Review

Unmasking Kabuki syndrome


The identification of de novo dominant mutations in KMT2D (MLL2) as the main cause of Kabuki syndrome (KS) has shed new light on the pathogenesis of this well-delineated condition consisting of a peculiar facial appearance, short stature, organ malformations and a varying degree of intellectual disability. Mutation screening studies have confirmed KMT2D as the major causative gene for KS and have at the same time provided evidence for its genetic heterogeneity. In this review, we aim to summarize the current clinical and molecular genetic knowledge on KS, provide genotype–phenotype correlations and propose a strategic clinical and molecular diagnostic approach for patients with suspected KS.

Conflict of interest

The authors declare no conflict of interest.

In 1981, the groups of Niikawa and Kuroki reported for the first time a syndrome of intellectual disability, short stature and a peculiar facial gestalt, which reminded the authors of the make-up of actors in the traditional Japanese Kabuki theater (1, 2). The striking facial features of Kabuki syndrome (KS; MIM 147920) provide a high recognizability in typical cases; however, these features may not at all be present even in cases with genetically confirmed KS. Other findings like short stature, microcephaly, renal and cardiac malformations, repeated infections, hearing loss and persistent fetal fingertip pads are characteristic, but are unspecific symptoms of KS. KS is believed to be a rare disease. Estimates of birth prevalence vary between 1:32,000 for the Japanese population (3) and 1:86,000 for the populations of New Zealand and Australia (4). The molecular cause of KS had long been unknown until, in 2010, Ng et al. reported the identification of heterozygous mutations in KMT2D (MLL2; MIM 147920) as the major genetic cause of KS (5). Subsequent mutation screenings in KS cohorts found a mutation in KMT2D in 56% to 75% of patients (6, 7). The majority of mutations identified were private de novo mutations, although familial cases with autosomal dominant inheritance have occasionally been described (8, 9). A second gene for KS was described by Lederer et al. showing heterozygous whole-gene or larger partial-gene deletions of KDM6A (UTX, MIM 300128) in three patients, highlighting the significance of array-comparative genomic hybridization (array-CGH) as a tool for differential diagnosis in patients without a mutation in KMT2D (10). Different non-recurrent structural chromosomal aberrations have also been reported for patients with a Kabuki phenotype. For a large number of patients (approximately 30%), however, the underlying genetic cause remains unidentified. The clinical variability and genetic heterogeneity of this disorder as well as the occurrence of mosaicism, may make the diagnosis challenging, clinically as well as molecularly. Here, we give an overview of the clinical and molecular spectrum of KS and propose an up-to-date diagnostic approach.

Clinical features

Craniofacial anomalies

The main and most recognizable feature of KS is its very distinctive facial gestalt. The long palpebral fissures with an evasion of the lower eyelid, long,
dense eyelashes and arched eye-brows are the common characteristic features of KS (Fig. 1). The ears are prominent, with simply formed or hypoplastic helices, and pre-auricular pits can be observed in some patients. The tip of the nose is typically depressed because of a short columella. The mouth has a thin upper and full lower lip, and the corners of the mouth slant downwards. Prognathism and symmetrical nodules of the lower lip may be observed.

Of late, it has become evident that this classical facial appearance is observed more often in patients carrying a KMT2D mutation than in patients without a KMT2D mutation. We performed a meta-analysis of the available comparative phenotype data from recent mutation screening studies in cohorts of patients with KS (6–8, 11) and found a significantly higher frequency of typical facial features ($p = 0.002$) in patients with a KMT2D mutation compared with patients without a mutation in KMT2D (Table 1; Fig. 1a,b). However, not all patients who carry a mutation in KMT2D show all of the typical facial criteria (6). Banka et al. applied a facial dysmorphology score to evaluate how likely the facial gestalt of KS patients predicts their KMT2D mutation status (11). They found that all but one patient (93%) from the mutation-negative group were predicted mutation-negative and therefore concluded that patients without a KMT2D mutation might have a related overlapping condition that should be referred to as Kabuki-like syndrome. On the other hand, only 72% of patients who carried a mutation in KMT2D were scored accordingly. This finding illustrates that while most mutation-negative cases have atypical KS, so do approximately 30% of the mutation-positive cases. Moreover, there seems to be a change in facial appearance with age. In our own experience and that of other authors KS is most easily recognized in early childhood, while both in early infancy and in adulthood the clinical diagnosis based on dysmorphic features can be difficult (11, 12). Therefore, we believe that molecular genetic testing of KMT2D is warranted also for atypical and Kabuki-like cases.

Table 1. Phenotypic differences between KMT2D mutation carriers and non-carriers, meta-analysis

<table>
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<tr>
<th>Author</th>
<th>Mutation detection rate (%)</th>
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<tr>
<td></td>
<td>Hannibal et al. (8)</td>
<td>73.6</td>
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<td>Paulussen et al. (7)</td>
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<td>Banka et al. (11)</td>
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<th>Li et al. (6)</th>
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<td>Short stature</td>
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<td>24/25</td>
<td>11/19</td>
<td>49/70</td>
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<td>Developmental delay</td>
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<td>19/20</td>
<td>18/19</td>
<td>92/93</td>
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<td>Typical facial gestalt</td>
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<td>29/34</td>
<td>17/19</td>
<td>46/53</td>
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<tr>
<td>Cleft palate</td>
<td>29/72</td>
<td>8/18</td>
<td>3/19</td>
<td>49/131</td>
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<td>Urogenital anomalies</td>
<td>31/66</td>
<td>2/14</td>
<td>7/17</td>
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<td>31/33</td>
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<tr>
<td>Feeding problems</td>
<td>–</td>
<td>–</td>
<td>13/19</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>9/19</td>
<td>34/56</td>
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</table>

aData from the study of Micale et al. were included in the calculation of overall mutation detection rate (15).

bValues in bold have a $p$-value < 0.05.
Apart from the typical facial gestalt the craniofacial phenotype of KS can include cleft lip/palate and abnormal dentition. Cleft lip/palate has been reported with a variable frequency between 12% and 50% of patients (3, 6, 13). The clefts are mostly isolated palatal or submucous, while combined cleft lip/palate and isolated cleft lip are rare (14). Dentition abnormalities, including widely spaced teeth and/or hypodontia, have been frequently reported (53–100%) (7, 13, 15). Paulussen et al. did not find any statistically significant difference in the occurrence of hypodontia between groups with and without a KMT2D mutation (7).

Whether or not craniosynostosis is more frequent in KS patients than in the general population remains unclear. So far, it has rarely been described in patients with KS (14, 16) but it might be underreported. We have observed a metopic craniosynostosis in 2 of 42 patients with KMT2D mutation (not reported in our previous study).

Body weight
KS patients are typically born with normal body measures, but fail to thrive in infancy because of feeding problems. Feeding difficulties, caused by poorly coordinated suck and swallowing, as well as gastroesophageal reflux, in some patients necessitating nasogastric tube or even gastrostomy feeding, have been documented in 65–74% of cases (6, 13). White et al. noticed that while two thirds of their patients manifested failure to thrive in infancy, 57% of them developed an elevated body mass index in the overweight and obese range after the age of five (4). This observation should be taken into account when counseling affected families in order to promote awareness and early dietary intervention.

Growth
Post-natal proportionate short stature is a common finding in KS that is reported in approximately 58% of cases (cumulative frequency) (6–8, 11). Interestingly, genotype–phenotype comparisons revealed a significantly higher frequency of short stature in patients with a KMT2D mutation than in patients without a mutation in KMT2D (p < 0.0001; Table 1). Patients with a mosaic MLL2 mutation might less frequently show short stature (17).

Neurological symptoms and behavior
Intellectual disability of a varying degree has been reported for approximately 90% of KS patients (3, 7, 8). White et al. found that head size did not correlate with the degree of intellectual disability in their cohort (4), still, 29–56% of patients with KS show a post-natal microcephaly (6, 15). It is equally frequent in patients with and without a KMT2D mutation. The degree of intellectual disability ranges from mild to severe with the majority of patients being mildly affected (8). No statistically significant difference in the occurrence of intellectual disability could be found, neither between patients with and without a KMT2D mutation, nor between patients with truncating vs missense mutations in KMT2D (8). Apart from the intellectual disability the psychomotor development of KS patients may be complicated by hypotonia and seizures. Muscular hypotonia is common, described in 51–98% of patients (7, 13). Hypotonia appears to be most prominent in the neonatal period and to improve over time. Schrander-Stumpel et al. reported in a literature review that 24% of cases of KS are complicated by seizures (7). In a study of 10 patients diagnosed with epilepsy Verrotti et al. observed mainly partial seizures with predominant involvement of the tempororoccipital areas (18). They reported a good anti-epileptic drug responsiveness and an overall favorable outcome. Lodi et al. reported a predominance of focal seizures in the frontocentral region, equally with a favorable outcome (19). In our recent publication we observed seizures significantly more often in patients without a KMT2D mutation (1/19 vs 7/15, p < 0.05) (6). However, structural abnormalities of the brain, namely ventricular dilatation, brain atrophy, agenesis/hypoplasia of the corpus callosum and white matter abnormalities, were seen almost equally frequently among these groups (46 vs 60%, p > 0.05).

Ho and Eaves described autistic-like behavior in three of four male Kabuki patients from Vancouver and suggested KS as a differential diagnosis in dysmorphic patients with autism spectrum disorder (20). White et al. painted a more differentiated picture, pointing out that although 44% of their patients with KS tended to avoid eye contact, general socialization skills were good and the children had an affable, affectionate disposition (4).

Endocrinological findings
Early breast development has been noted in girls with KS (43%) (7, 21–23). however, this symptom is not necessarily associated with other signs of precocious puberty. Banka et al. found early breast development in 41% of patients with a mutation in KMT2D vs 4% of mutation-negative patients (p < 0.05) (11). Growth hormone (GH) deficiency, hypothyroidism, hyperinsulinism and diabetes insipidus, as well as abnormal pituitary findings on magnetic resonance imaging have been rarely reported, with GH deficiency being the most common finding (4, 7, 18, 24). Although few in number, these reports warrant endocrinological evaluation of patients with KS in routine care. The benefits of GH therapy for patients with short stature need further investigation in larger patient cohorts.

Cardiac malformations
Congenital heart defects are common in patients with KS. The reported frequencies vary between approximately 40% and 50% in different literature reviews (24, 25). The most frequent malformations are atrial septal defects, ventricular septal defects and aortic coarctation (26). Heart defects can be found equally frequently in patients with and without a mutation in KMT2D (p > 0.05; Table 1).
Bögershausen and Wollnik

Urinary tract abnormalities

Urinary tract abnormalities can be found in approximately 30–40% of patients. Hydronephrosis seems to be common, but also abnormalities of renal position and ascent can be found (6, 8, 11, 27). Of note, renal malformations are significantly more common in patients with a KMT2D mutation (p < 0.0001, Table 1). The most frequent genital anomalies are cryptorchidism and hypospadias in males (7, 28).

Hepatic complications

Involvement of the liver is rare in KS. Biliary atresia has been reported in three patients, hepatic fibrosis and sclerosing cholangitis in one patient, respectively (29–33). In our cohort we have observed one patient with a mutation in KMT2D with a yet unexplained hepatopathy with hepatomegaly and elevated transaminases.

Otological findings

Interestingly, patients with KS have a susceptibility to middle ear infections (6, 7), which can lead to conductive hearing loss and can aggravate problems in speech acquisition. While conductive hearing loss appears to be the most frequent hearing impairment, a sensorineural type of hearing loss has also been reported. Barozzi et al. performed audiometry and vestibular function tests on 10 patients with KS and found hearing loss in 7 of 10 patients (34). In their study, hearing loss was either conductive or mixed and in most patients because of serous and/or chronic otitis media resulting in complications such as tympanosclerosis and tympanoossicular fixation. One patient had unilateral aural atresia. Sensorineural hearing loss was not observed in this study. Vestibular function was normal in 95% of the examined ears. In contrast, Igawa et al. reported Mondini dysplasia of the inner ear with sensorineural hearing impairment in three patients with KS (35). Involvement of the inner ear has also been shown in a study by Tekin et al., who identified two patients with KS among a large cohort of patients with an established diagnosis of severe to profound sensorineural deafness (36).

Ophthalmological symptoms

Severe visual impairment is rare in KS; nevertheless, ocular abnormalities can be found in 38–61% of patients. These include strabismus, blue sclera, ptosis, coloboma of the iris and retina, refractive anomalies, Peter’s anomaly and congenital corneal staphyloma (7, 27, 37).

Skeletal findings

Skeletal abnormalities include rib anomalies, malformations of the vertebrae, scoliosis and abnormalities of the fingers, especially brachydactyly and/or clinodactyly of the fifth digit. Taken together, patients with KS show some skeletal abnormality in about 80% of cases (25), brachydactyly being the most common. Cleft hand has been reported once (38).

Skin and connective tissue

A distinctive symptom of KS is the presence of persistent fetal finger pads. This characteristic is present in up to 92% of patients who carry a mutation in KMT2D, but only in 71% of patients without mutation (p < 0.05; Table 1). Another common finding that has been reported in up to 96% of patients (not tested for KMT2D mutation) is an abnormality of the dermatoglyphic pattern, such as absence of the digital triradius ‘c’ and ‘d’ and presence of interdigital triradii, as well as hypothenar and interdigital ulnar loop patterns (25).

Involvement of the connective tissue in KS has been proposed because of the combination of facial laxity, joint hyperlaxity and joint dislocations. Initially described by Burke et al (12), connective tissue involvement has been supported by various authors including Armstrong et al. (26), who noted hyperelastic skin in addition to the above mentioned symptoms. Joint hyperlaxity and hip dislocation have been reported with very varying frequencies ranging from 6% to 97% and 6% to 37%, respectively (6, 7, 13). These contrasting numbers suggest a certain bias of ascertainment. Banka et al. found habitual joint dislocations in 32% of patients with a mutation in KMT2D vs 9% in patients in whom no KMT2D mutation was detected (p < 0.05) (11).

KS and cancer

Although somatic truncating mutations in KMT2D have been implicated in childhood medulloblastoma and are frequently present in non-Hodgkin lymphoma (39, 40), no clear association has yet been established between KS and an increased risk for cancer. Cancer has been reported in seven individuals with KS: two suffered from neuroblastoma, one presented with hepatoblastoma, one developed low-grade fibromyxoid sarcoma, one had acute lymphocytic leukemia, another EBV-positive Burkitt’s lymphoma and one was diagnosed with synovial sarcoma (41–46). The KMT2D mutation status of these reported patients is unknown. One study showed that in a large cohort of pediatric cancer patients the incidence of monogenic syndromes was higher than expected in the general population and that the diagnoses of these syndromes had frequently been missed. However, only 1 patient of 1073 was diagnosed with KS (41). Thus, the hypothesis that KS predisposes to malignancies remains questionable.

Autoimmunity

KS may present with autoimmune disease, in particular idiopathic thrombocytopenic purpura and/or hemolytic anemia in rare cases (27, 29, 30, 47, 48). Vitiligo has been reported in two patients (47, 49).
A susceptibility to infection leading to recurrent infections of the upper airway tract and the middle ear is a known and common feature of KS. It is found in 55% of patients and slightly more often in patients with a KMT2D mutation (60% vs 47%) (5, 11), although this difference is not statistically significant. Hoffmann et al. reported decreased IgA levels in 15 of 19 (79%) individuals with KS, 8 of them (42%) showing low total IgG levels in addition. IgG subclass abnormalities were found in 6 of 13 (46%). They proposed that the antibody abnormalities seen in children with KS resembled common variable immune deficiency (CVID). Hence, careful hematological evaluation and surveillance is necessary for each patient diagnosed with KS (50).

Molecular pathogenesis and diagnostics

Mutations in KMT2D

In 2010, mutations in KMT2D (MLL2; MIM 602113; NM_003482) were identified in patients with KS by means of whole exome sequencing (5). KMT2D was subsequently confirmed as the major causative gene in several mutation screening studies with mutation detection rates between 56% and 75% (6, 7). The cumulative mutation detection rate from five yet published mutation screening studies is approximately 67% (6–8, 11, 15). Since the publication of our initial study on KMT2D mutations, we have tested 88 additional patients with a clinical diagnosis of KS and found a KMT2D mutation in 42 of them, reflecting a mutation detection rate of approximately 48% (patients from our previous study are not included). This detection rate is further supported by a very recent study by Banka et al. also describing a mutation detection rate in routine molecular testing of 48% (17). This reduced mutation detection rate in a clinical setting might reflect a broader patient selection for molecular testing.

All mutations identified so far are dominant heterozygous mutations, most of them truncating, leading most likely to haploinsufficiency of the KMT2D protein (5–8, 11, 15). The most frequent mutation types are stop mutations, identified in 89 patients, and frameshifting mutations, found in 82 patients. Missense mutations were found in 38 and splice-site mutations in 21 patients. Six patients had in-frame deletions or duplications, and three of these patients had the recurrent single amino acid deletion c.16489_16491delATC in exon 53 (Table 2). No correlation could yet be established between mutation type and phenotypic expression of KS. Most patients carry a private mutation in KMT2D. However, an increasing number of recurrent mutations have been described. For example the frameshifting mutation p.Y2199IfsX65 in exon 31 has been identified in seven patients so far (6–8, 11, 15). Presumably, the number of recurrent mutations will rise with the number of patients analyzed. The majority of reported KMT2D mutations are de novo mutations found in sporadic cases, but also a few familial cases have been described. Hannibal et al. presented three families with proven KMT2D mutations, including two parent–offspring pairs and one pair of monozygotic twins (8). Kokitsu-Nakata et al. described a mother and daughter with KS and a missense mutation within the highly conserved SET-domain of lysine methyltransferase 2D (KMT2D) likely to impair KMT2D function (9). A possible reason for the small number of dominant families might be a reduced fertility rate in patients. However, nothing is yet known about the fertility status in individuals with a mutation in KMT2D. A role in spermatogenesis, oocyte survival and fertility has been ascribed to Kmt2b (NM_029274; termed Mll2 in the publications) (51, 52), but there is no evidence for a similar function of Kmt2d.

Distribution of KMT2D mutations

Mutations in KMT2D appear to be overrepresented toward the C-terminus; however, mutations show no significant clustering in a certain exon or a known protein-domain in terms of a mutational hot spot. In order to analyze the distribution of mutations we reviewed the localization of all described KMT2D mutations (Fig. 2) (5–8, 11, 15, 17). We divided the whole coding sequence into a 5′- and 3′-part, 8307 bp each, with the border located within exon 33 (reference sequence according to the hg 19 assembly on the UCSC browser, http://genome.ucsc.edu/), and found that 65.7% of all mutations (155 of 236) are located within the 3′-half of the gene. At the same time 62.7% of all mutations are found in exons 10, 11, 31, 34, 39 and 48, i.e. the six largest exons of KMT2D representing 62% of the entire coding sequence. Thus, although at first glance the largest exons appear as mutational hot spots, it becomes obvious that this accumulation is attributable to their size. Moreover, 60% of all 54 coding exons harbor at least one described mutation. Interestingly, missense mutations seem to cluster to a certain extent in exon 48 (16 of 38, 42%), which codes for part of a plant homeo domain (PHD) finger, the N-terminal phenylalanine/tyrosine rich (FYRN) domain and part of the C-terminal phenylalanine/tyrosine rich (FYRC) domain. Further five missense mutations and the recurrent single amino acid deletion, c.16489_16491delATC, lie within exons 52 and 53, which code for the SET domain. Contrary to our expectation, some missense mutations are located within protein regions of unknown function. Summing up, we here show that mutations are found more frequently in the C-terminal half of the protein, without any mutational hot spot.

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<th>Mutation type</th>
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<th>Frequency (%)</th>
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<tr>
<td>Frameshift</td>
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<td>9</td>
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<tr>
<td>In-frame del/ins</td>
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</table>
Mosaic mutations in KMT2D

Recently, the presence of mosaic mutations has been described in three patients with KS, two frameshift mutations and one whole-gene deletion of KMT2D (17). The mosaic rate was determined as low as 15% for the whole-gene deletion and one of the frameshift mutations. It is important to note that mosaics were found in almost 10% of KMT2D mutations in that study, highlighting the need of careful interpretation of Sanger sequencing results in the molecular diagnostic lab. In our own cohort, we observed one mosaic frameshift mutation in KMT2D and we confirmed the mosaicism via next generation sequencing (NGS) and found a frequency of the mutated allele of only 15%. Currently, we can only speculate how many KMT2D mutations might have been missed by normal Sanger sequencing because of a rate of mosaicism of less than 10%. The use of deep sequencing using NGS techniques in routine diagnostics is the subject of an ongoing debate.

Copy number changes and mutations in KDM6A

The recent discovery of heterozygous deletions in KDM6A in patients with KS underscored the importance of copy number changes as a cause of this heterogeneous syndrome. Lederer et al. identified KDM6A deletions in 3 of 22 KMT2D mutation-negative patients, showing a detection rate of 12.5% (10). However, in a second study including 120 KMT2D mutation-negative patients not a single copy number change was identified in KDM6A (53). Point mutations in KDM6A were found neither by Lederer et al. nor by Hannibal et al. both in 22 patients (8, 10). Surprisingly, a recent report showed two nonsense mutations and one 3-basepair deletion of KDM6A in 3 of 32 KMT2D mutation-negative KS patients (54). This finding indicates a need to sequence KDM6A in routine diagnostics, although KDM6A mutations seem to be a very rare cause of KS.
chromosomal rearrangements (Table 3) (55–58). Hannibal et al. performed an array-CGH-based screening in 26 KMT2D mutation-negative KS patients and found structural chromosomal variations in four patients, in one case suggesting a different diagnosis (a deletion on chromosome 5 including the NSD1 gene suggesting Sotos syndrome). In the remaining three cases they found a 977 kb deletion of chromosome 19q13 in one patient, a complex translocation involving chromosomes 8 and 18 in another, and a probable mosaic aneuploidy for the whole chromosome 12 in the third patient (8).

Given the low frequency of small copy number changes in KMT2D on the one hand and the higher number of chromosomal aberrations in patients with Kabuki-like phenotypes on the other, we believe that high-resolution array-CGH is the appropriate second-line diagnostic tool for patients in whom no mutation in KMT2D could be detected. Moreover, high-resolution array-CGH would detect even copy number changes of the size of described KDM6A and KMT2D deletions.

Genetic heterogeneity

In the attempt to find additional causative genes for KS several candidates have been screened by different groups. In a recent study, we have sequenced KMT2A (MLL) as well as some important members of H3K4 methyltransferase multiprotein complexes (SMAD1, CXXC1, ASC2, ASH2L, RBBP5, WDR5, DPY30, and PTIP) and DKK1 (a known target gene of KMT2D) in 15 patients without a KMT2D mutation, but did not detect any pathogenic sequence variant (6). Maas et al. found a 250 kb deletion in a patient with KS that disrupted the MACROD2 gene, but screening of 19 additional patients did not reveal any further mutations (59). Kuniba et al. screened 41 patients for mutations in TRPM3, KLF9, SMC5 and MAMDC2, i.e. four genes enclosed in a deletion on chromosome 9q21.11-q21.12 in a patient with KS, equally without any success (Table 4) (60). Although these candidate genes screened by Sanger sequencing in KS cohorts

<table>
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gene approaches have failed to identify further causative genes for KS, future studies applying new technologies and elaborate bioinformatic strategies will certainly discover more genes for KS in the near future.

Kabuki-like mosaic Turner syndrome

It is an astonishing observation that a Kabuki-like phenotype can sometimes be seen in females with Turner syndrome. This association was mostly described in females with a mosaic 45,X/46,X,r(X) karyotype (Table 3) (61–67), but also for a patient with a 45,X karyotype (68), one patient with a mosaic isochromosome of the long arm of the X chromosome (69), and one patient with mosaic trisomy X (70). Dennis et al. noted a facial appearance reminiscent of KS in 6 of 47 patients with a 45,X/46,X,r(X) karyotype (64). The authors speculated that this impression resulted mainly from the presence of long palpebral fissures and heavy eye-lashes. On the other hand, the majority of their patients did not exhibit Kabuki-like traits. Leppig et al. did not recognize a Kabuki-like phenotype in a cohort of 21 subjects with a ring X (71). Attempts to correlate the phenotypic variability and the severity of mental retardation with the presence of an inactive or active state of the ring chromosome, and/or its size have failed. Although the facial features of these patients might be suggestive of KS because of the long palpebral fissures, arched eye-brows and heavy eye-lashes, there are also slight, but distinct differences from the typical facial gestalt of KS. The face has a coarser aspect, the tip of the nose seems more bulbous, the upper lip is fuller and the eye-brows are, although arched, more bushy than those of typical Kabuki patients.

Current knowledge on the molecular pathogenesis

*KMT2D* encodes a 5537 amino acid methyltransferase that is required for H3K4 di- and trimethylation, a hallmark of active transcription state. This group of enzymes is characterized by the presence of a SET domain, which is responsible for the methyltransferase activity. The H3K4 methyltransferases act in stable multi-protein complexes that contain various shared and some distinct components that contribute to the specific function of each complex. The best studied member of this group of enzymes is KMT2A, the corresponding gene being first discovered in 1992 in the context of translocations and duplications at chromosome 11q23 involved in human acute leukemia (72, 73). Because of its implication in acute leukemia, the gene was called *ALL*, and the gene name was later changed to mixed lineage leukemia (*MLL*). MLL bears homology to the *Drosophila* protein TRX (trithorax), therefore, these enzymes are also referred to as the trithorax group proteins. The trithorax group proteins are involved in epigenetic regulation of transcription during development and in the adult organism, where they act as the counterpart of the repressive polycomb group proteins. *KMT2D* (*MLL2*) was identified and characterized in 1997 in the context of a search for further SET domain containing genes, and termed *ALR* (ALL-1 related gene) (74) until it was approved as *MLL2* by the HUGO Gene Nomenclature Committee (HGNC) in 1998. This large protein contains a C-terminal SET domain, eight PHD domains, a high mobility group (HMG)-binding motif, a FYRC, a FYRN motif and a post-SET domain (75).

Expression studies showed that *KMT2D* is expressed in a variety of human and murine tissues, and a space and time-specific expression during embryonic development has been shown in mouse embryos (74). The KMT2D complex has also been shown to be implicated in transcriptional activation of constitutively expressed genes through H3K4 trimethylation (76). KMT2D is essential not only for direct methyltransferase activity, but also for binding properties of its complex (also referred to as ASCOM) to target genes (76). The complex component KDM6A is a H3K27 demethylase. Agger et al. put forth a model where ASC-2 complex (ASCOM) acts through the removal of repressive epigenetic marks, polycomb displacement and positioning of activating methylation marks (Fig. 3) (77).

The identification of *KMT2D* as the causative gene for a multiple congenital malformation syndrome underscored the importance of time and space-specific transcriptional regulation through the orchestrated interplay of different epigenetic marks during embryonic development in humans. This notion is further supported by the very recent identification of *de novo* dominant mutations in *KMT2A* (*MLL*; MIM 159555) in Wiedemann–Steiner syndrome (78), a dominant mutation in *KMT2C* (*MLL3*; MIM 606833) in Kleefstra syndrome (79), and mutations in the polycomb group gene *EZH2* (MIM 601573) as a cause of autosomal dominant Weaver syndrome (80, 81), to name only a few examples. Currently, our knowledge on genes transcriptionally regulated by the KMT2D complex during development is limited and, therefore, the underlying pathogenesis of KS remains unclear. To understand it, we need to know more about the specific target genes of the KMT2D complex and their particular functions during cell differentiation and growth.

Gene nomenclature

During the preparation of this manuscript it became apparent that the interpretation of scientific results of *KMT2* group genes and encoded proteins is complicated by a confusion in gene nomenclature, which arises from both *KMT2B* and *KMT2D* being referred to as *MLL2* and/or *MLL4* in humans and, correspondingly, *Mll2* and *Mll4* in mice. A more detailed discussion of this conflicted topic is given in our mini-review ‘Skirting the pitfalls: a clear-cut nomenclature for H3K4 methyltransferases’, Bögershausen et al. in this issue. To avoid confusion for the present review, we have chosen to use the nomenclature system for chromatin modifying enzymes proposed by Allis et al. (82) and supported by the HGNC and the Mouse Genomic Nomenclature Committee (MGNC).
Fig. 3. Schematic representation of KMT2D complex function. (a) Repressed chromatin. H3K27 is trimethylated by polycomb repressor complex 2. (b) Activated chromatin. KMT2D-complex removes repressive H3K27 marks and deposits activating H3K4 methylation marks leading to recruitment of the RNA polymerase II complex.

**Methods**

We systematically reviewed all currently available literature on KS. The number, type and locations of mutations were taken directly from the available figures and tables within the original articles presenting mutation screening data (5–8, 11, 15). The phenotypic information was correlated with genotype as given in the articles. Only patients with available clinical information were counted. We are aware that data collection is incomplete because of differences in study set up, and that the evaluation of certain features, such as facial gestalt, may be subject to bias of ascertainment. Statistical significance was tested using the Graphpad software for a two tailed Fisher’s exact test (http://graphpad.com/quickcalcs/contingency1/).

We reviewed only those articles presenting functional data on KMT2D that use a clear nomenclature or give accession numbers for the investigated gene/protein.

**Conclusions**

KS is a highly recognizable syndrome with a characteristic facial gestalt and various associated symptoms. Comparison of patients with and without a mutation in KMT2D revealed a number of differences between both the groups. Patients with a KMT2D mutation show a significantly higher frequency of short stature, typical facial features, persistent fetal finger pads, renal abnormalities and feeding problems compared with patients without a mutation in KMT2D. This information will help clinical geneticists to distinguish between typical and atypical cases of KS and be useful in establishing a reasonable algorithm for molecular genetic testing. Presently, as mutations in KMT2D are the most frequent genetic cause of KS and because mutations can be found even in atypical cases, it is reasonable to perform sequence analysis of KMT2D as a standard molecular genetic test in patients with suspected KS. For mutation-negative patients, array-CGH should be performed. We suggest MLPA for KMT2D and KDM6A as a third-line diagnostic tool after Sanger sequencing of KMT2D and array-CGH. As an alternative, high-resolution array-CGH might be a powerful diagnostic tool to detect structural chromosomal aberrations as well as specific deletions in KMT2D and KDM6A. The finding of low-level mosaic mutations in KMT2D might lead to the establishment of deep sequencing strategies for KMT2D molecular diagnostics in the near future. These NGS-based strategies will also facilitate the sequencing of KDM6A in parallel to KMT2D, in order to detect rare single nucleotide alterations.

The identification of KMT2D as the major causative gene for KS has brought us one step closer to understand the pathogenesis of this complex developmental phenotype. However, as long as the gene sets regulated by the KMT2D complex during development are not well characterized, we will not understand the exact mechanism leading to the various symptoms of the disease. The molecular genetic data published to date emphasize the genetic heterogeneity of this condition. We therefore conclude that the fascinating story of KS is not yet at its end.

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