Kabuki Syndrome: Identification of Two Novel Variants in *KMT2D* and *KDM6A*

Mehrnoosh Khodaeian^a Ehsan Jafarinia^b Fatemeh Bitarafan^a
Shohreh Shafeii^c Navid Almadani^d Mohammad Ali Daneshmand^e
Masoud Garshasbi^b

^aDepartment of Medical Genetics, DeNA Laboratory, Tehran, Iran; ^bDepartment of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Teheran, Iran; ^cDr. Shafeii Genetic Counseling Center, Arak, Iran; ^dDepartment of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran; ^eDaneshmand Pathology Laboratory, Arak, Iran

Established Facts

- About 50–80% of the Kabuki syndrome cases are caused by mutations or large deletions in the *KMT2D* gene.
- Most of the mutations in the *KMT2D* gene are de novo and act in dominant form.
- The main causative mutations reported in *KMT2D* are protein-truncating variants.
- Kabuki syndrome is also caused by point mutations or microdeletions in *KDM6A* which is located at Xp11.3.
- Both of the *KMT2D* and *KDM6A* proteins are histone modifiers, and they play an important role in the regulation of chromatin remodeling and transcription activation of downstream genes.

Novel Insights

- The Glu1661Serfs*61 frameshift variant in the *KMT2D* gene that we identified in patient 1 is expected to result in a truncated protein.
- The identified missense X-linked variant, Glu1101Lys, in patient 2 is affecting a highly conserved domain of *KDM6A*.
- Both of the variants are not reported in dbSNP version 150, 1K genome project, ExAC, ESP, gnomAD, Iranome, HGMD, or ClinVar databases.

Keywords

KMT2D · *KDM6A* · Kabuki syndrome · Whole-exome sequencing · Novel variants

Abstract

Kabuki syndrome (KS) is a rare genetic disorder characterized by the following 5 crucial symptoms: dysmorphic facial features, growth retardation, skeletal abnormalities, intellectual disability, and dermatoglyphic malformations. Studies

show that most of the KS cases are caused by mutations or large deletions in the *KMT2D* gene, while the other cases show mutations in *KDM6A*. We studied 2 patients with suspected KS in 2 unrelated families by whole-exome sequencing to identify the possible genetic cause(s) and by Sanger sequencing to validate the identified variants and check the segregation in other members of the families. Finally, the potential effects of the variants on the structure and function of respective proteins were tested using in silico predictions. Both affected members of the families showed typical manifestations of KS including intellectual disability, developmental delay, and abnormal facial characteristics. A novel heterozygous frameshift variant in the *KMT2D* gene, c.4981del;p.(Glu1661Serfs*61), and a novel hemizygote missense variant in the *KDM6A* gene, c.3301G>A;p.(Glu1101Lys), were detected in patients 1 and 2, respectively. The frameshift variant identified in the first family was de novo, while in the second family, the mother was also heterozygous for the missense variant. The frameshift variant in *KMT2D* is predicted to lead to a truncated protein which is functionally impaired. The Glu1101 residue of *KDM6A* (UTX) affected in the second patient is located in a conserved region on the surface of the Jumonji domain and predicted to be causative. Our findings provide evidence on the possible pathogenicity of these 2 variants; however, additional functional studies are necessary to confirm their impacts.

© 2021 S. Karger AG, Basel

Introduction

Kabuki syndrome (KS) is a rare genetic disorder characterized by recognizable dysmorphic facial features, prenatal and postnatal growth retardation, skeletal abnormalities, mild to moderate intellectual disability, and dermatoglyphic malformations [Cheon and Ko, 2015; Bögershausen et al., 2016]. Additionally, other clinical features such as seizure, deafness, vision impairment, or microcephaly have been observed in some KS patients [Lehman et al., 2017]. It has also been reported that visceral, endocrinological, and hematologic abnormalities can occur in some KS cases [Moon et al., 2018].

The incidence of KS has been estimated to be 1 in 32,000 births [Cheon et al., 2014]. It has been reported that about 50–80% of the KS cases are caused by mutations or large deletions in the *KMT2D* gene (OMIM 602113; previously known as *MLL2*). Most mutations are de novo and inherited as autosomal dominant [Banka et al., 2015]. The *KMT2D* gene is located at 12q13.12

[Paděrová et al., 2016]. It consists of 54 coding regions and encodes a histone H3 lysine 4 (H3K4)-specific methyltransferase. The H3K4 belongs to a trithorax group and acts in multi-protein complexes. During embryonic development, multi-protein complexes could help in *HOX* gene activation [Liu et al., 2015; Roma et al., 2015]. The main changes occurring in *KMT2D* are reported to be protein-truncating variants including small deletions and nonsense mutations [Hannibal et al., 2011].

KS is also caused by point mutations or microdeletions in the *KDM6A* gene (OMIM 300128; previously known as *UTX*) which is located at Xp11.3 [Sobreira et al., 2017]. *KDM6A* comprises 30 exons and has a crucial role in demethylation of di- and trimethyl-lysine 27 on histone H3, in interaction with *KMT2D* [Micale et al., 2014].

KDM6A escapes from X-inactivation; however, *KDM6A* paralog on the Y chromosome has also been shown in males. Mutations in *KDM6A* are mostly reported to be nonsense mutations, small deletions, and missense variants. Besides, large deletions and duplications/insertions have been also reported. In contrast to *KMT2D*, splice-site mutations have been reported more frequently in *KDM6A* [Guo et al., 2018].

Both of the *KMT2D* and *KDM6A* proteins are histone modifiers, and they play an important role in the regulation of chromatin remodeling and transcription activation of downstream genes. Therefore, loss of function of these genes leads to the dysregulation of certain developmental genes which in turn could cause multiple complications in KS such as neurological anomalies [Karagianni et al., 2016; Kurahashi et al., 2017].

Case Presentations

Here, we report 2 unrelated Iranian families suspected of having KS who were referred to the DeNA Laboratory, Department of Medical Genetics, Tehran, Iran (Fig. 1A, Fig. 2A).

Patient 1

The first case was an 11-year-old female patient with suspected KS with congenital facial abnormalities including receding anterior hairline, broad and hairy forehead, triangular face with micrognathia, mild bilateral ptosis with long and wide palpebral fissures, long eyelashes with sparse eyebrows, posteriorly rotated and large prominent ears, wide nasal bridge, oligodontia, short upper lip with cleft palate (which was corrected), hypoplastic clavicles,

Fig. 1. **A** Pedigree of the first family. Selected patient for exome sequencing is indicated by an arrow. White symbols: unaffected; black symbol: affected; squares: men; circles: females; parallel lines: consanguineous marriage. WT, wild-type allele; VAR, c.4981_4981delG variant. **B** Sanger sequencing chromatograms representing nucleotide sequences of *KMT2D* in the regions of c.4981_4981delG. **C** Comparison of the normal and altered orga-

nization of the *KMT2D* gene (NM_003482.3) and *KMT2D* protein showing the position of the c.4981_4981delG and p.(Glu1661Serfs*61) variants, respectively. PHD, plant homeotic domain; HMG-box, high mobility group-box; FYRN, phenylalanine/tyrosine-rich N-terminal domain; FYRC, phenylalanine/tyrosine-rich C-terminal domain; SET, Su(var)3-9, enhancer-of-zeste and trithorax domain.

brachydactyly, fetal finger pads, and short phalanges. She showed autistic behavior with neurodevelopmental delay and intellectual disability. Congenital hypothyroidism, maxillary chronic sinusitis, and recurrent upper respiratory tract infection were also seen.

Patient 2

The second case was a 7-year-old boy whose parents were not consanguine. He presented with intellectual disability, developmental delay, hypotonia, and large auricle of the ears (but without any facial manifestations). He

Fig. 2. A Pedigree of the second family. Selected patient for exome sequencing is indicated by an arrow. White symbols: unaffected; black symbol: affected; squares: men; circles: females; WT, wild-type allele; VAR, c.3301G>A variant. **B** Brain MRI without contrast media and MR spectroscopy represents an extensive signal abnormality in both periventricular regions with the involvement of corpus callosum, thinning of the corpus callosum, and subcortical white matter involvement. **C** Sanger sequencing chromatograms representing nucleotide sequences of *KDM6A* in the regions

of c.3301G>A which is found in the family. **D** Nucleotide alignment using the UCSC database indicating high conservation of the codon residue in vertebrates which encodes Glu1101. Conservation scores for the amino acid residue affected by the missense variant were calculated using the ConSurf server. Scores ranged from 1 to 9, where a score of 9 represented a highly conserved residue. **E** 3D modeling of the *KDM6A* protein structure showing the position of Glu1101 on the surface of the Jumonji domain.

Table 1. Primer sequences used to confirm the variants by Sanger sequencing

Sample ID	Gene	Variant	Primers	Tm
Family I, V ₁	KMT2D	c.4981_4981delG p.(Glu1661Serfs*61)	F: 5'-ATGAGGAGGCAGAGTTGTGG-3'	60.3°C
			R: 5'-GGGCTACATTGCCACTCAG-3'	59.3°C
Family II, IV ₄	KDM6A	c.3301G>A p.(Glu1101Lys)	F: 5'-TCAACAGGAGTCAAACCTTGTC-3'	60.1°C
			R: 5'-AAAAGAGACACAGTATTAGAAACATGC-3'	59.4°C

also had feeding problems in infancy. He showed difficulties in sitting since birth and loss of head control. Although hypertonic muscles of upper and lower extremities were seen, electromyography was normal with no evidence of myopathy, anterior horn cell, or nerve root lesion. Motor and sensory nerve conduction were normal and peripheral neuropathies were not observed. Brain MRI showed thinning of the corpus callosum and cystic encephalomalacia in both temporal lobes. Extensive signal abnormality was also present in both periventricular regions with the involvement of corpus callosum. MRI findings suggested leukodystrophies with temporal lobe cystic encephalomalacia (Fig. 2B).

Materials and Methods

DNA Extraction

Peripheral blood samples were obtained from both patients and their parents. Genomic DNAs were extracted by a High Pure PCR template preparation kit (Roche; Product No, 11814770001). The concentration of DNA was measured by Thermo Scientific™ Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA).

Whole-Exome Sequencing

About 1 µg of genomic DNA of the patients was subjected to WES. Agilent's SureSelect Human. All Exon V6 kit was used to enrich approximately 60 Mb of the Human Exome from fragmented genomic DNA. The generated library was sequenced on an Illumina HiSeq 4000 platform to obtain an average coverage depth of ~100×. Typically, ~97% of the targeted bases were covered >10×.

Bioinformatics Analysis

The raw sequencing data was primarily filtered to remove adapters, low-quality reads, and probable artifacts using FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and Trimmomatic tools [Bolger et al., 2014]. These trimmed reads were then aligned to the GRCh37/hg19 human genome assembly using Bowtie2 software [Langdon, 2013]. The GATK software was used for base quality recalibration and variant calling processes [McKenna et al., 2010]. The ANNOVAR software applied to annotate each variant, including gene names, allele frequencies, and their functions [Wang et al., 2010].

Six major steps were taken to evaluate and prioritize all annotated variants. In the first step, the low-quality variants (read depth <10×, genotype quality score <20, QualByDepth score <2) were excluded from subsequent analysis. In the next step, since only one patient existed in the families, both heterozygous and homozygous variants were considered. Thirdly, all previously identified SNPs with a minor allele frequency higher than 1% in public databases such as dbSNP150 (<http://www.ncbi.nlm.nih.gov/projects/SNP>), 1000 Genomes Project [Siva, 2008], Exome Aggregation Consortium (ExAC) [Karczewski et al., 2017], Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS>), gnomAD [Collins et al., 2019], and the local Iranome database [Fattahi et al., 2019] were excluded. Then, as we focused on coding exons along with flanking +/-20 intronic bases, variants within intergenic, intronic, untranslated regions, and synonymous alternations were attentively excluded. In the fifth step using Phenolyzer [Yang et al., 2015] and HPO Phenomizer [Köhler et al., 2009] tools, genotype-phenotype correlation was utilized. Finally, the previously reported variants in ClinVar [Landrum et al., 2016] and HGMD [Stenson et al., 2009] databases were considered. Several databases including SIFT [Ng and Henikoff, 2003], PolyPhen-2 [Adzhubei et al., 2013], MutationTaster [Schwarz et al., 2010], PROVEAN [Choi and Chan, 2015], FATHMM [Shihab et al., 2013], and CADD [Rentzsch et al., 2019] were used to predict the pathogenic consequences of the detected variants. The Genomic Evolutionary Rate Profiling (GERP) and phyloP scores were implemented to evaluate the evolutionary conservation status of the variants [Cooper, 2005].

Additionally, the evolutionary conservation in the region of detected variants was analyzed by aligning the amino acids and nucleotide sequences from several species using ConSurf (<http://www.consurf.tau.ac.il>) [Glaser et al., 2003] and UCSC database [Kuhn et al., 2009].

Segregation Analysis

Samples from all available family members were subjected to Sanger sequencing to reveal whether the potential variants in the causative genes cosegregate with the Kabuki phenotype. Primers surrounding the regions of the identified variants were designed by using the Primer3Plus (<https://primer3plus.com/cgi-bin/dev/primer3plus.cgi>) web-based server (Table 1). OligoAnalyzer Tool (<https://www.idtdna.com/calc/analyzer>) was used to analyze primer oligos. Lack of SNPs in the genomic region corresponding to the 3' ends of primers was checked by looking through the dbSNP database (<https://www.ncbi.nlm.nih.gov/snp>). The primer specificity was checked by the in silico PCR tool in the UCSC genome browser (<https://genome.ucsc.edu/cgi-bin/hgPcr>) and NCBI Primer blast (<https://www.ncbi.nlm.nih.gov/tools/primer-blast>). PCR was per-

Table 2. Coverage statistics of WES performed on the patients

Patient	Average	Target bp covered, %					
		0×	≥1×	≥5×	≥10×	≥20×	≥50×
Family I, V ₁	138.82	0.37	99.63	99.36	99.05	97.87	89.21
Family II, IV ₄	112.34	0.11	99.89	99.66	99.19	97.32	84.29

formed in a standard condition, and PCR products were sequenced by ABI 3100 with the ABI PRISM BigDye Terminator v. 3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA). Finally, sequence traces were analyzed using the CodonCode Aligner v. 8.0.2 (CodonCode Corp, Centerville, MA, USA).

Three-Dimensional Structure Modeling

The identified missense variant in the *KDM6A* gene, c.3301G>A; p.(Glu1101Lys), resides in exon 22. To predict the possible effects of p.(Glu1101Lys) on protein stability and protein folding, a BLAST sequence search against the protein data bank (PDB) was performed to select the template structure with the closest sequence similarity to the domain of *KDM6A*, and finally, the crystal structure of UTX protein (PDB ID: 6FUK) was employed to build the favorite model. The protein structure and the possible impact of the variant on protein flexibility and stability were analyzed by PyMOL software [DeLano, 2002].

Prediction of Single Point Variations on Protein Stability

The I-mutant2.0, which computes the $\Delta\Delta G$ values of the protein variant, was utilized to predict and annotate the effect of the single nonsynonymous variant in *KDM6A*, p.(Glu1101Lys), on the protein stability from its sequence. I-mutant2.0 (<https://www.folding.biofold.org/i-mutant/i-mutant2.0.html>) is based on the Support Vector Machine and ProTherm database [Bava, 2004] and is trained to predict the thermodynamic free energy change upon single-point variations in protein.

Results

Molecular Findings

We applied WES on 2 patients from 2 unrelated families to identify the probable genetic causes explaining the patients' phenotypes. The average depth of 100× was achieved, and 97% of targeted bases were covered at least 20× (Table 2). A total number of 22,316 and 22,335 variants were identified in patient 1 and 2, respectively.

We focused only on variants with a frequency of less than 1% according to dbSNP150, 1000 Genomes Project, ExAC, ESP, and gnomAD public databases as well as the local Iranome database. Therefore, the number of variants was reduced to 1,777 and 1,890 variants in patient 1 and 2, respectively. Then, variants located in the exonic and splicing regions were selected and synonymous variants

were removed which resulted in 37 homozygous and 148 heterozygous variants, and 35 homozygous, 244 heterozygous and 20 hemizygous variants in patient 1 and 2, respectively. Variants in genes related to the patients' phenotype reported in ClinVar and HGMD databases were given a higher priority. Functional prediction using SIFT, PolyPhen-2, MutationTaster, PROVEAN, FATHMM, and CADD databases and evolutionary conservation assessment utilizing GERP++ and phyloP scores were implemented to identify the possible causative variants.

After all of these considerations, we ended up with a heterozygous variant in patient 1, c.4981_4981delG; p.(Glu1661Serfs*61) in *KMT2D* (NM_003482.3) in chromosome 12, and a hemizygous variant (c.3301G>A; p.(Glu1101Lys) in *KDM6A* (NM_001291415.1) in chromosome X which affects a highly conserved domain of *KDM6A* encoded by exon 22, and 2 heterozygous (*SATB2* and *TFAP2A*) variants in patient 2. We investigated both heterozygous variants in *SATB2* and *TFAP2A* which lead to Glass syndrome and branchiooculofacial syndrome, respectively. None of them cosegregated with the disease in this family (Table 3). Finally, based on the American College of Medical Genetics and Genomics (ACMG) guidelines [Richards et al., 2015] using InterVar [Li and Wang, 2017] Software, we classified the 2 identified variants into likely pathogenic and uncertain significance categories in patient 1 and 2, respectively.

Both of the identified variants were not reported in dbSNP version 150, 1K genome project, ExAC, ESP, gnomAD, Iranome, HGMD, or ClinVar databases. Sanger sequencing confirmed the novel frameshift variant in patient 1 (c.4981del; p.Glu1661Serfs*61, exon 20, NM_003482.3), but neither of her parents had this variant; therefore, it was considered as a de novo frameshift variant in *KMT2D* (Fig. 1B). In patient 2, Sanger sequencing results verified the missense variant in *KDM6A* (c.3301G>A; p.Glu1101Lys, exon 22, NM_001291415.1). His mother was also heterozygous, but his father had the wild-type allele (Fig. 2C).

Discussion

KS is a rare genetic syndrome which is characterized by 5 major clinical symptoms: facial anomalies, abnormal skeletal features, mild to moderate intellectual disability, postnatal developmental delay, and dermatoglyphic malformations [Miyake et al., 2013].

Exome sequencing in 10 individuals with different ancestry has shown that mutations in *MLL2/KMT2D* are the

Table 3. Online databases used to predict the pathogenicity of the variants in the *KMT2D* and *KDM6A* genes

Variant	Gene/ genomic location	Zygosity			Allele frequencies	Effect
		Index	Mother	Father		
NM_003482.3:c.4981_4981delG NP_003473.3:p.(Glu1661Serfs*61)	KMT2D; g.49438288del	Het	Hom (WT)	Hom (WT)	gnomAD: NR	PROVEAN: deleterious
					1000 genome: NR	MutationTaster: NA
					ESP: NR	FATHMM: damaging
					Iranome: NR	PolyPhen: probably damaging
NM_001291415.1:c.3301G>A NP_001278344.1:p.(Glu1101Lys)	KDM6A; g.44941821 G>A	Hemi (MT)	Het	Hom (WT)	gnomAD: NR	PROVEAN: deleterious
					1000 genome: NR	MutationTaster: disease causing
					ESP: NR	FATHMM: damaging
					Iranome: NR	PolyPhen: benign

Hemi, hemizygote; Hom, homozygote; Het, heterozygote; WT, wild type; MT, mutant; NR, not reported; NA, not available.

main cause of the disease [Ng et al., 2010]. About 9–14% of the cases have been reported to have *KDM6A* mutations [Dentici et al., 2015].

H3K4 methylation at the immunoglobulin heavy chain locus is catalyzed by the *KMT2D* protein. Also, *KMT2D* plays a crucial role in B cell terminal differentiation; therefore, mutations in *KMT2D* result in dysregulation of terminal B cell differentiation and humoral immune deficiency [Lindsley et al., 2016].

The N-terminal of the *KMT2D* protein includes 6 plant homeotic domains categorized in 2 clusters, while the C-terminal includes a SET domain which has a crucial role in the H3K4 methyltransferase activity (Fig. 1C) [Ruthenburg et al., 2007]. Protein stability of *KMT2D* in cells is also maintained by the SET domain [Dorigi et al., 2017]. In vitro studies have shown that plant homeotic domains 4–6, located in the second cluster, play an important role in the methylation of *KMT2D*-catalyzed nucleosome by recognizing H4 tails on nucleosomes [Dhar et al., 2012]. Reports have shown the coactivator role of *KMT2C* and *KMT2D* at enhancers and their important role in enhancer activation through CREB-binding protein (CBP) and/or p300 [Lai et al., 2017]. In association with *KMT2C* and *KMT2D*, *KDM6A* plays its role in demethylation of the H3K27 site followed by its acetylation through recruitment of CBP and/or p300 [Froimchuk et al., 2017].

KDM6A is located in the X chromosome but interestingly escapes X inactivation. This gene is 5.4 kb and contains 29 exons which encode a 154-kDa protein containing 1,401 amino acids. *KDM6A* is known to have 2 functional domains. Demethylation of lysine 27 on histone H3

(H3K27) is catalyzed by *KDM6A* which mediates the expression of different genes with crucial roles in developmental processes. *KDM6A* also plays an important role in gene silencing by removing the H3K27me3 mark [Van Laarhoven et al., 2015]. The other functional domain of *KDM6A* is involved in chromatin remodeling by interacting with the switch/sucrose complex. Altogether, *KDM6A* and *KMT2D* play significant roles in regulating the expression of various genes involved in embryogenesis and development [Lederer et al., 2012].

Although missense variants are reported in various studies [Lu et al., 2016; Schott et al., 2016; Lepri et al., 2017; Cocciadiferro et al., 2018], indel frameshift, nonsense, or splice-site mutations causing truncating changes are the main mutations that have been reported in *KMT2D*. It has been shown that the majority of truncating mutations, most of them affecting highly conserved domains, if translated, will result in shorter forms of the *KMT2D* protein [Micale et al., 2011].

The Glu1661Serfs*61 frameshift variant in the *KMT2D* gene that we identified in patient 1 is also expected to result in a truncated protein (Fig. 1C). Patient 1 presented with typical characteristics of KS including postnatal developmental delay, intellectual disability, facial characteristics such as palpebral fissures, large prominent ears, cleft palate, and skeletal anomalies such as fetal finger pads and brachydactyly. The heterozygous state for the Glu1661Serfs*61 variant in the patient and its absence in the parents was confirmed by Sanger sequencing, indicating de novo state in the index patient (Fig. 1B). Also, in silico predictions showed the variant to be damaging (Table 3).

The identified missense X-linked variant Glu1101Lys in patient 2 (Table 3) is affecting a highly conserved domain of KDM6A. The male patient presented with intellectual disability, developmental delay, and feeding problems in infancy. His brain MRI was suggestive for encephalomacia with leukodystrophies (Fig. 2B). The identified variant in this patient was confirmed by Sanger sequencing, and also his mother was shown to be a carrier (Fig. 2C). Multiple alignments of amino acids and nucleotide sequences in different species by Consurf and UCSC databases indicated that this variant affects a highly conserved residue of the KDM6A protein (Fig. 2D). Three-dimensional modeling of KDM6A showed that the Glu1101 residue is located on the surface of the Jumonji domain which is the catalytic domain, and binds and demethylates methyllysine (Fig. 2E) [Sengoku and Yokoyama, 2011]. Prediction based on I-mutant2.0 showed that this substitution may decrease protein stability (DDG: −1.25). These findings suggest that this variant causes conformational changes in the Jumonji domain and probably affects the function of KDM6A and its interactions with other proteins.

Conclusion

In this study, 2 novel variants in the *KMT2D* (c.4981del) and *KDM6A* (c.3301G>A) genes were identified as a possible cause for KS in 2 Iranian families. This is the first report on KS patients in the Iranian population. Our findings support the possible malfunction due to these variants. However, additional functional studies are strongly suggested to confirm their pathogenicity.

References

- Adzhubei I, Jordan DM, Sunyaev SR. Predicting Functional Effect of Human Missense Mutations Using PolyPhen-2. *Curr Protoc Hum Genet*. 2013;Chapter 7(1):Unit7.20.
- Banka S, Lederer D, Benoit V, Jenkins E, Howard E, Bunstone S, et al. Novel KDM6A (UTX) mutations and a clinical and molecular review of the X-linked Kabuki syndrome (KS2). *Clin Genet*. 2015;87(3):252–8.
- Bava KA, Gromiha MM, Uedaira H, Kitajima K, Sarai A. ProTherm, version 4.0: thermodynamic database for proteins and mutants. *Nucleic Acids Res*. 2004;32(Database issue):D120–1.
- Bögershausen N, Gatinois V, Riehmer V, Kayserili H, Becker J, Thoenes M, et al. Mutation Update for Kabuki Syndrome Genes *KMT2D* and *KDM6A* and Further Delineation of X-Linked Kabuki Syndrome Subtype 2. *Hum Mutat*. 2016;37(9):847–64.
- Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–20.
- Cheon CK, Ko JM. Kabuki syndrome: clinical and molecular characteristics. *Korean J Pediatr*. 2015;58(9):317.
- Cheon CK, Sohn YB, Ko JM, Lee YJ, Song JS, Moon JW, et al. Identification of *KMT2D* and *KDM6A* mutations by exome sequencing in Korean patients with Kabuki syndrome. *J Hum Genet*. 2014;59(6):321–5.
- Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015;31(16):2745–7.
- Cocciadiferro D, Augello B, De Nittis P, Zhang J, Mandriani B, Malerba N, et al. Dissecting *KMT2D* missense mutations in Kabuki syndrome patients. *Hum Mol Genet*. 2018;27(21):3651–68.
- Collins RL, Brand H, Karczewski KJ, Zhao X, Alföldi J, Khera AV, et al. An open resource of structural variation for medical and population genetics. *bioRxiv*. 2019;578674.
- Cooper GM, Stone EA, Asimenos G, NISC Comparative Sequencing Program, Green ED, Batzoglu S, et al. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res*. 2005;15(7):901–13.
- DeLano WL. Pymol: An open-source molecular graphics tool. *CCP4 Newsl Protein Crystallogr*. 2002;40:82–92.
- Dentici ML, Di Pede A, Lepri FR, Gnazzo M, Lombardi MH, Auriti C, et al. Kabuki syndrome: Clinical and molecular diagnosis in the first year of life. *Arch Dis Child*. 2015;100(2):158–64.

Acknowledgments

We would like to thank the families for their participation that made this work possible. We sincerely appreciate the staff of DeNA laboratory for helping us in this research.

Statement of Ethics

The authors declare that they have conducted the project ethically in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants or their respective guardians. Participants also provided written informed consent for publication of their related information included in this paper.

Conflict of Interest Statement

The authors declare that they have no competing interests.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Designed the project: M.G. Data collection and analysis: M.G., M.K., E.J., and F.B. Conducted the experiments: M.K., E.J., and F.B. Clinical evaluation: N.A., S.S., and M.D. Wrote the paper: M.K., M.G., E.J., and F.B. All authors read and approved the final manuscript.

- Dhar SS, Lee SH, Kan PY, Voigt P, Ma L, Shi X, et al. Trans-tail regulation of MLL4-catalyzed H3K4 methylation by H4R3 symmetric dimethylation is mediated by a tandem PHD of MLL4. *Genes Dev.* 2012;26(24):2749–62.
- Dorigi KM, Swigut T, Henriques T, Bhanu NV, Scruggs BS, Nady N, et al. Mll3 and Mll4 Facilitate Enhancer RNA Synthesis and Transcription from Promoters Independently of H3K4 Monomethylation. *Mol Cell.* 2017; 66(4):568–576e4.
- Fattahi Z, Beheshtian M, Mohseni M, Poustchi H, Sellars E, Nezadi SH, et al. Iranome: A catalog of genomic variations in the Iranian population. *Hum Mutat.* 2019;40(11):1968–84.
- Froimchuk E, Jang Y, Ge K. Histone H3 lysine 4 methyltransferase KMT2D. *Gene.* 2017;627: 337–42.
- Glaser F, Pupko T, Paz I, Bell RE, Bechor-Shental D, Martz E, et al. ConSurf: Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic Information. *Bioinformatics.* 2003;19(1):163–4.
- Guo Z, Liu F, Li HJ. Novel KDM6A splice-site mutation in kabuki syndrome with congenital hydrocephalus: a case report. *BMC Med Genet.* 2018;19(1):206.
- Hannibal MC, Buckingham KJ, Ng SB, Ming JE, Beck AE, Mcmillin MJ, et al. Spectrum of MLL2 (ALR) mutations in 110 cases of kabuki syndrome. *Am J Med Genet A.* 2011; 155A(7):1511–6.
- Karagianni P, Lambropoulos V, Stergidou D, Fryssira H, Chatziioannidis I, Spyridakis I. Recurrent giant cell fibroblastoma: Malignancy predisposition in Kabuki syndrome revisited. *Am J Med Genet A.* 2016;170A(5):1333–8.
- Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, et al. The ExAC browser: Displaying reference data information from over 60 000 exomes. *Nucleic Acids Res.* 2017;45(D1):D840–5.
- Köhler S, Schulz MH, Krawitz P, Bauer S, Dölken S, Ott CE, et al. Clinical Diagnostics in Human Genetics with Semantic Similarity Searches in Ontologies. *Am J Hum Genet.* 2009;85(4):457–64.
- Kuhn RM, Karolchik D, Zweig AS, Wang T, Smith KE, Rosenbloom KR, et al. The UCSC Genome Browser Database: update 2009. *Nucleic Acids Res.* 2009;37(Database issue):D755–61.
- Kurahashi N, Miyake N, Mizuno S, Koshimizu E, Kurahashi H, Yamada K, et al. Characteristics of epilepsy in patients with Kabuki syndrome with KMT2D mutations. *Brain Dev.* 2017; 39(8):672–7.
- Lai B, Lee JE, Jang Y, Wang L, Peng W, Ge K. MLL3/MLL4 are required for CBP/p300 binding on enhancers and super-enhancer formation in brown adipogenesis. *Nucleic Acids Res.* 2017;45(11):6388–403.
- Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipirala S, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016;44(D1):D862–8.
- Langdon WB. Which is faster. *Bowtie2.* 2013; 1301–5187.
- Lederer D, Grisart B, Digilio MC, Benoit V, Crespin M, Ghariani SC, et al. Deletion of KDM6A, a Histone Demethylase Interacting with MLL2, in Three Patients with Kabuki Syndrome. *Am J Hum Genet.* 2012;90(1): 119–24.
- Lehman N, Mazery AC, Visier A, Baumann C, Lachesnais D, Capri Y, et al. Molecular, clinical and neuropsychological study in 31 patients with Kabuki syndrome and KMT2D mutations. *Clin Genet.* 2017;92(3):298–305.
- Lepri F, Cocciaferro D, Augello B, Alfieri P, Pes V, Vancini A, et al. Clinical and Neurobehavioral Features of Three Novel Kabuki Syndrome Patients with Mosaic KMT2D Mutations and a Review of Literature. *Int J Mol Sci.* 2017;19(1):82.
- Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet.* 2017;100(2): 267–80.
- Lindsley AW, Saal HM, Burrow TA, Hopkin RJ, Shchelochkov O, Khandelwal P, et al. Defects of B-cell terminal differentiation in patients with type-1 Kabuki syndrome. *J Allergy Clin Immunol.* 2016;137(1):179–187e10.
- Liu S, Hong X, Shen C, Shi Q, Wang J, Xiong F, et al. Kabuki syndrome: a Chinese case series and systematic review of the spectrum of mutations. *BMC Med Genet.* 2015;16:26.
- Lu J, Mo G, Ling Y, Ji L. A novel KMT2D mutation resulting in Kabuki syndrome: A case report. *Mol Med Rep.* 2016;14(4):3641–5.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20(9):1297–303.
- Micale L, Augello B, Fusco C, Selicorni A, Loviglio MN, Silengo MC, et al. Mutation spectrum of MLL2 in a cohort of kabuki syndrome patients. *Orphanet J Rare Dis.* 2011;6:38.
- Micale L, Augello B, Maffeo C, Selicorni A, Zucchetti F, Fusco C, et al. Molecular Analysis, Pathogenic Mechanisms, and Readthrough Therapy on a Large Cohort of Kabuki Syndrome Patients. *Hum Mutat.* 2014;35(7):841–50.
- Miyake N, Koshimizu E, Okamoto N, Mizuno S, Ogata T, Nagai T, et al. MLL2 and KDM6A mutations in patients with Kabuki syndrome. *Am J Med Genet A.* 2013;161A(9):2234–43.
- Moon J-E, Lee S-J, Ko CW. A de novo KMT2D mutation in a girl with Kabuki syndrome associated with endocrine symptoms: a case report. *BMC Med Genet.* 2018;19(1):102.
- Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003;31(13):3812–4.
- Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet.* 2010;42(9):790–3.
- Paděrová J, Holubová A, Simandlová M, Puchmajerová A, Vlčková M, Malíková M, et al. Molecular genetic analysis in 14 Czech Kabuki syndrome patients is confirming the utility of phenotypic scoring. *Clin Genet.* 2016; 90(3):230–7.
- Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* 2019;47(D1): D886–94.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405–24.
- Roma D, Palma P, Capolino R, Figà-Talamanca L, Diomedi-Camassei F, Lepri FR, et al. Spinal ependymoma in a patient with Kabuki syndrome: a case report. *BMC Med Genet.* 2015; 16(1):80.
- Ruthenburg AJ, Allis CD, Wysocka J. Methylation of Lysine 4 on Histone H3: Intricacy of Writing and Reading a Single Epigenetic Mark. *Mol Cell.* 2007;25(1):15–30.
- Schott DA, Blok MJ, Gerver WJ, Devriendt K, Zimmermann LJ, Stumpel CT. Growth pattern in Kabuki syndrome with a KMT2D mutation. *Am J Med Genet A.* 2016;170(12): 3172–9.
- Schwarz JM, Rödelberger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods.* 2010;7(8):575–6.
- Sengoku T, Yokoyama S. Structural basis for histone H3 lys 27 demethylation by UTX/KDM6A. *Genes Dev.* 2011;25(21):2266–77.
- Shihab HA, Gough J, Cooper DN, Stenson PD, Barker GL, Edwards KJ, et al. Predicting the Functional, Molecular, and Phenotypic Consequences of Amino Acid Substitutions using Hidden Markov Models. *Hum Mutat.* 2013; 34(1):57–65.
- Siva N. 1000 Genomes project. *Nat Biotechnol.* 2008;26(3):256.
- Sobreira N, Brucato M, Zhang L, Ladd-Acosta C, Ongaco C, Romm J, et al. Patients with a Kabuki syndrome phenotype demonstrate DNA methylation abnormalities. *Eur J Hum Genet.* 2017;25(12):1335–44.
- Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The Human Gene Mutation Database: 2008 update. *Genome Med.* 2009;1(1):13.
- Van Laarhoven PM, Neitzel LR, Quintana AM, Geiger EA, Zackai EH, Clouthier DE, et al. Kabuki syndrome genes KMT2D and KDM6A: functional analyses demonstrate critical roles in craniofacial, heart and brain development. *Hum Mol Genet.* 2015;24(15):4443–53.
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38(16):e164.
- Yang H, Robinson PN, Wang K. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. *Nat Methods.* 2015; 12(9):841–3.