developmental delay and at age 18 months she does not walk and her speech is delayed. Cytogenetic analysis and X-rays of the skull were normal.

Family History

This was the first child of the couple. The mother (Fig. 5; IV-5) presents some facial patterns resembling her daughter. The father (Fig. 5; IV-4) is normal. The paternal grandfather (Fig. 5; III-1) had cleft lip. The mother has a normal dizygotic twin sister (Fig. 5; IV-6) and two cousins in second-degree (Fig. 5; IV-12, IV-13) with cleft lip and palate and cleft lip, respectively.

Physical examination of the mother (Fig. 3A-B; Fig. 4) revealed a similar facial appearance, including eyebrows with dispersed lateral half, long and upward slanting palpebral fissures, lateral incisor agenesis on the right, retrognathia, dysplastic and posteriorly rotated ears, skin hyperpigmentation in the preauricular area at left, prominent finger pads, clubfeet, brachydactyly of feet, learning difficulties, and psychomotor development delay. Additional abnormalities included speech delay, hypernasal voice, and congenital dislocation of the hips. Because of several orthopedic procedures for the hip dislocation, she was late to walk (four years age). At age 19 years, her weight was 38 kg (<3rd centile), stature was 155 cm (10thcentile), and head circumference was 52.5 cm (2nd centile). Renal ultrasound scans showed agenesis of the left kidney. Cytogenetic analysis was normal.

Molecular Genetics Report

Materials and Methods—Templates for sequencing of all exons of *MLL2* were generated by polymerase chain reaction (PCR) in an Applied Biosystem Gene Amp PCR System 9700 (Applied Biosystem, Foster City, CA). Sequencing then was performed at Functional Biosciences, Inc, Madison, WI (<http://www.functionalbio.com/index.htm>) following in-house protocols.

Results

PCR amplification and sequence analysis of all 54 coding regions in the proband revealed a novel missense mutation in exon 51 of the *MLL2* gene. PCR amplification and sequencing of this coding region with the mother's DNA confirmed the presence of the same mutation changing the amino acid arginine (R) to glutamic acid (Q) at the position 5432 (R5432Q) in the middle of the exon 51 and therefore within the SET domain of the *MLL2* protein. No other mutations were found.

Genetic analysis of the family showed a single missense mutation (R5432Q) c.16295G>A in the *MLL2* gene of both the proband and her mother (Fig. 6), DNA from grandparents was not available for analysis. The missense mutation was not found in any sample present in the 1000 genomes database (<http://www.1000genomes.org/>) nor the Exome Variant Server (<http://evs.gs.washington.edu/EVS/>) that includes data of over 2000 samples suggesting that it is a rare event with potential etiological effect. Analysis of functional effect prediction with Polyphen revealed an unknown effect of the mutation to the protein, however analysis made with SIFT predicts that Arginine (R) is the only aminoacid tolerated in this position of the protein, therefore any change can be damaging. The location of this mutation within the methyl transferase conferring SET domain of the *MLL2* protein not only supports an autosomal dominant pattern of inheritance in Kabuki syndrome, but also that *MLL2* mutations are a major cause of Kabuki syndrome as suggested by some authors Ng et al. [2010] and Li et al. [2011].

Discussion

The cardinal characteristics of the Kabuki syndrome include moderate-to-severe mental retardation, postnatal growth retardation, and facial dysmorphism. Multiple cases with

Kabuki syndrome have now been reported with most as sporadic cases. Autosomal dominant inheritance of this condition was suggested firstly by Kuroki et al. [1981] and later by McKusik [1986] and Niikawa et al. [1988]. The first familial occurrence reported of the Kabuki syndrome was described by Halal et al. [1989]. These authors reported familial occurrence of Kabuki syndrome in which father and his son and daughter were affected, suggesting autosomal dominant inheritance. Posteriorly, other familial cases with Kabuki syndrome suggesting autosomal dominant inheritance pattern were described in the literature [Silengo et al., 1991; Say et al., 1993; Ilyina et al., 1995; Galán-Gomez et al., 1995; Kobayashi and Sakuragawa, 1996; Tsukahara et al., 1997; Wilson, 1998; Courtens et al., 2000; Frediani et al., 2001; Shotelersuk et al., 2002; How, 2004; Pottinger et al., 2009]. Therefore, to our knowledge sixteen familial cases have been reported so far, showing large inter-and intrafamilial clinical variability in this condition. Interestingly, cleft lip and palate was observed in unique case reported by Hou [2004].

On the other hand, cleft palate was the predominant orofacial cleft type observed in some described cases [Ilyina et al., 1995; Tsukahara et al., 1997; Wilson, 1998; Shotelersuk et al., 2002; How, 2004; Pottinger et al., 2009].

Our case presents typical facial appearance and clinical findings of Kabuki syndrome associated with cleft lip and palate. Phenotypic similarities were observed in her mother, supporting the evidence for a dominant autosomal trait. Although the mother does not have cleft lip and palate, she presents other clinical features, beyond those in common with her daughter, including kidney agenesis, and congenital dislocation of the hips. Niikawa et al. [1988] reported clefts of all types in their patients. According to report of Burke and Jones [1995] and Schrandt-Stumpel et al. [2005], most patients had cleft palate (51%) and cleft lip/palate was rare. It is possible that cleft lip and palate present in our proband is part of the phenotypic characteristic of Kabuki syndrome. On the other hand should not be ruled out the possibility of the purpose had cleft lip and palate as multifactorial inheritance, since there are relatives with this anomaly.

Our case represents the first familial Brazilian case reported, and also an additional case in the literature emphasizing the possibility of autosomal dominant inheritance with variable expressivity in Kabuki syndrome.

Although the prediction of functional effect with Polyphen revealed an unknown effect of the mutation to the protein, the location of this mutation within the methyl transferase conferring SET domain of the *MLL2* protein not only supports an autosomal dominant pattern of inheritance in Kabuki syndrome, but also that *MLL2* mutations are a major cause of Kabuki syndrome as suggested by some authors Ng et al. (2010) and Li et al (2011).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
A-B: Frontal and lateral view of the proband at age 5 months.

Figure 2.
A-D: Clinical aspects of proband at age 13 months. Note facial aspects, brachydactyly of hands and feet, prominent finger pads, and nail hypoplasia.

Figure 3.
A-B: Frontal and lateral view of the proband's mother.

Figure 4.
Note similar facial features of the proband and her mother.

Figure 5.
Pedigree of the family.

Figure 6.
Chromatogram showing the R5432Q mutation.