

Case Report

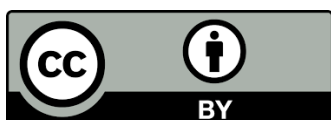
Unraveling and Expanding the Genotypic Spectrum of Kabuki Syndrome with Identification of de Novo Protein-Truncating Mutations in the *KMT2D* Gene: Insights into the Role of Premature Stop Codons in the Etiology of the Disorder

Mohammad-Reza Ghasemi ^{1, 2}, Maryam Mirahmadi ^{3, 4}, Hadi Bayat ⁵, Mohammad Miryounesi ^{1, 2}, Reza Mirfakhraie ^{1, 6}, Shadab Salehpour ⁷, Raheleh Tangestani ², Faezeh Sherafat ^{2, 8}, Hasan Roudgari ^{2, *}, Milad Gholami ^{9, *}

1. Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran; E-Mails: M.r.ghasemi@sbmu.ac.ir; Miryounesi@sbmu.ac.ir; r.mirfakhraie@sbmu.ac.ir
2. Center for Comprehensive Genetic Services, Shahid Beheshti University of Medical Sciences, Tehran, Iran; E-Mails: tangestani212@yahoo.com; sh.faezeh22@gmail.com; hroodgary@yahoo.com
3. Department of Medical Genetics, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran; E-Mail: m.mirahmadi@modares.ac.ir
4. Department of Exomine, PardisGene company, Tehran, Iran
5. Biochemical Neuroendocrinology, Montreal Clinical Research Institute (IRCM), affiliated to McGill University, Montreal H2W 1R7, Canada; E-Mail: hadi.bayat@mail.mcgill.ca
6. Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
7. Department of Pediatrics, Clinical Research Development Unit, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran; E-Mail: shadab.salehpour@gmail.com
8. Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
9. Department of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran; E-Mail: Dr.gholami@arakmu.ac.ir

* **Correspondences:** Hasan Roudgari and Milad Gholami; E-Mails: hroodgary@yahoo.com; Dr.gholami@arakmu.ac.ir

Academic Editor: Fabrizio Stasolla



© 2025 by the author. This is an open access article distributed under the conditions of the [Creative Commons by Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium or format, provided the original work is correctly cited.

Abstract

Kabuki Syndrome (KS) is a rare, multisystem congenital disorder with five foremost clinical manifestations encompassing dysmorphic facial characteristics, postnatal growth constraint, craniofacial/skeletal anomalies, mild to moderate intellectual disability, and dermatoglyphic abnormalities. We analyzed two unrelated Iranian patients suspected of having KS using whole-exome sequencing. *An in silico* analysis was performed to evaluate the potential effects of the discovered variants on the structure and function of the corresponding protein. A novel *de novo* heterozygous protein-truncating mutation was found in patient 1, NM_003482.4: c.13818C>G (p.Y4606X), and a *de novo* heterozygous premature stop codon was identified in patient 2, NM_003482.4: c.16360C>T (p.R5454X). These mutations cause haploinsufficiency in *KMT2D* and are absent in the proband's parents. Although these mutations provide evidence of the potential pathogenicity of *KMT2D* in KS, further functional studies are necessary to confirm their impact. Our observations not only provide evidence for the potential pathogenicity of the two identified protein-truncating mutations but also have important implications for the accurate diagnosis and potential treatment of KS. These findings underscore the significance of genetic testing in diagnosing this disorder and expand our understanding of the role of *KMT2D* mutations in its pathogenesis.

Keywords

Kabuki syndrome; whole exome sequencing; *KMT2D*; novel mutation

1. Introduction

Kabuki syndrome (KS) (known as Niikawa-Kuroki syndrome or Kabuki-makeup syndrome) is a rare monogenic syndrome with common autosomal dominant and X-linked dominant inheritance patterns. Moreover, KS is also considered a pleiotropic syndrome with an estimated incidence of 1:32000 in newborns [1]. Approximately 70-75% of the detected pathogenic variants are located in *KMT2D* (formerly known as *MLL2*, *MLL4*, or *ALR*; NM_003482.3; OMIM: 602113; cytogenetic location: 12q13.12) or *KDM6A* (also known as *UTX*; NM_001291415.1; OMIM: 300128; cytogenetic location: Xp11.3) [2, 3]. Initially, KS was described in two Japanese studies. Niikawa et al. [4] and Kuroki et al. [5], as a multisystem congenital disorder with five foremost clinical manifestations of KS encompassing dysmorphic facial characteristics (arched and broad eyebrows; depressed nasal tip; long palpebral fissures; and cupped ears; albeit many of them are not apparent in early infancy), postnatal growth constraint, craniofacial/skeletal anomalies (such as fifth digit clinodactyly and brachymesophalangy), mild to moderate Intellectual disability, and dermatoglyphic anomalies (routinely persistent fingertip pads) [6]. Likewise, other manifestations such as deafness, microcephaly, seizure, vision impairment, and endocrinological, visceral, and hematologic

abnormalities are observed in some KS patients. A diverse combination of those above cardinal clinical observations could be considered a promising diagnostic tool for KS [7, 8].

KMT2D comprises 54 coding exons and encodes a specific methyltransferase (methylating histone H3 lysine 4). Protein-truncating variants, such as small deletions and nonsense mutations, are the main variants in *KMT2D* [9]. Makrythanasis et al. described a phenotypic scoring system for predicting KS individuals who probably had a pathogenic mutation in *KMT2D*. By comparing clinical scores and genetic testing results, it was observed that subjects with a mutation in *KMT2D* had a significantly higher mean score (6.1) than those without a mutation in this gene [10]. Although it has been revealed that 70-75% of KS patients have mutations in *KMT2D*, the underlying causes of 20-45% of KS patients have not been identified, suggesting a broad spectrum of mutations in *KMT2D* and possible genetic heterogeneity [11].

Massively parallel DNA sequencing technologies have a significant impact on medical genetics research and health improvement approaches. Whole-exome sequencing (WES) is used to identify mutations in the coding and splicing regions. It is estimated that 85% of disease-causing variants are confined to coding regions and that WES can cover more than 95% of these areas [12]. Over the past decade, whole-exome sequencing and genome-wide DNA methylation profiling technologies have facilitated the identification of various KS-causing mutations in several genes, especially *KMT2D*, in most patients. These mutations disrupt histone modifications and chromatin remodeling, which can influence genomic DNA methylation patterns [13, 14].

In this study, we identified two *de novo* protein-truncating mutations in *KMT2D* using WES, which were confirmed by Sanger sequencing. *KMT2D*, a member of the methyltransferase family, is involved in chromatin remodeling. Haploinsufficiency of *KMT2D* causes the dysregulation of several developmental factors, resulting in various neurological and structural complications in patients with KS.

2. Methods

2.1 DNA Extraction

To extract genomic DNA (gDNA), peripheral blood samples were collected from probands and their parents. gDNA was extracted using the salting-out method [15]. Thermo Scientific™ Nanodrop 2000 (Thermo Fisher Scientific) was used to evaluate the quality and quantity of gDNA.

2.2 Whole-Exome Sequencing (WES)

To perform WES, one µg of gDNA from each proband was used. SureSelectXT2 V6 kit was applied to enrich the exomes from other fragmented gDNA. Illumina HiSeq4000 was used to sequence the generated library with a read length of 101 bp and a coverage of 100×.

Fastq read files are produced by the manufacturer's software on the sequencing platform. Trimming the Fastq file and using the Burrows-Wheeler Aligner to match it to the human reference genome hg19 allowed for the removal of the adaptor and low-quality reads (BWA) [16]. Following variant calling evaluation using SAMtools and Picard, variant annotation using ANNOVAR software was performed on BAM data [17-19]. Additionally, the frequency of variation in gnomAD, dbSNP138, 1000 Genome Projects, and Iranome databases (<https://gnomad.broadinstitute.org/>, <https://www.ncbi.nlm.nih.gov/snp/>), as well as other public databases, was analyzed. Only low-

frequency variations with a minor allele frequency (MAF) under 0.01 throughout the review period were chosen. Uncommon and new variations were prioritized using non-synonymous, indel, and potential splice sites. Predictor tools such as Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<https://sift.bii.a-star.edu.sg/>), MutationTaster (<http://www.mutationtaster.org/>), and most importantly, CADD software, have been used for in silico evaluation to determine the pathogenicity of candidate variants. Along with the internal database, worldwide mutation and polymorphism databases were used to analyze the final sequencing results.

2.3 Segregation Analysis

To confirm the cosegregation of the variant in the probands with KS, DNA samples from the probands' parents were subjected to Sanger sequencing. Primers were designed using the Primer3 web-based server (<https://primer3.ut.ee/>) (exon 41: forward 5'-CGTCAGCAATTCCTCAAGT-3' and reverse: 5'-TTGGTGAGCTCCCGAAAGAA-3'; exon 52: forward: 5'-CTCACCTCCTCTCCTTTGGG-3' and reverse: 5'-ACAACGTGTACCTGGCTCG-3'). The quality of the designed primers was validated using OligoAnalyzer (<https://www.idtdna.com/pages/tools/oligoanalyzer>). Primer specificity was controlled using NCBI primer BLAST software (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi>). PCR products were sequenced using an automated ABI PRISM 3130XL (Applied Biosystems). CodonCode Aligner v. 8.0.2 offline software was used for analyzing the sequence traces.

2.4 Prediction of the Protein Structure

The protein sequences of the upstream and downstream sites of the causative mutation were introduced into Iterative Threading ASSEmbly Refinement (I-TASSER), a hierarchical approach to predict protein structure and plausible structure-based functions. Structural templates from the protein data bank (PDB) were identified using the multiple-threading approach LOMETS (<http://zhang.bioinformatics.ku.edu/LOMETS>). Then, by re-threading the 3D models using BioLiP (<http://zhanglab.ccmb.med.umich.edu/BioLiP/>), functional insights into the corresponding target were derived.

2.5 Ethics Statement

This study was approved by the Medical Ethics Committee of Arak University of Medical Sciences (Approval no: IR.ARAKMU.REC.1400.354). Written informed consent was obtained from both parents to publish this report under the journal's patient consent policy.

3. Results

3.1 Case Presentation

3.1.1 Patient 1

The proband was the only child of a consanguineous healthy parent. A 9-year-old Iranian female with a history of neurodevelopmental delay and mild intellectual disability was referred to our center. She was born early at 33 weeks of pregnancy via spontaneous vaginal delivery due to

premature rupture of membranes (PROM). She weighed approximately 1200 g and was admitted to the neonatal intensive care unit (NICU) for seven days due to prematurity. She was diagnosed with four fundamental deficits: a neurodevelopmental disorder without seizures, walking delay, mild intellectual disability, and mild speech delay with slight stuttering. Furthermore, she started to walk around the age of three years, and since then, she has never had any walking problems. Moreover, the metabolic panel results were expected. The family pedigree is shown in Figure 1.

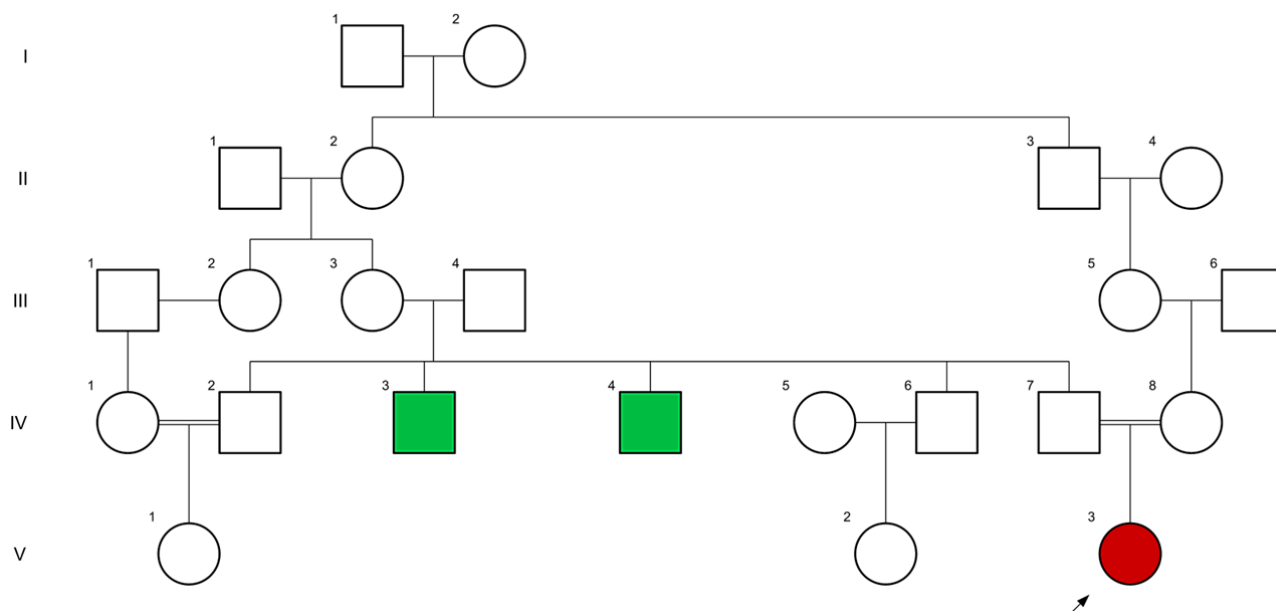


Figure 1 The pedigree of patient 1. The proband is indicated by the arrow. Red and green indicate KS and intellectual disability, respectively.

3.1.2 Patient 2

The Proband was the only child of a non-consanguineous healthy parent Figure 2. An 85-day-old Iranian girl with a history of failure to thrive (FTT). She was born full-term (37 weeks of pregnancy) through cesarean section from a polyhydramnios pregnancy. Her birth weight was 2285 g, head circumference was 37 cm, and blood ammonia and lactate levels were mildly elevated after birth. These values were typical in the following tests: although her fundamental deficit was a mild motor delay, the metabolic panel was standard.

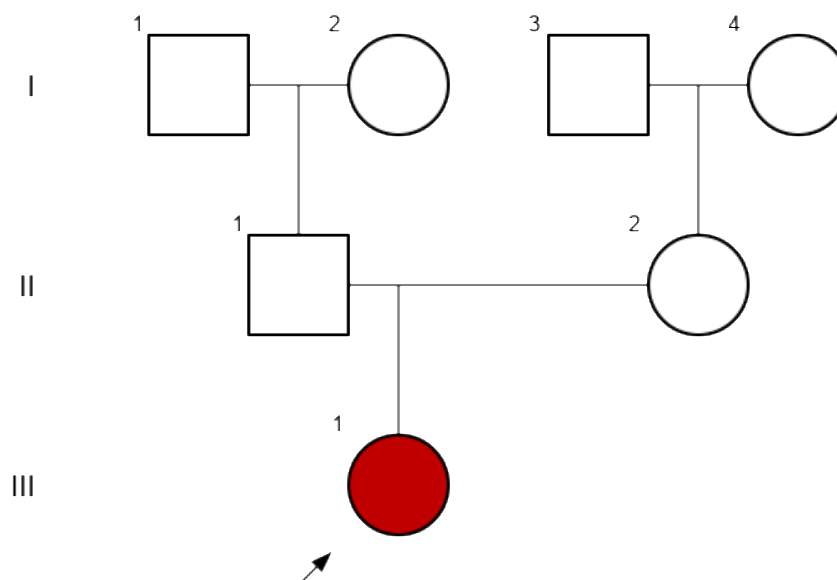


Figure 2 The pedigree of patient 2. The proband is indicated by the arrow. Red indicates KS.

Both patients were evaluated using the international consensus diagnostic criteria for KS [3], which confirmed the diagnosis based on cardinal clinical features and molecular findings. Clinical evaluations prioritized neurodevelopmental and growth parameters. While classical dysmorphic facial features of KS (e.g., arched eyebrows, long palpebral fissures, or depressed nasal tip) were not explicitly documented in these cases, both patients exhibited key clinical features of KS, including neurodevelopmental delay, growth abnormalities, and intellectual disability (Patient 1). Genetic testing confirmed pathogenic variants in *KMT2D*, consistent with the molecular diagnosis of KS under the international consensus criteria [3]. Observed Phenotypes in Reported Cases: Patient 1: Neurodevelopmental delay (walking at 3 years, speech delay with stuttering), Mild intellectual disability, Postnatal growth constraint (preterm birth, low birth weight: 1200 g), and Normal metabolic panel, ruling out alternative diagnoses; Patient 2: Failure to thrive (FTT), Mild motor delay, Elevated neonatal ammonia/lactate (transient), and Polyhydramnios during pregnancy.

To identify potential variants in the two patients suspected of having KS, we performed WES. Using WES, a total read base of 7 million base pairs was obtained for each case. In total, approximately 90000 variants were detected in each proband. The allele frequency-based filtration resulted in approximately 1500 variants. The variations were prioritized according to the severity of pathogenicity, including the splicing region, stop gain, and frameshift mutations. Several amino acid change predictors have been used to prioritize the pathogenic severity of non-synonymous mutations. Finally, WES analysis revealed novel variants in the heterozygous state of *KMT2D*. To be exact, variants with a frequency lower than 1% for the 1000 Genomes Project (<https://www.internationalgenome.org/>), gnomAD (<https://gnomad.broadinstitute.org/>), dbSNP150 (<https://genome.ucsc.edu/cgi-bin/hgTrackUi?db=hg19&g=snp150>), ESP (<https://bio.tools/esp>), and ExAC public databases (<https://www.exac.ca/en/>) were selected for further consideration. Variants reported in the Human Gene Mutation Database (HGMD; <https://www.hgmd.cf.ac.uk/ac/index.php>) and ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) were considered causative. For the conservation assessment of causative variants, the

PhyloP100way, PhastCons100way, fitCons-gm, and SiPhy29way data were used (<https://varsome.com/>). For the pathogenicity score assessment, BayesDel noAF, BayesDel addAF, FATHMM-MKL, MutationTaster, and EIGEN (<https://varsome.com/>) were used. Accordingly, we identified a novel nonsense heterozygous variant in Patient 1, *KMT2D* (NM_003482.4): c.13818C>G/p. Y4606X, and another nonsense heterozygous variant in patient 2, *KMT2D* (NM_003482.4): c.16360C>T/p. R5454X, which affects exons 42 (according to UniProt) and 53 (in the SET domain according to UniProt). Based on the ACMG/AMP guidelines [20], both variants were classified as pathogenic based on the presence of the PVS1 and PS2 criteria. Specifically, the protein-truncating nature of these variants (PVS1) in *KMT2D*, combined with their *de novo* occurrence (PS2), strongly supports their pathogenic roles. No variant was reported in gnomAD, the 1000 Genome Project, HGMD, ExAC, Iranome, ESP 6500, TOPMed Bravo, GME Variome, 4.7KJPN, GenomeAsia, or the Mexican DB. Based on ClinVar and ACMG classifications (<https://www.acmg.net/>), these causative variants were categorized as pathogenic variants [20] (Table 1).

Table 1 Pathogenicity and conservation scores according to online databases.

Variant	Zygosity			Pathogenicity	Conservation
	Proband	Father	Mother		
<i>KMT2D</i> (NM_003482.4): c.13818C>G (p.Tyr4606X)	Het	Hom (WT)	Hom (WT)	BayesDel noAF: Damaging BayesDel addAF: Damaging FATHMM-MKL: Damaging MutationTaster: Disease causing automatic EIGEN: Pathogenic BayesDel noAF: Damaging BayesDel addAF: Damaging FATHMM-MKL: Damaging MutationTaster: Disease causing automatic EIGEN: Pathogenic	PhyloP100way: 6.892 PhastCons100way: 1 fitCons-gm: 0.7025 SiPhy29way: 10.9497 Bstatistic: 412 PhyloP100way: 9.855 PhastCons100way: 1 fitCons-gm: 0.6979 SiPhy29way: 17.7533 Bstatistic: 446
<i>KMT2D</i> (NM_003482.4): c.16360C>T (p.Arg5454X)	Het	Hom (WT)	Hom (WT)		

WT: wild-type.

Sanger sequencing confirmed our observed variants in patients 1 and 2 but not in their parents (Figures 3A and 3B, respectively). This observation revealed that the causative nonsense variants were *de novo* and were inherited in an autosomal dominant pattern.

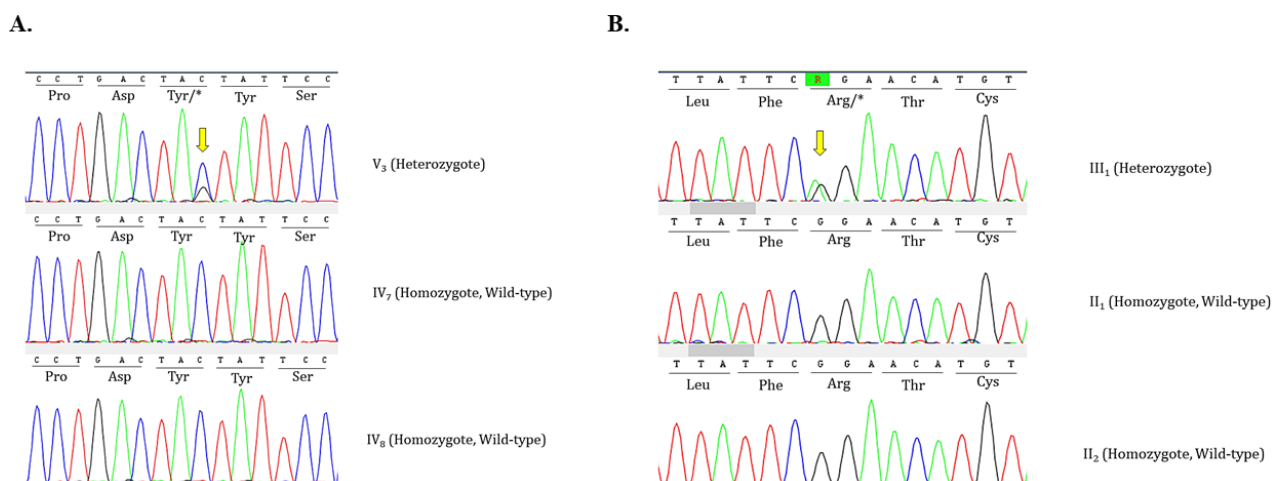


Figure 3 Sanger sequencing was performed to confirm the variants detected. **(A)** Patient 1 (V₃) had a heterozygous mutation, but her parents were wild-type homozygotes. **(B)** Patient 2 (III₁) had a heterozygous mutation, whereas her parents were wild-type homozygotes. The mutation sites are indicated by yellow arrows.

We did not find any predicted protein domain structure validated by nuclear magnetic resonance (NMR) or crystallography for the mutation sites in the *KMT2D* protein in the PDB. Hence, we decided to depict the predicted structure for the mutation sites using the I-TASSER online software [21]. Between the top 10 threading templates, I-TASSER selected PDB hit: 3javA with a Z-score of 5.39, as a template with the highest significance in alignments. To predict the final model, I-TASSER applied the SPICKER program [22] to cluster decoys based on the similarity in the pairwise structure. In this line, the protein model with a C-score of 0.47 (the highest score between the predicted structures), template Modeling (TM; the global structural similarity between two protein structures, ranging from 0 to 1, with higher scores indicating better structural matches)-score = 0.78 ± 0.10 , and the Root Mean Squared Deviation (RMSD; the average distance between the atoms of a predicted structure and a known, native structure, used to assess the accuracy of the prediction; A lower RMSD value indicates a better structural match) = 8.7 ± 4.6 Å, was selected for future analysis (Figure 4A). The simulated protein domain structures after the introduction of premature stop codons are shown (Figures 4B and 4C). To find a similar protein structure, I-TASSER used the TM-align program to match the simulated structure with the closest structure in the PDB library. PDB Hit, 3javA, with a TM score of 0.973, showed the highest match, and consequently may have a function similar to that of the simulated structure. The predicted function based on the COFACTOR and COACH algorithms suggested that this simulated domain had a ligand-binding site identical to that of PDB Hit: 4EVAa with a C-score of 0.05. The name of the proposed ligand for binding to this simulated domain is URE [23].

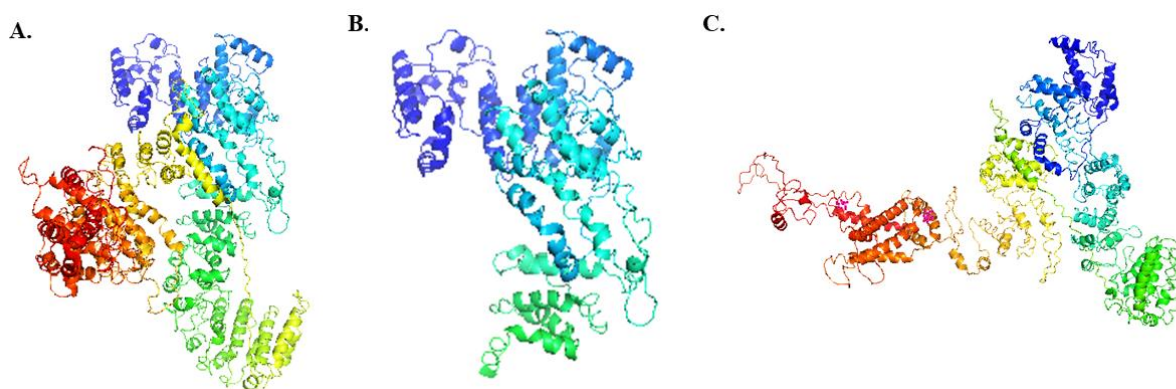


Figure 4 Protein domain structures depicted using I-TASSER. **(A)** Structure of the normal domain. **(B)** Structure of the domain containing the p.Y4606X mutation. **(C)** Structure of the domain with the p.R5454X mutation.

4. Discussion

KS is a congenital mental retardation syndrome, with an approximate prevalence of 1 in 32000. KS is commonly associated with additional features such as peculiar distinct facial dysmorphism (resembling the makeup of the Japanese theatrical form, Kabuki), large prominent earlobes, depressed nasal tip, high-arched palate, persistence of finger pads, scoliosis, recurrent otitis media in infancy, and radiographic abnormalities of the vertebrae [2]. KS is frequently caused by pathogenic mutations in *KMT2D* and *KDM6A* [3]. The diagnosis of KS can be challenging because the clinical signs can vary and overlap with those of other genetic disorders. However, advances in genetic testing have improved the diagnosis and genetic counseling of patients with KS.

KMT2D and *KDM6A* are members of the methyltransferase family and affect the expression of various downstream genes by modifying chromatin remodeling [24]. Downregulation of the ortholog *KMT2D* in zebrafish results in brain, craniofacial, and cardiac anomalies associated with the KS phenotype [25]. Recent studies have shown that mutations in *KMT2D* (*MLL2*) are frequently associated with KS. *KMT2D* encodes a histone methyltransferase, crucial in epigenetic regulation and embryonic development. Mutations in *KMT2D* have been found to affect the development of multiple organs, including the central nervous system, heart, and immune system. KS is a complex disorder caused by various mutations in *KMT2D*, including missense mutations, frameshifts, splice sites, and premature stop codon mutations. The type of mutation can affect the severity and diversity of the clinical features observed in patients with KS. For example, frameshift mutations often lead to a more severe phenotype than missense mutations, whereas a premature stop codon may result in a truncated protein that impairs development across multiple organ systems. Recently, a few cases of KS caused by premature stop codons in *KMT2D* have been reported. These premature stop codons introduce haploinsufficiency in the protein, leading to developmental delays, intellectual disabilities, and other clinical manifestations. In our study, both mutations in *KMT2D* were identified as heterozygous premature stop codons, leading to haploinsufficiency of the *KMT2D* protein. This emphasizes the variability of clinical features in patients with KS and highlights the need for an accurate genetic diagnosis and individualized treatment [26]. The identification of two novel de novo heterozygous protein-truncating mutations, c.13818C>G (p.Y4606X) and c.16360C>T (p.R5454X), in *KMT2D* among our KS patients is consistent with the existing literature that highlights

the prevalence of such loss-of-function variants in this gene. Previous studies, such as those by Hannibal et al. (2011) and Makrythanasis et al. (2013), have consistently documented a spectrum of *KMT2D* mutations, predominantly protein-truncating, distributed throughout the gene's coding region [9, 10]. The localization of our identified mutations, p.Y4606X and p.R5454X, within the latter portion of the *KMT2D* protein further supports the observation that mutations across the gene, rather than being confined to specific domains, contribute to the KS phenotype. Furthermore, the *de novo* nature of these mutations, as observed in our patients, aligns with the established understanding of KS pathogenesis, wherein many cases arise from sporadic rather than inherited genetic events.

Our I-TASSER modeling analysis revealed that introducing premature stop codons into the *KMT2D* protein led to notable structural deviations from the 3javA template, as quantified by TM-score and RMSD metrics. Additionally, the predicted ligand-binding site, similar to that of 4EVAa, indicates a potential functional disruption due to the introduced mutations. These structural changes are hypothesized to compromise the native functional integrity of the *KMT2D* protein, particularly in terms of its ligand-binding capacity, potentially contributing to the KS phenotype. The observation of a high TM-score alignment with 3javA for the simulated structure, despite the presence of mutations, suggests that while the global protein fold is conserved, localized structural perturbations induced by premature stop codons are likely to disrupt ligand interactions. Consequently, disruption of functional ligand-binding domains has emerged as a potentially critical factor in the pathogenesis of KS. Future research should aim to identify the specific ligands involved and elucidate their roles in *KMT2D*-mediated signaling pathways.

Whole-exome sequencing in 10 unrelated subjects affected by KS from different ethnic groups validated nonsense or frameshift mutations in 7 patients. In a follow-up study, 66% of the patients with KS had a mutation in *KMT2D*, all of which occurred *de novo*. Moreover, in two families where KS was transmitted from parent to child, a pathogenic mutation was observed in *KMT2D* [13]. In another study considering 110 kindreds with KS, 74% of the patients had a mutation (commonly nonsense or frameshift) in *KMT2D*. Remarkably, 25 cases presented *de novo* mutations, and 2 of 3 familial cases showed mutations in *KMT2D* [9]. Truncating mutations may cause haploinsufficiency in *KMT2D*. Truncating mutations can be distributed alongside this gene, whereas non-truncating mutations are mostly confined to the functional domains. Miyake et al. reported that 61.7% of KS patients in their study had a mutation in *KMT2D*, among which 70% were protein-truncated [27]. Micale et al. used multiplex ligation-dependent probe amplification (MLPA) and direct sequencing to find pathogenic mutations in 303 KS patients. They identified 133 mutations in the *KMT2D*, of which 62 were *de novo*. They also demonstrated that these mutations in patients' lymphoblastoid and skin fibroblast cell lines resulted in dysregulation of *KMT2D* target genes [28]. In the present study, we identified two distinct protein-truncating mutations in exons 42 and 53 that caused haploinsufficiency in *KMT2D*.

Genotype-phenotype correlation studies indicated that patients with mutations in *KMT2D* had typical KS facial features. Moreover, KS patients with *KMT2D* mutations have kidney anomalies, joint dislocations, feeding problems, early breast bud development, and palatal malformations compared to KS patients without *KMT2D* mutations [11]. Miyake et al. reported that KS patients harboring the truncating mutation in *KMT2D* had facial features that were more typical than those of cases originally considered as KS. In addition, short fifth fingers, high-arched eyebrows, and infantile

hypotonia were mainly observed in KS patients with *KMT2D* mutations compared with those with *KDM6A* mutations.

In our reported cases, Patient 1 was born early term due to PROM and had four fundamental deficits: neurodevelopmental disorders without seizures, walking delay, mild intellectual disability, and mild speech delay with slight stuttering. Patient 2 was born full-term through cesarean section from a polyhydramnios pregnancy and had a history of failure to thrive (FTT) but showed mild motor delay. Both patients had regular metabolic panels.

Both cases demonstrate the complex nature of KS and the importance of identifying genetic mutations to provide accurate diagnoses and potential treatments. The observation of two cases with differing parental consanguinity highlights the variable genetic backgrounds encountered in KS, further emphasizing the importance of considering both genetic and environmental factors when evaluating patients with developmental delays. In other words, these cases highlight the importance of identifying genetic mutations in patients with KS to provide accurate diagnosis, prognosis, and potential treatments.

Although there is no cure for KS, identifying specific genetic mutations can help guide the management and treatment options. For example, patients with mutations in *KMT2D* may benefit from early interventions such as physical therapy, speech therapy, and educational support to maximize their overall development.

5. Conclusion

In this study, we identified two novel protein-truncating mutations in *KMT2D* (c.13818C>G and c.16360C>T) in two unrelated Iranian families likely to cause KS. The cases presented in this article add to the growing literature on KS caused by premature stop codons in *KMT2D*. Our findings provide insights into the role of premature stop codons in *KMT2D* in the etiology of KS and highlight the importance of genetic testing in diagnosing KS. Further research is required to understand better the mechanisms underlying this complex disorder. Therefore, understanding the role of epigenetic regulation, including both histone modification and DNA methylation, in human development through *KMT2D* mutations can provide insights into the development of more effective treatments for affected patients.

Acknowledgments

We would like to thank all the patients and their families for their participation in this research.

Author Contributions

Mohammad-Reza Ghasemi: Conceptualization, Investigation, Methodology, Validation, Formal analysis, writing-original draft; Maryam Mirahmadi: Investigation, Methodology, Formal analysis, writing-original draft; Hadi Bayat: Investigation, Formal analysis, writing-review and editing; Mohammad Miryounesi: Investigation, Methodology, Validation, Resources; Reza Mirfakhraie: Investigation, Methodology, Validation, Resources; Shadab Salehpour: Methodology; Raheleh Tangestani: Methodology; Faezeh Sherafat: Investigation, Methodology; Hasan Roudgari: Conceptualization, Investigation, Methodology, Validation, Resources, supervision, writing-review and editing; Milad Gholami: Conceptualization, Investigation, Methodology, Validation, Resources,

supervision, Project administration, Funding acquisition, writing-review and editing. All the authors have read and agreed to the published version of the manuscript.

Funding

This study was funded by Arak University of Medical Sciences (Grant Number: 4123).

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

References

1. Boigontier J, Tacchella JM, Lemaître H, Lehman N, Saitovitch A, Gatinois V, et al. Anatomical and functional abnormalities on MRI in kabuki syndrome. *Neuroimage Clin.* 2019; 21: 101610.
2. Salguero MV, Chan K, Greeley SA, Dyamenahalli U, Waggoner D, Del Gaudio D, et al. Novel *KDM6A* Kabuki syndrome mutation with hyperinsulinemic hypoglycemia and pulmonary hypertension requiring ECMO. *J Endocr Soc.* 2022; 6: bvac015.
3. Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, et al. Kabuki syndrome: International consensus diagnostic criteria. *J Med Genet.* 2019; 56: 89-95.
4. Niikawa N, Matsuura N, Fukushima Y, Ohsawa T, Kajii T. Kabuki make-up syndrome: A syndrome of mental retardation, unusual facies, large and protruding ears, and postnatal growth deficiency. *J Pediatr.* 1981; 99: 565-569.
5. Kuroki Y, Suzuki Y, Chyo H, Hata A, Matsui I. A new malformation syndrome of long palpebral fissures, large ears, depressed nasal tip, and skeletal anomalies associated with postnatal dwarfism and mental retardation. *J Pediatr.* 1981; 99: 570-573.
6. Yap KL, Johnson AE, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: Clinical and molecular characterization of 10 affected individuals. *Genet Med.* 2019; 21: 233-242.
7. 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: 298-305.
8. Moon JE, Lee SJ, 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: 102.
9. 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; 155: 1511-1516.
10. Makrythanasis P, Van Bon BW, Steehouwer M, Rodríguez-Santiago B, Simpson M, Dias P, et al. *MLL2* mutation detection in 86 patients with Kabuki syndrome: A genotype-phenotype study. *Clin Genet.* 2013; 84: 539-545.

11. Banka S, Veeramachaneni R, Reardon W, Howard E, Bunstone S, Ragge N, et al. How genetically heterogeneous is Kabuki syndrome?: *MLL2* testing in 116 patients, review and analyses of mutation and phenotypic spectrum. *Eur J Hum Genet.* 2012; 20: 381-388.
12. Rabbani B, Tekin M, Mahdih N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet.* 2014; 59: 5-15.
13. 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: 790-793.
14. Montano C, Britton JF, Harris JR, Kerkhof J, Barnes BT, Lee JA, et al. Genome-wide DNA methylation profiling confirms a case of low-level mosaic Kabuki syndrome 1. *Am J Med Genet A.* 2022; 188: 2217-2225.
15. Banan M, Bayat H, Azarkeivan A, Mohammadparast S, Kamali K, Farashi S, et al. The *XmnI* and *BCL11A* single nucleotide polymorphisms may help predict hydroxyurea response in Iranian β -thalassemia patients. *Hemoglobin.* 2012; 36: 371-380.
16. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2010; 26: 589-595.
17. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. *Bioinformatics.* 2009; 25: 2078-2079.
18. Costanza P, Herzeel C, Verachtert W. A comparison of three programming languages for a full-fledged next-generation sequencing tool. *BMC Bioinformatics.* 2019; 20: 301.
19. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010; 38: e164.
20. 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: 405-423.
21. Zheng W, Zhang C, Li Y, Pearce R, Bell EW, Zhang Y. Folding non-homologous proteins by coupling deep-learning contact maps with I-TASSER assembly simulations. *Cell Rep Methods.* 2021; 1: 100014.
22. Zhang Y, Skolnick J. *SPICKER*: A clustering approach to identify near-native protein folds. *J Comput Chem.* 2004; 25: 865-871.
23. Zhang C, Freddolino L, Zhang Y. COFACTOR: Improved protein function prediction by combining structure, sequence and protein-protein interaction information. *Nucleic Acids Res.* 2017; 45: W291-W299.
24. 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: 1335-1344.
25. 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: 4443-4453.
26. Barry KK, Tsapalis M, Hoffman D, Hartman D, Adam MP, Hung C, et al. From genotype to phenotype-a review of Kabuki syndrome. *Genes.* 2022; 13: 1761.
27. 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; 161: 2234-2243.

28. 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: 841-850.