Immunol Res
DOI 10.1007/s12026-015-8707-4

INTERPRETIVE SYNTHESIS REVIEW ARTICLE

Epigenetic control of the immune system: a lesson from Kabuki syndrome

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Abstract Kabuki syndrome (KS) is a rare multi-systemic disorder characterized by a distinct face, postnatal growth deficiency, mild-to-moderate intellectual disability, skeletal and visceral (mainly cardiovascular, renal, and skeletal) malformations, dermatoglyphic abnormalities. Its cause is related to mutations of two genes: *KMT2D* (*histone-lysine N-methyltransferase 2D*) and *KDM6A* (*lysine-specific demethylase 6A*), both functioning as epigenetic modulators through histone modifications in the course of embryogenesis and in several biological processes. Epigenetic regulation is defined as the complex of hereditable modifications to DNA and histone proteins that modulates gene expression in the absence of DNA nucleotide sequence changes. Different human disorders are caused by mutations of genes involved in the epigenetic regulation, and not surprisingly, all these share developmental defects, disturbed growth (in excess or defect), multiple congenital organ malformations, and also hematological and immunological defects. In particular, most KS patients show increased susceptibility to infections and have reduced serum immunoglobulin levels, while some suffer also from autoimmune manifestations, such as idiopathic thrombocytopenic purpura, hemolytic anemia, autoimmune thyroiditis, and vitiligo. Herein we review the immunological aspects of KS and propose a novel model to account for the immune dysfunction observed in this condition.

Keywords Kabuki syndrome · *KMT2D* (*MLL2*) · *KDM6A* (*UTX*) · Autoimmunity · Epigenetic regulation · Thrombocytopenia · Antibody deficiency

Introduction

Kabuki syndrome (KS, OMIM#147920), also known as Niikawa–Kuroki syndrome, is a rare protean multi-systemic disorder firstly described in Japan in 1981 by two independent research groups [1, 2]: The disease has an incidence of 1 per 30.000–40.000 births, though these data are probably underestimated. Most cases described in the literature are sporadic [3], and familial occurrence, consistent with autosomal dominant inheritance, has also been reported [4–6].

In the majority of cases, KS is due to mutations in the *KMT2D* (*histone-lysine N-methyltransferase 2D*, OMIM *602113) gene, also known as *MLL2* (*myeloid/lymphoid or mixed-lineage leukemia 2*) [4–6]; however, mutations in the *KDM6A* (*lysine-specific demethylase 6A*, OMIM*300867) gene have been described in a minority of patients [7, 8].

This syndrome is characterized by a peculiar face, reminiscent of the makeup used by actors of the Kabuki drama (a Japanese traditional theatrical form), which is defined by long palpebral fissures with eversion of the lateral third of the lower eyelids, sparseness of eyebrows'

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Published online: 28 September 2015

lateral sides, short columella with broad and depressed nasal tip, and prominent ears [1, 2] (Fig. 1).

Mild-to-moderate developmental delay, a variable degree of hypotonia, early infancy feeding difficulties, and a set of malformations, such as cleft palate, left-sided cardiac defects, skeletal, renal, or anorectal malformations, represent the phenotype clinical core of KS.

Additional features, useful as diagnostic handles, are the typical persistence of fetal fingertip pads, brachydactyly, hypoplastic fingernails, some dermatoglyphic abnormalities, postnatal growth delay with short stature, and microcephaly [1, 2].

Although recurrent otitis media has been consistently reported since the initial descriptions of KS, immunological abnormalities were systematically analyzed for the first time only in 2005 [9]. In particular, low serum immunoglobulin levels and reduced memory T and B lymphocytes have been reported [10]. Furthermore, while anatomic and/or functional anomalies in the palate, velopharyngeal structure, and urinary tract may contribute to the increased susceptibility to respiratory and urinary tract infections in patients with KS, the increased occurrence of autoimmune manifestations, such as idiopathic thrombocytopenic purpura (ITP), hemolytic anemia, autoimmune thyroiditis, and vitiligo [11], suggest that both *KMT2D* and *KDM6A* genes may play a relevant, still undisclosed, role in the immune homeostasis.

In this regard, it is interesting to note that somatic mutations of the *KMT2D* and *KDM6A* genes have been reported in patients with lymphomas and leukemia [12, 13]. Furthermore, a role of histone modifications, associated with KMT2D and KDM6A function, has been clearly demonstrated in the immune response [14, 15].

No studies have analyzed in detail the functional consequences of *KMT2D* and *KDM6A* mutations on the immune system of patients with KS, and no mechanistic models accounting for such abnormalities have been proposed.

Herein we review the immunological abnormalities that have been reported in the medical literature for patients with KS. Papers have been identified through a systematic search on PubMed, EMBASE, and Cochrane databases (from 1981 to July 2015): All published studies (written in English) concerning KS, *KMT2D* or *KDM6A* genes were retrieved entering the words: "Kabuki," "Kabuki syndrome," "*KMT2D*," "*KDM6A*," and "immune system." Searches were augmented by manually reviewing the reference lists of all original articles and all systematic review articles, with each study being evaluated for inclusion. In the end, we propose a mechanistic model to account for the immunological abnormalities associated with KS.

A comprehensive description of the immunological defects associated with this syndrome and a better understanding of the pathophysiology of immune abnormalities in these patients may help define the most appropriate strategies for clinical and laboratory monitoring of KS patients and offer novel therapeutic perspectives, while improving our understanding of the epigenetic control of the immune response.

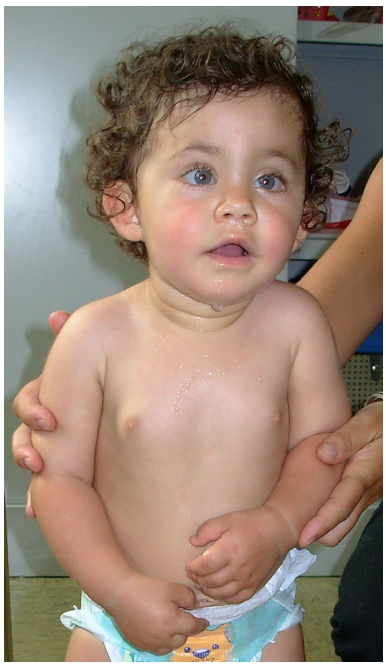

Fig. 1 Distinctive facial features in one patient with Kabuki syndrome

The genetic basis of Kabuki syndrome

The genetic basis of KS has been recently elucidated through whole exome sequencing. In particular, heterozygous mutations in the *KMT2D* gene (12q12-q14) cause KS type 1 (KS1) in approximately 70 % of affected individuals [5, 16–19], whereas in roughly 5 % of patients mutations are found in the *KDM6A* gene (Xp11.3) [19–21]. This last variant is also known as KS type 2 (KS2). Males and females can be equally affected by both variants. Importantly, in approximately 25 % of patients with KS the underlying genetic defect remains unknown [22].

The *KMT2D* gene, also known as *MLL2 or MLL4/ALR*, comprises 54 coding exons and encodes for a ubiquitously expressed lysine H3K4-specific histone methyltransferase protein, containing 5.537 amino acids [23], organized into several functional domains. In particular, at its C-terminus, the KMT2D protein contains a histone-lysine N-methyltransferase domain, which characterizes all members of the MLL (mixed-lineage leukemia)-like family of proteins. MLL proteins participate at the formation of multi-protein

complexes and play a critical non-redundant role in the development, as demonstrated by the embryonic lethality of the respective knockout mouse models [24].

KMT2D heterozygous point mutations, occurring de novo or transmitted with an autosomal dominant pattern of inheritance, have been reported in 55–80 % of patients [4–6, 16, 18]. Less frequently, *KMT2D* mosaic mutations and intragenic deletion duplications may cause KS1 [25]. The *KMT2D* nonsense and frameshift mutations observed in KS1 patients occur throughout the entire coding region, while missense mutations are preferentially clustered in exons coding for the protein C-tail. Although there is no obvious genotype–phenotype correlation in mutant *KMT2D*-positive patients and between mutant *KMT2D*-positive and *KMT2D*-negative patients, facial features are more pronounced in patients with *KMT2D* truncations, whereas organ malformations are more frequent in *KMT2D*-mutated patients [26].

It has been postulated, and recently demonstrated, that in most cases KS1 results from *KMT2D* haploinsufficiency, as transcripts carrying nonsense and frameshift mutations, are destructed by nonsense-mediated decay, resulting in decreased levels and reduced activity of the KMT2D protein, as assessed by the expression of transcription factor HOXC6 target gene in KS1 cell lines [27]. Alternatively, *KMT2D* missense mutations may disrupt the protein conformation and cause altered protein–protein interaction or affect posttranslational modifications. Because KMT2D is part of a multi-protein complex, it is possible that missense mutations of the *KMT2D* gene may cause disease through a dominant-negative effect of the mutant protein; finally, splice site mutations have been also reported [18].

A great deal of information about KMT2D functions has emerged through studies in cancer, as *KMT2D* is a common target of somatic mutations in a variety of tumors, including lymphoma, medulloblastoma, and gastric adenocarcinoma. In these cases, *KMT2D* mutations generally disrupt the catalytic domain of the protein. Interestingly, occurrence of tumors has been reported in seven KS patients out of a total of approximately 300 patients described, suggesting that KS may represent a condition predisposing to cancer. However, the heterogeneity of tumor types (acute lymphoblastic leukemia, Burkitt lymphoma, fibromyxoid sarcoma, synovial sarcoma, neuroblastoma, and hepatoblastoma) does not prompt a change in the surveillance strategy of these patients, other than raising clinicians' awareness [28].

Lederer et al. and Miyake et al. [7, 8] described, respectively, deletions and point mutations of the *KDM6A* gene as a cause of KS2. The *KDM6A* gene, also known as *UTX*, is located at Xp11.3: It comprises 29 exons and encodes for the lysine demethylase 6A protein, which demethylates di- and trimethyl lysine 27 on histone H3, "erasing" a repressor mark and allowing chromatin opening and active transcription [29]. Significantly, the KDM6A protein interacts with the C-terminal region of KMT2D. Both proteins are therefore part of a multi-protein complex, and the majority of KDM6A target genes are also coregulated by KMT2D [30]. In particular, *KDM6A*-catalyzed demethylation of trimethylated H3K27 and *KMT2D*-mediated trimethylation at H3K4 occur interdependently at cotarget genes of *KDM6A* and *KMT2D* [31]. Because *KMD6A* partially escapes X chromosome inactivation, also female carrying heterozygous mutations may be affected by KS. These data indicate that females physiologically require higher levels of expression (roughly 30 % more) of KMD6A protein as compared to males [8].

Zebrafish *kmt2d* and *kmd6a* morphants confirm the role of these proteins in craniofacial, heart, and brain development and provide further support for a role played by the KMT2D and KDM6A proteins in the etiology of KS [31]. In KS mouse model-derived fibroblasts, there is a reduction in histone 3 lysine 4 trimethylation (H3K4me3), as expected because of the reduced KMT2D enzymatic activity, but also in histone 4 acetylation. Interestingly, both these activities were normalized in response to a histone deacetylase inhibitor (HDAC), corroborating a model in which imbalance between open and closed chromatin is central to the pathogenesis of KS. These results also suggest that chromatin-activating agents, such as HDAC, might have a therapeutic potential. In vivo, the deficiency of H3K4me3 in the dentate gyrus granule cell layer of mice with KS correlated with reduced neurogenesis and hippocampal memory defects. These abnormalities improved upon postnatal treatment of KS mice with HDAC, suggesting that a reversible deficiency in postnatal neurogenesis may underlie the intellectual disability of KS [32]. Whether this treatment may also improve immunity in patients with KS remains to be evaluated.

Immunological characteristics of Kabuki syndrome

Patients with KS suffer from recurrent bacterial respiratory infections, especially otitis and pneumonia [9]. As KS is a multi-systemic disorder, children may come to clinicians' attention at birth because of heart congenital defects or cleft palate, during infancy because of variable hypotonia, feeding difficulties with gastroesophageal reflux and/or urinary tract anomalies (clinically symptomatic or detected by routine ultrasound investigation), or later because of a developmental delay. The recurrence of respiratory infections may easily be overlooked, because recurrent infections are common in infancy and in preschool children. Moreover, each episode typically responds to antibiotic

therapy and complications are rare. Finally, a higher rate of infections in patients with KS has been often related to the presence of other comorbidities. Only a few studies have explored the immune status and function in patients with KS. The most relevant literature consists of approximately 30 case reports and four cohort studies with a minimum of five patients (Table 1). No significant differences appear to exist between patients who harbor *KMT2D* mutations and those who do not in terms of risk and rate of infections [26]. Recurrent or chronic otitis media was observed in the majority of patients (50–100 % in various series), whether or not they also had cleft palate, and it was mostly accompanied by hearing impairment with a predominant transmissive component. Bronchitis and pneumonia were also reported with increased frequency in KS: Some of these episodes may be due to severe gastroesophageal reflux and/or ab ingestis phenomena. In any case, these complications were especially common in dysgammaglobulinemic KS patients (Table 1).

The lack of opportunistic or complicated viral infections indicates that severe T and/or NK cell defects are not part of the syndrome. However, Epstein–Barr virus infection is associated with a risk of lymphoproliferation and autoimmune cytopenias, which is higher than in the general population ([19, 33], Lapi's personal unpublished observation).

Granulomatous lymphocytic interstitial lung disease (GLID), a rare inflammatory condition usually complicating common variable immune deficiency, has been described in two patients with KS. Both patients were females of 14 and 18 years of age, respectively, who had a past medical history characterized by recurrent respiratory infections and thrombocytopenia. The youngest had absent IgA, lack of protective specific antibody response, and a defect of isotype switching of specific antibodies following booster immunizations; she suffered from a series of complications related to GLID immunosuppressive treatment and died at the age of 17 due to chronic renal failure [34]. The other patient had low levels of IgA, but also very low levels of IgG and IgM; in addition, she had no measurable antibody level to diphtheria, tetanus, measles, mumps, and rubella. After 3 years since the onset of GLID, she was alive, but symptomatic, as she could not tolerate intravenous immunoglobulin (IVIG) replacement therapy, which is the elective treatment for GLID [35].

In a cohort of KS patients from Brazil [36], four out of seven patients were reported to have allergic manifestations (three rhinitis and one asthma, accompanied in three cases by atopic dermatitis). However, allergy is not mentioned in other patients with KS, with the exception of one patient with cow milk protein intolerance and eczema [37]. Nevertheless, because allergy is a very common disorder (affecting 5–20 % of the pediatric population), it may be an underreported feature, mainly considered unrelated to the underlying syndromic condition.

There are no reports of lymphoid hyperplasia (such as tonsillar and/or adenoid hypertrophy, or splenomegaly) in patients with KS. However, palatal tonsils, thymus, and spleen volumes were explicitly evaluated and found to be normal in only one patient [38]. By contrast, a 10-month-old boy with KS, who died for cardiac arrhythmia, was severely lymphopenic, and severe lymphoid depletion was documented at the postmortem examination of both thymus and spleen [39].

With regard to laboratory data, a variable degree of hypogammaglobulinemia is detected approximately in half of KS patients, with defects of serum IgA levels identified in up to 80 % in the largest cohort study [9]. However, data regarding serum immunoglobulins were often unavailable or incomplete, and IgE in particular was rarely evaluated [39]. Furthermore, no longitudinal studies have ever been performed, and therefore, a possible decline of immunoglobulin levels over time may have been missed. Out of 65 KS patients with clinical history and signs of immune dysfunction and in whom serum immunoglobulin levels were tested at least once, 41 (63 %) had IgA deficiency, with an apparent bimodal trend, as 76, 38, and 86 % of patients aged 0–5 years, 5–10 years, and 10–20 years, respectively, displayed low IgA levels (Table 1). Based on these data, we suggest that periodic examination of serum immunoglobulins, and IgA in particular, may be helpful in the management of these patients, as a low level might represent an early marker of immunological derangement.

Significant improvement in both severity and frequency of infections has been noted following administration of IVIG in a small number of KS patients [9, 10, 40]. However, caution should be used in IVIG replacement therapy, because of the risk of adverse reactions, especially in IgA-deficient patients [9, 35, 38]. In such cases, hence, when administration of IVIG is indicated as replacement therapy or to counteract the effects of immunosuppressive therapy, preparations with low IgA content should be chosen, and slow rate of infusion and appropriate premedication with antihistamines should also be used.

The count and distribution of T, B, and NK cells are largely normal in patients with KS. Furthermore, normal levels of T cell receptor excision circles (TRECS) and kappa-deleting recombination excision circles (KRECS) indicate normal output of naïve T and B cells from the thymus and bone marrow, respectively [10]. In vitro studies of T cell proliferation after stimulus with mitogens are normal in KS [38, 41]. Furthermore, protective levels of specific antibodies in response to immunizations (both with polysaccharide and protein antigens) have been documented in the majority of the cases studied [9, 36], though

Table 1 Summary of all immunological characteristics reported for patients with Kabuki syndrome

Age (years)	Sex	IgA	IgG	Autoimmunity	Infection history	Genetic analysis	References	Years
1	M	NA	NA	Sclerosing cholangitis	OM, UTI	NA	[52]	1998
1	F	↓↓	nl	–	PN	NA	[44]	2002
1	F	↓	nl	–	OM, UTI	NA	[9]	2005
1	F	↓↓↓	↓↓	–	–	NA	[9]	2005
1	M	↓	nl	–	OM, PN	NA	[9]	2005
1	M	↓	nl	–	OM, PN	NA	[9]	2005
1	M	↓↓↓	nl	–	OM	KMT2D: P2550Rfs2604X	[10]	2014
1	M	nl	nl	–	OM	KMT2D: Q5379X	[10]	2014
2	M	↓↓↓	nl	–	OM	KMT2D: C5109F	[10]	2014
2	M	↓	↓	–	PN	NA	[9]	2005
3	M	NA	NA	–	OM	KDM6A: c.2515_2518del	[7]	2012
3	F	↓↓↓	↓	VT, ITP, neutropenia	Chronic diarrhea	NA	[37]	2004
4	F	↓	nl	–	–	NA	[9]	2005
4	M	nl	nl	ITP, AIHA, leukopenia	–	NA	[11]	2005
4	F	nl	nl	Anemia (AIHA?)	UTI	NA	[36]	2009
4	M	nl	nl	ITP	OM	NA	[42]	2001
5	F	nl	↓	–	OM	NA	[9]	2005
5	M	nl	nl	–	OM	NA	[9]	2005
5	M	nl	nl	–	PN	NA	[9]	2005
5	M	nl	nl	ITP	OM	NA	[9]	2009
5	F	NA	NA	Hyperthyroidism	OM	KMT2D: R1252X	[10]	2014
5	F	↓↓	NA	HT, VT	–	NA	[52]	1998
6	M	nl	↓G2	–	OM, UTI	NA	[36]	2009
6	M	nl	nl	ITP	OM	NA	[36]	2009
6	M	nl	nl	Arthritis?	PN, OM	NA	[61]	2014
6	F	nl	↓	–	OM	KMT2D: R5500 W	[10]	2014
6	M	↓↓	nl	–	OM, PN, UTI	NA	[9]	2005
7	F	↓↓	↓↓	ITP	–	NA	[11]	2005
7	F	nl	↓	–	OM, PN	NA	[36]	2009
7	M	nl	↓	ITP	PN	NA	[36]	2009
7	F	↓↓↓	nl	ITP, neutropenia	OM, RTI, UTI	NA	[44]	2002
8	F	↓↓	↓	–	OM, PN	NA	[9]	2005
8	M	↓↓	↓	–	OM	NA	[9]	2005
8	F	↓↓↓	↓↓↓	ITP	OM, RTI, UTI	NA	[38]	1996
9	F	nl	nl	VT	OM	NA	[42]	2001
9	F	↓↓	↓	–	OM, S, UTI	NA	[9]	2005
9	M	NA	NA	Crohn's disease	–	NA	[53]	2009
9	M	nl	nl	ITP, leukopenia	–	KMT2D: 5462H	[47]	2014
9	M	↓↓	↓↓	–	OM	NA	[9]	2005
10	F	↓↓	–	ITP, AIHA	–	KMT2D: wt/NA	[47]	2014
10	F	↓	nl	–	OM	KMT2D: Q4013X	[10]	2014
10	M	↓	nl	–	OM	KMT2D: E5425 K	[10]	2014
10	F	↓	↓↓	Type III glomerulonephritis	PN, OM	NA	[56]	2014
11	F	↓	nl	–	OM, S	NA	[9]	2005
12	M	↓↓↓	NA	VT	RTI	NA	[58]	2007
12	F	↓↓	↓↓	ITP	–	NA	[51]	1997
13	M	NA	NA	ITP, AIHA	–	NA	[11]	2005
13	M	↓↓	↓↓	ITP	–	NA	[11]	2005

Table 1 continued

Age (years)	Sex	IgA	IgG	Autoimmunity	Infection history	Genetic analysis	References	Years
13	F	nl	nl	HT?	OM	NA	[36]	2009
13	F	↓↓	NA	ITP	OM	NA	[41]	1994
14	F	↓↓↓	↓↓	ITP, AIHA	Interstitial PN	NA	[34]	1999
14	F	nl	nl	–	OM	KMT2D: Y1998C	[10]	2014
14	M	↓	nl	–	OM	KMT2D: R2915X	[10]	2014
14	M	↓↓	↓	–	OM, S	NA	[9]	2005
15	M	↓↓	nl	Chorea with aPLs	–	NA	[46]	2007
16	F	nl	nl	–	OM	NA	[36]	2009
17	M	↓	nl	–	OM	NA	[9]	2005
18	F	↓↓↓	↓↓	ITP?	UTI, OM, GLID	NA	[35]	2012
18	M	↓↓↓	↓↓↓	–	RTI, OM, PN	NA	[76]	1998
19	M	NA	NA	ITP, neutropenia	OM	NA	[48]	2004
19	M	NA	NA	ITP	PN, UTI	NA	[48]	2004
20	F	↓↓↓	nl	–	OM, UTI	NA	[9]	2005
21	M	nl	nl	–	OM	KMT2D: T5464M	[10]	2014
22	F	nl	nl	–	OM	KMT2D: R5432X	[10]	2014
24	F	nl	↓	–	OM	KMT2D: R1757X	[10]	2014
27	M	nl	nl	–	OM	KMT2D: T5464M	[10]	2014
29	F	↓	nl	T1DM	RTI	NA	[60]	2003
31	M	nl	nl	T2DM	OM	KMT2D: T5464M	[10]	2014
31	M	↓↓↓	↓↓↓	ITP	OM, PN, S, UTI	NA	[9]	2005
35	–	↓↓	NA	AIHA	OM	NA	[50]	1984
NA	F	NA	NA	VT	–	NA	[59]	1993
NA	F	NA	NA	Celiac disease	–	NA	[37]	2004
NA	M	NA	NA	VT	–	NA	[11]	2005
NA	M	NA	NA	ITP, AIHA	–	KMT2D: S1632X	[45]	2012
NA	M	NA	NA	VT	RTI, OM	KMT2D: K5490RFsX21	[45]	2012
NA	M	NA	NA	VT	–	NA	[37]	2004

– not reported, ↓↓↓ absent, ↓↓ <50 % of the lower limit normal for age, ↓ slightly decreased, *nl* normal (ranges according to age and laboratory), *AIHA* autoimmune hemolytic anemia, *aPLs* anti-phospholipid antibodies, *GLID* granulomatous lymphocytic interstitial lung disease, *HT* Hashimoto's thyroiditis, *ITP* idiopathic thrombocytopenia, *NA* not available, *OM* otitis media, *PN* pneumonia, *RTI* respiratory tract infection, *S* sinusitis, T1/T2 DM, type 1/type 2 diabetes mellitus, *UTI* urinary tract infection, *VT* vitiligo

with several exceptions [9, 10, 38, 39]. In one study dealing with 9 *KMT2D*-mutated patients, after immunization against hepatitis B, seven patients displayed undetectable levels of anti-HBs antibodies [10]. In another study, a lack of antibody response to tetanus has been reported in 1 out of 12 tested patients [9]. Finally, no patients have shown complement deficiency, neutropenia, or neutrophil defective superoxide production [10, 36]. Altogether, these data suggest that KS is not basically characterized by significant abnormalities of the immune system. However, a closer look at the immune system of these patients may reveal unanticipated abnormalities. In particular, a significant reduction in memory B (CD19+ CD27+) and T (CD4+ CD45R0+) cells was documented in a whole cohort of 12 KS patients with a molecularly confirmed diagnosis (heterozygous *KMT2D* mutations) [10]. These data may help explain the variable (interindividual and temporal) occurrence of dysgammaglobulinemia, which may increase the risk of infections. Moreover, a reduced generation of memory T cells may be at the basis of the lack of delayed-type hypersensitivity response (including to PPD and candidin), documented in four out of five KS patients from a Brazilian cohort [36]. This is particularly significant because BCG vaccine is routinely administered at birth in Brazil, and candidin antigen is ubiquitous.

While preliminary, these data suggest that KS may be characterized by an inability to generate or maintain immunological memory, which is a defining feature of the adaptive immunity: the ability of the immune system to respond more rapidly and effectively to previously encountered antigens. The capacity of memory B and T

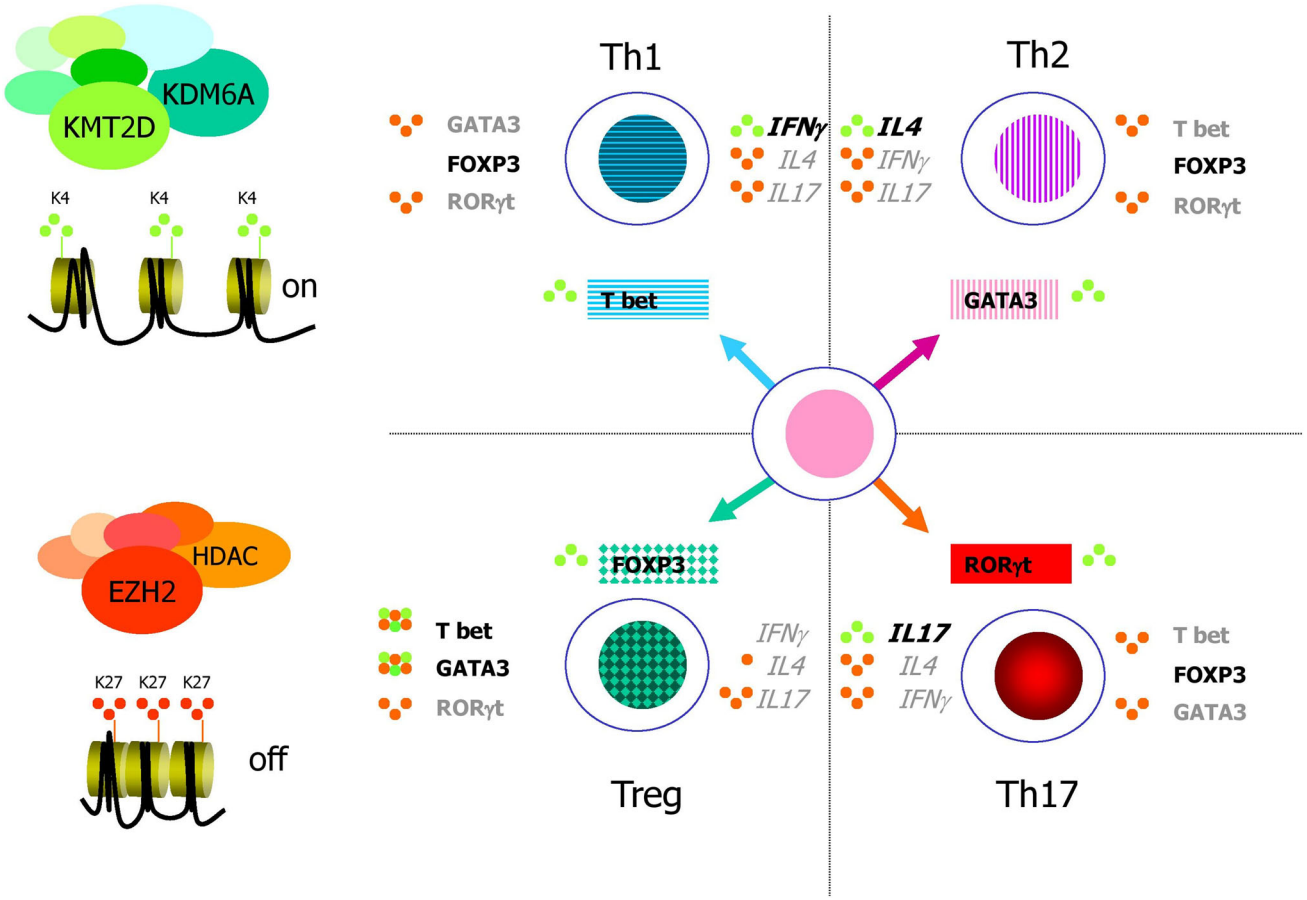

Fig. 2 The *green* multi-protein complex (containing KMT2D and KDM6A) promotes active chromatin conformation, whereas the *red* complex (containing EZH2 and HDAC) promotes transcription repression. *Green dots* represent H3K4 trimethylation, *red dots* H3K27 trimethylation. Naïve CD4+ T cells clonally expand upon antigen encounter and—depending on signals from innate immune cells—differentiate into one of several fates, including different effector, e.g., T helper 1 (Th1), Th2, Th17 cells, or regulatory (Treg) subsets, whose phenotype is preserved during subsequent rounds of cell division. A naïve CD4 lymphocyte which may differentiate into different effector cells, according to the combination of epigenetic marks at critical transcription factors genes, is in the *center*. The effector cells have a defining pattern of cytokines which relies on the epigenetic marks at their genes. The presence of "bivalent" (that is both active and repressive) marks, such as those shown for T-bet and GATA3 in Treg cells confers plasticity to the cell itself, which has the ability to further and rapidly differentiate toward a Th1 or a Th2 phenotype (Color figure online)

cells to "remember" previous cellular responses to specific antigens ultimately lies in their unique patterns of gene expression, which is ensured by a combination of epigenetic marks, as we will discuss later in more detail. An imbalance of these marks deriving from a defective *KMT2D* or *KDM6A* function might compromise the generation or the maintenance of B and T cell terminal differentiation. Further characterization of both B and T cell subsets in KS, in particular data regarding switched and unswitched B cells, T helper (Th) 17 and T regulatory (Treg) cells is warranted to explore this issue further.

An increased rate of autoimmune manifestations has been documented in KS. Idiopathic thrombocytopenic purpura (ITP) is rare in the general population, with an overall incidence inferior than 1/10.000, whereas more than 20 cases (Table 1) have been described so far in patients with KS of variable age, from early childhood to adulthood [9, 34, 36–38, 41–48]. Moreover, in a number of these, ITP had a more severe chronic or relapsing course, and in some patients, it was complicated by pancytopenia. In at least four patients, ITP was life-threatening, poorly controlled by conventional therapy, and required splenectomy or anti-CD20 monoclonal antibody ([9, 42, 49], Lapi's personal unpublished observation). Splenectomy was performed in two patients, but was effective only in one [48]. Anti-CD20 monoclonal antibody was successfully administered in a 5-year-old patient with KS and refractory ITP [49]. In our experience, anti-CD20 therapy was able to increase the platelet count, but this result was not maintained over time. Mycophenolate mofetil was then introduced and proved effective to control the disorder.

Patients with KS might also display positive Coombs tests or a clinically overt autoimmune hemolytic anemia [34, 45, 47, 50], autoimmune thyroiditis, and vitiligo [35, 37, 38, 41, 42, 45, 46, 49–58] (Table 1). In a mutational analysis referred to 12 Korean patients with KS, Cheon et al. [19] reported one 51.8-month-old child with Hashimoto thyroiditis, though negative to the *KMT2D* mutation analysis. In a large study of Lin et al. [10], one patient had hyperthyroidism. Vitiligo has been reported in six patients with KS, and in all of them, clinical features and course were typical [37, 42, 45, 52, 58, 59]. A patient with both thyroiditis and vitiligo was reported by Ewart-Toland et al. [52].

Moreover, two cases of diabetes mellitus have also been reported in patients with KS [10, 60], as well as one case of chorea with anti-phospholipid antibodies [46], arthritis [61], celiac disease [37], and Crohn's disease [53].

Finally, an 11-year-old Japanese girl with KS, with a history of repeated respiratory infections and hypogammaglobulinemia, developed a membranoproliferative glomerulonephritis type 3 (which is due to immune complexes deposited in the subepithelial space, with disruption of glomerular basement membrane), causing proteinuria and microscopic hematuria: Infections were excluded, autoantibodies were negative, and complement levels were normal. The patient was successfully treated with high-dose pulsed corticosteroids for 3 days, followed by prednisolone on alternate days and angiotensin receptor blocker [56]. An 18-year-old woman with KS and IgA deficiency has been recently found to present moderate proteinuria, with antinuclear antibody positivity, but no serum complement reduction (Gulino's personal unpublished observation). It has been already observed that this kind of immune-mediated kidney damage may affect patients with primary hypogammaglobulinemia. Hence, urinalysis should be regularly performed in KS patients.

Synopsis about the role of histone methylation in the immune system

Besides KS, several other human disorders are caused by mutations of genes involved in the epigenetic regulation, including immunodeficiency centromeric instability facial syndrome 1 (OMIM#242860), Rett syndrome (OMIM# 312750), Rubinstein–Taybi syndrome (OMIM#180849 and #613684), Sotos syndrome (OMIM#117550), alpha-thalassemia/mental retardation X-linked syndrome (OMIM# 301040), and CHARGE syndrome (OMIM#214800). Moreover, mutations in other genes (*KMT2A*, *KMT2C*, and *EZH2*) coding for proteins with histone methyltransferase activity have been identified in patients with Wiedeman-Steiner (OMIM#605130), Kleefstra (OMIM#610253), and Weaver syndromes (OMIM#277590), respectively. All these conditions share developmental defects, disturbance of growth (in excess or defect), multiple congenital malformations, and variable degrees of poorly characterized hematological and immunological defects.

In eukaryotic cells, transcription occurs in the context of chromatin, a complex formed between the genome and histone protein (namely H1, H2A, H2B, H3, and H4) octamers (2xH2A, 2xH2B, 2xH3, and 2xH4), termed nucleosomes, around which the DNA is wound. The histone aminoterminal tails protrude from the nucleosome and can be modified in multiple ways through acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and citrullination, which cause a change in chromatin conformation. Of these, crucial are acetylation (which promotes the euchromatic state) and methylation, which is associated with both active and repressed DNA conformations in a context-dependent manner. In particular, methylation on lysine 4 (H3K4) of histone 3, which is catalyzed by some MLL proteins (including KMT2D), is found in actively transcribed genes, whereas methylation on lysine 27 (H3K27), catalyzed by EZH2, is associated with transcriptional repression. Moreover, methylation can be also found with different "valencies," i.e., mono-, di-, and trimethylation (me1, me2, and me3, respectively) [62–65]. The epigenetic information associated with these posttranslational modifications is transduced by proteins containing motifs capable of docking to these marks, such as acetyl-lysine-binding bromodomains, and lysine methyl-binding chromodomains. In addition, the dynamic nature of the system is achieved by enzymes able to "erase" acetyl marks (histone deacetylases or HDACs) and methyl marks (histone demethylases, or HDMs, such as KDM6A). The result of the contributions of these enzymatic and effector activities is a highly dynamic system of paramount importance to control the response of eukaryotic cells to environmental cues, resulting in cell differentiation, activation, and proliferation [62–65].

In particular, H3K4 methylation is one of the mechanisms through which adult somatic cells activate and/or maintain a specific differentiation program, hence building the base for "memory" processes, both in the brain and in the immune system [66, 67]. Within the immune system, upon antigen encounter, naïve CD4+ T cells clonally expand and, depending on signals from innate immune cells, differentiate into one of several fates, including different effectors, e.g., Th1, Th2, and Th17 cells [68–72] or Treg subsets [73], whose phenotype is preserved during subsequent rounds of cell division (Fig. 2). These effector and regulatory CD4+ T cell lineages are defined by their cytokine expression signature, display a characteristic pattern of receptors and other products, which specifically confer protection toward various pathogenic agents, and

control inflammation or self-tolerance. Th1 cells produce interleukin (IL)-2 and interferon (IFN)-γ to sustain cell-mediated immunity; Th2 cells, which secrete IL-4, IL-5, and IL-13, enhance humoral immunity; Th17 cells, which produce the proinflammatory IL-17, are involved in the pathogenesis of autoimmune disorders and acute transplant rejection. Finally, Treg cells inhibit proliferation and cytokine production by both CD4+ and CD8+ T cells and control immunoglobulin production by B cells, cytotoxic activity of natural killer (NK) cells, and maturation of dendritic cells, thereby promoting the immunological tolerance. The induction of this maturation process is ultimately determined by the activation of specific transcription factors (TFs): T-box21 (also known as Tbet) for Th1, Gata 3 for Th2, RORγt and RORα for Th17, and FOXP3 for Treg cells. Expression of these TFs is induced downstream of a signaling cascade generated from the combined engagement of membrane receptors. TFs are crucial in regulating cellular differentiation, as exemplified in humans by IPEX (OMIM# 304790), a severe congenital immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, caused by FOXP3 deficiency and hence absence of Treg cells [74]. However, the fine tuning and proper orchestration of cell differentiation depend on epigenetic mechanisms, and KS might represent a precious model to shed light on this topic.

It is well established that histone modifications correlate with gene transcription in T cells [75–77]. Th1 and Th2 cells show different epigenetic modifications in a number of signature cytokine loci, such as activating marks (H3K4me) on *IFNγ* locus in Th1 cells [78] and on *IL4* locus in Th2 cells [68], respectively, associated with repressive epigenetic marks (H3K27me3) in the "opposing cytokine" gene, which generate two stable distinct lineages, "terminally differentiated." However, recent data suggest considerable plasticity of Th17 and Treg cells with respect to their capacity of producing cytokines and even switching to a different phenotype. At the core of this flexibility is the aforementioned combination of different epigenetic modifications at TF and cytokine genes. For example, a "bivalent" modification at TFs, such as that of H3K27me3 marks with retained H3K4me3 association (Fig. 2), suggests that TFs are "poised" for expression and may contribute to CD4+ T cell plasticity, when alternative responses are required. In murine Treg cells, bivalent marks of the *Tbx21* and *Gata3* loci have been documented, associated with the absence of H3K27me3 marks in the *IFNγ* locus and a minimal H3K27me3 in *IL4* locus, suggesting that it should be possible to induce IFN-γ production or IL-4 in these cells, if necessary. On the contrary, the *IL17* locus is strongly repressed in polarized Th1, Th2, and Treg cells. Finally, the *FOXP3* locus has no repressive marks in Th1, Th2, and Th17 cells, which theoretically would allow any kind of T cell to "become" Treg [67], opening interesting sceneries for future therapies. These observations highlight the crucial role that the dynamic control of the methylation state of chromatin plays in the regulation of lymphocyte "identity" and function.

As KS patients present a consistent reduction in circulating memory T cells, identified by CD45R0+ surface marker, this finding seems to be compatible and suggests a possible defect of T cell memory generation or maintenance in KS. It would be extremely interesting to analyze more in-depth peripheral blood T lymphocytes of patients with KS and study T cell differentiation in vitro. In the B cell compartment, resting naïve B cells display genome-wide DNA hypermethylation. In particular, most regions within the immunoglobulin heavy chain (Igh) locus exist in a closed chromatin state, devoid of activating histone modifications and enriched in repressive histone modifications (e.g., H3K27me3) [79, 80], with the exception of active marks (H3K4me) displayed by VDJ-CHμ, which encodes the surface B cell receptor (BCR), by other genes such as Pax5, Spib, and Ebf1 (B cell specific identity TFs) and by B cell marker genes, such as Cd19 [81, 82]. Circulating B naïve cells are identified by cytofluorimetry by positivity for staining CD19, IgM, and IgD (CD19+ IgM+ IgD+). Upon activation by antigens, in secondary lymphoid organs, CD19+ IgM+ IgD+ cells undergo genome-wide DNA demethylation and histone modifications, which drive B cell proliferation (through activation of the TF Bcl6) and differentiation into germinal center cells [83] (Fig. 3). These events are accompanied by molecular changes necessary for the maturation of the antibody response, mediated by the enzyme activation-induced deaminase (AID) [79, 84]: somatic hypermutation (SHM, that is the insertion of point mutations in Igh V(D)J DNA to generate higher affinity antibodies) and class switch recombination (CSR, a DNA editing process consisting in the elimination of the intervening DNA sequence through a double-strand break (DSB) in a donor switch region (Sμ) upstream the constant heavy (CH) chain of IgM (CHμ) and a DSB in an acceptor S region preceding a CH with different biological effector functions (e.g., IgG, IgA, IgE)).

Finally, germinal center cells give rise either to long-lived plasma cells or to memory B cells. Upregulation of Blimp-1 (encoded by the *Prdm1* gene) is central to B cell differentiation into plasma cells, which is characterized by epigenetic inhibition of Bcl6, Pax5, and Spib. Plasma cells, then, generally have undergone SHM and CSR: These cells do not proliferate, but secrete clono-specific antibodies at high rates (10^7 molecules/hour) [85]. On the other hand, memory B cells present active transcription of CD27 (memory B cell hallmark), maintain Pax5 and Spib transcription, and, similarly to T cell memory cells, are likely in a "poised" state, marked by active and repressive

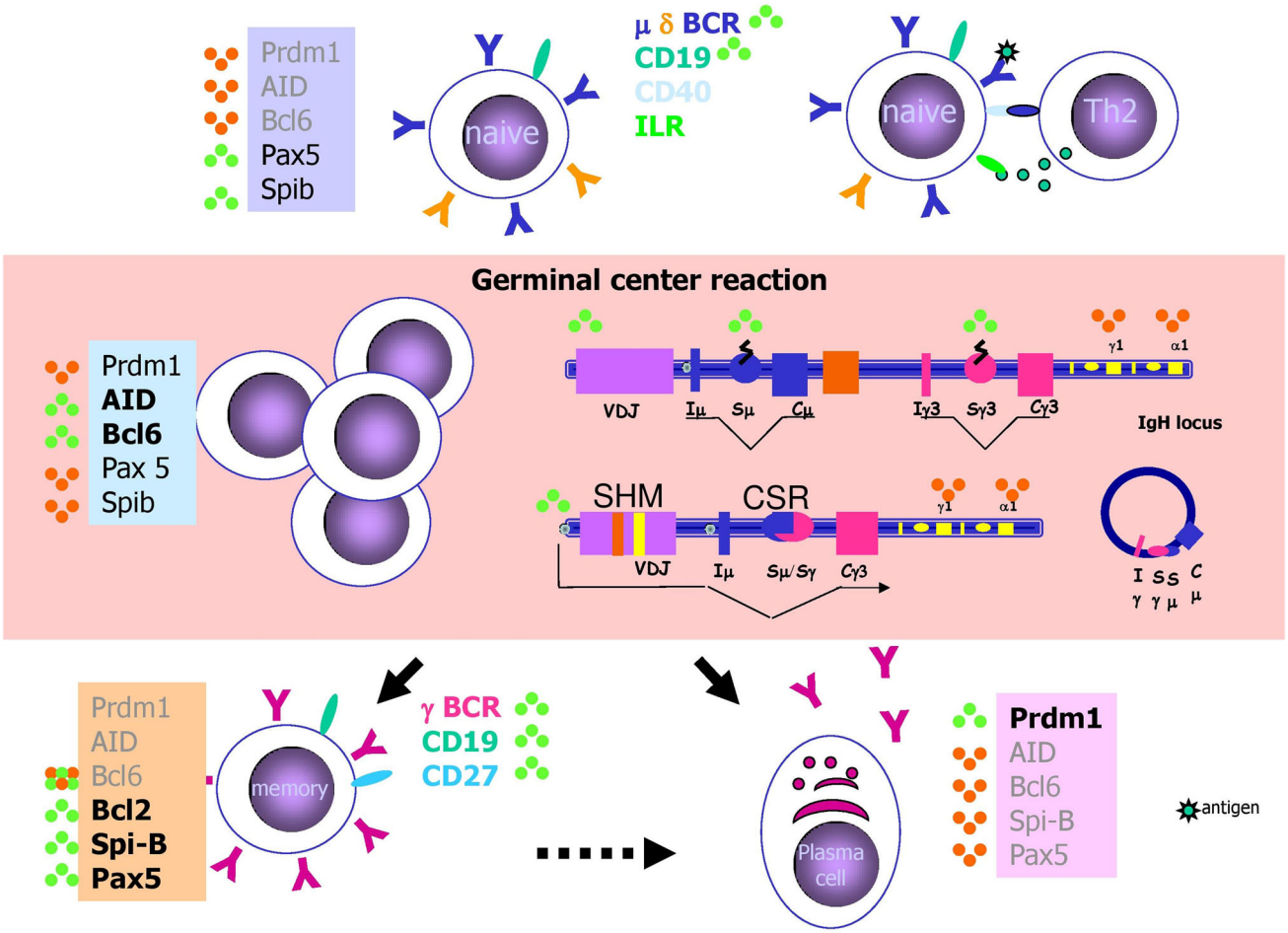

Fig. 3 In secondary lymphoid organs, upon activation by antigens, naïve B cells (CD19+ IgM+ IgD+) undergo genome-wide DNA demethylation and histone modifications, which drive cell proliferation through activation of the transcription factor Bcl6 and differentiation into germinal center (GC) cells. These events are accompanied by molecular changes necessary for the maturation of the antibody response, mediated by the enzyme activation-induced deaminase (AID). Somatic hypermutation (SHM) is the insertion of point mutations in Igh V(D)J DNA to generate higher affinity antibodies. Class switch recombination (CSR) is a DNA rearrangement between two S (switch) regions that give rise to Ig with different biological effector functions (e.g., IgG, IgA, IgE)

histone modifications in a balance, therefore displaying the potential for rapid activation upon antigen reencounter. Poised genes include those needed for proliferation (such as Ki67, Ccnd2, Ccne1, and E2f1) or reinitiation of SHM or CSR (e.g., Bcl-6).

In quiescent memory B cells, Aicda (the gene coding for AID) and Prdm1 (which encodes Blimp1) are silenced to preserve BCR integrity and prevent differentiation into plasma cells, respectively. While some memory B cells are IgM+ (un-switched), most memory B cells are class-switched (IgG+ or IgA+ or IgE+ IgD-CD27+) and express somatically hypermutated V(D)J gene segments [86]. Upon reactivation, memory B cells differentiate into plasma cells to mediate an anamnestic response. Reactivated memory B cells can undergo further SHM and/or CSR before differentiating into plasma cells or converting back to memory B cells.

However, chromatin accessibility enabled by histone modifications not only controls transcription, but may also affect more global biological processes, such as DNA repair, DNA replication, alternative splicing, and chromosome condensation [87]. Indeed, looking at the T and B lymphocytes development, successful antigen receptor DNA rearrangements—a process known as variable diversity joining (VDJ) recombination—which imply strictly controlled chromatin opening, double-strand breaks (DSBs), and repair, are fundamental to generate both T and B cells, as exemplified by the severe combined immune deficiency with lack of T and B cells in the Omenn syndrome (OMIM#603554), which is caused by defective RAG genes orchestrating VDJ recombination [88].

KS patients do not certainly suffer from a severe combined immune deficiency, and as we have already shown, circulating T and B cell numbers are normal, and TREC

and KREC analyses are normal. These findings exclude a gross defect in naïve T and B lymphocyte generation and show that KMT2D/KM6A are dispensable or that residual function is sufficient for a proper VDJ recombination in KS.

Rather, KS patients show a defective B cell memory compartment, which might arise from an impaired generation or maintenance of CD19+ CD27+ cells due to distorted epigenetic regulation of TF, or alternatively it might stem from an upstream CSR defect. Induction of CSR requires both a T–B cell interaction trough CD40L-CD40 and B cell receptor (BCR) engagement by pathogen antigenic epitopes. The cytokine environment determines the selection of the acceptor S region, so that for instance IL-4 promotes switch to IgG1 or IgE, or TGF-β to IgG2 and IgA, through the induction of specific germline transcription and activating S region histone modifications. Immunoglobulin class switch recombination deficiencies (CSR-Ds, also known as hyper-IgM syndromes) are rare primary immunodeficiencies characterized by low switched isotype (IgG/IgA/IgE) production due to defective T cell–B cell interaction (CD40-CD40L-mediated signaling), intrinsic B cell mechanisms (e.g., impaired AID function), and DNA repair machineries defects, including uracil-*N*-glycosylase, mismatch repair, and non-homologous end-joining repair (NHEJ) pathways, plus other mechanisms yet to be defined. Patients with intrinsic B cell CSR-D do well on IVIG replacement therapy, but one-third of them suffer from complications, mainly autoimmune disorders. Moreover, AID-deficient patients typically show lymphoid hyperplasia and, in some cases, a predisposition to lymphoid malignancy [89]. Hence, before CSR-D identification, these were typical patients diagnosed as affected with common variable immune deficiency. Modified histones, together with DNA transcription, open the chromatin in S regions, thereby allowing for access of CSR factors. In vitro experiments using murine cell lines indicate that an intact KMT2D function is required for CSR [90–93]. In KS B cells KMT2D haploinsufficiency might reduce CSR efficiency and KS immune defect could be a subtle form of CSR-D. Interestingly, histone modifications (such as H4K20me2) have been shown to enable the recruitment of DNA repair factors, such as p53-binding protein 1 (53BP1), to DSBs at S regions. H4K20 is catalyzed by the methyltransferase MMSET, encoded by WSHC1. Abrogation of MMSET expression impairs H4K20me2 enrichment and 53BP1 recruitment to the Igh locus S regions, resulting in defective CSR. WHSC1 gene is considered to be responsible for the core phenotype of Wolf–Hirschhorn disease (WHS, OMIM#194190), which is characterized by developmental delay, growth deficiency, and different organ malformations. In addition, WHS patients might display deficiency of antibodies [94]. In summary, KS immune defects may depend on a loss of H3K4 methylation at crucial TFs, which dysregulates T and B lymphocytes differentiation. KMT2D loss of function might also cause a direct alteration of the antibody maturation process, reducing the efficiency of CSR factors transcription and targeting. Autoimmunity may derive from a defective Treg generation or from an intrinsic B cell tolerance breakage. Nevertheless, alternative or concurrent mechanisms might be hypothesized, as it has been shown that KMT2D may act as nuclear receptor coactivator. Indeed, KMT2D associates with retinoic acid (RA) receptor [95] and with estrogen receptor (ER) [96, 97], facilitating these nuclear receptor-mediated gene activation. Both estrogens and RA profoundly affect immunity: In particular, RA impacts on T cell lineage commitment and plasticity, whereas estrogens have prominent effects on the innate immunity, which contribute to the reported sex differences in innate immune pathways. These complex mechanisms are reviewed elsewhere [98, 99], but it cannot be excluded that a defect of KMT2D may impair RA and/or ER activities.

Conclusions and future directions

In conclusion, KS immunological phenotype might be considered similar to that of patients with common variable immune deficiency, which is a clinically and molecularly heterogeneous disorder characterized by recurrent bacterial infections, hypogammaglobulinemia, and impaired antibody responses, whose onset varies from early childhood to adulthood. Similarly to common variable immune deficiency, KS patients may develop inflammatory and autoimmune disorders, malignancies as well as gastrointestinal problems [100]. KS provides clues to elucidate the effects of both *KMT2D* and *KDM6A* genes on lymphocyte differentiation and regulation of the immune response. From the therapeutic viewpoint, whether regular IVIG supplementation in younger patients decreases the frequency of otitis media and other respiratory infections or mitigates the severity of hearing impairment remains to be determined in large-scale prospective studies. Moreover, the effect of IVIG supplementation on the frequency of autoimmune phenomena should be cautiously evaluated in these patients. The alterations of *KMT2D* and *KDM6A* functions suggest new therapeutic opportunities and highlight an urgent need to better understand their exact functional mechanisms and interactions at a cellular level.

Take-home messages

Kabuki syndrome (KS) is a rare multi-systemic disorder characterized by peculiar facial dysmorphisms, postnatal

growth deficiency, multiple congenital skeletal and visceral anomalies, and mild-to-moderate developmental delay, in combination with a host of immunological abnormalities.

Today these patients may be diagnosed to have mutations in the *KMT2D* (*histone-lysine N-methyltransferase 2D*) or *KDM6A* (*lysine-specific demethylase 6A*) genes, both functioning in various signaling pathways as epigenetic modulators in the course of embryogenesis.

An abnormal regulation of immunity pathways may occur in children with KS, who might display hypogammaglobulinemia, increased susceptibility to infections, and even autoimmune disorders.

KS immune defects may depend on a loss of H3K4 methylation at crucial transcription factors, which dysregulates T and B lymphocytes differentiation: KMT2D loss of function might also cause a direct alteration of the antibody maturation process, reducing the efficiency of class switch recombination, while autoimmune phenomena may derive from a defective Treg generation or from an intrinsic B cell tolerance breakage.

Because there is heterogeneity in terms of frequency and severity of immune abnormalities in KS, it is not possible to suggest a pre-scheduled program of surveillance for every patient, though regular blood cell counts, serum IgA levels, and urinalysis could represent a minimum set of investigation for earlier detection of any immune dysfunction.

Patients with severe otitis and low levels of serum immunoglobulins should be evaluated by an immunologist, and intravenous immunoglobulins should be considered only with low content of IgA, with antihistamine premedication, with slow rate of infusion, and with experienced personnel available.

Acknowledgments Thanks are due to Prof. L.D. Notarangelo for his critical reading of the manuscript and to Dr. A. Pilotta for sharing clinical data.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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