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ORIGINAL ARTICLE

Molecular characterization of an embryonal rhabdomyosarcoma occurring in a patient with Kabuki syndrome: report and literature review in the light of tumor predisposition syndromes

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Abstract

Kabuki syndrome is a well-recognized syndrome characterized by facial dysmorphism and developmental delay/intellectual disability and in the majority of patients a germline variant in *KMT2D* is found. As somatic *KMT2D* variants can be found in 5–10% of tumors a tumor predisposition in Kabuki syndrome is discussed. So far less than 20 patients with Kabuki syndrome and a concomitant malignancy have been published. Here we report on a female patient with Kabuki syndrome and a c.2558_2559delCT germline variant in *KMT2D* who developed an embryonal rhabdomyosarcoma (ERMS) at 10 years. On tumor tissue we performed DNA-methylation profiling and exome sequencing (ES). Copy number analyses revealed aneuploidies typical for ERMS including (partial) gains of chromosomes 2, 3, 7, 8, 12, 15, and 20 and 3 focal deletions of chromosome 11p. DNA methylation profiling mapped the case to ERMS by a DNA methylation-based sarcoma classifier. Sequencing suggested gain of the wild-type *KMT2D* allele in the trisomy 12. Including our patient literature review identified 18 patients with Kabuki syndrome and a malignancy. Overall, the landscape of malignancies in patients with Kabuki syndrome was reminiscent of that of the pediatric population in general. Histopathological and molecular data were only infrequently reported and no report included next generation sequencing and/or DNA-methylation profiling. Although we found no strong arguments pointing towards KS as a tumor predisposition syndrome, based on the small numbers any relation cannot be fully excluded. Further planned studies including profiling of additional tumors and long term follow-up of KS-patients into adulthood could provide further insights.

Keywords Kabuki syndrome · KMT2D · Tumor predisposition · Rhabdomyosarcoma · Methylation

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Introduction

Kabuki syndrome (KS), also known as Niikawa-Kuroki syndrome or Kabuki-make-up syndrome, is a syndrome where affected persons present with a characteristic face, including arched eyebrows with a sparse lateral one-third and long palpebral fissures with eversion of the lower eyelids. Other features are hypotonia and developmental delay/intellectual disability [1]. In the majority of the patients a variant in *KMT2D* (KS1, OMIM #147920) or *KDM6A* (KS2, OMIM #300867, X-linked) can be identified [2–4]. In case of some rare syndromes, for patients, their parents and professionals involved, a key question is whether besides the syndromic features also a tumor predisposition exists [5]. This may guide tumor surveillance strategies including follow-up of the primary tumor and early detection of secondary malignancies [6]. A tumor predisposition has been established in a number of rare syndromes including Noonan syndrome type 1 (juvenile myelomonocytic leukemia), Gorlin syndrome (basal cell carcinoma, medulloblastoma) and PTEN Hamartoma Tumor (Cowden) Syndrome (predominantly breast- and thyroid carcinoma) [5, 7]. Such a predisposition is less clear for KS. Along the same lines, patients with Li-Fraumeni syndrome, Neurofibromatosis type 1, DICER1 syndrome, Costello syndrome, Noonan(-like) syndrome and Beckwith-Wiedemann syndrome are known to have an increased risk of developing rhabdomyosarcoma [8, 9]. For KS the question of a potential tumor predisposition is of special interest as somatic *KMT2D* variants are observed in approximately 5–10% of all cancers [10–13]. This frequency is even higher, up to 90%, in adult follicular lymphoma [14–18] and mutations in *KMT2D* are supposed to be driver events in various tumor types [19]. To date few (case) reports of malignancies occurring in patients with KS have been published [4, 20–35]. KMT2D fulfills as a histone 3 lysine 4 (H3K4) methyltransferase important roles in many aspects of normal development [36] and is in KS associated with a distinct DNA methylation signature [37, 38]. Also in cancer the role of KMT2D mediated DNA methylation has received increased attention [39]. Nevertheless, detailed molecular data of (epi-)genomic alterations occurring in tumors from patients with KS is lacking or sparse. Here we report on a 10-year female patient with KS who developed an embryonal rhabdomyosarcoma. On tumor material, we performed extensive molecular analyses including exome sequencing and DNA-methylation profiling. In addition we performed a review of literature focusing on the clinical-, pathological as well as molecular features of malignancies occurring in patients with KS.

Methods

Patient

The clinical history of the patient is described in the results section. The parents of the patient provided written informed consent for the use of archival tissue for further analyses and consent for publication.

Histopathology and immunohistochemistry

Histopathological, immunohistochemical and FISH analyses with *FOXO1* and *EWSR1* break-apart probes were conducted as part of routine clinical diagnostics and the former re-evaluated by two bone- and soft-tissue pathologists (M.v.d.H and Ra.S.).

Molecular and bio-informatic analyses

For the present study, after consent from the parents of the patient, additional molecular analyses were performed on fresh-frozen tumor material from the primary biopsy. For this DNA was extracted from fresh-frozen tumor tissue with a Promega Maxwell RSC DNA FFPE kit (Promega, Madison, WI, USA) according to manufacturer's instructions.

DNA-methylation profiling: For DNA methylation analysis of the tumor DNA the Illumina Infinium® array technology (Illumina Inc., San Diego, CA, USA) using the Infinium Methylation EPIC BeadChip (850K array) was used following the manufacturer's instructions. Raw methylation data was processed as analogous to Wagener et al. and further described in the Supporting methods [40, 41]. For methylation-based sarcoma classification, the DNA methylation profile of the current case was analysed using the DKFZ-sarcoma classifier (v12.2) available at https://www.molecularneuropathology.org/msp/ [42]. DNA methylation changes at the imprinted region of the Beckwith-Wiedemann-Syndrome locus at 11p15.5 in the ERMS was compared to five controls which were processed analogous to Bens et al. [43]. For copy number variant (CNV) analysis raw methylation data was normalized using the R-package minfi [44]. Subsequently, CNV data were extracted from the methylation data using the R-package conumee [45].

Exome sequencing was performed as described in the Supporting methods. In brief, tumor-only exome sequencing was performed on a NextSeq (Illumina, San Diego, Ca., USA) with Illumina Nextera™ Exome Kit. For data-analysis evaluation was restricted to a virtual gene panel of 95 cancer predisposition related genes (according to the TruSightCancer-Panel, Illumina) with addition of *KMT2D* and *KDM6A*, as well as 10 genes recurrently mutated in embryonal and/or fusion negative rhabdomyosarcoma (Supporting Table S1)

[46–48]. The primers used for Sanger-sequencing of the germline variant in tumor material are listed in Supporting Table S2.

Literature review

The English literature published till September 1st 2021 was searched for publications with patients with a diagnosis of KS and a concomitant malignancy. The search strategy is outlined in detail in the Supporting Methods.

Results

Clinical history of the patient

A 10-year old girl had a clinical and molecular diagnosis of KS with a c.2558_2559delCT pathogenic variant in *KMT2D* (g.49444907_49444908) predicted to result in a p.(Pro853Argfs*3) change at the protein level (NM_003482.3, NP_003473.3). She had the classical facial features of the syndrome as assessed by an expert dysmorphologist (C.T.R.M.S.). Her height growth followed -2 SD and she had a nasal speech. She was included in a clinical trial investigating the metabolic effect of growth hormone in children with KS [49]. However, within the first weeks of inclusion she was diagnosed with a retroperitoneal rhabdomyosarcoma and was treated with chemotherapy followed by surgical removal of the tumor. For this reason she was, according to the study protocol, excluded from the study. No causal association between the development of the rhabdomyosarcoma and initiation of growth hormone therapy was assumed.

Histopathological and fluorescence in situ hybridization (FISH) analysis

The pre-treatment core needle biopsy and post-chemotherapy excisional specimen were histopathologically analysed (Fig. 1a–d) and neither of the specimens showed an alveolar growth pattern and/or anaplastic features. With FISH analysis the tumor was *FOXO1* and *EWSR1* break negative.

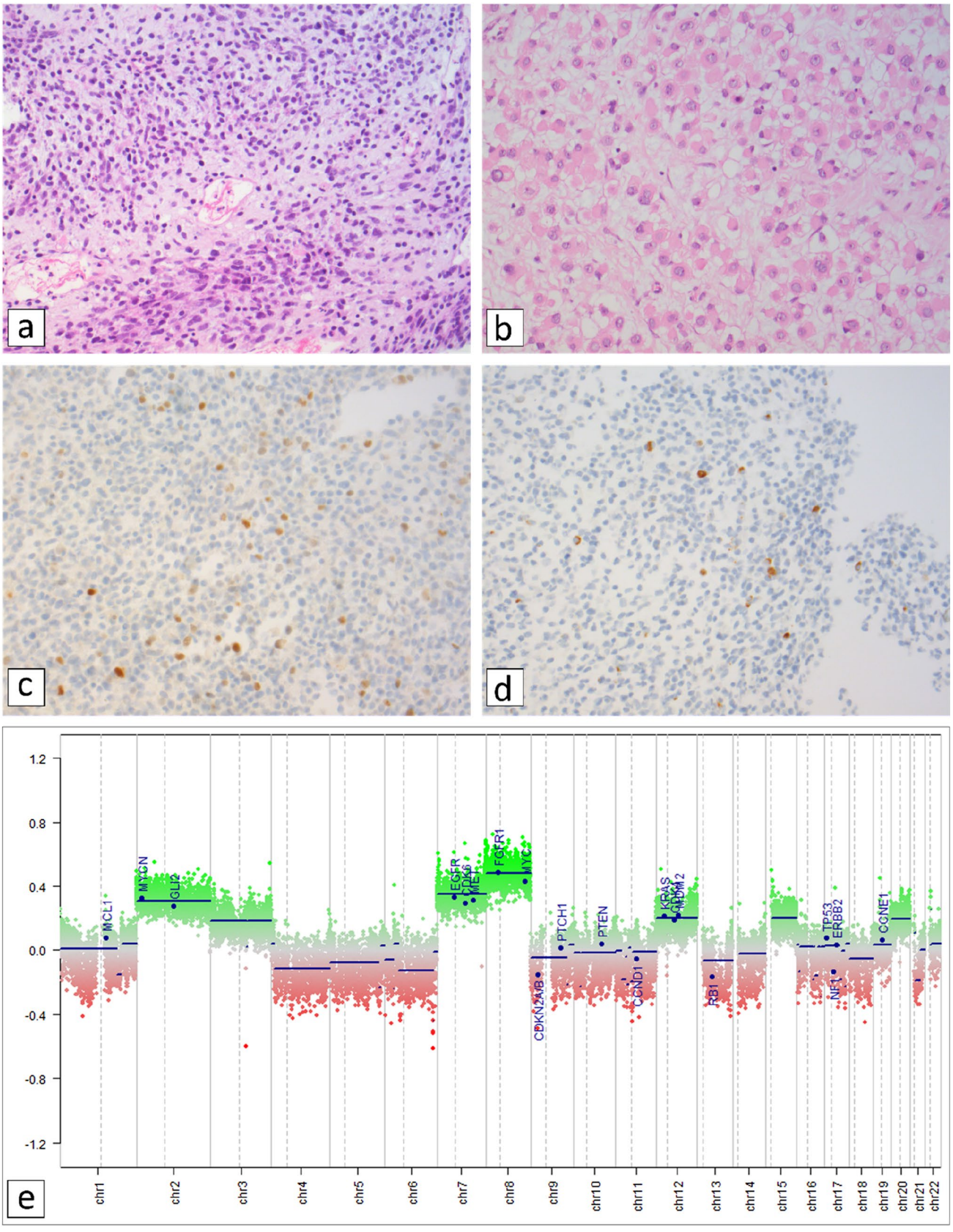
Molecular analysis

DNA-methylation analyses: the methylation profile obtained with genome-wide epigenomic profiling of the current case was analysed using the "DKFZ-sarcoma classifier" and showed a methylation class of (embryonal)rhabdomyosarcoma (calibration score 0.99) confirming the histopathological diagnosis. DNA-methylation based CNV-analysis showed amongst others, (partial) gains of chromosomes 2, 3, 7, 8, 12, 15, and 20 and 3 focal losses in chromosome 11p (Fig. 1e). The latter deletions at cytogenetic bands 11p15.1-p13, 11p12 and 11p11.2-q12.1 are located more centromeric than the Beckwith-Wiedemann Syndrome (BWS) locus at 11p15.5-p15.4. As patients with BWS have an increased risk of developing rhabdomyosarcomas [50] and 11p15 (epi)genetic effects are recurrent in rhabdomyosarcoma [47, 51–53], we analyzed the DNA methylation at the BWS locus in detail using the EPIC array data. Here a hypermethylation of *H19* / imprinting center 1 (IC1)/differentially methylated region 1 (DMR1) and a hypomethylation of *KCNQ1*/*KCNQ1OT1* imprinting center 2 (IC2)/differentially methylated region 2 (DMR2) was seen (Supporting Fig. S1).

Exome sequencing revealed, amongst others, sequence variants in *ERCC5* and *TP53*. In *KDM6A* one synonymous and in *KMT2D* one synonymous and one intronic variant were observed (Supporting Table S3). The germline variant in *KMT2D* was not identified in the tumor-DNA as a result of low-coverage at this position (exon 10, overall coverage *KMT2D* 95%) but confirmed with Sanger sequencing (Supporting Fig. S2). The Sanger sequence, moreover, suggests a gain of the wild-type and not the mutated allele in the trisomy 12 (Supporting Fig. S2, Fig. 1e). For the variants on chromosome 11 there was—in contrast to the other chromosomes—a strong bias towards variants present in a homozygous state pointing to an uniparental disomy (UPD). In contrast, the diagnostic SNP-array (trio analysis of the proband and parents) was normal (arr snp (1–22,X) × 2).

Literature review

With literature review we identified, including the patient from the present study, 18 patients with a clinical and/or molecular diagnosis of KS who developed a malignancy (Table 1). In 10/11 patients from which DNA was subjected for mutational analysis a *KMT2D* (n = 9) or *KDM6A* (n = 1) variant was identified. One patient, patient no.8, had a clinical diagnosis of KS but was negative for *KMT2D* and *KDM6A* variants by exome sequencing and array-CGH [27]. In other reports the mutational status was not reported or were published before the identification of *MLL2* (*KMT2D*) and *KDM6A* as cause of KS in 2010 and 2012/2013 [54–56]. In eight patients without mutational status or variant (patients 2,3,7,8,10,12,13 and 14) the provided clinical features and/or photographs were compatible with a clinical diagnosis of KS. In one other patient no clinical information was provided except mentioning of the diagnosis of KS: this patient (S1) is included in Supporting Table S4. Two other patients with KS (clinical diagnosis [57] and not specified [58]) and neuroblastoma and non-Hodgkin lymphoma respectively were excluded from the literature review and discussion due to complete lack of (clinical) data of the patient, e.g. sex and age not reported.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◂**Fig. 1** Histopathological- and molecular characterization of the tumor. **a** HE-section (X200) of the diagnostic pre-treatment core-needle biopsy showing hypercellular and less cellular areas of a small blue round cell tumor with a myxoid stromal component in the latter. **b** the excision specimen 3 months later after neoadjuvant chemotherapy was partly necrotic. Vital areas were less cellular than the primary biopsy and characterized by a more abundant myxoid matrix with tumor cells showing prominent rhabdoid differentiation as is commonly observed in embryonal rhabdomyosarcoma (ERMS) after chemotherapy [145]. In neither of the specimens an alveolar growth pattern and/or anaplastic features were seen. **c** partially positive nuclear staining for MYF4/Myogenin (**d**) and focal expression of Desmin. **e** Methylation-array based CNV profile. Gains are depicted in green, losses in red. Blue lines represent flattened profiles. Focal copy number (CN) aberrations may not be visible in the figure and genomically distinct CN aberrations laying in close proximity may not be visible as separate but instead of single genomic events. (Color figure online)

For completeness, these patients (S2 and S3) are included in Supporting Table S4.

The reported malignancies in patients with a clinical and/or molecular diagnosis of KS are bone- and soft-tissue tumors (n = 5), hematologic malignancies (n = 5), embryonal tumors (n = 4) and tumors not belonging to any of the aforementioned malignancy groups (n = 4). Among the bone- and soft-tissue tumors, 3 of the 5 cases were reported as sarcoma (embryonal rhabdomyosarcoma, synovial sarcoma, low-grade fibromyxoid sarcoma). The most frequent hematologic malignancy was Burkitt lymphoma which was seen in 3 patients. Precursor B-cell acute lymphoblastic leukemia (pre-B-ALL) and Hodgkin's lymphoma were seen in one patient each. Two patients presented with a neuroblastoma, and Wilms tumor, fetal-type hepatoblastoma, ependymoma, hepatocellular carcinoma, carcinoma of unknown primary origin and endometrial cancer were seen in one patient each.

In addition, the reports were screened for potential tumor predisposing and/or contributing factors other than KS. In case 10 [29], with pre-B-ALL, there was a positive family history with an uncle with leukemia at the age of 3½ years which *could* point to a familial predisposition for leukemia. Patient 16 [34], hepatocellular carcinoma (HCC), had a history of hepatic adenomatosis and use of (high-dose) oral contraceptives which could have lead or contributed to the development of HCC. Three malignancies were Epstein-Barr virus positive (EBV +): patient 7 [26] had a EBV + Burkitt lymphoma, patient 9 [28] developed an EBV + Hodgkin lymphoma under immune suppression and in patient 17 [35] an EBV-associated carcinoma of unknown primary (CUP) was diagnosed.

Although histopathological features were reported in 10 of the 16 patients (not including patient 18 with a known *KDM6A* variant) most reports lacked a detailed description. (Molecular)cytogenetic data were provided only in three published cases. For patient 6 [24], Burkitt lymphoma, it was shown that the *KMT2D* variant was present in both germ-line and tumor DNA in a heterozygous state. Apart from the case included in the present study, in none of the cases next-generation sequencing and/or methylation profiling on the tumor were performed. Although most of the tumors do not meet (current) World Health Organization (WHO) Classification of Tumours diagnostic criteria for the reported tumor entities and therefore caution has to be made with drawing conclusions based on (some of the) provided diagnoses, it appears that overall the clinical presentation of the tumors in the patients does not seem to be very unusual with regard to age and sex distribution, site of presentation and histopathological characteristics (for references, see Supporting Methods). Although patient 18 (*KDM6A* variant) developed endometrial cancer (subtype not provided) at a young age (≤ 31 years), approximately ≤5% of endometrial cancers are reported to occur in women younger than 40 years [59]. Patient 2 (synovial sarcoma) experienced a local relapse at 4 months and for patient 4 (giant cell fibroblastoma), as commonly observed for this entity [60, 61], local recurrence was reported. Further no second malignant neoplasms, bilateral-, multifocal- or meta-synchronous cancers were reported (Supporting Table S5).

Discussion

In our study we present the history of a patient with Kabuki syndrome (KS) with a germline *KMT2D* variant who developed an embryonal rhabdomyosarcoma. On tumor-DNA we performed exome sequencing and DNA-methylation profiling and conducted a literature review focusing on the clinical-, pathological- and molecular characteristics of other malignancies occurring in patients with KS. Although patient number 18 carried a *KDM6A* variant and we cannot exclude the presence of *KDM6A* variants in the patients from the literature review in which no mutational analysis was performed or no variant was identified, a detailed discussion about the role of KDM6A in malignancies goes beyond the scope of the present study. For a detailed discussion about the (in vitro) oncogenic potential of KMT2D and its role in cancer we refer to recent articles [62–68] and reviews [10, 36, 39, 69, 70]. Also of interest in the light of tumor predisposition is the functional link between KS and RASopathies [71], a disease family with known germline predisposition to a variety of hematologic and solid cancers [72, 73]. Although based on our present analyses and literature review no definitive conclusion regarding tumor predisposition in KS can be drawn, we would like to point out several observations.

Table 1 Summary of literature review of patients with Kabuki syndrome and a malignancy[a]

Pt	Sex, age	*KMT2D*/*KDM6A* variant	COSMIC/SOM	Clinical diagnosis (variant neg/NA)[b]	Malignancy[c]	Histopathological features	Molecular tumor features	Common/general (clinical) presentation for reported tumor entity?[d]	Other potential predisposing or contributing factors?[e]	Ref. case
Bone- and soft-tissue tumors (n = 5)										
1	F (10)	*KMT2D*, c.2558_2559delCT, p.(Pro853Argfs*3)	NO		Embryonal rhabdomyosarcoma (ERMS, small pelvis)	Small blue round cell tumor with spindle cell features. No alveolar or anaplastic characteristics. desmin+, Myf4±, keratin±, CD99+, Myod1-, synaptophysin-	ERMS-methylation profile, typical ERMS CNVs GL-variant *KMT2D* confirmed (Het.)	Typical age for ERMS. Small pelvis less common site and presentation	None[f]	*this study*
2	F (16)	NR. Normal karyotype, subtelomeric study and 22q11.2 FISH		(compatible with) Kabuki syndrome	Synovial sarcoma (SS) (right lung mass > 10 cm)	NR	NR	Common age. SS most common at extremities, trunk and head/neck. Thorax/lung is unusual but reported primary site	NR	[20]
3	F (10)	NR, clinical diagnosis. G-banded karyotype normal		(compatible with) Kabuki syndrome	Low-grade fibromyxoid sarcoma (right posterolateral chest wall)	Myxoid background, spindle cell population, curvilinear blood vessels. Vimentin+, S100-, desmin-, SMA-, CD34-	NR	Most common in young adults, slight male preponderance. 10 yrs is typical pediatric age, Trunk common presentation	NR	[21]
4	F (10 & 12)	*KMT2D*, c.7307_7308insT (frameshift), p.(Ser2438Ilefs*11)	NO		Giant cell fibroblastoma[g] (neck right-side)	10 yr: mesenchymal mass, vascular malformations, spindle cells, CD34+, SMA-, S-100-, GFAP-, EMA-, Ki-67 15%	NR	Pediatric tumor. Overall male preponderance. Head- and neck region uncommon but reported. Local recurrence is common	NR	[22]

Table 1 (continued)

Pt	Sex, age	*KMT2D*/*KDM6A* variant	COSMIC/SOM	Clinical diagnosis (variant neg/NA)[b]	Malignancy[c]	Histopathological features	Molecular tumor features	Common/general (clinical) presentation for reported tumor entity?[d]	Other potential predisposing or contributing factors?[e]	Ref. case
						12 yr: mesenchymal mass, vascular malformations, spindle cells, multinucleated giant cells, CD34+, Vimentin+, SMA-, S-100-, CD99-, CD31-, EMA-, Ki-67 15%	NR			
5	F (10)	*KMT2D*, c.11233C > T, p.(Gln3745*)	YES/NO		Aggressive desmoid fibromatosis (occipital bone)	Locally invasive fibroblastic proliferation. Spindle-shaped cells, SMA+	NR	For pediatric age-group typical age. Head- and neck region less common but recurrently involved site	NR	[23]
Hematologic malignancies (n = 5)										
6	M (5)	*KMT2D*, c.511-1G > A, p.?	YES/YES		Burkitt lymphoma (rhino-pharyngeal mass)	"Starry-sky" pattern, CD20+, CD79+, CD10+, CD38+, BCL6+, BCL2-, CD3-, TdT-, Ki-67 > 98%	t(8;14)+[h] c.511-1G > A *KMT2D* heterozygous	Typical age and sex	NR	[24]
7	M (3)	NR, clinical diagnosis. Normal karyotype[i]		(compatible with) Kabuki syndrome	Burkitt lymphoma (abdominal, mesenterium/ upper right quadrant, mesenteric lymphadenopathy)	Diffuse proliferation of lymphocytes. "Starry-sky" pattern, CD20+, CD22+, EBER-1+(ISH), LMP-, EBNA-2-	NR	Typical age and sex	EBV+	[26]
8	M (3)	*KMT2D* and *KDM6A* negative		(compatible with) Kabuki syndrome	Burkitt lymphoma (abdominal)	NR	NR	Typical age and sex	NR/insufficient information	[27]
9	M (≥ 32)	*KMT2D*, c.12985C > T, p.(Gln4329*)	YES/YES		Hodgkin lymphoma	NR	NR	Age distribution and % EBV+ vary according to (c)HL subtype	EBV+ (under immunosuppression)	[28]

Table 1 (continued)

Pt	Sex, age	*KMT2D/KDM6A* variant	COSMIC/SOM	Clinical diagnosis (variant neg/NA)[b]	Malignancy[c]	Histopathological features	Molecular tumor features	Common/general (clinical) presentation for reported tumor entity?[d]	Other potential predisposing or contributing factors?[e]	Ref. case
10	F (2)	NR; 46,XX; no 22q11 or 4p16,3 microdeletion, normal subtelomeric FISH, normal CGH		(compatible with) Kabuki syndrome CHARGE syndrome can not be fully excluded based on clinical features	Pre-B-ALL (BM)	CD19+, CD24+, CD13-, CD23-, CD65S- (BM smear)	diploid (DNA index 1.00), *BCR/ABL*, *MLL/AF4*, *TEL/AML1*, *MLL/ENL* negative	Typical age for pre- B-ALL, B-ALL > > T-ALL	Maternal uncle with leukemia at age: 3 1/2 years	[29]
Embryonal tumors (n = 4)										
11	F (3)	*KMT2D*, c.13285C > T, p.(Gln4429)*	NO		Wilms tumor/ nephroblastoma	Mixed-type nephroblastoma, no anaplastic features. Blastemal and epithelial elements	NR	Typical pediatric renal tumor and age	NR	[30]
12	F (0)	NR, clinical diagnosis. *PTPN11* negative, normal array-CGH		(compatible with) Kabuki syndrome	Neuroblastoma (adrenal mass)	NR	no *MYCN* amplification	Most common extracranial pediatric tumor, majority diagnosed < 5 years (median ≈18 M.)	NR	[32]
13	F (NR)	NR, clinical diagnosis		(compatible with) Kabuki syndrome	Neuroblastoma	NR	NR		NR	[31]
14	M (6)	NR, clinical diagnosis		(compatible with) Kabuki syndrome	Fetal-type hepatoblastoma	NR	NR	Most common malignant pediatric liver tumor, majority diagnosed < 5 years (median ≈ 18 M.)	NR	[32]
Other (n = 4)										
15	F (23)	*KMT2D*, c.16085_16086delAG, p.Lys5362Serfs*96	NO		Ependymoma (lumbar endocanalar mass, filum terminale)	Oval/elongated cells, mild nuclear atypia, eosinophilic fibrillary stroma, fascicular/ vaguely perivascular growth pattern. GFAP+, EMA+, Ki-67 3–5%	NR	Intracranial ependymoma's are more prevalent in the pediatric age group, spinal more typical presentation for adults	NR	[33]

Table 1 (continued)

Pt	Sex, age	*KMT2D*/*KDM6A* variant	COSMIC/SOM	Clinical diagnosis (variant neg/NA)[b]	Malignancy[c]	Histopathological features	Molecular tumor features	Common/general (clinical) presentation for reported tumor entity?[d]	Other potential predisposing or contributing factors?[e]	Ref. case
16	F (15)	*KMT2D*, c.8594dupC	NO		Hepatocellular carcinoma (HCC)	Hepatic adenomatosis, macrovesicular steatosis, extramedullary hematopoiesis. Well-differentiated HCC with a hepatic adenoma (> 8 cm), > 10 satellite lesions	NR	OC use is associated with hepatic adenomatosis and hepatic adenomas may transform to HCC. HCC is a clinical and molecular highly heterogeneous disease	Hepatic adenomatosis, high-dose estrogen OC	[34]
17	F (16)	*KMT2D* c.2871dupA, p.(Glu958Argfs*11)	NO		Carcinoma, unknown primary origin. Lymphadenopathy from neck to abdomen, sternal, liver and kidney lesions	Cervical lymph node: poorly differentiated carcinoma, EBV-associated	NR	NA	EBV-associated?	[35]
18	F (≤ 31)[j]	*KDM6A*, c.1846dupA, p.Thr616Asnfs*5	NO		Endometrial cancer	NA	NA	≤5% of endometrial cancer diagnosed in women ≤ 40 years	NA	[4]

B-ALL B-cell acute lymphoblastic leukemia, *BAP* break-apart probe, *BCL* B-cell lymphoma, *BM* bone marrow, *CD* cluster of differentiation, *CGH* comparative genomic hybridization, *CHARGE syndrome/MIM#214800* coloboma, heart defects, choanal atresia, retardation of growth/development, genital- and ear abnormalities, *CNV* copy number variation, *COSMIC* catalogue of somatic mutations in cancer, *EBNA-2* Epstein-Barr nuclear-antigen 2, *EBV* Epstein-Barr virus, *EMA* epithelial membrane antigen, *FISH* fluorescence in situ hybridization, *GFAP* glial fibrillary acidic protein, *GL* germline, *Het* heterozygous, *ISH* in situ hybridization, *Ki-67* Kiel clone 67, *M* months, *Myf4* myogenin, *MYOD1* myogenic differentiation 1, *NA* not analyzed/available *Neg* negative, *NR* not reported, *OC* Oral contraceptive, *Pt* patient, *SMA* smooth muscle actin, *SOM* reported as somatic variant in COSMIC database, *TdT* terminal deoxynucleotidyl transferase, *T-ALL* T-cell acute lymphoblastic leukemia, *WHO* World Health Organization, *Yr* year

[a]Patients S1 with Burkitt lymphoma with insufficient clinical information regarding Kabuki syndrome is not present in this table but is included in Supporting Table S4

[b]Clinical diagnosis based on assessment of clinical features presented in the individual manuscripts by the author's of the present study, see the "supplementary materials and methods" section

[c]Diagnosis, clinico- and histopathologic features as provided in the original manuscript. These may not fulfil the present WHO-criteria for the respective tumors

[d]References for common/general (clinical) presentation of individual tumor entities in Supporting data

[e]Includes (other)potential predisposing (genetic) factors for the reported malignancy

[f]Morphology does not show anaplastic features suggestive of a *TP53* germline variant [143, 144]

[g]Initial tumor diagnosed as spindle cell hemangioma, 2nd tumor at same site as giant cell fibroblastoma

[h]Diagnosed by long-distance polymerase chain reaction (PCR)

[i]Karyotype at time of tumor diagnosis (peripheral blood)

[j]Age at last examination

The landscape of malignancies in patients with Kabuki syndrome

First, from a pediatric-oncology perspective it seems that the landscape of reported malignancies occurring in patients with KS broadly resembles that of the pediatric population in general. In the literature review we identified 18 patients with KS presenting with in total 19 malignancies. This included solid tumors in 13 patients (bone- and soft-tissue tumors, n = 5; embryonal tumors, n = 4; "other" tumors, n = 4) and hematologic malignancies (n = 5). The one additional patient with KS but without accompanying clinical information had a diagnosis of Burkitt lymphoma [25] (Supporting Table S4). Although having to take publication bias and the small number of cases into account this seems at least broadly in line with the different groups of malignancies occurring in the pediatric age groups in general [74]. Along the same lines, *KMT2D* would, if being a genuine tumor predisposition gene (TPG), predispose to a rather wide range of malignancies including bone- and soft-tissue tumors, hematological malignancies, embryonal tumors and carcinoma's. Although many TPGs predispose to a single or limited types of tumors (e.g., *ATM*, 11p15/*CDKN1C*, *CDH1*, *PAX5*, *PTPN11*, *SMARCB1*), based on the observed tumor spectrum, *KMT2D* would belong to a group of TPGs predisposing to a broader spectrum of tumours like seen for *TP53*, *PTEN*, *STK11* and *DICER1*.

Of interest, although hematologic malignancies are common in both patients with KS and the general pediatric population the distribution of reported malignancies is different: for KS (including patient S1 from Table S4) 4 patients with Burkitt lymphoma [24–27] and 1 patient with B-ALL [29] have been published. In contrast, the incidence of B-ALL in the pediatric population (far) outnumbers that of Burkitt lymphoma [75, 76]. Publication bias could play a role in this, however, than one would expect this to be also the case for B-ALL and malignancies in general and not specifically for Burkitt lymphoma alone. Of interest, somatic *KMT2D* variants are recurrent but not highly frequent in Burkitt lymphoma occurring in ≤ 15% patients [77–81]. Intriguing in the light of the postulated cell-of-origin in Burkitt lymphoma—a germinal center B-cell poised to express IgA [79, 82]—and it's pathogenesis are the reduced serum IgA levels in patients with KS and mouse models [83–85] and the smaller and reduced number of Peyer's patches reported in one study [83]. In line with the findings of two *Kmt2d*-loss mouse models which showed (after immunization) an enhanced germinal-center response with an increase in the number of germinal-center B-cells with increased proliferation [86, 87] it might be speculated that in KS germline *KMT2D* variants may not have a direct classic tumor predisposition effect but may instead increase the chance of developing Burkitt lymphoma by an increased germinal-center response and proliferation.

As three EBV + malignancies (Hodgkin lymphoma, Burkitt lymphoma and a carcinoma of unknown primary) were reported it might be speculated that the combination of immune deficiency in patients with KS and EBV infection could—in analogy to other inborn errors errors of immunity—contribute to an increased susceptibility to develop EBV + lymphoproliferations and tumors [88, 89]. However, it has to be acknowledged that the number of EBV + malignancies in patients with KS is small and EBV-status has not been routinely reported.

Somatic *KMT2D* variants in malignancies in patients with Kabuki syndrome and cancer in the general population

Second, (also) for other malignancies reported in patients with KS, besides Burkitt lymphoma, somatic *KMT2D* variants are mostly only infrequently reported. In contrast, malignancies in which somatic *KMT2D* variants are highly recurrent typically do not or only infrequently occur in patients with KS. E.g., somatic variants involving *KMT2D* are only infrequently reported in (embryonal) rhabdomyosarcoma [46–48, 90], in less than 10% of Hodgkin lymphoma [91–95] and on average in ≤ 15% of (pediatric) Burkitt lymphomas [77–81]. Hepatocellular carcinoma is a molecularly and clinically highly heterogeneous disease were *KMT2D* variants can be identified in approximately 5% [96–99]. In pre-B-ALL the frequency of *KMT2D* variants varies strongly between individual genetic subgroups and is high(er) in e.g. the *ERG*/*DUX4* and *ZNF384* [100, 101] rearranged subgroups but is overall, not taken these subgroups into account, low [18, 100–103]. Moreover, in the typical pediatric cancers including Wilms tumor [104–106], neuroblastoma [104, 107, 108] and pediatric hepatoblastoma [104, 109–111] somatic *KMT2D* variants only (very) infrequently occur. In contrast, in other cancers including, amongst others, pediatric- and adult diffuse large B-cell lymphoma (DLBCL) (20–35%) [11, 15, 112, 113], adult follicular lymphoma (70–90%) [14, 15], nodal marginal zone lymphoma (≈20–30%) [114–116], (non)small cell lung cancer/lung squamous cell carcinoma (≈20–30%) [11, 65, 117], upper tract urothelial carcinoma/bladder cancer (≈25–45%) [11, 118–120], esophageal (squamous cell) carcinoma (≈10–25%) [11, 121, 122] and pediatric- and adult medulloblastoma (overall ≈5–30%, large differences between individual molecular subgroups) [123–125] somatic *KMT2D* variants are (highly) recurrent but these cancers have not been reported in patients with KS (yet). However, with a lack of longitudinal studies it remains unclear whether KS patients reach the ages at which many of these tumor

types are most prevalent. In cancer truncating *KMT2D* variants have been reported with varying frequencies [39, 70, 126]. A recent in-depth study analyzing germline and somatic *KMT2D* variants found 80% of the germline variants causing KS to be protein truncating. On the other hand, somatic *KMT2D* variants in cancer where predominantly missense with only 35% were predicted to be protein truncating [126]. Missense variants in patients with KS and somatic variants in cancer showed an overlapping but also different distribution across KMT2D protein domains [126]. Whereas in patients with KS many missense variants have a loss-of-function (LoF) effect (by impaired methyltransferase activity and/or loss of protein–protein interactions) [127] it can not be excluded that some missense variants in cancer may have a gain-of-function [126] or, in analogy to selected germline *KMT2D* missense variants [128] a dominant-negative effect [126]. Finally, it should be noted that the across different cancer types the percentages of missense variants varies widely [39]. Moreover, for many of the reported (presumed) somatic *KMT2D* variants no variant classification is provided [129] and depending on the cancer type may act either as (early) driver or may arise only later in the process of malignant transformation [19, 39, 130–134]. Finally, not in all studies the "true" somatic origin of the *KMT2D* variants has been reported which might be relevant considering the (relatively) frequent germline origin of *KMT2D* missense variants initially detected with sequencing of tumor material [108, 135]. Regarding germline and somatic *KMT2D* variants non-mutually exclusive parallels can be drawn with the situation for *ARID1A/B*- and *SMARCB1*. E.g. for *ARID1A/B* truncating germline variants cause Coffin-Siris-Syndrome but may not predispose to cancer although truncating somatic variants are frequently present in cancer [136, 137]. In case of *SMARCB1* both the type (truncating versus non-truncating missense) and location in the gene determine the phenotype (low-grade malignancies, malignant rhabdoid tumors, Coffin-Siris syndrome) [137].

(Epi)genetic analysis of the embryonal rhabdomyosarcoma

Third, when interpreting the molecular data it should be taken into account that, in contrast to some other tumor predisposition genes, a functional read-out like bi-allelic involvement (e.g. Lynch syndrome) is not useful for *KMT2D* as overall in cancer bi-allelic variants are rare and most variants are present in heterozygous state [70]. In addition, although we did not identify a second (likely)pathogenic variant in *KMT2D* we cannot exclude such variant because of incomplete coverage (95%) of the gene. The genome wide epigenetic profiling (methylation profile, methylation changes at 11p15.5) and CNV-analysis (gains of chromosomes 2,7,8 and 12) revealed a for ERMS typical aberrations and profile [42, 47, 138]. Unfortunately, in the light of the observed trisomy 12 (Fig. 1e), we were not able to analyse the percentage of mutant versus wild-type reads at the position of the germline *KMT2D* variant due to insufficient coverage at this position and in this region. However, we confirmed the variant with Sanger sequencing. Although taking the intrinsic limitations of quantification and Sanger sequencing into account the pattern of the peak-heights of the wild-type and mutated-sequence is suggestive for a gain of the wild-type and not the mutated allele.

Conclusions

In conclusion we present the first exome wide genomic and genome wide epigenomic analyses of a malignancy occurring in a patient with KS. Our molecular findings and observations from the literature neither prove nor rule out a potential tumor predisposition for KS. Regarding the tumor spectrum and age of onset of the tumor in patients with Kabuki syndrome it was observed that this broadly resembled that of the pediatric population in general. However, even an in vitro or in vivo oncogenic effect of KMT2D perturbation might not directly translate to a clinical relevant tumor predisposition. Regarding the latter, one should consider that if in KS the cancer-frequency would exceed a reasonable threshold for tumor surveillance (e.g. $\geq 1 \sim 5\%$ for other pediatric cancer predisposition syndromes [139]) and if the (dis)advantages of imaging modalities like whole-body MRI (e.g. false-positive findings, required general anesthesia) outweigh the benefits [140, 141] for pediatric patients especially when they suffer from developmental delay. Moreover, considering that Burkitt lymphoma appears rather frequent in patients with KS and has a doubling time of approximately 24 h only long-term interval surveillance would not be effective. Alternatively, liquid-biopsy-based surveillance strategies might overcome some of these hurdles in paediatric patients with a cancer predisposition syndrome [142]. The (epi-)genetic analysis revealed a typical ERMS methylation- and copy number profile. Although we found no strong arguments pointing towards KS as a tumor predisposition syndrome, based on the small numbers any relation cannot be fully excluded. Further planned studies including exome- and genome-wide (epi)genetic profiling of additional tumors in patients with KS and long term follow-up of patients with KS into adulthood could provide further insights into the pathogenesis of these rare but challenging tumors.

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Author contributions Performed and/or supervised pathological, molecular and/or bioinformatical analysis: SG, MFCMvdH, SD, MJB, MH, JK, RaS, RS Provision of clinical data: DAS, CTRMS Design of the study: SMA, RS and CTRMS. Analyzed and interpreted data: SMA, SG, RS and CTRMS. Wrote the manuscript: SMA, RS and CTRMS.

Data availability Data available in article Supporting Information.

Declarations

Conflict of interest The author's have no relevant conflict of interest.

Informed consent The parents of the patient provided written informed consent for the use of archival tissue for further analyses and consent for publication.

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