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Molecular insights of KMT2D and clinical aspects of Kabuki Syndrome Type 1

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Abstract

Background—Kabuki Syndrome Type 1 (KS1), a rare multisystem congenital disorder, presents with characteristic facial features, intellectual disability, persistent fetal fingertip pads, skeletal abnormalities, and postnatal growth delays. KS1 results from pathogenic variants in the *KMT2D* gene, which encodes a histone methyltransferase protein involved in chromatin remodeling, promoter and enhancer regulation, and scaffold formation during early development. KMT2D also mediates cell signaling pathways, responding to external stimuli and organizing effector protein assembly. Research on KMT2D's molecular mechanisms in KS1 has primarily focused on its histone methyltransferase activity, leaving a gap in understanding the methyltransferase-independent roles in KS1 clinical manifestations.

Methods—This scoping review examines KMT2D's role in gene expression regulation across various species, cell types, and contexts. We analyzed human pathogenic *KMT2D* variants using publicly available databases and compared them to research organism models of KS1. We also conducted a systematic search of healthcare and governmental databases for clinical trials, studies, and therapeutic approaches.

Results—Our review highlights KMT2D's critical roles beyond methyltransferase activity in diverse cellular contexts and conditions. We identified six distinct groups of KMT2D as a cell signaling mediator, including evidence of methyltransferase-dependent and -independent activity. A comprehensive search of the literature, clinical databases, and public registries emphasizes the need for basic research on KMT2D's functional complexity and longitudinal studies of KS1 patients to establish objective outcome measurements for therapeutic development.

Conclusion—We discuss how KMT2D's role in translating external cellular communication can partly explain the clinical heterogeneity observed in KS1 patients. Additionally, we summarize the current molecular diagnostic approaches and clinical trials targeting KS1. This review is a resource

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The authors declare no conflicts of interest.

for patient advocacy groups, researchers, and physicians to support KS1 diagnosis and therapeutic development.

Keywords

Kabuki Syndrome; KMT2D; COMPASS; chromatin; cell signaling pathways; molecular diagnosis

1. INTRODUCTION

Kabuki syndrome (KS, Kabuki Make-Up Syndrome, Niikawa-Kuroki Syndrome) is a rare genetic disorder with an estimated incidence of 1 in 32,000 (Adam et al., 2019; Dugan, 2021). KS was first diagnosed independently by Norio Niikawa and Yoshikazu Kuroki in 1981 (Kuroki et al., 1981; Niikawa et al., 1981). The clinical phenotypes of KS were initially described as characteristic craniofacial features, mild to moderate intellectual disabilities, hearing loss, growth retardation, and susceptibility to infections (Bokinni, 2012). This list has since expanded to include a spectrum of multisystemic features that range in severity across patients (Adam et al., 2019; Boniel et al., 2021).

KS is caused by heterozygous pathogenic variants in one of two chromatin modifying genes, *KMT2D* (OMIM #147920; KS Type I, KS1) (Fig. 1. A–C), or the X-linked gene *KDM6A* (OMIM #300867; KS Type II, KSII) (Wang et al., 2019). These genes encode epigenetic proteins associated with opening chromatin, enabling active gene expression (Bokinni, 2012). KMT2D deposits active methyl signatures onto H3K4, while KDM6A removes repressive methyl signatures from H3K27 (Wang et al., 2019). This review will focus primarily on KS1, as pathogenic variants in KMT2D are known to cause the majority of KS cases (Adam et al., 2021).

Historically, KMT2D is referred to by multiple names, some of which pertain to orthologs or paralogs in different species. To avoid confusion, Table 1 provides the nomenclature and alternative names for *KMT2D* based on its gene location in human, mouse, and fish. While some of the work cited in this review examines KMT2D in non-human species, we have chosen to utilize the human gene and protein designations for KMT2D throughout our analysis to maintain consistency.

The KMT2 methyltransferase family in humans is composed of six members that range in size from 1707 amino acids (KMT2F) to 5537 amino acids of its larger member, KMT2D. KMT2 homologues are subdivided into three groups based on their similarity with the *Drosophila melanogaster* orthologs: trithorax (Trx-like; KMT2A and KMT2B), trithorax-related (Trr-like; KMT2C and KMT2D), and Set1 (Set1-like; KMT2F and KMT2G) (Reviewed in Zhai and Brownell, 2021). The human 19.4-kb *KMT2D* transcript encodes a large 593-kDa protein with several structural domains that confer functional diversity (Fig. 1. D): seven plant homeodomain fingers (PHD) that recognizes unmodified or methylated lysines; one high mobility group 1 (HMG) domain that facilitates DNA-protein interactions with low selectivity to specific DNA structures; five LXXLL consensus motifs known to be essential for transcriptional regulation through nuclear receptors and protein-protein interactions; FYRC/FYRN domains that mediate the heterodimerization of the C-terminal

end, and a SET domain with catalytic methyltransferase activity that is stabilized by a short post-SET domain (Banka et al., 2012; Poreba et al., 2022).

KMT2D functions as a part of a multi-subunit complex termed the COMplex of Proteins ASsociated with Set1 (COMPASS). KMT2D-COMPASS deposits tri- and monomethylation marks on promoters and enhancers, respectively, supporting active transcription of thousands of targets across the genome depending on the cell type and developmental stage (Hu et al., 2013; Piunti & Shilatifard, 2016; Wang et al., 2016; Wang et al., 2018) (Fig. 2. A, B). The function of KMT2D-COMPASS is determined by its interaction with two types of subcomplexes: (1) the WRAD subcomplex (including proteins WDR5, RBBP5, ASH2L, DPY30) that stabilizes the SET domain and increases its enzymatic activity by up to 600-fold (Lavery et al., 2020), and (2) non-WRAD unique subcomplexes, such as KDM6A, PTIP, PA1, and NCOA6, that expand KMT2D's role in the cell by increasing the number of proteins and DNA it interacts with (Lavery et al., 2020). Thus, the composition of the COMPASS complex can modulate the function of KMT2D, including its substrate specificity, recruitment to chromatin, and catalytic activity. Due to the diversity of functional domains and interacting partners, it is reasonable to propose that KMT2D serves a broader role in the cell beyond its traditional function in chromatin modification (Fig. 2. C, D).

Emerging evidence suggests that KMT2D can regulate processes other than chromatin methylation, including transcriptional initiation in *Drosophila* (Dorighi et al., 2017; Herz et al., 2012), DNA damage response in mice (Chaudhuri et al., 2016), and epithelial-mesenchymal transition in human immortalized mammary epithelial cells (Zhang et al., 2022). In this review, we propose a new perspective on how KMT2D mediates cell-type transcriptional networks in response to signaling pathways and the resulting impact on nuanced heterogenic clinical presentations. We also briefly discuss the current state of molecular diagnostic techniques and therapeutic development in this field. Additionally, as research on the role of KMT2D in mediating developmental signaling pathways is limited, we hope this review offers insights for future primary research in this area.

2. KMT2D AS A MEDIATOR OF CELL SIGNALING

External environmental stimuli can directly regulate chromatin through interactions between chromatin modifiers, such as KMT2D, and signaling networks within a cell (Badeaux & Shi, 2013). Most studies in this field describe KMT2D's role in promoting transcriptional changes during cell-fate transitions by poising enhancers with H3K4me1 marks (Lavery et al., 2020). However, KMT2D has recently emerged as a pleiotropic protein involved in various cellular processes beyond H3K4 methylation across several species, including human, mouse, and zebrafish (Fagan & Dingwall, 2019; Froimchuk et al., 2017; Pang et al., 2021; Serrano et al., 2019). Furthermore, its ubiquitous expression at different developmental times suggests interactions between signaling pathways and target gene sets that are unique to the cell type and context (Fig. 2).

While KMT2D-mediated methylation of non-histone proteins has not yet been demonstrated, KMT2D is implicated in several nuclear receptor-mediated signaling cascades that affect a range of cellular responses to external stimuli, including cell growth, migration,

cycling dynamics, and differentiation (Fagan & Dingwall, 2019; Froimchuk et al., 2017; Pang et al., 2021; Serrano et al., 2019). Additionally, another member of the KMT2 family, KMT2F (also known as SET1A), has been shown to methylate non-histone proteins in a human lung cancer cell line (Fang et al., 2018). In this study, KMT2F methylated YAP protein, a key effector of the Hippo pathway, repressing its nuclear export and enhancing its transcriptional activity. Similarly, a recent study demonstrated a direct interaction between purified extracts of recombinant P53 and KMT2B proteins, suggesting that P53 could be a potential substrate of KMT2B (Y. Li et al., 2020).

Most research focuses on understanding the role of KMT2D in responding to signaling cues, particularly in the context of cancer (Maitituoheti et al., 2020; Xiong et al., 2018; Lv et al., 2018; Toska et al., 2017). However, some studies have also examined how KMT2D integrates signaling cues to drive differentiation. KMT2D has been shown to respond to signaling cues through direct binding to transcription factors (TFs) and through binding with other COMPASS proteins (C. Guo et al., 2012, Cho et al., 2007, Wu et al., 2013, Poreba et al., 2022). Thus, KMT2D likely mediates different signaling pathways depending on the cell type and stage of development, as these features often differ across such contexts. More research is needed to fully understand the role of KMT2D in various signaling pathways beyond its histone methyltransferase activity, including its interactions with pathway effectors, the conditions under which these interactions occur, the impact on target gene expression throughout development, and its relevance to the KS phenotype.

2.1 KMT2D and Notch Pathway

Effective cell-cell communication often requires the targeted activation of transcription factors, which must interact with chromatin-modifying proteins to initiate transcription. One example is the Notch signaling pathway, which controls various cellular processes such as proliferation, migration, survival, fate specification, and tissue patterning (Wang, 2011). To properly specify cell fate, the Notch pathway must be spatiotemporally controlled during development. Several groups have provided evidence of an interaction between the Notch pathway and KMT2D, such as Oswald et al. demonstrating that SHARP, an RBP-J corepressor, binds to the NCoR-repressive or KMT2D-activating complexes to regulate the transcription of Notch target genes in pre-T lymphoma cells (Oswald et al., 2016). Additionally, the *Drosophila* KMT2D ortholog, *Trr,* negatively regulates tissue growth in eye imaginal discs by controlling the expression of Notch receptors and cyclin B, a mitotic protein (Kanda et al., 2013).

More recent studies have demonstrated that KMT2D is critical in regulating Notch-induced signaling during cardiovascular patterning. Serrano et al. showed in zebrafish that loss of KMT2D results in increased expression of the Notch pathway effector Rbpj, leading to dysregulated endocardial patterning, hypoplastic ventricle, and ectopic blood vessels sprouting (Serrano et al., 2019). Additionally, a study based on scRNAseq data analysis of human fetal cells and iPSC-derived endothelial cells from a patient with hypoplastic left heart syndrome (HLHS) suggests that KMT2D-dependent Notch regulation contributes to coronary abnormalities in HLHS by impairing the proliferation of endothelial cells in the human fetal heart with hypoplastic ventricle (Yu et al., 2022).

2.2 KMT2D and Wnt Signaling

Studies have demonstrated that KMT2D is associated with Wnt signaling, an essential developmental pathway that also affects cell proliferation, migration, fate transition, and tissue patterning. Using ChIP-seq, Hu et al. found that genes located within 50-kb of KMT2D peaks in the human colon cancer cell line, HCT116, are related to several signaling pathways, including Wnt, MAPK, and ErbB (Hu et al., 2013). Additionally, when KMT2D was knocked down in the murine dental epithelial tooth cell line, LS8, Pang et al. observed a reduction in the adhesive properties, proliferation, and cycling of these cells through S/G2 phase. Bulk RNA-seq data from this work further revealed that eight Wnt signaling effectors were downregulated, such as Lgr4, Wnt9a, Wnt10b, Tle2, Tle6, Axin2, Lef1, and β-catenin (Pang et al., 2021). From an immunological perspective, Satgi and colleagues suggest a potential mechanistic role of KMT2D-mediated histone methylation and Wnt pathway nuclear receptors, RORα and RORγt, in driving aberrant helper T cell 17 (Th17) differentiation (Stagi et al., 2016). These findings propose an association between KMT2D activity and Wnt pathway effector expression across different cell types, species, and developmental timepoints.

2.3 KMT2D and Nuclear Receptor Signaling

Nuclear receptors (NR) are transcription factors activated by steroid hormones or lipid-soluble molecule ligands, such as retinoic acid. They regulate transcription by binding to the promoter regions of target genes and recruiting corepressor and coactivator protein partners via the recognition of a highly conserved LXXLL motif, also known as the NR box (Heery et al., 1997; Savkur & Burris, 2004; Sever & Glass, 2013). Both KMT2D and specific subunits of the COMPASS complex have been reported to interact with LXXLL motif-bearing nuclear receptors and transcription factors (Rao & Dou, 2015). This suggests that KMT2D and the COMPASS complex play a versatile role in regulating gene expression and cell identity through their interactions with nuclear receptors and transcription factors. Additionally, the scaffolding role of KMT2D specifies the composition of the COMPASS complex in response to different cellular conditions, subsequently allowing it to interact with different regulatory proteins, target different genes, and ultimately influence cellular behavior and fate acquisition.

Retinoic Acid (RA) regulates several developmental processes via direct transcriptional regulation during early vertebrate embryogenesis. In RA signaling, retinoic acid receptors and retinoid X receptors (RAR/RXR), both members of the NR family, heterodimerize and bind to retinoic acid response elements (RAREs) on target genes to recruit nuclear receptor coactivators (NCOA) or repressor (NCOR) complexes (Cunningham & Duester, 2015). KMT2D has six predicted NR boxes, shown previously to directly bind to estrogen receptors, to estrogen receptor coactivating complex, and to the COMPASS member ASC2 (also called NCOA6) across human and mouse cell contexts (Lee et al., 2008; Mo et al., 2006; Wang et al., 2017). Specifically, Lee et al utilized yeast-2-hybrid screens to demonstrate physical interactions between KMT2D and estrogen receptor alpha (ERα) via two of its NR boxes, NR1 and NR5 (Lee et al., 2008). They further confirm a reduction in this binding event upon ASC2 knockdown in MEF and HeLa cells, suggesting that ASC2 recruits and tethers KMT2D to the LXR-RXR heterodimer, which is typically bound to an

LX-RE target. This study additionally reveals that two members of COMPASS respond to signaling input, highlighting the notion that the composition of COMPASS could impact which signaling pathways it responds to at any given time. In MCK-7 cells, two C-terminal LXXLL motifs on KMT2D were shown to bind the complex in coactivating ERα (Mo et al., 2006). Wang et al. treated HEK293 cells with retinoic acid and ran the nuclear extracts over immobilized RAR/RXR substrates to reveal that KMT2D binds to these receptors (Wang et al., 2017). Altogether the numerous interactions between KMT2D, members of COMPASS, and NRs could provide additional time needed for KMT2D to methylate chromatin and encourage downstream transcriptional activation of target genes.

2.4 KMT2D and the HIF Pathway

KMT2D has also been shown to play a role in regulating the hypoxia-inducible factor (HIF) pathway. The HIF pathway is essential for triggering transcriptional responses to hypoxia, impacting several physiological and cellular programs necessary for adaptation to low oxygen (Weidemann & Johnson, 2008). Carosso and colleagues demonstrated that KMT2D supports neuronal progenitor maintenance during cortical neurogenesis in mice by enabling HIF1A transcriptional responses to hypoxia (Carosso et al., 2019). This study uses a *Kmt2d*$^{+/\beta geo}$ mouse model and hippocampal mouse neuron cell lines hosting homozygous and heterozygous *KMT2D* variants, respectively, in the SET domain. Both systems employ a truncated version of the KMT2D protein that is still present in the nucleus, but lacks the SET domain, allowing the assessment of KMT2D's methyltransferase activity only. In this context, the KMT2D-SET truncated neurodevelopmental models failed to activate HIF1A targets, including VEGFA, BNIP2, DDIT3, CDKN1A, leading to abnormal hypoxia, decreased proliferation, and upregulation of pro-neural genes in neural progenitor cells (Carosso et al., 2019). Moreover, downregulation of HIF1A targets and upregulation of pro-neural genes was also observed in iPSC and neuronal stem progenitor cells derived from a KS1 patient hosting a *KMT2D* heterozygous variant in exon 32 that codes for the domain PHA03247 (Atrophin-1 superfamily domain) (Carosso et al., 2019).

2.5 KMT2D-COMPASS interactions with other pathways effectors

KMT2D engages both directly and indirectly with other signaling pathways to facilitate cell responses. Downstream of PI3K, the kinase AKT1 can recognize and phosphorylate the RXRXXS/T motif present before the PHD4 domain on KMT2D,attenuating its function (Wang et al., 2021). In a KMT2D SET conditional knockout (cKO) mouse model, Jang et al. used ChIP-seq to show a reduction of KMT2D peaks on enhancers bound with the adipogenic transcription factors CEBPα and PPARγ in preadipocytes (Jang et al., 2019). In a KMT2D heterozygous variant and cKO mouse model, KMT2D seemed to regulate growth hormone-releasing hormone (GHRH)-producing neurons in the developing hypothalamus (Huisman et al., 2021). Here ChIP-seq experiments confirmed that KMT2D was bound to the neuronal transcription factor, Nrf1, when promoting GHRH-neural gene expression. This suggests a role for KMT2D in permitting the secretion of growth hormones in these neurons (Huisman et al., 2021). In mouse hepatocytes, KMT2D has been shown to mediate transcriptome changes in response to overnutrition by associating with the nuclear receptor PPARγ2 (Kim et al., 2016). Here, ABL1 kinases were stimulated by a high-fat diet, which

led to PPARγ2 phosphorylation and its association with KMT2D, opening chromatin and activating the transcription of steatotic target genes, including *Pparγ2* itself.

Several studies have also demonstrated that KMT2D mediates cell signaling through its COMPASS members. Because of this, it follows that the absence of certain members of COMPASS may impact the phenotype differently across cell types. ASH2L is a member of the core WRAD complex that facilitates efficient KMT2D-SET enzymatic activity. A mouse cKO model of ASH2L resulted in the downregulation of Wnt-related genes that affected the proliferative, migratory, and cycling capacities of neural progenitor cells (Li et al., 2019), suggesting that ASH2L-KMT2D is necessary for Wnt targets transcriptional regulation. In KMT2D KO or SET-null mouse embryonic stem cell mutants, KMT2D's role of transcriptional regulation during pluripotency transition occurred independently of its catalytic activity. In this context, COMPASS subunits recruited by KMT2D's N-terminus prevented lysine-specific demethylase (LSD1) silencing activity, which is known to repress genes that KMT2D activates (Cao et al., 2018). This expands KMT2D's functionality and suggests that its role in regulating transcription is not solely based on its enzymatic activity, but also on its ability to recruit other proteins that directly influence the activity of other transcriptional regulators.

Therefore, any heterogeneity in KMT2D variants along the protein could influence its ability to bind particular proteins that are critical in mediating cell processes during certain stages of cell development, independent of its C-terminal SET function. Likewise, if KMT2D acts downstream of signaling pathways that mediate stemness or cell fate transitions, its complete loss could prevent the recruitment and scaffolding of such proteins, preventing the overall integration of signaling events, resulting in the failure of executing well-orchestrated fate transitions during development.

2.6 KMT2D in Cancer

Tumorigenic alterations in KMT2D can link oncogenic proliferative and migration signaling pathways like Wnt, RTK/RAS, p53, TGFβ, and PI3K to transformed cells (Guo et al., 2013; Liao et al., 2022). KMT2D is one of the most frequently mutated genes across several cancers, including bladder cancer, breast cancer, non-Hodgkin's lymphoma (NHL), prostate cancer, medulloblastoma, colorectal cancer, esophageal squamous cell cancer, T-cell lymphoma, and acute myeloid lymphoma among others (Ansari et al., 2011; Ortega-Molina et al., 2015; Sze & Shilatifard, 2016). In certain contexts, KMT2D can act as a tumor suppressor. For example, in melanoma KMT2D negatively regulates insulin growth factor 1 receptor (IGF1R) signaling, which increases the expression of IGFBP5 ligands, upregulating glycolytic gene expression. In other contexts, KMT2D can promote tumorigenic pathways. Across melanoma, pancreatic, gastric, and breast cancers, KMT2D promoted the expression of genes that activate the PI3K/AKT pathway, like KLF4 and LIFR, which increases processes like proliferation and invasion (Dhar & Lee, 2021; Lin-Shiao et al., 2018). As mentioned earlier, such AKT and SGK1 kinases can regulate KMT2D activity through direct phosphorylation of KMT2D itself, reducing its SET activity. In human U2-OS and HEK293T cells, TGFβ-stimulated SMAD2/3 binding sites were enriched on enhancers occupied by KMT2D (Baas et al., 2017). UCSC genome

browser track analysis further demonstrated that SMAD3 and KMT2D colocalize on the TGFβ responsive genes SERPINE1 and PMEPA1, suggesting a general mechanism behind tumorigenic gene activation. In prostate cancer, KMT2D knockdown in mouse xenograft tumors resulted in reduced tumor growth and downregulation of LIFR and KLF4 genes, which regulate the PI3K/AKT and EMT pathway, respectively (Lv et al., 2018). These studies underscore the need to understand how KMT2D mediates cell signaling across specific cell populations and disease contexts, along with the potential to broadly apply such knowledge to areas of research beyond KS.

3. KS1 IN THE CLINIC

3.1 Clinical Features of KS1

KS1 is characterized by several clinical manifestations including, cardinal facial gestalts (long palpebral fissures, eversion of the lower lateral eyelids, arched eyebrows, depressed nasal tip, palate malformations, and large prominent ears), feeding difficulties, digital and hand anomalies, cardiovascular defects, skeletal and growth abnormalities, immune system dysregulation, anxiety and sleep difficulties, neonatal hypotonia, renal anomalies, and endocrine abnormalities (Adam et al., 2019; De Leon & Stanley, 2017; Niikawa et al., 1981; Rapp et al., 2022; Stagi et al., 2016; Yap et al., 2019). Other possible manifestations include orbital vascular malformations and cholestasis, cleft hand, epilepsy, microphthalmia, and pilomatricoma, among many others (Bernier et al., 2017; Bögershausen et al., 2016; Bruni et al., 2021; Huh et al., 2011; Kamada, et al., 2019; McVeigh et al., 2015).

Accurately diagnosing KS1 can be challenging due to the broad clinical spectrum that varies in severity and the affected organ systems across patients (Boniel et al., 2021; Montano et al., 2022; Sobreira et al., 2017). Additionally, mild developmental hallmarks often become evident within a year after birth, and KS1 is suspected earlier in life only if infants present with severe anomalies (Yap et al., 2019). The underlying heterogeneity of pathogenic variants in the *KMT2D* gene might partly explain the expansive phenotypic landscape of KS1 patients. Diverse variants of the same gene can alter distinct protein domains, which poses unique functional implications for the protein and may result in different clinical phenotypes (Banka et al., 2013; Barry et al., 2022; Cocciadiferro et al., 2018; Cuvertino et al., 2020; Schott et al., 2016). However, there is currently no clear correlation between a set of pathogenic variants in *KMT2D* and the resulting KS1 phenotype, meaning that a given variant may not lead to the same features in all patients. Mosaicism of *KMT2D variants* further complicate the KS1 phenotype by manifesting differently across tissues within the same patient, presenting in ways that do not include cardinal hallmarks and often resolving as mild phenotypes (Banka et al., 2013; L. Guo et al., 2022; Lepri et al., 2017; Manheimer et al., 2018; Montano et al., 2022; Razzaghy-Azar et al., 2022). To aid in diagnosis, Adam et al and Makrythanasis et al developed an international consensus diagnostic criteria and phenotypic scoring system (Adam et al., 2019). However, molecular confirmation of *KMT2D* pathogenic variants is essential to distinguish KS1 from other similarly presenting developmental disorders, such as CHARGE syndrome (Kasdon & Fox, 2012). For a more comprehensive review of KS clinical features, see Adam et al., Dugan, and Barry et al. (Adam et al., 2019; Dugan, 2021; Barry et al., 2022).

Longitudinal studies, such as natural history studies, can greatly advance our understanding of the developmental trajectory of KS patients also leading to accurate diagnoses and early interventions. In line with this, Ventola and colleagues performed a longitudinal case report of a KS1 patient from 8 to 27 years of age (Ventola et al., 2019). Evaluating this patient's cognitive, behavioral, and psychiatric presentation over time demonstrated that external support in friendships, schooling, and access to healthcare becomes critical to maintain through young adulthood. Furthermore, Theodore-Oklota et al expanded the holistic understanding of managing relatives or children with KS1 by assessing the unique challenges experienced by caregivers (Theodore-Oklota et al., 2020).

Current clinical studies, patient registries, and natural history studies are focused on determining the natural progression of KS and identifying objective outcome measures to facilitate the development of future clinical trials (National Library of Medicine, clinicaltrials.gov). For example, Rapp et al recently identified sleep disturbance as a specific feature shared by the majority of participants and correlated their sleep score with anxiety and several neurobehavioral domains (Rapp et al., 2022). Further, Boisgontier and colleagues suggest implementing specific memory tests that assess how episodic and working memory are impacted by the hippocampal substrate in these patients (Boisgontier et al., 2019). These outcome measures will help assess the effectiveness of intervention in clinical trials and allow for more accurate and reliable evaluations of KS.

3.2 Molecular Diagnostics of KS1

Evaluating the broad variant landscape across the *KMT2D* gene in KS patients is a critical component in diagnosing and understanding the molecular basis of this syndrome. In this section, we summarize several types of variants that have been identified in KS patients, the exons that have a higher frequency of variants, and the different types of genetic testing that are available for detecting these pathogenic variants.

Most *KMT2D* pathogenic variants are *de novo* and often result in truncations, splicing errors, and coding frameshifts (Banka et al., 2012, 2013; Guo et al., 2022; Stangler Herodež et al., 2020). However, the entire variant landscape includes missense, nonsense, splice-site, deletions, insertions, duplications, mosaic, and frameshifts, all of which are associated with KMT2D haploinsufficiency (Baldridge et al., 2020; Bögershausen & Wollnik, 2013; Coocciadiferro, 2018; Dentici et al., 2015; Guo et al., 2022; Hannibal et al., 2011; Montano et al., 2022; Murakami et al., 2020; Paděrová et al., 2016; Sobreira et al., 2017). Although variants are positioned throughout the entire *KMT2D* gene, missense variants often localize within or nearby the C-terminus, specifically at the FYRN/FYRC and SET functional regions, while truncating variants are distributed across the coding region (Bögershausen & Wollnik, 2013; Micale et al., 2014; Murakami et al., 2020). Additional reports of high frequency variants are located within the LXXLL domain (exon 48), coil-coil domains (exons 31, 32, 33, 40, 38, and 39), and SET domain (exons 52 and 53) (Banka et al., 2013; Hannibal et al., 2011; Paděrová et al., 2016). Variants located in deep intronic regions, large intragenic deletions, and duplications between exons and exon-intron boundaries in *KMT2D* have also been described (Adam et al., 2021; Banka et al., 2013). Finally, *KMT2D* is associated with variants of unknown significance (VUS), which are sequence alterations

of unknown functional significance not yet recorded in any public database. Nearly 40% of patients with KS are estimated to contain a VUS in *KMT2D* (Banka et al., 2013). Because of this, it can be difficult for patients with clinical features of KS to be accurately diagnosed. Without a molecular diagnosis, these patients have historically been misdiagnosed with other rare disorders that present similar clinical features. Future research should focus on the genetic pathomechanisms that drive such diverse phenotypic features observed across KS patients (Boniel et al., 2021).

Although there are approximately 3,000 variants annotated for KMT2D (Fig. 1), roughly 600 variants have been described in KS. Of those, 55–80% are pathogenic and impart C-terminal loss-of-function truncations (Cheon et al., 2014; Guo et al., 2022). Implementation of next-generation sequencing techniques such as whole exome sequencing, trio-whole exome sequencing, and targeted sequencing used alongside sanger sequencing offer improved resolution in detecting a variety of genetic alterations in *KMT2D*, such as insertions/deletions, single nucleotide variants, and copy number variations (Adam et al., 2019; Aref-Eshghi et al., 2019; Banka et al., 2013; Boniel et al., 2021; Cheon et al., 2014; Guo et al., 2022; Hannibal et al., 2011; Montano et al., 2022; Murakami et al., 2020; Sobreira et al., 2017; Stangler Herodež et al., 2020; Wang et al., 2019). Despite this, the genetic basis for an estimated 20–45% of KS patients remains unknown (Banka et al., 2013; Guo et al., 2022; Van Laarhoven et al., 2015).

To help address this gap, two powerful diagnostic tools were established in addition to targeted genetic testing. A phenotypic scoring system developed by Makrythanasis and colleagues aims to assist clinicians in determining if patients are likely to retain a variant in *KMT2D* and, therefore, is often utilized as a reliable starting point for diagnosis (Adam et al., 2021; Makrythanasis et al., 2013). Using this scoring system, physicians have since determined the mean scores of variant-positive patients being 6–7.5, while the mean scores of variant-negative patients patients is roughly 4.8 (Adam et al., 2019; Banka et al., 2012, 2013; Makrythanasis et al., 2013; Paděrová et al., 2016; Stangler Herodež et al., 2020). Additionally, DNA methylation status can be used to identify unique episignatures in KS1 patients, regardless of the variant classification (nonsense, frameshift, missense, deletion, duplication, and insertion) (Aref-Eshghi et al., 2019; Gooch et al., 2022; Montano et al., 2022; Sobreira et al., 2017). Importantly, these tools can increase access to reaching a diagnosis for patients and families that do not have immediate access to advanced genetic testing clinics. Finally, advances in sequencing technology coupled with Makrythanasis's phenotypic scoring system have enabled more precise molecular diagnoses for KS patients across a wide range of variants (Cheon et al., 2014; Guo et al., 2022; Holubová et al., 2016).

3.3 Therapeutic Advancements of KS1

There are currently no active therapies that directly address KS1. Current interventions target the complications that arise from developmental abnormalities and aim to maximize the quality of life of patients. These include occupational, speech, feeding, behavioral, and physical therapies that comprehensively address the needs of each patient (Adam et al., 2021). In the United States, several clinics offer services directed towards KS patients; Maryland: Kennedy Krieger Institute and Johns Hopkins; Massachusetts: Roya

Kabuki Clinic at Boston Children's Hospital; Pennsylvania: Kabuki Syndrome Clinic at Children's Hospital of Philadelphia; Ohio: Epigenetic Syndromes Clinic at Cincinnati Children's; California: ASXL-Related Disorders and Chromatinopathies Clinic at UCLA; and Washington: Seattle Children's Hospital.

Over the past 14 years, several studies related to KS have been reported on clinicaltrials.gov, a global database housing clinical studies. While most of the reported studies are observational, one is a clinical trial (National Library of Medicine [NLM], NCT04722315) and another introduces a preclinical investigation of a possible intervention (NLM, NCT03855631). . Importantly, many preclinical studies related to KS1 are not reported on clinicaltrials.gov. Although this database is an important resource that captures several clinical study characteristics related to trial design, outcome, results, and more, we hope to clarify that much ongoing research behind the basic science and therapeutic development for KS1 are not fully accounted for on this website alone.

The only ongoing recruiting clinical trial (NLM, NCT04722315) utilizes the Atkins ketogenic diet as an intervention that inhibits histone deacetylase activity. The observational patient registration study (NLM, NCT01793168), established by the Coordination of Rare Diseases at Stanford Research, advances research in >7,000 rare diseases by connecting individuals, researchers, and advocacy groups in a centralized patient registry site (Stanford Health, 2023). A 2018 trial (NLM, NCT01314534) examined the memory index of children aged 6–16 years old and reported dysregulation among the full-scale intellectual quotient, verbal comprehension index, perceptive reasoning index, processing speed index, and working memory index (Lehman et al., 2017).

Two other registered studies have since been completed but did not openly publish or report the results. The observational study (NLM, NCT01314534) aimed to uncover the frequency of known and unknown clinical symptoms and complications by capturing epidemiological and MRI-based morphological data across 110 participants. Secondly, a recently completed intervention study (NLM, NCT03855631) aimed to establish a therapeutic pipeline that could correct a *KMT2D* variant in patient-derived mesenchymal stem cells using a CRISPR/Cas9 gene therapy approach. However, this group did not perform any interventional investigations in human subjects. While the data from these studies are not released, providers or individuals can contact the research staff furnished by the sponsor contact details on clinicaltrials.gov for more information.

Interventional clinical research will likely increase as a greater understanding of the molecular basis underlying KS continues to build, highlighting the critical need to invest in the basic research that mechanistically reveals the role of KMT2D, specifically in disease. Despite the limitations that come with rare disorders, the combination of clinical studies and trials has been instrumental in advancing the collective medical knowledge related to KS. However, the rarity of KS results in low participant enrollment within clinical research, which can make it difficult to generalize findings to other KS patients. Additionally, as much progress remains to be made in developing effective therapies for KS, the methods and repeated clinical studies, albeit with a low overall participant count, provide valuable data to the KS community (Fig. 2. E).

4. SUMMARY

KMT2D is increasingly becoming recognized as having many functions outside of methyltransferase activity alone. In this review, we discussed multiple examples where KMT2D mediates a range of signaling interactions to influence gene expression across several species, cell types, and biological contexts. Notably, KMT2D's response to shared signaling cues that induce cell-type specific transcriptional programs throughout development may also support the shared clinical manifestations of other developmental disorders. Indeed, the nucleosome remodeling complex commonly affected in CHARGE syndrome, CHD7, also regulates cell-fate decisions by binding to lineage-determining transcription factors following retinoic acid signaling in rodent neural stem cells (Micucci et al., 2014). Understanding the entirety of KMT2D functions will undoubtedly increase the understanding of the various phenotypic manifestations and therapeutic potential for advancing KS as a treatable disorder. Early diagnosis and symptom management are vital to reducing the physical and psychological toll that KS patients and families may experience. As molecular testing for KS improves and awareness increases throughout physician and public communities, the true incidence of KS is likely higher than the current estimation. There is a great need for expanding the basic research on chromatin-modifying proteins like KMT2D and how their altered function may impact childhood development.

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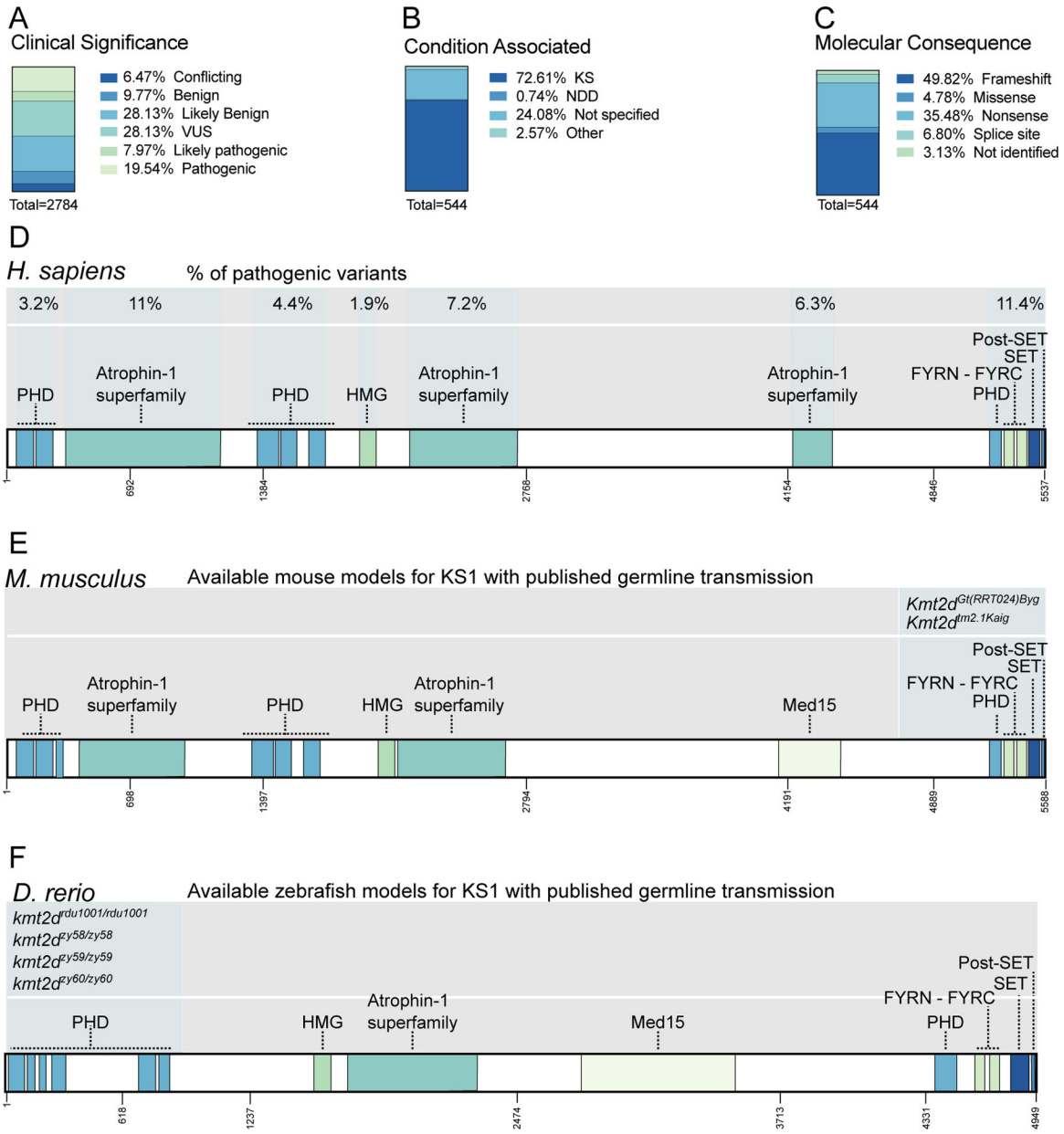

Figure 1. Pathogenic variants distribution in KMT2D protein and available animal models.

A) KMT2D variants annotated in ClinVar database. From 2788 registered variants, 544 are considered pathogenic. B) 72.6% of ClinVar annotated pathogenic variants are associated with Kabuki Syndrome 1. C) Distribution of the different types of variants in all annotated pathogenic variants of KMT2D. Data analysis was performed from information available at https://www.ncbi.nlm.nih.gov/clinvar/. D) Human KMT2D protein. Domains were identified using *KMT2D-201* transcript on human genome assembly GRCh38.p13. Pathogenic variants were extracted from ClinVar and annotated in the domain they affect. E) Mouse KMT2D protein. Domains were assigned based on transcript *KMT2D-201* on mouse genome assembly Mouse (GRCm39). Available mouse null/knock-out lines with published germline transmission are depicted in the domain affected by

genetic manipulation. Mouse lines information source: Mouse Genome Informatics (MGI, https://www.informatics.jax.org/). F) Zebrafish KMT2D protein. Domain information was retrieved from transcript *KMT2D-201*, genome assembly Zv9 archive version. The current version (GRCz11) does not have the full transcript annotated. Zebrafish mutant lines with a published germline mutation are shown. These lines host mutations that affect the N-terminus PHD domains. Information about zebrafish mutant lines was extracted from the Zebrafish Information Network (ZFIN, https://zfin.org/). KS, Kabuki Syndrome; NDD, Neurodevelopmental Disorder; VUS, Varian of Uncertain Significance; PHD, Plant Homeodomain; Atrophin-1 superfamily, also seen as PHA03247; HMG, High Mobility Group; Med15, ARC105 or Med15 subunit of Mediator complex non-fungal; FYRN/C, "FY-rich" domain N-terminal (FYRN) and "FY-rich" domain C-terminal (FYRC) sequence motif domains; SET, Su(var)3–9, Enhancer-of-zeste and Trithorax domain.

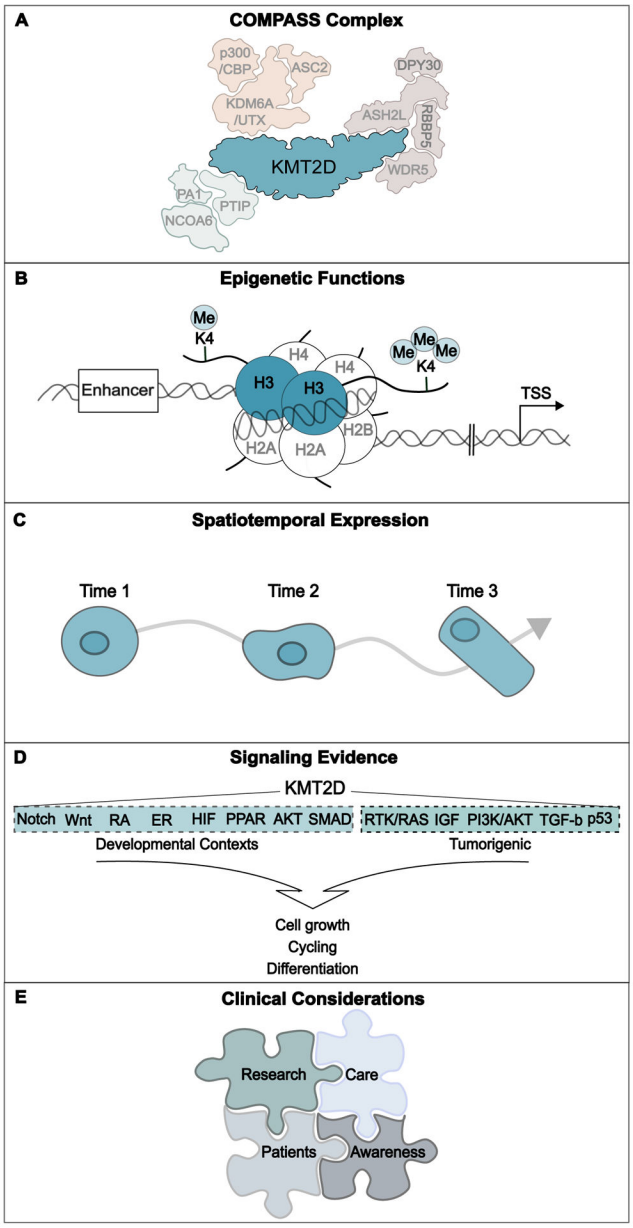

Figure 2. Cellular, molecular, and clinical foundations of KMT2D biology.

A) KMT2D is part of the COMPASS complex, a multi-subcomplex unit that targets various regions of the genome to open chromatin. B) KMT2D functions by mono- and tri-methylating H3K4 to activate enhancers and promoters, respectively. C) The output of KMT2D's activity depends on several factors, including the cell type, time of expression, and developmental stage of the organism. D) Recent work centralizes KMT2D as a mediator of external signaling cues established during development and under tumorigenic circumstances. Dashed lines represent the limited understanding of KMT2D in this context. E) Building engaging partnerships between scientists, clinicians, and patient advocates will

improve outcomes for KS patients and provide unique perspectives on the unmet needs often overlooked throughout benchtop research.

Table 1.

Summary of *KMT2D* nomenclature

(National Center for Biotechnology Information, 2016).

Gene symbol	Species	Gene location †	Alternative names
KMT2D	*Homo Sapiens*	12:49,018,975-49,060,794	*ALR, GAGL114, MLL2, MLL4, TNRC21, KABUK1, AAD10, KMS*
Kmt2d	*Mus musculus*	15:98,729,550-98,769,085	*C430014K11Rik, Mll2, Mll4*
kmt2d	*Danio rerio*	23:27,756,984-27,793,877	*Im:7157663, mll2*

†Gene locations were mapped in the following genome versions: Human, GRCh38.p13; Mouse, GRCm39; Zebrafish, GRCz11