Kabuki syndrome: clinical and molecular diagnosis in the first year of life

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ABSTRACT

Objective To review the clinical and molecular genetic characteristics of 16 patients presenting a suspected diagnosis of Kabuki syndrome (KS) in the first year of life, to evaluate the clinical handles leading to a prompt diagnosis of KS in newborns. Clinical diagnosis of KS can be challenging during the first year of life, as many diagnostic features become evident only in subsequent years.

Methods All patients were clinically investigated by trained clinical geneticists. A literature review was performed using the Pubmed online database and diagnostic criteria suggested by DYSCERNE–Kabuki Syndrome Guidelines (2010) were used (a European Network of Centres of Expertise for Dysmorphology, funded by the European Commission Executive Agency for Health and Consumers (DG Sanco), Project 2006122). Molecular analysis of the known causative genes of KS, *KMT2D/MLL2* and *KDM6A*, was performed through MiSeq-targeted sequencing platform. All mutations identified were validated by Sanger sequencing protocols.

Results Mutations in *KMT2D* gene were identified in 10/16 (62%) of the patients, whereas none of the patients had *KDM6A* mutations. Facial dysmorphisms (94%), feeding difficulties (100%) and hypotonia (100%) suggested the clinical diagnosis of KS. No significative differences in terms of facial features were noticed between mutation positive and negative patients of the cohort. Brachydactyly, joint laxity and nail dysplasia were present in about 80% of the patients. Other congenital anomalies were most commonly present in the mutated group of patients, including left-sided cardiac abnormalities, skeletal, renal and anorectal malformations and hypertricosis.

Conclusions We present an overview of patients with KS diagnosed during the first year of life. Early diagnosis is serviceable in terms of clinical management and for targeted genetic counselling.

What is already known on this topic

- Kabuki syndrome (KS) is a heterogeneous genetic, multiple congenital anomaly syndrome, characterised by distinct facial dysmorphisms, postnatal growth deficiency and mild–moderate intellectual disability.
- Suggested minimal criteria for KS diagnosis includes the characteristic eye conformation with long palpebral fissures and broad-arched eyebrows with lateral sparseness.
- Is extremely difficult to formulate a KS diagnosis in infants. Clinical guidelines prompts to revaluate infants at further follow-up, before attaching a definite KS 'label'.

What this study adds

- This is the first assay of a infant Kabuki syndrome (KS) cohort, since its genetic background discovery, in which KS clinical suspect could be confirmed by molecular testing.
- An accurate phenotypical observation of certain dysmorphic features, although subtle, can direct to KS diagnosis in infants, particularly when associated with multiple congenital anomaly, hypotonia, feeding difficulties.
- The essential role of the clinical geneticist in assessing infant patients in which KS diagnosis leads towards early molecular characterisation is crucial for prompt prospective care.

INTRODUCTION

Kabuki syndrome (KS; OMIM 147920) is a disorder first described by Niikawa *et al*[1] and Kuroki *et al*[2] in Japan. Clinical features include distinct facial anomalies, developmental delay, growth retardation, skeletal abnormalities and various organ malformations.[3–9] KS is a heterogeneous condition, two causative genes having been identified so far. Mutations in MLL2/*KMT2D* (MIM# 602113) are found in 55%–80% of the patients,[10–18] whereas deletions or mutations of *KDM6A* (MIM# 300128) have been reported in 9%–14% of *KMT2D*-negative individuals.[16 19–22] Clinical diagnosis of KS is often challenging in the first months of life because the phenotype tends to evolve over time[4] and characteristic facial features, such as long palpebral fissures with everted lower lid, become more evident during childhood.[4 23]

We analysed the clinical and molecular features of 16 subjects with KS suspected in infancy, to outline the key features for early recognition of this disorder.

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PATIENTS

From January 2003 to December 2012, KS was clinically suspected in 19 patients evaluated in our hospital in their first 12 months of life. Physical examination for major and minor anomalies was carried out by trained medical geneticists (MLD, MCD and BD).

The diagnostic criteria suggested by Adam and Hudgins[8] and DYSCERNE–Kabuki Syndrome Guidelines (2010) were used (http://www.dyscerne.org). Information about family history, pregnancy, delivery and birth data was recorded. All patients underwent anthropometric measurements, cerebral ultrasound and/or MRI, auditory brainstem response, ophthalmological evaluation, chest X-ray examination, neurological assessment for hypotonia and developmental delay.

Genomic DNA was collected from circulating leukocytes and molecular testing performed in 16/19 patients. Three subjects lost at follow-up were discharged from this study. Standard chromosome analysis was normal in 15 patients, whereas a 47, XXY karyotype was diagnosed in P8. Clinical photographs were collected whenever possible, along with informed consent signed from the patients' legal guardians. This study was approved by the Bambino Gesù Paediatric Hospital Ethics Committee (Number 599LB).

METHODS

The patient's genomic DNA was extracted from circulating leukocytes according to the standard procedures. *KMT2D* and *KDM6A* genes were analysed in all patients by targeted resequencing, using the MiSeq sequencing platform (Illumina, San Diego, California, USA). Probe design was performed by entering target genomic regions into Design Studio (DS) online software (Illumina, San Diego, California, USA). A list of amplicons (short regions of amplified DNA) was visualised and the quality of each amplicon design was predicted based on the success score provided by DS. The only exon with a low success score (<60%) was *KMT2D*-exon 15, which was excluded from the TruSeq Custom Amplicon (TSCA) panel and was analysed by Sanger sequencing. The design generated a panel of 135 amplicons 500 bp long with coverage of 99% of the cumulative region.

Library preparation and sequencing

The TSCA kit generates targeted amplicons with the necessary sequencing adapter and indices for sequencing on the MiSeq system. Library preparation and sequencing runs were performed according to the manufacturer's procedure.

Data analysis

MiSeq Reporter software performed secondary analysis on the base calls and Phred-like quality score (Qscore) generated by real-time analysis software during the sequencing run. The TSCA workflow in Miseq evaluated amplicons for variants through the alignment of reads against a 'manifest file', provided by Illumina, specified while starting the sequencing run. A 200X as a minimum threshold for covering all bases of the targeted panel was used, reliable for the identification of mosaicism.[24]

Sanger sequencing validation

All mutations identified by Miseq were validated by Sanger sequencing and, when available (3 cases), DNA of the parents' patients with mutations was screened to investigate whether the change was de novo or inherited.

In silico analysis

The potential functional effects of the novel missense variant identified in *KMT2D* protein was predicted by three servers: Polyphen-2 (HumVar model) (http://genetics.bwh.harvard.edu/pph2/),[25] SIFT (http://sift.bii.a-star.edu.sg/)[26] and MutationTaster (http://www.mutationtaster.org/).[27]

RESULTS

Two study cohorts were included in the study. A first group presented 10 sporadic infants with a full-blown Kabuki phenotype, confirmed by molecular testing. A second group comprised six subjects exhibiting a phenotype that was suggestive for the disorder, but negative for molecular testing. Among the molecular negative cohort, some patients presented many KS key features, whereas others showed atypical facial appearance (figure 1). Clinical features of both groups are summarised in table 1.

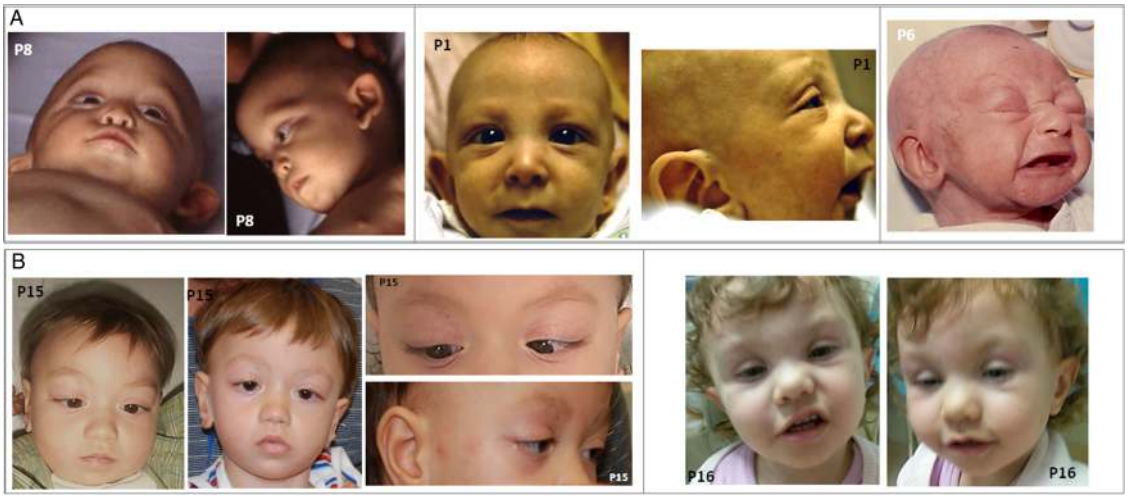

Figure 1 Representative facial images of our cohort of Kabuki syndrome (KS) patients. (A) Neonatal patients displaying typical features and facial dysmorphisms of KS, with nonsense *KMT2D* mutations (P1, P8) and novel missense *KMT2D* variations (P6). Note long palpebral fissures with eversion of the lateral eyelids which appears more evident on the lateral view of P1 and P8 newborn with KS; short columella with depressed nasal tip, prominent ears that are retroverted and preauricular pit are also visible in P1. (B) Patients with atypical KS facial features in which mutation neither in *KMT2D* nor in *KDM6A* has been found.

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Table 1 Clinical features of 16 patients with KS (10 KMT2D mutation-positive and 6 mutation-negative patients with KS) of the present cohort

	First group (*KMT2D*-mutated positive patients)											Second group (molecular negative patients)							
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Total	P11	P12	P13	P14	P15	P16	Total	Total (%)
Age at diagnosis (months)	1	1	1	1	1	1	1	3	10	12	3.2	1	2	2	2	3	9		
Sex	M	F	F	M	M	M	F	M	M	F	6M/4F	M	F	M	M	M	F	4M/2F	10M/6F
Polyhydramnios (P)	No	No	No	No	Yes	No	No	No	No	Yes	2/10	No	Yes	No	Yes	No	OH	2/6	4/16 (25)
Single umbilical artery	No	No	No	No	Yes	No	No	No	Yes	No	2/10	No	No	No	No	Yes	No	1/6	3/16 (19)
Birth weight <10%	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	7/10	No	Yes	Yes	No	Yes	Yes	4/6	11/16 (69)
Birth length <10%	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	7/10	No	Yes	Yes	No	Yes	Yes	4/6	11/16(69)
Birth OFC <3rd centile	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	7/10	No	Yes	Yes	No	Yes	Yes	4/6	11/16 (69)
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	yes	yes	yes	yes	yes	yes	6/6	16/16 (100)
Feeding difficulties	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	Yes	Yes	Yes	Yes	Yes	Yes	6/6	16/16 (100)
Seizures	No	No	No	No	No	No	No	No	No	No	0	No	No	No	No	No	No	0	0
Facial dysmorphism	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	Yes/no	Yes	Yes	Yes	Yes	Yes	5/6	15/16 (94)
Long palpebral fissures	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	No	Yes	Yes	Yes	Yes	Yes	5/6	15/16 (94)
Lateral eversion of lower eyelid	No	No	Yes	No	No	Yes	No	Yes	No	Yes	4/10	No	No	No	Yes	No	No	1/6	5/16 (31)
High-arched eyebrows	No	No	No	Yes	No	No	Yes	No	Yes	No	3/10	No	Yes	No	Yes	Yes	No	3/6	6/16 (37)
Palpebral ptosis	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	8/10	No	Yes	No	Yes	No	Yes	3/6	11/16 (69)
Blue sclerae	No	No	Yes	Yes	No	No	No	Yes	Yes	No	4/10	No	No	Yes	No	No	Yes	2/6	6/16 (39)
Strabismus	Yes	Yes	No	Yes	No	No	Yes	Yes	No	No	5/10	No	Yes	Yes	Yes	Yes	Yes	5/6	10/16 (62)
Epicanthal folds	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No	5/10	No	Yes	No	Yes	Yes	Yes	4/6	9/16 (56)
Prominent/large ears	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	Yes	Yes	Yes	Yes	Yes	Yes	6/6	16/16 (100)
Preauricular pits	Yes	No	No	No	No	Yes	No	Yes	No	No	3/10	No	Yes	Yes	No	Yes	No	3/6	6/16 (37)
Broad nose with depressed tip	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	7/10	No	Yes	Yes	Yes	Yes	Yes	5/6	12/16 (75)
Cleft palate	Yes	No	No	No	No	Yes	No	No	No	No	2/10	No	Yes	No	Yes	No	Yes	3/6	5/16 (31)
Everted lower lip	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	7/10	No	Yes	Yes	Yes	Yes	Yes	5/6	12/16 (75)
Lower lip pits	No	No	No	No	No	No	No	Yes	No	No	1/10	No	No	No	No	No	Yes	1/6	2/16 (12)
Nuchal skin or low hairline	No	No	No	No	No	No	Yes	Yes	No	Yes	3/10	No	Yes	Yes	No	Yes	Yes	4/6	7/16 (44)
Skeletal Anomalies	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	No	Yes	Yes	Yes	Yes	No	4/6	14/16 (87)
Brachydactyly (hands)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/10	No	Yes	Yes	Yes	Yes	No	4/6	14/16 (87)
Hypoplastic fingernails	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	9/10	No	Yes	Yes	Yes	Yes	No	4/6	13/16 (81)
Persistent fetal finger pads	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	8/10	No	Yes	Yes	Yes	Yes	Yes	5/6	13/16 (81)
Congenital hip dislocation	No	No	No	Yes	No	No	No	No	No	No	1/10	No	No	No	No	No	No	0	1/16 (6)
Vertebral anomalies	No	No	No	Yes	No	No	No	No	BV	No	2/10	No	No	No	No	No	No	0	2/16 (12)
Congenital heart	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	9/10	Yes	Yes	Yes	Yes	No	No	4/6	13/16 (81)
CoA of the aorta	No	No	Yes	No	No	Yes	No	No	Yes	No	3/10	Yes	No	No	No	No	No	1/6	4/16 (25)
ASD/VSD	VSD	No	No	VSD	VSD	No	No	ASD	VSD	PFo	6/10	VSD/PFo	VSD	ASD	PFo	No	No	4/6	10/16 (62)
Bicuspid aortic valve	Yes	Yes	No	No	No	No	No	No	No	No	2/10	No	No	No	No	No	No	0	2/16 (12)
Other	Bi-rV; MD					MD		No		No	2/10	TB				MD	No	2/6	4/16 (25)
Diaphragmatic defect	No	No	No	No	No	No	No	No	No	No	0	No	No	No	No	No	No	0	0
Renal anomalies	No	URA	No	DCS	No	ptosis	No	No	No	No	3/10	No	No	No	No	No	No	0	3/16 (19)
Anorectal malformation	No	Yes	No	No	Yes	No	No	No	No	No	2/10	No	No	No	No	No	No	0	2/16 (12)
Genital anomalies (males)	No	NA	NA	No	No	No	NA	No	Cryp	NA	1/6	Hyp	NA	No	No	No	NA	1/4	2/10 (20)

Continued

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Table 1 Continued

	First group (*KMT2D*-mutated positive patients)											Second group (molecular negative patients)								
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Total	P11	P12	P13	P14	P15	P16	Total	Total (%)
Hypertrichosis	No	No	No	Yes	No	No	Yes	Yes	Yes	No	4/10	No	No	No	No	No	No	0	4/16 (25)
Joint hyperlaxity	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	8/10	Yes	Yes	Yes	Yes	Yes	No	5/6	13/16 (81)
Breast prominence	No	No	No	No	No	No	No	No	Yes	Yes	2/10	No	No	No	No	No	No	0	2/16 (12)
Other	DW	No	No	No	No	HypoT	HypoG	XXY	No			No		No	No		CMP		
KMT2D mutation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes		No	No	No	No	No	No		10/16 (62)

APVR, anomalous pulmonary venous return; Bi-rV, bicamerated right ventricle; BV, butterfly vertebrae; CMP, camptodactyly; Cryp, cryptorchidism; DCS, duplicated collecting system; DW, Dandy Walker; OFC, Occipital Frontal Circumference; Hyp, hypospadia; HypoG, hypoglycemia; HypoT, hypothyroidism; KS, Kabuki syndrome; MD, mitralic dysplasia; NA, not applicable; OH, oligohydramnios; PFo, patent foramen ovale; Sao, subaortic; TB, truncus bicarotideus; URA, unilateral renal agenesis; VSD/ASD, ventricular/atrial septal defects.

Mean age at the time of clinical diagnosis was 110 days. Medical complications during pregnancy were identified in 5/16 (31%) patients, with polyhydramnios being recorded in four cases. All patients were born at term, with the exception of P10 delivered at 32 weeks. Birth weight and length were below the 10th centile in 11 (69%) cases.

KMT2D gene mutations were found in 10/16 (62%) patients, including four frameshift, four nonsense and two missense. No somatic mosaicism nor *KDM6A* mutations were detected. All variants identified were confirmed by Sanger sequencing. Of 10 mutations, 7 were novel. Parents were analysed in 3/16 cases (table 2). Of the two missense variations identified, one (Arg5179Cys) was previously described as pathogenic, whereas for the missense C5092Y it was not possible to verify inheritance. Hence, a definite causative effect cannot be ascribed to this variant even if it was considered most likely pathogenic, based on a combination of homology modelling and in silico analysis in which three servers consistently assigned potentially important functional effect (see online supplementary material).

The frequency of clinical features found in the present cohort is summarised in table 3 along with the frequencies reported in published studies including newborns[23] and in patients with a wider age distribution.[28 29]

DISCUSSION

The spectrum of KS clinical features in the infant period is wide, including distinctive facial anomalies and a variety of malformations. Newborns display milder facial anomalies and more organ malformations (table 3) when compared with older individuals. This can be explained by assuming that the suspicion of a syndromic disorder in the first months of life is based often on the presence of major structural defects. Similarly, Vaux *et al*,[23] by studying 16 newborns with a clinical diagnosis of KS, inferred that their cohort of patients was biased, and the true prevalence of malformations in KS in a broader observation period was lower.

Facial features. Vaux *et al*[23] suggested that several distinct facial features of KS are present in the neonates, although they appear less pronounced. Accordingly, their cases were not diagnosed in the infant period, and their report was retrospective. In this study, the diagnosis of KS was suspected clinically at a median age of 3 months and 20 days of life. No significative differences were revealed between mutation positive and negative patients of the present cohort, as the crucial handle for the diagnosis was the facial gestalt (table 1). However, in some patients the dysmorphisms were quite mild. Elongated palpebral fissures were found in all of our mutated patients, and in general this feature was better appreciable at lateral inspection (figure 1). Eyebrows were often thin and sparse, and, as a consequence, the characteristic high-arched interrupted eyebrows were found only in 3/10 of our *KMT2D*-positive newborns, compared with the 80% of general KS population. Also, eversion of the lower lid was less common in our mutated cohort (4/10), compared with 87%–90% of the KS population. Broad nose with flat nasal tip was recorded in 7/10 of cases, while large ears were invariably present. Excess nuchal skin and/or low posterior hairline occurred in 3/10 of cases, a figure slightly lower comparable with the 65% recorded by Vaux *et al*[23] in the KS neonatal patients. Palatal defects, including cleft palate, high-arched palate, bifid uvula with submucous cleft palate occurred in 2/10 of our mutated patients, with respect to the 40% recorded in the general KS population.[28] This figure was significantly lower ($p<0.01$) compared with the Vaux's neonatal series (63%).[23] While lower lip pits are often considered a distinctive feature of KS, they were found only in one of our *KMT2D*-positive infants.

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Table 2 Missense, nonsense and frameshift KMT2D variants identified in 16/18 patients of the present cohort

Mutation type	ID	Exon	Mutation	AA change		Inheritance	Reference
Frameshift	P18	6	c.721del	p.(Leu241Cysfs*20)	L241CfsX260	De novo	Novel
	P1	10	c.1345_1346del	p.(Leu449Valfs*5)	L449VfsX5		Micale *et al*[12]
	P9	11	c.3161_3171del	p.(Pro1054Hisfs*10)	P1054Hfs1063X		Novel
	P2	11	c.3318dup	p.(Ser1107Glnfs*8)	S1107QfsX8		Novel
Nonsense	P3	11	c.3532C>T	p.(Gln1178*)	Q1178X		Novel
	P4	26	c.5707C>T	p.(Arg1903*)	R1903X	De novo	Novel
	P10	32	c.8160G>A	p.(Trp2720*)	W2720X	De novo	Novel
	P5	39	c.13450C>T	p.(Arg4484*)	R4484X		Paulussen *et al*[13]; Makrythanasis *et al*[29]
Missense	P6	47	c.15275G>A	p.(Cys5092Tyr)	C5092Y		Novel
	P7	48	c.15535C>T	p.(Arg5179Cys)	R5179C		This study, Ng *et al*[10]; Hannibal *et al*[11]

Polyhydramnios. It was reported in 4/16 (25%) of our total cases. These four patients had congenital heart defect (CHD), including two with ventricular septal defects and two with patent foramen ovale (table 1).

Growth and nutrition. More than two-third of our patients displayed at birth growth parameters below the 10th centile and all were evaluated for severe hypotonia and feeding difficulties. These characteristics were found in the 65% of KS neonates studied by Vaux *et al*[23] and in the 70% of large KS cohorts.[5] Thirteen (81%) of our patients required nasogastric tube placement for poorly coordinated suck and swallow, or even gastrostomy feeding (2/16=12%).

Epilepsy. Different seizure types and EEG changes are found in up to 50% of the KS individuals.[28] None of our patients developed seizures in the first year of life, compared with the 6% in the Vaux's neonatal cohort.[23] Therefore, while onset of epilepsy in KS may occur in the neonatal period, it more likely manifests in later stages of life, but EEG abnormalities tend to decrease during adolescence.[30]

Musculoskeletal Brachydactyly and hypoplastic fingernails are found in many KS subjects.[6] In our series, joint hypermobility occurred in 81% of patients with no differences between positive and negative to molecular testing subjects, with respect to the 44% frequency recorded in another infant cohort.[23] This high figure may be explained by the fact that in KS, similar to in other genetic disorders, joint hypermobility tends to improve with age. Hypoplastic nails, primarily affecting the fifth digit, and prominent fetal fingertip pads were seen in the majority of patients of both cohorts, in agreement with the opinion that they represent one of the five cardinal manifestations of KS. Vertebral anomalies, expected in about 20% of patients with KS,[28] were present in two patients of our first group (12%), manifesting butterfly vertebrae, segmentation defects and sacral dysgenesis, while no malformations were detected in the molecular negative group.

Congenital heart defects. CHDs were observed in 81% of the present cohort, with respect to a 90% recorded in another infant series (90%).[23] These defects are found in the 55% of general KS population.[28] They included left-sided obstructions (coarctation of the aorta (CoA) and bicuspid aortic valve (BAV)) and ventricular/atrial septal defects (VSD/ASD) alone or in association. Surgical repair was required in 6/13 (46%) of the cases. Mitral valve anomalies were also found, and one patient (P11) presented Shone complex with VSD and truncus bicarotideus, previously reported as an anatomical marker of KS.[31] Interestingly, the frequency of CHDs, particularly left-sided defects such as CoA and BAV, was higher in the mutated cohort with respect to the negative group (table 1).

Diaphragmatic defects. Diaphragmatic defects are uncommon in patients with KS.[1 5 32] However, diaphragmatic eventration was recorded in four infant KS cases.[23] In the present series, none had congenital diaphragmatic abnormalities (table 1).

Genitourinary. Genitourinary defects in KS include dysplastic horseshoe kidney,[5] cryptorchidism and bifid and shawl scrotum in men.[9] All of our patients underwent renal ultrasound that revealed anomalies in 3 (19%) cases, including unilateral renal agenesis, renal ptosis and duplicated collecting system.

Renal and anorectal anomalies, breast prominence and hypertrichosis were significantly more common in the first group of patients with respect to the molecular negative group (table 1).

Diagnostic management: Comprehensive management guidelines are available for KS (http://www.dyscerne.org). Dyscerne clinical guidelines, in agreement with Adam and Hudgins[8] suggestions, point to difficulty of clinical diagnosis in the neonates and recommend the assessment of the infants at a later time, before attaching a definite label of KS. However, these guidelines have been written when the molecular defect underlying KS was still unknown and no laboratory test was available to corroborate the clinical diagnosis. We recommend that molecular testing of *KMT2D* and *KDM6A* genes is promptly performed in neonates in which KS is suspected. In the present study, 62% of the patients were heterozygous for a *KMT2D* mutation. This figure is in line with previous studies.[10–15 18] In the infancy, more severe pattern of malformations in patients with KS may be a handle to an early diagnosis. Notably, 81% of our patients have received intensive care for severe CHD, hypotonia and feeding difficulties.

Among 16 patients with a clinical diagnosis of KS, 10 presented a *KMT2D* mutation with the majority being nonsense and frameshift. Only two missense mutations were identified and included in the presents study. While the mutation Arg5179Cys was established as pathogenic as previously shown,[10 11] for the novel missense mutation (Cys5092Tyr) the causative nature is inferred from informatic tools and argues for a pathogenic effect (see online supplementary material), although a definite role cannot be ascertained.

In conclusion, (1) the spectrum of clinical features associated with KS in the infant period is wide, including a great variety of malformations, often severe. (2) No statistically significant differences in facial dysmorphisms arise between patients mutated in *KMT2D* gene and those negative to molecular screening The facial phenotype is less marked in the infancy, with many distinct features, such as arched eyebrow, less pronounced. Nevertheless, some clinical characteristics, such as large prominent ears and elongated palpebral fissures could prompt the clinical suspect of KS, particularly when associated with hypotonia, feeding difficulties and malformations. (3) The frequency of congenital malformations (left-sided cardiac abnormalities and skeletal, renal and anorectal

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Table 3 Comparison between phenotypical features in patients with a clinical diagnosis of KS in the first year of life (present study, Vaux *et al*[23]) and in patients diagnosed at various ages of life[28 29]

Clinical features	This study (KMT2D positive patients)	This study (molecular negative patients)	This study (general KS population)	Vaux *et al*[23]	Hennekam *et al*[28]	Makrythanasis *et al*[29]
Patients (n)	10	6	16	16	~200	86
Sex	6M/4F	4M/2F	10M/6F	9M/7F	NR	
Polyhydramnios	20%	33%	25%	31%	NR	
Single umbilical artery	20%	17%	19%	31%	NR	
Birth weight <10%	70%	67%	69%	25%	30%	
Birth length <10%	70%	67%	69%	27%	30%	57%
Birth OFC <3rd c	70%	67%	69%	66%	25%–33%	41%
Hypotonia	100%	100%	100%	32%	75%	80%
Feeding difficulties	100%	100%	100%	65%	50%	
Seizures	0	0	0	6%	Up to 50%	
Facial dysmorphism	100%	83%–100%	94%		100%	
Long palpebral fissures	100%	83%	94%	20%	100%	99%
Lateral eversion of lower eyelid	40%	17%	31%	0	90%	87%
High-arched interrupted eyebrows	30%	50%	37%	0	80%	78%
Palpebral ptosis	80%	50%	69%	0	35%	40%
Blue sclerae	40%	33%	39%	6%	30%	30%
Strabismus	50%	83%	62%	0	50%–60%	
Epicanthal folds	50%	67%	56%	6%	50%–60%	
Prominent/large ears	100%	100%	100%	0/30%	85%	79%
Preauricular pits	30%	50%	37%	44%	25%	
Pits of lower lip	10%	17%	12%	NR		35%
Broad nose with depressed tip	70%	83%	75%	56%	80%	62%–80%
Cleft palate	20%	50%	31%	63%	40%	66%*
Everted lower lip	70%	83%	75%	NR	occasional	76%
Nuchal skin or low hairline	30%	67%	44%	65%		
Skeletal anomalies	100%	67%	87%			
Brachydactyly (hands)	100%	67%	87%	50%	80%	84%
Hypoplastic fingernails	90%	67%	81%	75%	occasional	
Persistent fetal finger pads	80%	83%	81%	18%	80%	89%
Congenital hip dislocation	10%	0	6%	12%	20%	26%
Vertebral anomalies	20%	0	12%	18%	20%	
Congenital heart	90%	67%	81%	90%	55%	42%
Diaphragmatic defect	0	0	0	25%		
Renal anomalies	30%	0	19%	19%	>25%	40%
Anorectal malformation	20%	0	12%	25%	15%	NR
Genital anomalies (males)	36%	25%	20%	44%	25%	40%
Hypertrichosis	40%	0	25%	NR		
Joint hyperlaxity	80%	83%	81%	44%	75%	75%
Breast prominence	20%	0	12%	NR		28%
Other						

Report of 16 patients with KS during the first year of life.[23] Hennekam *et al*[28] and Makrythanasis *et al*[29] reported 200 and 86 patients, respectively, at various ages of life.
*Includes high palate.
KS, Kabuki syndrome; NR, not reported.

malformations) and hypertrichosis appears higher among *KMT2D* mutation-positive patients compared with individuals negative for KS molecular testing. Additional observations are necessary to support this association because statistical differences may be influenced by the numerical limited survey. (4) Genetic referral is crucial for the identification of subtle clinical findings of KS in infant patients. Early diagnosis of the disease is useful in terms of prospective care of the associated medical problems, and for genetic counselling, leading towards early molecular characterisation.

Contributors MCD, BD, RC and MLD recorded the clinical data. ADP, MHL, CA, AB and AD were involved in intensive neonatal care of patients. FRL, MG, SP, EP and AA performed molecular genetic studies. EB provided functional analysis. MLD drafted the manuscript; the final version was completed by MLD, MCD and BD.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethical Committee of Bambino Gesù Children's Hospital.

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