OPEN ACCESS This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/ by-nc-nd/4.0), which permits copy and redistribute the material in any medium or format, provided the original work is properly cited.

sciendo

128 ENDOCRINE REGULATIONS, Vol. 57, 128–137, 2023

doi:10.2478/enr-2023-0016

A narrative review on pathogenetic mechanisms of hyperinsulinemic hypoglycemia in Kabuki syndrome

Evelina Maines [1], Arianna Maiorana [2], Letizia Leonardi [1], Giovanni Piccoli [3], Massimo Soffiati [1], Roberto Franceschi [1]

[1]Division of Pediatrics, S. Chiara General Hospital, APSS, Trento, Italy; [2]Division of Metabolism and Research Unit of Metabolic Biochemistry, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [3]CIBIO - Department of Cellular, Computational and Integrative Biology, Università degli Studi di Trento, Italy
E-mail: evelina.maines@apss.tn.it

Objective. Kabuki syndrome (KS) is associated with hyperinsulinemic hypoglycemia (HH) in 0.3–4% of patients, thus exceeding the prevalence in the general population. HH association is stronger for KS type 2 (KDM6A-KS, OMIM #300867) than KS type 1 (KMT2D-KS, OMIM #147920). Both the disease-associated genes, KMD6A and KMT2D, modulate the chromatin dynamic. As such, KS is considered to be the best characterized pediatric chromatinopathy. However, the exact pathogenetic mechanisms leading to HH in this syndrome remain still unclear.

Methods. We selected on the electronic database PubMed all articles describing or hypothesizing the mechanisms underlying the dysregulated insulin secretion in KS.

Results. The impact on the gene expression due to the KDM6A or KMT2D function loss may lead to a deregulated pancreatic β-cell differentiation during embryogenesis. Moreover, both KMT2D gene and KDM6A gene are implicated in promoting the transcription of essential pancreatic β-cell genes and in regulating the metabolic pathways instrumental for insulin release. Somatic KMT2D or KDM6A mutations have also been described in several tumor types, including insulinoma, and have been associated with metabolic pathways promoting pancreatic cell proliferation.

Conclusions. The impact of pathogenic variants in KDM6A and KDM2D genes on β-cell insulin release remains to be fully clarified. Understanding this phenomenon may provide valuable insight into the physiological mechanisms of insulin release and into the pathological cascade causing hyperinsulinism in KS. The identification of these molecular targets may open new therapeutic opportunities based on epigenetic modifiers.

Key words: Kabuki syndrome, hyperinsulinism, hypoglycemia, KMT2D, KDM6A, review

Congenital hyperinsulinism (CHI) is the most frequent cause of persistent hypoglycemia in pediatric patients. CHI includes heterogeneous biochemical disorders characterized by transient or genetically determined alterations in the cellular pathways of insulin secretion leading to unsuppressed insulin secretion during hypoglycemia (hyperinsulinemic hypoglycemia, HH). CHI patients are at high risk of significant neurological sequelae such as epilepsy, cerebral palsy, and cognitive impairment (Demirbilek and Hussain 2017; Maiorana and Dionisi-Vici 2017).

The known genetic forms originate from mutations in genes regulating insulin secretion. Four main categories have been described. The first category includes gene coding for potassium channels (ABCC8, KCNJ11). The second and third categories encompass gene coding for enzymes (such as GLUD1, GCK, HADH, UCP2, HK1, PGM1, PMM2, ADK),

Corresponding author: Evelina Maines, Pediatric Unit, "S. Chiara" Hospital, Largo Medaglie d'oro 9, 38122 Trento, Italy; phone: +390461903538; fax: +39 0461903824; e-mail: evelina.maines@apss.tn.it.

transporters (SLC16A1) or transcription factors (such as HNF4A, HNF1A, FOXA2), all impinging on the regulation of insulin secretion (Maiorana and Dionisi-Vici 2017; Galcheva et al. 2019). Lastly, the fourth category is associated with a broad spectrum of genetic syndromes, accounting for a significant proportion of patients with CHI (Galcheva et al. 2019).

HH is a clinical feature in a wide range of syndromes, such as Kabuki syndrome (KS), Turner syndrome, Beckwith-Wiedemann syndrome, Sotos syndrome, Costello syndrome, Simpson-Golabi-Behmel syndrome, Ondine syndrome, Usher syndrome, Perlmann syndrome, Timothy syndrome, trisomy 13, and the congenital disorders of glycosylation (Gole et al. 2016; Kostopoulou and Shah 2019). The list of syndromes associated with HH is rapidly expanding.

KS is a rare congenital syndrome first described by Niikawa and Kuroki in Japan (Niikawa et al. 1981; Kuroki et al. 1981). It was named after the characteristic facial features of the patients, recalling the actors of the Kabuki theatre (Niikawa et al. 1981). KS prevalence is 1:32000 in Japan and 1:86000 in western countries (Gole et al. 2016). The syndrome is clinically and genetically heterogeneous and characterized by variable manifestations. Such a clinical picture may reflect the epigenetic role played during the embryonic development of the two genes associated with KS, KMT2D and KDM6A, responsible for KS type 1 (OMIM #147920) and KS type 2 (OMIM #300867), respectively.

More than 75% of patients with KS harbor pathogenic loss-of-function variants in KMT2D (12q13.13, previously known as MLL2). Typically, KMT2D mutations occur de novo and only in rare cases are inherited and associated with autosomal dominant KS. 5–8% of patients have pathogenic loss-of-function variants in KDM6A (located at Xp.11.3, previously known as UTX). Almost all KDM6A mutations happen de novo and cause X-linked dominant KS. 15–20% of patients with a clinical diagnosis of KS may not carry variants in either KMT2D or KDM6A genes (Yap et al. 2019). A recent publication suggests new genes and loci that potentially contribute to a Kabuki-like syndrome (Lintas and Persico 2018).

KMT2D and KMD6A encode for Lysine Methyltransferase 2D and Lysine Demethylase 6A, respectively, two enzymes involved in the epigenetic regulation of transcription. The two enzymes interact physically and functionally to control the transcriptional status of chromatin (Lintas and Persico 2018).

Epigenetics was initially proposed by Waddington in 1942, and its definition was finalized at the 2008 Cold Spring Harbor Conference as "a stable heritable phenotype caused by chromosomal changes without changing the DNA sequence" (Waddington 2012). The epigenetic status of chromatin is tightly influenced by histone modifications. In particular, the modification of histones can modulate the chromatin structure and regulate the accessibility to transcription factors. Chromatin can assume permissive or repressive conformation (Sharma and Gurudutta 2016; Boniel et al. 2021). Euchromatin (the open conformation) allows gene expression. Conversely, heterochromatin (the condensed conformation) hampers gene transcription (Sharma and Gurudutta 2016).

A broad panel of post-translational modifications involves histones. Acetylation, methylation, phosphorylation, ubiquitination, and other substitutions, creates a complex 'bar' code, which affects histone-histone and histone-DNA interactions and, eventually, dictates chromatin status. Histones acetylation/deacetylation and methylation/demethylation play a major role. Histone methylation is executed by histone methyltransferases (HMTs), so-called "writers," and removed by histone demethylases (HDMs), the "erasers" (Sharma and Gurudutta 2016).

KMT2D gene contains 55 exons and 54 introns and encodes the Lysine Methyltransferase 2D, one of the major histone H3 lysine 4 (H3K4) mono-methyltransferase. H3K4 methylation tags open chromatin (Froimchuk et al. 2017). The KDM6A gene codes for the histone demethylase specific for histone H3 Lysine 27 (H3K27me3) (Quilichini and Haumaitre 2015; Tran et al. 2020). The trimethylation of histone H3 at lysine 27 (H3K27me3) is a transcriptional repressive epigenetic mark. KMT2D and KMD6A cooperate to control chromatin status and are crucial players in the epigenetic control of gene transcription. As such, KS is considered the best characterized pediatric chromatinopathy.

An international consensus on the diagnostic criteria for KS was established in 2019 (Adam et al. 2019). A definite diagnosis of KS can apply to patients of any age with a history of infantile hypotonia, developmental delay, and showing at least one of the primary criteria: a pathogenic or likely pathogenic variant in KMT2D or KDM6A, a typical dysmorphic features (including long palpebral features, eversion of the lower eyelid and two more among arched and broad eyebrows, with notching or sparseness short columella and depressed nasal tip, large, prominent or cupped ears, persistent fingertip pads). Further

clinical features, including short stature, microcephaly, cleft palate, lip pits, hearing loss, congenital heart defects, feeding difficulties, and immunological disorders may support the initial diagnosis (Adam et al. 2019).

Hypoglycemia was observed in up to 27.7% of patients with KS, either in the neonatal period or later in their lives (Subbarayan and Hussain 2014; Lintas and Persico 2018; Faundes et al. 2021). Hypoglycemia can be transient or persistent and has been linked to various causes such as augmented energy consumption, growth hormone deficiency, adrenal insufficiency and secondary to adrenocorticotropic hormone deficiency, and hyperinsulinism (HI) (Gole et al. 2016). In particular, KS is associated with hyperinsulinemic hypoglycemia (HH) in 0.3–4% of patients, which exceeds the prevalence in the general population (Hoermann et al. 2020). The prevalence of HH in KS could be even higher (Faundes et al. 2021) because mild or transient forms of HI cases may remain undetected (Lintas and Persico 2018).

A comparative meta-analysis concluded that KDM6A variants were more frequent in KS patients with HH than in patients without HH and that KDM6A-KS was more likely to be associated with HH than KMT2D-KS (Hoermann et al. 2020). This finding has been confirmed in other case series (Lintas and Persico 2018). KS patients usually manifest HH on the first day of life and are responsive to diazoxide, starting doses between 5 and 13 mg/kg/day (Hoermann et al. 2020). Nevertheless, Yap et al. (2019) have reported two cases of diazoxide-unresponsive HH.

In this article, we review the state-of-the-art knowledge on the molecular basis of HH in KS in order to highlight the known or hypothesized pathogenic mechanisms associated with hyperinsulinism in this syndrome.

Methods

To select articles, we searched the electronic database PubMed using the term "hyperinsulinism" OR "hyperinsulinaemic/hyperinsulinemic" AND "Kabuki"; "KMT2D" OR "KDM6A" AND "hyperinsulinism" OR "hyperinsulinaemic/hyperinsulinemic"; "KMT2D" OR "KDM6A" AND "pancreas" OR "beta-cell" OR "glucose". Since searches yielded few results on the role of the KMT2D gene, the search was further extended using the terms "H3K4me3" AND "pancreas".

We identified the most relevant publications written in English along the past twenty years. Abstracts were reviewed and the most relevant publications were used. Next, we screened the reference list of the selected studies.

Our strategy identified a total of 130 papers. After carefully reviewing titles and abstracts, we excluded 110 records reporting outcomes not relevant for our analysis. Upon the examination of the full text, we further excluded 10 studies. We added other 10 studies by screening the reference cited, for a total of 20 studies included in this review (Figure 1).

Review

Evidence on KDM6A gene

Gole et al. (2016) have postulated that the loss-of-function (LOF) mutations of KDM6A may alter the H3K27me3 mark and, eventually, impacts pancreatic β-cell differentiation during embryogenesis. KDM6A regulates endoderm differentiation from human embryonic stem cells (hESCs) (Jiang et al. 2013). The disruption in the gene expression dynamic consequent to KDM6A LOF can lead to endodermal differentiation defects, including an

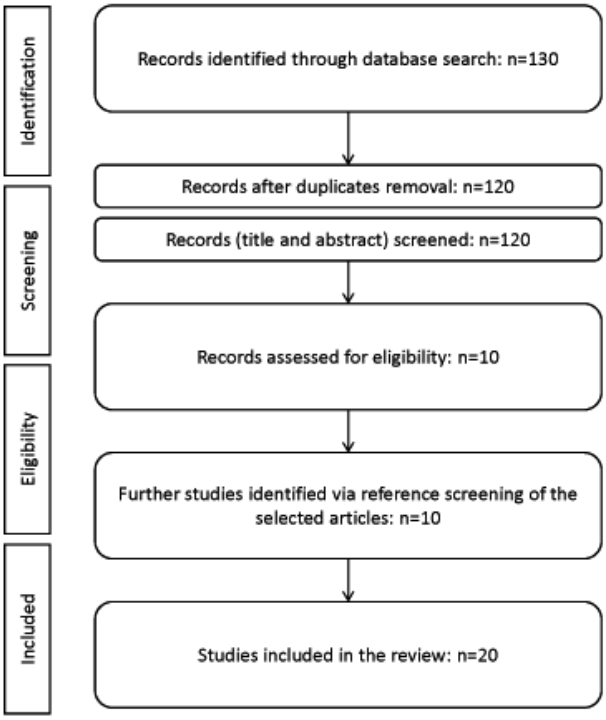


Figure 1. PRISMA Flow-Diagram indicating the processing of the individual records. Records excluded: n=110. Out of scope: n=105. Languages other than English: n=5

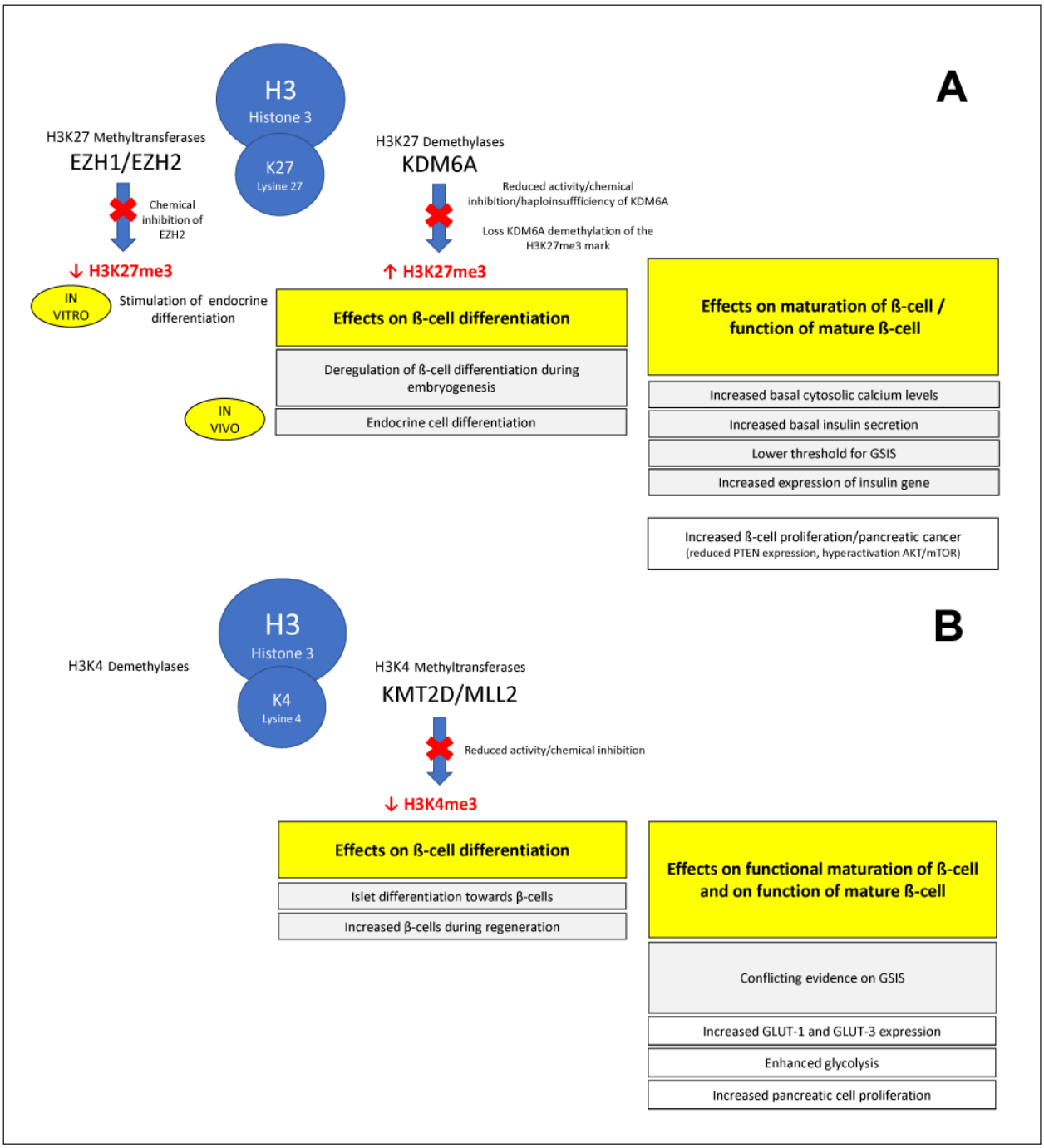


Figure 2. Primary potential pathogenic mechanism of β-cell deregulated insulin secretion in Kabuki syndrome (KS). **A**) Evidence on mechanisms related to KMD6A loss; **B)** Evidence on mechanisms related to KMT2D loss. In eukaryotic cells, chromatin is composed of DNA wrapped around histone proteins, forming the basic building block of nucleosomes. The nucleosomes are further compacted into higher-order chromatin architecture, organized into condensed compartments or heterochromatin domains and open compartments or euchromatin domains. The nucleosome structural unit consists of approximately 146 base pairs of DNA wrapped around an octamer, encompassing two copies of each of the four core histone proteins (H2A, H2B, H3, and H4). Histones have an unstructured N-terminal tail that undergoes different post-translational modifications, such as methylation (arginine/R and lysine/K on H3 and H4), which alter the functional properties and structures of chromatin (Kaimala et al. 2022). Histone methyltransferases (HMTs) are enzymes that catalyze histone methylation by transferring one to three methyl groups (monomethylation – me1, dimethylation – me2, and trimethylation – me3) from s-adenosyl methionine to the lysine or arginine residues of the histone tails. Histone methylation is a reversible process catalyzed by histone demethylases (HDMs).

altered development of β-cells (Hemming et al. 2014; Gole et al. 2016) (Figure 2A).

In vitro evidence shows that the H3K27me3 mark is crucial to suppressing pancreatic progenitor differentiation (van Arensbergen et al. 2010). These results are consistent with the observation that the chemical inhibition of the histone H3K27 methyltransferase EZH2 increases endocrine cell differentiation in complementary murine pancreatic models (Xu et al. 2014). *In vitro*, the H3K27me3 signature declines during embryonic stem cell differentiation. Instead, H3K27me3 tags increase along with pancreatic differentiation *in vivo* (Xu et al. 2014). In KS type 2, the reduction of KDM6A activity may increase the H3K27me3 marks in the genome and, eventually, may promote an aberrant development of embryonic progenitors towards endocrine cells (Boniel et al. 2021).

KDM6A LOF may also directly perturb insulin release in mature β-cell. Gibson et al. (2018) have described HH in Turner syndrome patients. Turner syndrome is a rare chromosomal disorder caused by the loss of an X-chromosome or structural abnormalities in the X-chromosome. Interestingly, at the histopathological analysis, the pancreas showed scattered islet cell nucleomegaly and a normal lobular parenchymal architecture, typical of diffuse hyperinsulinism due to KATP defects (Gibson et al. 2018). The authors assessed insulin secretion on pancreatic islets isolated from the patients. The islets displayed a peculiar response, different from what observed in KATP hyperinsulinism. In particular, the basal cytosolic calcium fell between the values observed in normal control islets and the ones reported in KATP channel LOF hyperinsulinism. Basal insulin secretion was higher than in control islets.

Moreover, the islets gathered from Turner syndrome patients showed a lower threshold for glucose and amino acids-stimulated insulin secretion than control human islets. Patients' cells demonstrated a prominent "off" response in insulin release upon glucose withdrawal and had a normal response to glyburide, a KATP channel antagonist. The islets from a patient with KATP hyperinsulinism did not respond to glucose, they were hypersensitive to amino acids, and unresponsive to glyburide. In order to assess the role of KDM6A LOF, Gibson et al. (2018) studied insulin secretion in isolated mouse islets exposed to a well-established inhibitor of KDM6A, GSK-J1 (Heinemann et al. 2014). GSK-J1 increased insulin secretion upon glucose and cytosolic calcium levels (Gibson et al. 2018). Altogether, these findings suggest that the KDM6A haploinsufficiency may cause HH.

The impact of KDM6A on basal cytosolic β-cell calcium levels remains still elusive, but it may arise from KATP channel-independent mechanisms.

Several reports have discussed the overlapping features of Turner and KS, including the high frequency of HH (Gibson et al. 2018). Unfortunately, reports on pancreatic histopathology of patients with KS with HH are missing.

KDM6A is a potent pancreatic tumor suppressor (Kalisz et al. 2020; Revia et al. 2022; Yi et al. 2022). KDM6A LOF is necessary and sufficient to trigger an insulinoma (Wang et al. 2017). KDM6A-deficient pancreatic adenocarcinomas show an increased mTORC1 signaling (Revia et al. 2022) and the activation of the noncanonical p38-dependent activin A pathway (Yi et al. 2022).

KDM6A regulates the expression of the tumor suppressor PTEN. KDM6A promotes PTEN expression and eventually decreases AKT and mTOR phosphorylation (Jiang et al. 2020). Interestingly, somatic or germline PI3K/AKT/mTOR signaling pathway defects have been associated with overgrowth and hypoglycemia (Maines et al. 2021) (Figure 2A).

Evidence on KMT2D gene

Differentiated α-cells exhibit a higher density of H3K4me3/H3K27me3 marks than β-cells or exocrine cells (Bramswig et al. 2013). An unspecific histone methyltransferase inhibitor may induce a partial reprogramming of α to β cell fate in cultured pancreatic islets (Bramswig et al. 2013). Therefore, KMT2D LOF could promote the differentiation towards β-cells via the reduction of H3K4me3. This hypothesis has been confirmed in an experimental model of pancreatic regeneration, where the reduction of H3K4me3 marks increased β-cell proliferation (Dhawan et al. 2009) (Figure 2B).

The functional relevance of H3K4 methylation marks in HH beyond the endocrine pancreatic differentiation is still debated. Moreover, evidences on H3K4 mark effects on glucose stimulated insulin secretion (GSIS) are conflicting.

Campbell et al. (2021) have found an elevated fed and fasting glycemia and impaired glucose tolerance in mice whose histone methyltransferase activity was abolished in the endocrine progenitors. The transcriptome analysis enlightened a downregulation of genes involved in GSIS and an upregulation of genes typical of immature β-cell. The authors concluded that H3K4 trimethylation might be necessary for the maturation of pancreatic islet endocrine cells

(Campbell et al. 2021). Indeed, H3K4me3 marks control the expression of genes such as Ins1/2 and Glut2, crucial for the maturation of endocrine cells (Chakrabarti et al. 2003; Maganti et al. 2015).

On the contrary, the lysine demethylase inhibitor GSK-J4, increasing the level of H3K4 methylation, improved β-cell function in mice and reduced the cytokine-induced destruction of β-cells (Backe et al. 2019). In particular, GSK-J4 ameliorated GSIS, both *in vitro* and *in vivo* (Backe et al. 2018, 2019). An increased expression of olfactory and glutamate receptors in β-cells, both boosting insulin secretion, may explain this outcome (Backe et al. 2019).

Pacelli et al. (2020) have characterized KMTD2 knockout mouse embryonic fibroblasts and fibroblasts from KS patients harboring KMT2D heterozygous pathogenic variants. The authors demonstrated a significant reduction of the mitochondrial oxygen consumption rate without any compensatory increase in the glycolytic flux (Koutsioumpa et al. 2019). Unfortunately, no studies have been conducted on mature β-cells.

Increased glycolysis, lactate production, and glucose uptake have been documented in pancreatic cancer cells harboring LOF mutations of KMT2D (Koutsioumpa et al. 2019). KMT2D silencing increases the expression of SLC2A1 (glucose transporter-1, GLUT-1) and SLC2A3 (Solute Carrier Family 2 Member 3, GLUT-3), suggesting an increase in glucose metabolism (Koutsioumpa et al. 2019) (Figure 2B).

Discussion

The present review summarizes the potential mechanisms underlying the HH observed in both KDM6A- and KMT2D-KS (Figure 3).

Current evidence for KS, including clinical data, is stronger for the KDM6A gene. Haploinsufficiency of KDM6A has also been linked to HH in Turner

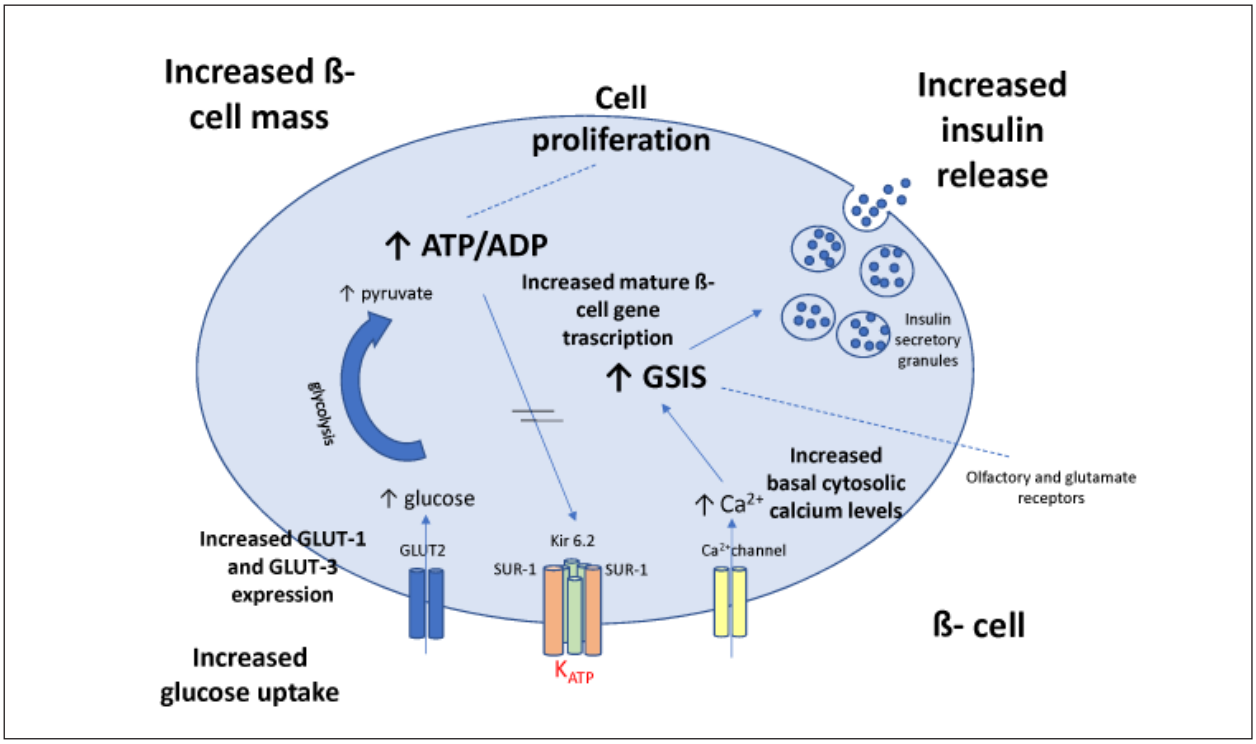


Figure 3. Summary of cellular pathways involved in deregulated insulin secretion of KMD6A/KMT2D - hyperinsulinemic hypoglycemia (HH). Physiology of insulin secretion: blood glucose is taken up by glucose transporter 2 (GLUT2). Upon phosphorylation by glucokinase, glucose is converted in glucose-6 phosphate and enters the glycolytic flux. Pyruvate is the terminal product of the pathway. The mitochondrial oxidation of pyruvate generates ATP. Such increased cytosolic ATP concentration triggers the closure KATP. Eventually, this induces the depolarization of the β-cell membrane. This change in potential opens voltage-dependent calcium channels (Ca^{2+} channel) and leads to calcium influx. The subsequent increase in the cytosolic Ca^{2+} concentration stimulates the release of insulin (Maiorana et al. 2017). The glycolysis pathway is also required for the production of biomass needed for mitosis and cell proliferation.

syndrome (Gibson et al. 2018). Nevertheless, the sex distribution did not significantly differ between patients with KS and HH and KS patients without HH (females 66.7% vs. males 52.0%, p=0.207) (Hoermann et al. 2020). In addition, the phenotypic features of KS patients with HH due to mutations in either KMT2D or KMD6A did not present marked differences. In particular, the onset of hypoglycemia and the responsiveness to diazoxide did not significantly differ between KS-children with pathogenic variants in KDM6A and KMT2D (Hoermann et al. 2020).

The increased risk of HH in KMD6A-KS patients may arise from the strong impact of KDM6A on insulin release in mature β-cells. Pathogenic mechanisms downstream or independent of KATP channel (Backe et al. 2018; Gibson et al. 2018), and activation or repression of target gene transcription are plausible mechanisms (Lien et al. 2021).

The observation that KDM6A and KMT2D are among the most frequently mutated epigenetic regulators in cancer strongly supports the direct role of both genes in the β-cell proliferation (Wang et al. 2017; Koutsioumpa et al. 2019). Increased glycolysis in aerobic conditions, known as the Warburg effect, is a major characteristic of cancer cells. Such aerobic glycolysis confers a proliferation advantage by producing ATP and glucose-derived metabolites necessary for the biosynthesis of nucleotides, lipids, and proteins (Ma et al. 2021). Interestingly, KMT2D-deficient cells are characterized by an increased expression of glucose transporter GLUT-3 and GLUT-1 and a glucose uptake independent glycolysis (Koutsioumpa et al. 2019). Further studies are needed to understand whether the alteration of pancreatic physiology is a direct effect of KDM6A/KMT2D loss of function or is a secondary compensatory mechanism. Increasing evidence suggests that multifactorial external stimuli lead to changes in the epigenetic modification of genes and transportable elements in cancer (Tzika et al. 2018).

A deeper understanding of the pathogenic mechanisms underlying HH in Kabuki syndrome may open new therapeutic opportunities exploiting epigenetic modifiers. The fifth multistakeholder Pediatric Strategy Forum recently focused on epigenetic modifier therapies for children and adolescents with cancer. It underlined that targeting epigenetic mechanisms will become an essential therapeutic approach. Eight classes of drugs were discussed and prioritized based on the current level of science to support an early evaluation in children, including inhibitors of EZH2 (Pearson et al. 2020). In gastroenteropancreatic neuroendocrine tumors, inhibitors of DNA methyltransferase (azacytidine) and histone deacetylation (butyrate, valproic acid, trichostatin A and MS-275) elicited growth stop, apoptosis, cell cycle arrest, and expression of neuroendocrine markers *in vitro* (Larsson 2013).

If heterochromatin plays a role in KS pathogenesis, agents that promote open chromatin states, such as histone deacetylase inhibitors (HDACis), lysine demethylase inhibitors or promoters may be potential novel treatments also for KS patients.

In particular, beta-hydroxybutyrate (BHB), a ketone body that is the natural end-product of hepatic fatty acid beta-oxidation, retains a histone deacetylation inhibitor activity. Benjamin et al. (2017) have demonstrated that treatment with a ketogenic diet (KD) for 2 weeks in a mouse model of KS normalizes the global histone modification state and corrects the deficiency of neurogenesis observed in the granule cell layer of the dentate gyrus. Histone deacetylase inhibition also rescues structural and functional brain deficits in a mouse model of KS (Bjornsson et al. 2014). These observations open the horizon that dietary manipulation, including a KD, may be a feasible strategy for KS.

Based on the analogies between HH and GLUT1 deficiency, both presenting with neuroglycopenia and a lack of alternative cerebral energy sources, KD has already been tried in patients with drug-unresponsive HH due to activating glucokinase (GCK) mutations. In these patients, KD determined the recovery from epilepsy and intellectual disabilities and averted the need for a near-total pancreatectomy (Maiorana et al. 2021). However, ketone bodies act not only as energy substrates but also as signaling molecules. The histone deacetylase inhibition activity of BHB links hepatic lipid metabolism and epigenetics (Longo et al. 2019). The mechanisms of action of KD in epilepsy have been recently revealed, and includes an epigenetic modulation via an increase of the adenosine level in the brain and the consequent inhibition of DNA methylation (Chen et al. 2019).

Our increasing knowledge of the relationship between epigenetic switches and metabolism will surely help addressing some human diseases in the near future (Bjornsson et al. 2014; Benjamin et al. 2017; Kaimala et al. 2022). Nevertheless, many issues are still obscure. The expression of genes involved in epigenetic modifications is tissue- and cell type-specific, and the downstream events of KMT2D/KM6DA deficiency could be different depending on tissue, cell type (Xie et al. 2013), differentiation stages (Xie et al. 2013; Kalisz et al. 2020; Boniel et al. 2021),

and age (Kalisz et al. 2020). Furthermore, signaling *in vitro* and *in vivo* may differ because of the different environments or cell type (endocrine cells derived vs. functionally mature cells differentiated *in vivo*) (Xie et al. 2013). Finally, many observations came from the study of endocrine tumors. Cancer cells may jeopardize the experimental outcome: some pathways may be restricted only to cancer cells (Revia et al. 2022).

Conclusions

Understanding the molecular mechanisms of hyperinsulinism in KS may provide a valuable insight into the normal physiological mechanisms regulating insulin release and the cellular pathogenetic mechanisms associated with hyperinsulinism. Moreover, the characterization of the mechanisms related to HH in KMT2D- and KMD6A-KS signatures may provide the rationale for developing of new therapeutic strategies based on the epigenetics. Epigenetic modifiers, including a ketogenic diet, hold promising therapeutic potentiality in KS. As epigenetic modifiers are relatively early in the development in pediatrics, there is a clear opportunity to shape the landscape of therapies targeting the epigenome.

Strong evidence supports the role of KMT2D and KDM6A in driving the β-cell differentiation. Nevertheless, how pathogenetic variants in KDM6A and KDM2D genes perturb insulin release is not fully clarified yet. Mouse models and large cohorts of KS patients with HH are necessary to investigate the molecular mechanisms leading to HH, as current findings on this topic remain still limited.

Conflict of interest: *Authors declare no conflict of interest.*

References

Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, Kawame H, Lanpher BC, Lindsley AW, Merla G, Miyake N, Okamoto N, Stumpel CT, Niikawa N; Kabuki Syndrome Medical Advisory Board. Kabuki syndrome: International consensus diagnostic criteria. J Med Genet 56, 89–95, 2019.

Backe MB, Andersson JL, Bacos K, Christensen DP, Hansen JB, Dorosz JJ, Gajhede M, Dahlby T, Bysani M, Kristensen LH, Ling C, Olsen L, Mandrup-Poulsen T. The lysine demethylase inhibition protects pancreatic beta cells from apoptosis and improves beta cell function. Mol Cell Endocrinol 460, 47–56, 2018.

Backe MB, Jin C, Andreone L, Sankar A, Agger K, Helin K, Madsen AN, Poulsen SS, Bysani M, Bacos K, Ling C, Perone MJ, Holst B, Mandrup-Poulsen T. The lysine demethylase KDM5B regulates islet function and glucose homeostasis. J Diabet Res 2019, 5451038, 2019.

Benjamin JS, Pilarowski GO, Carosso GA, Zhang L, Huso DL, Goff LA, Vernon HJ, Hansen KD, Bjornsson HT. A ketogenic diet rescues hippocampal memory defects in a mouse model of Kabuki syndrome. Proc Natl Acad Sci U S A 114, 125–130, 2017.

Bjornsson HT, Benjamin JS, Zhang L, Weissman J, Gerber EE, Chen YC, Vaurio RG, Potter MC, Hansen KD, Dietz HC. Histone deacetylase inhibition rescues structural and functional brain deficits in a mouse model of Kabuki syndrome. Sci Transl Med 6, 256ra135, 2014.

Boniel S, Szymanska K, Smigiel R, Szczaluba K. Kabuki syndrome – clinical review with molecular aspects. Genes (Basel) 12, 468, 2021.

Bramswig NC, Everett LJ, Schug J, Dorrell C, Liu C, Luo Y, Streeter PR, Naji A, Grompe M, Kaestner KH. Epigenomic plasticity enables human pancreatic α to β cell reprogramming. J Clin Invest 123, 1275–1284, 2013.

Campbell SA, Begin J, McDonald CL, Vanderkruk B, Stephan TL, Hoffman BG. 3H3K4 trimethylation is required for postnatal pancreatic endocrine cell functional maturation. Diabetes 70, 2568–2579, 2021.

Chakrabarti S, Francis J, Ziesmann SM, Garmey JC, Mirmira RG. Covalent histone modifications underlie the developmental regulation of insulin gene transcription in pancreatic beta cells. J Biol Chem 278, 23617–23623, 2003.

Chen F, He X, Luan G, Li T. Role of DNA methylation and adenosine in ketogenic diet for pharmacoresistant epilepsy: Focus on epileptogenesis and associated comorbidities. Front Neurol 10, 119, 2019.

Demirbilek H, Hussain K. Congenital hyperinsulinism: diagnosis and treatment update. J Clin Res Pediatr Endocrinol 9, 69–87, 2017.

Dhawan S, Tschen SI, Bhushan A. Bmi-1 regulates the Ink4a/Arf locus to control pancreatic β-cell proliferation. Genes Dev 23, 906–911, 2009.

Faundes V, Goh S, Akilapa R, Bezuidenhout H, Bjornsson HT, Bradley L, et al. Clinical delineation, sex differences, and genotype-phenotype correlation in pathogenic KDM6A variants causing X-linked Kabuki syndrome type 2. Genet Med 23, 1202–1210, 2021.

Froimchuk E, Jang Y, Ge K. Histone H3 lysine 4 methyltransferase KMT2D. Gene 627, 337–342, 2017.

Galcheva S, Demirbilek H, Al-Khawaga S, Hussain K. The genetic and molecular mechanisms of congenital hyperinsulinism. Front Endocrinol (Lausanne) 10, 111, 2019.

Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, Bhatti T, Bamba V, Adzick NS, De Leon DD, Ganguly A, Stanley CA. Congenital hyperinsulinism in infants with Turner syndrome: possible association with monosomy X and KDM6A haploinsufficiency. Horm Res Paediatr 89, 413–422, 2018.

Gole H, Chuk R, Coman D. Persistent hyperinsulinism in Kabuki syndrome 2: case report and literature review. Clin Pract 6, 848, 2016.

Heinemann B, Nielsen JM, Hudlebusch HR, Lees MJ, Larsen DV, Boesen T, Labelle M, Gerlach LO, Birk P, Helin K. Inhibition of demethylases by GSK-J1/J4. Nature 514, E1-E2, 2014.

Hemming S, Cakouros D, Isenmann S, Cooper L, Menicanin D, Zannettino A, Gronthos S. EZH2 and KDM6A act as an epigenetic switch to regulated mesenchymal stem cell lineage specification. Stem Cells 32, 802–815, 2014.

Hoermann H, El-Rifai O, Schebek M, Lodefalk M, Brusgaard K, Bachmann N, Bergmann C, Roeper M, Welters A, Salimi Dafsari R, Blankenstein O, Mayatepek E, Christesen H, Meissner T, Kummer S. Comparative meta-analysis of Kabuki syndrome with and without hyperinsulinaemic hypoglycaemia. Clin Endocrinol (Oxf) 93, 346–354, 2020.

Jiang W, Wang J, Zhang Y. Histone H3K27me3 demethylases KDM6A and KDM6B modulate definitive endoderm differentiation from human ESCs by regulating WNT signaling pathway. Cell Res 23, 122–130, 2013.

Jiang Q, Huang X, Hu X, Shan Z, Wu Y, Guangming Wu G, Lei L. Histone demethylase KDM6A promotes somatic cell reprogramming by epigenetically regulating the PTEN and IL-6 signal pathways. Stem Cells 38, 960–972, 2020.

Kaimala S, Kumar CA, Allouh MZ, Ansari SA, Emerald BS. Epigenetic modifications in pancreas development, diabetes, and therapeutics. Med Res Rev 42, 1343–1371, 2022.

Kalisz M, Bernardo E, Beucher A, Maestro MA, Del Pozo N, Millán I, Haeberle L, Schlensog M, Safi SA, Knoefel WT, Grau V, de Vas M, Shpargel KB, Vaquero E, Magnuson T, Ortega S, Esposito I, Real FX, Ferrer J HNF1A recruits KDM6A to activate differentiated acinar cell programs that suppress pancreatic cancer. EMBO J 39, e102808, 2020.

Kostopoulou E, Shah P. Hyperinsulinaemic hypoglycaemia – an overview of a complex clinical condition. Eur J Pediatr 178, 1151–1160, 2019.

Koutsioumpa M, Hatziapostolou M, Polytarchou C, Tolosa EJ, Almada LL, Mahurkar-Joshi S, Williams J, Tirado-Rodriguez AB, Huerta-Yepez S, Karavias D, Kourea H, Poultsides GA, Struhl K, Dawson DW, Donahue TR, Fernandez-Zapico ME, Iliopoulos D. The histone lysine methyltransferase 2D regulates pancreatic carcinogenesis through metabolic reprogramming. Gut 68, 1271–1286, 2019.

Kuroki Y, Suzuki Y, Chyo H, Hata A, Matsui I. A new malformation syndrome of long palpebral fissures, large ears, depressed nasal tip, and skeletal anomalies associated with postnatal dwarfism and mental retardation. J Pediatr 99, 570–573, 1981.

Larsson C. Epigenetic aspects on therapy development for gastroenteropancreatic neuroendocrine tumors. Neuroendocrinology 97, 19–25, 2013.

Lien YC, Lu XM, Won KJ, Wang PZ, Osei-Bonsu W, Simmons RA. The Transcriptome and epigenome reveal novel changes in transcription regulation during pancreatic rat islet maturation. Endocrinology 162, bqab181, 2021.

Lintas C, Persico AM. Unraveling molecular pathways shared by Kabuki and Kabuki-like syndromes. Clin Genet 94, 283–295, 2018.

Longo R, Peri C, Cricri D, Coppi L, Caruso D, Mitro N, De Fabiani E, Crestaniet M. Ketogenic Diet: A new light shining on old but gold biochemistry. Nutrients 9, 11, 2497, 2019.

Ma R, Wu Y, Li S, Yu X. Interplay between glucose metabolism and chromatin modifications in cancer. Front Cell Dev Biol 9, 654337, 2021.

Maganti AV, Maier B, Tersey SA, Sampley ML, Mosley AL, Ozcan S. Transcriptional activity of the islet beta cell factor Pdx1 is augmented by lysine methylation catalyzed by the methyltransferases Set 7/9. J Biol Chem 290, 9812–9822, 2015.

Maines E, Franceschi R, Martinelli D, Soli F, Lepri FR, Piccoli G, Soffiati M. Hypoglycemia due to PI3K/AKT/mTOR signaling pathway defects: two novel cases and review of the literature. Hormones (Athens) 20, 623–640, 2021.

Maiorana A, Dionisi-Vici C. Hyperinsulinemic hypoglycemia: clinical, molecular and therapeutical novelties. J Inherit Metab Dis 40, 531–542, 2017.

Maiorana A, Caviglia S, Greco B, Alfieri P, Cumbo F, Campana C, Bernabei SM, Cusmai R, Mosca A, Dionisi-Vici C. Ketogenic diet as elective treatment in patients with drug-unresponsive hyperinsulinemic hypoglycemia caused by glucokinase mutations. Orphanet J Rare Dis 16, 424, 2021.

Niikawa N, Matsuura N, Fukushima Y, Ohsawa T, Kajii T. Kabuki make-up syndrome: a syndrome of mental retardation, unusual facies, large and protruding ears, and postnatal growth deficiency. J Pediatr 99, 565–569, 1981.

Pacelli C, Adipietro I, Malerba N, Squeo GM, Piccoli C, Amoresano A, Pinto G, Pucci P, Lee JE, Ge K, Capitanio N, Merla G. Loss of function of the gene encoding the histone methyltransferase KMT2D leads to deregulation of mitochondrial respiration. Cells 9, 1685, 2020.

Pearson AD, Stegmaier K, Bourdeaut F, Reaman G, Heenen D, Meyers ML, et al. Paediatric Strategy Forum for medicinal product development of epigenetic modifiers for children: ACCELERATE in collaboration with the European Medicines Agency with participation of the Food and Drug Administration. Eur J Cancer 139, 135–148, 2020.

Quilichini E, Haumaitre C. Implication of epigenetics in pancreas development and disease. Best Pract Res Clin Endocrinol Metab 29, 883–898, 2015.

Revia S, Seretny A, Wendler L, Banito A, Eckert C, Breuer K, et al. Histone H3K27 demethylase KDM6A is an epigenetic gatekeeper of mTORC1 signalling in cancer. Gut 71, 1613–1628, 2022.

Sharma S, Gurudutta G. Epigenetic regulation of hematopoietic stem cells. Int J Stem Cells 9, 36–43, 2016.

Subbarayan A, Hussain K. Hypoglycemia in Kabuki syndrome. Am J Med Genet A. 164A, 467–471, 2014.

Tran N, Broun A, Ge K. Lysine demethylase KDM6A in differentiation, development, and cancer. Mol Cell Biol 40, e00341–20, 2020.

Tzika E, Dreker T, Imhof A. Corrigendum: Epigenetics and metabolism in health and disease. Front Genet 2020; 11:428. Erratum for: Front Genet 9, 361, 2018.

van Arensbergen J, Garcia-Hurtado J, Moran I, Maestro MA, Xu X, Van de Casteele M, Skoudy AL, Palassini M, Heimberg H, Ferrer J. Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. Genome Res 20, 722–732, 2010.

Waddington CH. The epigenotype. 1942. Int J Epidemiol 41, 10–13, 2012.

Wang H, Bender A, Wang P, Karakose E, Inabnet WB, Libutti SK, et al. Insights into beta cell regeneration for diabetes via integration of molecular landscapes in human insulinomas. Nat Commun 8, 767, 2017.

Xie R, Everett LJ, Lim HW, Patel NA, Schug J, Kroon E, Kelly OG, Wang A, D'Amour KA, Robins AJ, Won KJ, Kaestner KH, Sander M. Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. Cell Stem Cell 12, 224–237, 2013.

Xu CR, Li LC, Donahue G, Ying L, Zhang YW, Gadue P, Zaret KS. Dynamics of genomic H3K27me3 domains and role of EZH2 during pancreatic endocrine specification. EMBO J 33, 2157–2170, 2014.

Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml PJ, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of Kabuki syndrome: clinical and molecular characterization of 10 affected individuals. Genet Med 21, 233–242, 2019.

Yi Z, Wei S, Jin L, Jeyarajan S, Yang J, Gu Y, et al. KDM6A regulates cell plasticity and pancreatic cancer progression by noncanonical activin pathway. Cell Mol Gastroenterol Hepatol 13, 643–667, 2022.