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Investigation of genetic and phenotypic heterogeneity in 37 Turkish patients with Kabuki and Kabuki-like phenotype

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Abstract

Kabuki syndrome (KS) is a rare disorder characterized by distinct face, persistent fingertip pads, and intellectual disability (ID) caused by mutation in *KMT2D* (56%–76%) or *KDM6A* (5%–8%). Thirty-seven children aged 1–16 years who followed for median of 6.8 years were included in this study, which aimed to investigate the genetic and clinical characteristics of KS patients. *KMT2D* and *KDM6A* were evaluated by sequencing and multiplex-ligation-dependent probe amplification in 32 patients. Twenty-one pathogenic variants in *KMT2D*, of which 17 were truncated and nine were novel, one frame-shift novel variant in *KDM6A* were identified. The molecular diagnosis rate was 68.7% (22/32). In the whole-exome sequencing analysis performed in the remaining patients, no pathogenic variant that could cause any disease was detected. All patients had ID; 43.2% were severe and moderate. We observed that facial features that became more prominent with age were enough for a possible diagnosis of KS in infancy. The frequencies of facial features, cardiac and renal anomalies, short stature, microcephaly, and epilepsy did not differ depending on whether they had truncating or nontruncating variants or were in variant-negative KS-like group. This study has expanded clinical features of the disease, as well as identified new variants in genes causing KS.

KEYWORDS
facial features, Kabuki syndrome, Kabuki-like phenotype, *KDM6A*, *KMT2D*, MLPA

1 | INTRODUCTION

Kabuki syndrome (KS) [OMIM:147920 and 300867] is a disease characterized by long palpebral fissures with eversion of the lateral third of the lower eyelids, permanent fetal finger pads, a varying degree of intellectual disability (ID), short stature, congenital heart disease (CHD), and renal anomalies. It was described by Niikawa and Kuroki and named Kabuki due to the facial gestalt of these patients consisting of long eyelids and slight ectropion in one-third of the lower eyelid, similar to traditional Kabuki actors in Japan (Kuroki et al., 1981). The prevalence has been 1/32.000 birth in the Japanese population (Niikawa et al., 1988).

Heterozygous loss-of-function variants in lysine (K)-specific methyltransferase 2D (*KMT2D*; formerly *MLL2*; NM_003482.3) were discovered to cause KS (Ng et al., 2010). Two years later, it was revealed that *KDM6A* (NM_021140.3) also causes KS (Lederer et al., 2012). *KMT2D* is responsible for 56%–76% of the patients (Murakami et al., 2020) and encoding a methyltransferase responsible for dimethylation and trimethylation of histone 3 lysine 4 (H3K4), which are the epigenetic marks for active transcription and euchromatin (Froimchuk et al., 2017). *KDM6A* demethylates histone 3 lysine-27 (H3K27) responsible for 5%–8% of KS (Bögershausen et al., 2016). Most variants in *KMT2D* and *KDM6A* are truncating ones and eliminate enzyme activity (Bögershausen et al., 2015; Miyake et al., 2013).

Also, large gene deletions in *KMT2D* and *KDM6A* were defined in KS (Banka et al., 2013; Lederer et al., 2012). The genetic etiology of 20%–45% clinically diagnosed KS is still unknown (Banka et al., 2012).

The presence of typical facial features together with ID provides the clinical diagnosis of KS. Makrythanasis et al. (2013) developed a scoring system, called "Kabuki phenotype scoring list" with 23 individual phenotypic features, after their cohort study with 86 patients. They found the clinical score significantly higher in patients with the *KMT2D* variant, and these patients had more severe Kabuki phenotypes than those patients with the molecular-negative group. In 2019, the consensus of the international group of KS experts decided to use diagnostic criteria that would include patients with possible or probable KS, which could help support the clinical diagnosis of KS (Adam et al., 2019). They proposed that a definitive clinical diagnosis can be made in an individual of any age with a history of infantile hypotonia, developmental delay, and one or both of the following major criteria: (1) a pathogenic or likely pathogenic variant in *KMT2D* or *KDM6A*; and (2) typical dysmorphic features. Typical dysmorphic features include long palpebral fissures with eversion of the lateral third of the lower eyelid and ≥2 of the following signs: arched and broad eyebrows with sparseness in the lateral one-third, short columella with depressed nasal tip, large, prominent, or cupped ears, and persistent fingertip pads.

The aim of this study is to evaluate clinical features of KS in the Turkish cohort, to investigate the presence of variants in *KMT2D* and *KDM6A*, by next-generation sequencing (NGS), multiplex ligation-dependent probe amplification (MLPA), and whole-exome sequencing (WES).

2 | MATERIALS AND METHODS

2.1 | Study cohort

Thirty-four patients (18 girls and 16 boys) with clinically suspected KS and three girls with the Kabuki-like Turner syndrome phenotype were included in this study (Table S1). Clinical diagnosis was made according to the consensus criteria of the international KS group (Adam et al., 2019). Five patients who had typical clinical findings according to the consensus criteria of the international KS group, but could not undergo molecular analysis, were also included. Twenty-six patients have been followed up for 1–23 years (median: 6.8 years). Height, head circumference, and weight were measured by a pediatrician. Eye and hearing examinations, echocardiographic and urinary ultrasonographic investigations were performed on all patients. Denver Developmental Screening Test II and WISC-R test were used to assess developmental delay and ID. Cranial MRI was performed on 19 patients. Written consent to use both parents' and their children's clinical and laboratory data and to print the photographs was obtained from the parents.

2.2 | Genetic analysis

Initially, chromosomal analysis was performed in patients. Genomic DNA was extracted from peripheral blood samples of the patients according to standard procedures.

2.2.1 | Genetic test algorithm

KMT2D and *KDM6A* sequencing with NGS was performed in 32 patients at the first stage. MLPA for *KMT2D/KDM6A* and then WES were performed in 10 patients without *KMT2D* and *KDM6A* variants.

2.2.2 | *KMT2D* and *KDM6A* sequencing

Two genes were analyzed by targeted NGS on Illumina Mi-Seq platform. The variant interpretation was made according to the American College of Medical Genetics and Genomics (ACMG) practice guidelines (Richards et al., 2015). For Sanger sequencing of novel variants in parental DNA, reactions were performed by ABI PRISM 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA) and were analyzed using Cutepeaks (https://github.com/labsquare/cutepeaks).

2.2.3 | Multiplex ligation-dependent probe amplification

Large deletions and duplications of *KMT2D* and *KDM6A* genes were analyzed by MLPA using commercially available assays (P389-B1 *MIL2* and P445-A3 *KDM6A*; MRC-Holland).

2.2.4 | Whole-exome sequencing

Genomic DNA of the proband was captured using IDTxGen Exome Panel (Integrated DNA Technologies, Coralville, IA, USA), followed by 101 base paired-end sequencing on the Illumina NovaSeq 6000. Sequences were aligned to the human genome GRCh37/hg19 with BWA-MEM. Further processing was performed according to the best practices of the Genome Analysis Toolkit. Variant calls were made with GATK Haplotype Caller and annotated with ANNOVAR. The pathogenicity of the variants was interpreted according to ACMG guidelines (Richards et al., 2015).

2.2.5 | Statistical analysis

The frequency of clinical features among patients with truncating and nontruncating variant and the molecularly undiagnosed group were compared by Fisher's exact test. If the probability value (*P*-value) was <0.05, it was considered statistically significant.

3 | RESULTS

3.1 | Genetic studies

The karyotype analysis revealed 45, X in two patients and 45,X (54%)/46,X,r(X) (46%) in one patient in which patients were classified as KS-like Turner syndrome.

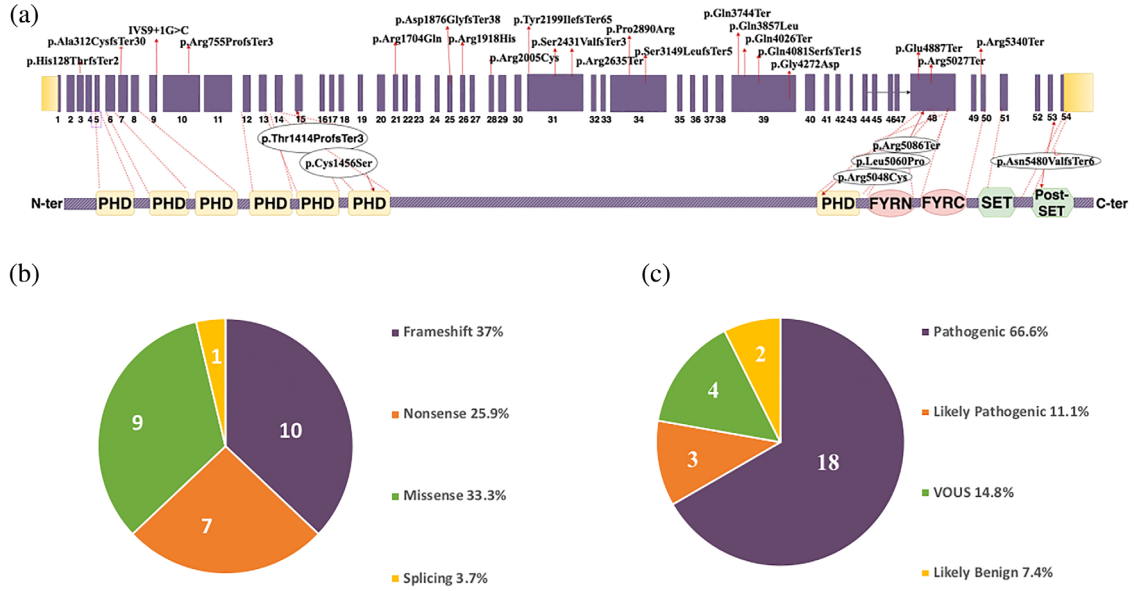

FIGURE 1 Overview of exon and protein distribution (a), variant type (b), and pathogenicity (c) of *KMT2D*-identified variants. (a) The upper panel indicates the genomic structure of *KMT2D* consisting of 54 exons, and the protein structure shows the protein domains and motifs including the PHD, FYRN, FYRC, Su(var)3–9, enhancer-of-zeste, Trithorax (SET) domain, and PostSET domain. Arrows indicate the exonic location of the variants identified in the patients. The lower panel indicates six variants in the functional domains. (b) Variant types of *KMT2D* were identified in this study. (c) Classification of all variants identified in this study according to the 2015 ACMG guidelines

Seventeen *KMT2D* variants (80.9%) were truncating (7 nonsense and 10 frameshift), one was splicing, and three were missense (Figure 1 and Table 1). All truncating variants and one splicing variant met the PVS1 + PM2 + PP3 criteria according to the ACMG classification and were classified as pathogenic. Three likely pathogenic missense variants met the PM1 + PM2 + PM3 criteria, two of which were novel, are located in the functional domain of *KMT2D* based on a prediction by SMART (http://smart.embl-heidelberg.de/) and the UniProtKB database (http://www.uniprot.org/uniprot/O14686). A novel frameshift variant was detected in *KDM6A* in one patient and classified as pathogenic (PVS1 + PM2). The variant detection rate was 68.7% (22/32).

Moreover, we identified additional six novel missense variants in *KMT2D* (Table 1); four of them (c.11570A>T, c.6013C>T, and c.5753G>A, c.12815G>A) were found together with pathogenic and likely pathogenic variants in Patients 6, 14, 18, and 21. When we classified them according to the ACMG guidelines, two (c.11570A>T and c.6013C>T) were uncertain significance (VOUS) (PM2 and/or PP3), and two (c.5753G>A and c.12815G>A) were likely benign (LB) and parentally inherited. Two heterozygous missense variants, which were inherited from their parents were also detected in two patients with no other pathogenic variants causing disease (Table 1). Although the facial findings of these patients were very typical of KS, their parents' facies did not resemble them and were therefore considered as nondisease-causing variants (polymorphisms).

We could not find any deletion or duplication in MLPA analysis for *KMT2D* and *KDM6A* in ten patients without any variant of these genes. In addition, in the WES analysis performed in these patients, no pathogenic variants were found in the genes associated with KS and KS-like syndromes.

3.2 | Clinical features

The age of clinical diagnosis was between 1 and 16 years (median 5 years). The clinical findings of variant-positive and -negative groups and those who could not perform genetic testing are compared in Table 2.

All of the patients had recognizable facial features such as long palpebral fissures with eversion of the lateral third of the lower eyelid, arched and broad eyebrows with sparseness in the lateral one-third, and either developmental delay or ID (Table 2 and Figures 2 and 3). The rates of common craniofacial findings of patients, such as large dysplastic ears, short columella with depressed nasal tip, broad nasal root, and high or cleft palate, were 78.3%, 81.8%, 93.9%, and 70.2%, respectively (Table 2). Permanent fingertip pads, a pathognomonic finding, were present in 90.9% of the patients. Other minor skeletal abnormalities such as brachydactyly and clinodactyly were seen in 54% and 45.9% of the patients, respectively. The common accompanying multisystem abnormalities were CHD (48.6%), renal anomalies (43.2%), ptosis (51.3%), strabismus (40.5%), epilepsy (37.8%), microcephaly (29.7%), and gastrointestinal anomalies (16.2%).

All patients had certain degrees of ID with 24.3% severe, 18.9% moderate, and 56.7% mild. The frequency of nonspecific central nervous system malformations such as Dandy-Walker malformation and

TABLE 1 Distribution of *KMT2D* and *KDM6A* variants according to their types, protein change, pathogenicity, and novelty

Patient No	*KMT2D* variant (NM_003482.3)	Type	Protein change	Exon	Domain	Novelty	Pathogenicity	Segregation
P1	c.5627_5630delACAG	Frameshift	p.Asp1876GlyfsTer38	25	NF	–	P	–
P2	c.15256C>T	Nonsense	p.Arg5086Ter	48	F(PHD2)	–	P	–
P3	c.6595delT	Frameshift	p.Tyr2199IlefsTer65	31	NF	–	P	–
P4	c.12241delC	Frameshift	p.Gln4081SerfsTer15	39	NF	+	P	De Novo
P5	c.9446_9449delCCCT	Frameshift	p.Ser3149LeufsTer5	34	NF	+	P	NA
P6	c.4367G>C	Missense	p.Cys1456Ser	15	F(PHD4)	+	LP	De Novo
	c.5753G>A[a]	Missense	p.Arg1918His	26	NF	+	LB	Inherited Pat
P7	c.1258 + 1G>C	Splicing	–	Intron 9	–	+	P	De Novo
P8	c.12076C>T	Nonsense	p.Gln4026Ter	39	NF	–	P	–
P9	c.7289dup	Frameshift	p.Ser2431ValfsTer3	31	NF	–	P	–
P10	c.15079C>T	Nonsense	p.Arg5027Ter	48	NF	–	P	–
P11	c.16018C>T	Nonsense	p.Arg5340Ter	50	NF	–	P	–
P12	c.932dupC	Frameshift	p.Ala312CysfsTer30	7	NF	+	P	NA
P13	c.2263dupC	Frameshift	p.Arg755ProfsTer3	10	NF	–	P	–
P14	c.14659G>T	Nonsense	p.Glu4887Ter	48	NF	–	P	–
	c.11570A>T[a]	Missense	p.Gln3857Leu	39	NF	+	VOUS	NA
P15	c.11230C>T	Nonsense	p.Gln3744Ter	39	NF	+	P	NA
P16	c.7903C>T	Nonsense	p.Arg2635Ter	31	NF	–	P	–
P17	c.16438_16441delAACT	Frameshift	p.Asn5480ValfsTer6	53	F(SET)	–	P	–
P18	c.15179T>C	Missense	p.Leu5060Pro	48	F(PHD2)	+	LP	NA
	c.6013C>T[a]	Missense	p.Arg2005Cys	28	NF	+	VOUS	NA
P19	c.15142C>T	Missense	p.Arg5048Cys	48	F(PHD2)	–	LP	–
P20	c.382delC	Frameshift	p.His128ThrfsTer2	3	NF	+	P	NA
p21	c.4239delC	Frameshift	p.Thr1414ProfsTer3	15	F(PHD3)	+	P	De novo
	c.12815G>A[a]	Missense	p.Gly4272Asp	39	NF	+	LB	Inherited Mat
P23	c.5111G>A	Missense	p.Arg1704Gln	21	NF	+	VOUS	Inherited Pat
P24	c.8669C>G	Missense	p.Pro2890Arg	34	NF	+	VOUS	Inherited Pat/Mat
***KDM6A* Variant (NM_021140.3)**								
P22	c.4068dupA	Frameshift	p.His1357ThrfsTer32	28	–	+	P	De novo

Abbreviations: F, functional; LB, likely benign; LP, likely pathogenic; Mat, maternal; NA, not available; NF, nonfunctional; P, pathogenic; Pat, paternal; PHD, plant homeobox domain; SET, Su(var)3–9, Enhancer-of-zeste, trithorax (SET) domain; VOUS, variant of uncertain significance.

[a]Detected together with another variant (P6, P14, P18, and P21).

TABLE 2 Clinical features in the patients with variant-positive and -negative and without a molecular diagnosis

	KMT2D/KDM6A variants (+) (n:22)					*KMT2D/KMD6A* variants (−) (n:10)						
Patients number	Truncating/ splicing (n:18)	Missense (n:3)	*P** value	KDM6A (n:1)	Total (n:22)	Turner (n:3)	Other (n:7)	Total (n:10)	*P*** value	No test (n:5)	*P**** value	Total (n:37)
Long palpebral fissures with everted lower eyelid	18/18	3/3	–	1/1	22/22	3/3	7/7	10/10	–	5/5	–	37/37
Arched eyebrows with sparse lateral third	18/18	3/3	–	1/1	22/22	3/3	7/7	10/10	–	5/5	–	37/37
Ptosis	10/18	2/3	1.000	0/1	12/22	1/3	4/7	5/10	1.000	2/5	0.648	19/37
Strabismus	9/18	1/3	1.000	0/1	10/22	0/3	3/7	3/10	0.467	2/5	1.000	15/37
Short columella with depressed nasal tip	13/15	2/3	0.442	1/1	16/19	1/3	5/6	6/9	0.352	5/5	1.000	27/33
Large dysplastic ears	15/18	2/3	0.489	1/1	18/22	3/3	4/7	7/10	0.648	4/5	1.000	29/37
Broad nasal root	15/15	3/3	–	1/1	19/19	2/3	5/6	7/9	0.095	5/5	–	31/33
High or cleft palate	16/18	2/3	1.000	1/1	19/22	2/3	5/7	7/10	0.681	2/5	0.136	28/37
High palate	11	2/3	1.000	1/1	14/22	2/3	5/7	7/10	1.000	2/5	0.370	
Cleft palate	5/18	0/3	0.549	0/1	5/22	0/3	1/7	1/10	0.637	0/5	0.547	
Persistent fetal pads	17/17	3/3	–	1/1	21/21	1/3	6/7	7/10	0.027	2/2	–	30/33
Hip dislocation	3/18	0/3	1.000	0/1	3/22	0/3	1/7	1/10	1.000	1/5	1.000	5/37
Brachydactyly	11/18	3/3	1.000	1/1	15/22	0/3	3/7	3/10	0.062	2/5	0.326	20/37
Clinodactyly	10/18	2/3	1.000	1/1	13/22	0/3	4/7	4/10	0.450	0/5	0.041	17/37
Joint laxity	8/18	1/3	1.000	0/1	9/22	0/3	1/7	1/10	0.114	0/5	0.136	10/37
Premature thelarche	1/18	1/3	0.271	1/1	3/22	0/3	0/7	0/10	0.534	0/5	1.000	3/37
CHD	13/18	1/3	1.000	1/1	15/22	2/3	5/7	7/10	0.446	0/5	0.060	22/37
PPS	1/18	0/3	1.000	0/1	1/22	0/3	1/7	1/10	0.534	0/5	1.000	
MVP	3/18	0/3	1.000	0/1	3/22	0/3	0/7	0/10	1.000	0/5	1.000	
BAV	2/18	0/3	1.000	0/1	2/22	1/3	1/7	2/10	0.572	0/5	1.000	
VSD	3/18	0/3	1.000	1/1	4/22	0/3	2/7	2/10	1.000	0/5	0.565	
ASD	4/18	1/3	1.00	0/1	5/22	0/3	1/7	1/10	0.637	0/5	0.547	
AAH	0/18	0/3	–	–	0/22	1/3	0/7	1/10	0.313	0/5	–	
ACo	0/18	0/3	–	–	0/22	1/3	0/7	1/10	0.313	0/5	–	
Renal anomalies	10/18	1/3	0.586	1/1	12/22	2/3	1/7	3/10	0.265	1/5	0.326	16/37
Gastrointestinal anomalies	5/18	0/3	0.549	0/1	5/22	0/3	1/7	1/10	0.637	0/5	0.547	6/37
Short stature	8/18	0/3	0.257	1/1	9/22	1/3	1/7	2/10	0.425	2/5	1.000	13/37
Microcephaly	8/18	0/3	0.257	0/1	8/22	0/3	1/7	1/10	0.210	2/5	1.000	11/37
Epilepsy	7/18	2/3	0.553	0/1	9/22	0/3	2/7	2/10	0.425	3/5	0.628	14/37

TABLE 2 (Continued)

Patients number	*KMT2D/KDM6A* variants (+) (n:22)					*KMT2D/KMD6A* variants (−) (n:10)					
	Truncating/ splicing (n:18)	Missense (n:3)	*P** value	KDM6A (n:1)	Total (n:22)	Turner (n:3)	Other (n:7)	Total (n:10)	*P*** value	No test (n:5)	*P**** value	Total (n:37)
Cranial MRI abnormality	7/12	1/1	1.000	–	8/13	0/1	2/3	2/4	1.000	1/2	1.000	11/19
Intellectual disability	18/18	3/3	–	1/1	22/22	3/3	7/7	10/10	–	5/5		37/37
Severe	4/18	0/3	1.000	1/1	5/22	0/3	1/7	1/10	0.637	3/5	0.136	9/37
Moderate	3/18	1/3	0.489	0/1	4/22	1/3	2/7	3/10	0.648	0/5	0.561	7/37
Mild	11/18	2/3	1.000	0/1	13/22	2/3	4/7	6/10	1.000	2/5	0.628	21/37

Abbreviations: AAH, aortic arch hypoplasia; ACo, Aortic coarctation; ASD, atrial septal defect; BAV, bicuspit aortic valve; CHD, congenital heart disease; MRI, magnetic resonance imaging; MVP, mitral valve prolapsus; PPS, peripheral pulmonary stenosis; VSD, ventricular septal defect.

*Shows the statistical analysis between truncating groups and nontruncating groups.

**Shows the statistical analysis of between *KMT2D/KDM6A* variants (+) groups and *KMT2D/KMD6A* variants (−) groups.

***Shows the statistical analysis of between *KMT2D/KDM6A* variants (+) group and clinically diagnosed group.

corpus callosum hypoplasia was 57.8% in 19 patients who underwent cranial MRI. Epilepsy was present in 37.8% of the patients and occurred prior to or at the age of 4 years, except for one.

Short stature was detected in 35.1% of the patients. Final height without growth hormone therapy ranged between 0.15 and −3.93 standard deviation (*SD*) in eight females and −0.68 *SD* in one male patient. After 3 years of growth hormone treatment, Patient 5's height *SD* score improved from −3.40 to −1.63.

Likewise, rare findings include hearing loss (8/37), cryptorchidism [4/16 (male)], coloboma (3/37), premature thelarche [3/21(female)], uterus didelphys [1/21 (female)], bicornuate uterus [1/21 (female)], and congenital hypothyroidism (2/37).

3.2.1 | Comparison of clinical features of patients with variant-positive and -negative groups

Typical facial features

The photographs of patients with *KMT2D* variants from the age of 5 months to 28 years are shown in Figure 2. In photographs of Patient 13, aged 1, 3, and 16 years (Figure 2a–c), and Patient 2, aged 5 and 28 years (Figure 2ç,d), it is seen that typical facial features become pronounced with increasing age, and ptosis becomes more severe (Figure 2a–d). When we compared the facial features in all patients, there was no statistical difference between the frequency of these findings in both the truncating (Figure 2a–u,y,z) and missense groups (Figure 2ü–x) and the molecular positive and negative groups ($P > 0.05$) (Table 2). The patient with the *KDM6A* variant also had typical facial features of KS and long halluces (Figure 2q–s). In the variant-negative group, there were 10 patients (Figure 3a–q), three of which had Kabuki-like Turner syndrome (Figure 3m–q). All of them demonstrated typical KS facial features. Dense eyelashes were present in our two patients with KS-like Turner syndrome.

Other clinical features

We observed an increased rate of persistent fetal pads in patients in the variant-positive group compared to the variant-negative group (*P* value: 0.027 < 0.05). The patient with *KDM6A* had premature thelarche, hypothyroidism, and skeletal anomalies such as clinodactyly, third and fourth metatarsal shortening on the left and bilateral long halluces. Interestingly, Patient 28 in the variant-negative group had resembling skeletal findings for the *KDM6A* phenotype (Figure 3f–h). While IQ scores in the variant-positive group ranged from 23 to 70 points (mean: 48), those in the variant-negative group ranged from 43 to 60 points (mean: 49). In the final examination of seven individuals, a decrease in the mean ID scores (between 71 and 42) was observed, of which four were in the truncating-variant positive group and three in the variant-negative group.

The rate of facial, skeletal findings, and visceral abnormalities of five patients without molecular analysis, were identical to those with *KMT2D* variants, except for clinodactyly finding ($P = 0.04 < 0.05$) (Figure 3r–v and Table 2).

FIGURE 2 Facial photographs of the variant-positive patients at different ages. Patient 13 at the ages of 1 year, 3 years, and 16 years (a–c); Patient 2 at the ages of 5 years and 28 years (ç,d); Patient 20 at the age of 7 years (e); Patient 16 at the age of 1 year (f); Patient 7 at the age of 16 months and 10 years (g,h); Patient 8 at the age of 3.5 years (ı), Patient 14 at the ages of 4 years and 10 years (i,j), Patient 11 at the age of 3.5 years (k); Patient 15 at the age of 5 months (l), Patient 4 at the age of 6 years and 10 years (m,n); Patient 5 at the age of 15 years and 20 years (o,ö); Patient 1 at the age of 1 year (p); Patient 22 with *KDM6A* variant, at the age of 2 years and 10 years (q,r). Note third and fourth metatarsal shortening on the left and bilateral long halluces (s); Patient 17 at the age of 11 years (ş), Patient 21 at the age of 9 years and 20 years (t,u); Patient 6 at the age of 7.5 months and 5 years (ü,v); Patients 18 at the age of 5 years(w), Patient 19 at the age of 5 years (x), Patient 3 at the age of 6 years and 14 years (y,z). Note that all patients had long palpebral fissures, eversion of the lateral third of the eyelid, arched, broad laterally sparsed eyebrows, short columella, depressed nasal tip, broad nasal root, and large dysplastic ears. Facial features become more pronounced with age (a–d). Patients with missense variants (ü–w) and patient with *KDM6A* variant (q–s) also had typical facial features

4 | DISCUSSION

4.1 | Molecular results

We detected 21 disease-causing *KMT2D* (65.6%) variants, and one *KDM6A* variant (3.1%), 10 of which were novel (Table 1). In large cohorts of KS, including 116, 81, 100, and 347 patients, the molecular diagnosis rate was reported as 63.8%, 67.9%, 80%, and 63%, respectively (Banka et al., 2012; Bögershausen et al., 2016; Miyake et al., 2013; Murakami et al., 2020).

To date, 1050 different variants in *KMT2D* have been reported (HGMD, http://www. hgmd.org). *KMT2D* variants were reported as 70%–80% truncating, 16%–30% missense type (Bögershausen et al., 2016; Miyake et al., 2013; So et al., 2020). Pathogenic variants mostly had been detected in the large *KMT2D* exons, 39 and 48 (Banka et al., 2012; Bögershausen & Wollnik, 2013; Bögershausen et al., 2016; Liu et al., 2015; Ng et al., 2010). The majority (80.9%) of the disease-causing variants detected in *KMT2D* in our study were truncating-type, six of which were novel; the exons 39 and 48 were commonly affected in *KMT2D* (Table 1 and Figure 1). Three missense

FIGURE 3 Facial photographs of the variant-negative patients at different ages (a–q): Patient 27 at the age of 15 months, 7 years, and 13 years (a–c); Patient 29 at the age of 16 years (d); Patient 26 at the age of 7.5 years (e); Patient 28 at the age of 13 years and 25 years. Note fetal finger pads, short fourth and fifth metacarpal bones and fourth metatarsal bones, overlapping toes (f–h); Patient 25 at the age of 10 years (i), Patient 24 at the age of 5.5 years and 18 years (j,l). Note typical fetal pad. Photographs of three patients with Kabuki-like Turner syndrome (m–q). Note that the facial features of these patients were similar to variant-positive patients: Patient 30 at the age of 2.5 years (m), Patient 32 at the age of 4 years (n,o), Patient 31 at the age of 17 months and 11 years (p,q). The patients without molecular testing: Patient 34 at the age of 3 years and fetal finger pads (r,s); Patient 33 at the age of 8 years (t); Patient 36 at the age of 4 years (u); Patient 37 at the age of 15 years (v). They have typical KS clinical features

variants (14.2%) were classified as likely pathogenic, two of which were novel and considered as disease-causing. The pathogenicity of missense variants in *KMT2D* and whether they cause KS is not yet clear. *KMT2D* consists of plant homeobox domain (PHD), FY-rich domain N-terminal region (FYRN), FY-rich domain C-terminal region (FYRC), Su(var)3–9, Enhancer-of-zeste, Trithorax (SET) domain, and PostSET domain. Truncating variants are distributed throughout the entire coding region of *KMT2D*, while pathogenic missense variants are located in functional domains (PHD4, FYRN, and SET), particularly at the carboxyl terminus of *KMT2D* (Miyake et al., 2013; Paulussen et al., 2011). Similarly, our study described three missense likely pathogenic variants in the functional region in three patients, one in PHD4 and two in the PHD2 domain, near the carboxyl terminus (Table 1 and Figure 1). Banka et al. (2012) examined the phenotypes of patients

with truncating variants together with missense variant, and suggested that these variants may contribute to the phenotype. In our study, four patients with pathogenic and likely pathogenic variants besides found a missense variant had a high rate of visceral anomalies. Besides, we detected two different missense variants, which were classified as VOUS in two patients in whom we could not find pathogenic variants that could cause disease. None of these variants were in the functional region, and both were inherited from healthy parents. For this reason, we did not think that it may have effects on the clinic of the patients.

KDM6A variants have been reported to occur in 3%–8% of patients (Bögershausen et al., 2016). In our case series, we identified the novel c.4068dupA variant in *KDM6A* in one girl. Whole-gene deletion, multiexon deletion, and intragenic multiexon duplications were reported in both genes causing KS (Banka et al., 2013; Lederer et al., 2012). When we investigated both *KMT2D* and *KDM6A* by MLPA analysis, neither deletions nor duplications were found in these genes in variant-negative patients.

4.2 | Clinical characteristics

It has been stated that it may be difficult to distinguish facial features in the neonatal period and adulthood, whereas they are most striking in toddlers and school-age children (Banka et al., 2012; Bögershausen & Wollnik, 2013; Bögershausen et al., 2016; So et al., 2020). Although the facial features of our patients were not typical enough to make a definite clinical diagnosis in infancy (Figure 2a,f,l), they had findings suggestive of a possible or probable KS diagnosis according to the criteria of the international KS group consensus (Adam et al., 2019). Besides, it has been reported that the eversion of the lower eyelid of some patients was resolved during adulthood (Bögershausen et al., 2016; So et al., 2020). In our patients over 12 years of age, eversion of the lower eyelids did not return to normal, as claimed, and other facial findings were no less typical. Miyake et al. (2013) suggested that the KS patients with *KMT2D* truncating variants had more typical facial features than the patients with missense variants. In our patient group, the facial findings were similar in both patients with truncating and missense variants (Figure 2). The frequency of fetal finger pads, which are pathognomonic findings of KS (90.9%), was consistent with the literature (Bögershausen et al., 2015; So et al., 2020).

The frequency of microcephaly in KS patients was reported to range from 17% to 52% (Murakami et al., 2020; So et al., 2020). It was present 29.7% of our patients, and 33.3% of them had nonspecific central nervous system malformations, which have been previously described in KS (Bögershausen & Wollnik, 2013; Liu et al., 2015). 37.8% of the patients we followed for a long time had epilepsy with a frequency similar to the literature (Kurahashi et al., 2017). The mean IQ score was 48 and 49 in our *KMT2D* variant-positive and -negative groups, respectively, while previously reported mean IQ scores in individuals with pathogenic variants of *KMT2D* ranged between high 50s and high 60s (Adam et al., 2011; Lehman et al., 2017). It is known that female patients with *KDM6A* pathogenic variants have milder cognitive impairment compared to male patients with *KDM6A* or *KMT2D* pathogenic variants (Bögershausen et al., 2016). Moreover, long halluces and large central incisors were seen in the patients with *KDM6A* variant, dissimilar to typical KS features (Banka et al., 2015; Di Candia et al., 2022; Lederer et al., 2012; Miyake et al., 2013; Wang et al., 2019). The girl with *KDM6A* variant presented here had severe short stature (−2.51 *SD*) and ID (IQ: 31), autism, premature thelarche, hypothyroidism, and long halluces (Figure 2s).

Cardiovascular and renal anomalies were reported to occur at a rate of 40%–50% and 30%–40%, respectively, in patients (Bögershausen & Wollnik, 2013; Hannibal et al., 2011; Li et al., 2011). So et al. (2020) found that all cases with congenital heart defects, gastrointestinal, and genitourinary anomalies belonged to the protein-truncating variant group. In our patient group, the frequencies of congenital heart and renal anomalies were 48.6% and 43.2%, respectively, and renal anomalies were more prevalent in the *KMT2D* variant positive group, whereas cardiac anomalies occurred regardless of variant status.

Short stature has been reported to be 44%–55% in previous KS cohorts (Miyake et al., 2013; So et al., 2020). In our cohort, 35.1% of patients had short stature, which was more prevalent in the variant-positive group as compatible with previous research (Bögershausen & Wollnik, 2013; Li et al., 2011). The mean adult height without growth hormone therapy was found between −2.99 *SD* and −1.08 *SD* in males and between −5.57 and −1.47 *SD* in females (Schott et al., 2016). In another study also, the mean height of KS individuals was observed to be −2 and −1.8 *SD* of parental target height for males and females, respectively (Ruault et al., 2020). Ten of our patients reached final height; while it was −1.63 *SD* in one male patient receiving growth hormone therapy, the final height in eight women without growth hormone treatment ranged from 0.15 to −3.93 *SD*.

Four of the patients had cryptorchidism, three patients had coloboma, and three patients had premature thelarche. Cryptorchidism was shown in 21% (Bögershausen et al., 2015), coloboma in 3% (Ming et al., 2003), premature thelarche in 4%–46% (Miyake et al., 2013; So et al., 2020). Despite the fact that malignancies have been reported in KS, none of our patients developed cancer, even though our oldest patient was 28 years old (Adam et al., 2011; Murakami et al., 2020). Likewise, this cohort contributes to the literature with these rare findings.

It has been reported that patients with KS-like Turner syndrome have some slight differences from the typical facial gestalt as coarse face, bulbous nose, full upper lip, dense eyelashes, and arched and bushy eyebrows (Bögershausen & Wollnik, 2013). Dense eyelashes were present in our patients with KS-like Turner syndrome. One study claimed that *KDM6A*, which normally escapes inactivation, will likely be expressed from an active X chromosome, thus deletion of *KDM6A* will manifest in affected individuals (Lederer et al., 2012). Interestingly, it was reported that in patients with Kabuki-like Turner syndrome who have an X-ring chromosome, *KDM6A* is also located within the ring chromosome; and thus, there must be two copies.

KDM6A variant screening was performed in patients with Kabuki-like Turner Syndrome and did not find any genetic variants that could explain the KS-like phenotype as in our patients (Bögershausen et al., 2016). Most of the genetic variants identified and associated with Kabuki-like patients have a similar functional role to *KMT2D/KDM6A* and share the same biological processes (Lintas & Persico, 2018). Bögershausen et al. (2015) demonstrated *RAP1A* and *RAP1B* variants in two patients diagnosed with KS-like phenotype and explained genetic and functional interaction with KS-related genes and products in the zebrafish model and patient cell line, and suggested that the pathophysiology of KS overlaps with RASopathies. *ANKRD11*, *KDM1A*, *HRNRPK*, *EPC1*, *ZNF852*, and certain *CNVs* were related to the Kabuki-like phenotype in 30% of patients in the literature, and these genes share a pathway with *KMT2D* in several metabolic and biosynthetic processes (Lintas & Persico, 2018). These results show that the causes of the KS-like phenotype need further investigation.

We also assessed the clinical phenotypes of all patients presented here according to the scoring system described by Makrythanasis et al. (2013); the mean score was 6.0 in the molecular-positive group and 5.0 in the molecular-negative group. So et al. (2020) evaluated the patients whose molecular positive using a phenotypic scoring system described by Makrythanasis et al. (2013) and found the mean score for their patients was 5.6. Paderova et al. (2018) observed that the mean score for variant-positive patients was 7.5, whereas 4.8 for variant-negative patients. They suggested that targeted gene analysis could be effective in patients with a KS phenotype score of 6 and above. In contrast, aCGH and exome analyzes may be helpful in patients with a KS phenotype score of 5 and below as considering KS-like phenotype. Our research also corroborates these findings, and all these results support the possibility of novel genes causing KS.

In fact, our study has several limitations. We could not perform molecular analyzes in five patients. All these patients had characteristic facial findings of KS. Their rate of visceral anomalies was similar to the patients who had the *KMT2D* variant. Another problem in molecular diagnosis is the uncertainty of missense variants. Recent studies have shown an abnormal methylation profile in patients without a molecular diagnosis (Aref-Eshghi et al., 2019; Sobreira et al., 2017). Sobreira et al. (2017) defined that patients with *KMT2D* loss of function and missense variants show distinct DNA methylation patterns. Therefore, the presence of nonpathogenic missense variants accompanying pathogenic variants could be affecting the phenotype via their current effect on the methylation or other regulatory and epigenetic effects.

5 | CONCLUSION

We detected the molecular diagnosis rate as 68.7% in the Turkish KS cohort. The facial features were enough for probable KS diagnosis in infancy that become pronounced with increasing age. Our study included the comparison of the phenotypic characteristics in-depth and observed that the frequencies of facial and skeletal findings and multiorgan anomalies were not statistically different in variant-positive and -negative groups, except for persistent fetal pads. Our patient profile highlights the need for further research in terms of missense *KMT2D* variants, and KS-like syndromes caused by unknown genes. We suggest that patients who do not have variants in known genes should be diagnosed clinically according to the KS criteria of the international group, and that should be followed up, and genetic counseling should be given.

AUTHOR CONTRIBUTION

Esra Usluer contributed to conceptualization, methodology, investigation, formal analysis, and writing-original draft. Gözde Yeşil Sayın developed software, validation, formal analysis, and resources. Nilay Güneş contributed to conceptualization, methodology, and investigation. Buşra Kasap contributed to molecular analysis, resources, and data curation. Beyhan Tüysüz developed conceptualization, methodology, formal analysis, investigation, resources, writing-review and editing, and supervision.

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CONFLICT OF INTEREST

The authors report no conflicts no interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

HUMAN ETHICS APPROVAL DECLARATION

The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association. Informed consent and permission for the publications of photos of children were obtained from all patients/parents. The study was approved by the Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Ethics Committee (09.08.2018/41511).

INFORMED CONSENT

Written informed consent was obtained from the patients or parents.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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