The original uploaded version contained characterization of the zebrafish phenotype from kmt2d and kdm6a/al mutants. These lines were lost during the covid shutdown, and upon revision for publication we were unable to validate these mutants or obtain another allele. Therefore we removed these data from this revised version of the manuscript.**

The role of Kabuki Syndrome genes KMT2D and KDM6A in development: Analysis in Human sequencing data and compared to mice and zebrafish

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Abstract

*KMT2D* and *KDM6A* are epigenetic regulators that have been implicated in Kabuki Syndrome, a rare congenital birth defect with multiple tissue and organ abnormalities, including craniofacial and heart defects. Our previous study identified human families with mutations in the epigenetic modifiers *KMT2D* and *KDM6A*, which is implicated in 32% and 10% of Kabuki Syndrome patients respectively. To understand the connection to Kabuki syndrome patients, and the transcriptional targets of *KMT2D* and *KDM6A* in humans, we performed RNA sequencing (seq) of lymphoblastoid cells from Kabuki Syndrome patients carrying mutations in *KMT2D* and *KDM6A*. We identified 1995 significant changes in transcriptional targets for *KMT2D* and 1917 for *KDM6A*, as compared to control. When compared with RNA-seq datasets obtained from other mouse and zebrafish studies, our analysis revealed *KMT2D* mutations affect the expression of 76 orthologous genes across all three datasets. Similariy, *KDM6A* affects the expression of 7 orthologous genes across three datasets. Despite the differences in cell types, stages, and species in the comparison between the transcriptomic datasets, there are common gene expression changes associated with *KMT2D* and *KDM6A* mutations. qPCR on novel zebrafish mutants confirmed the differentially expression in *KMT2D* or *KDM6A* mutant backgrounds. Taken together, our results show that *KMT2D* and *KDM6A* regulate common and unique genes across humans, mice, and zebrafish for early craniofacial and cardiac development and this information contributes to the understanding of epigenetic dysregulation during development of Kabuki syndrome.

Running Title:

Kabuki gene expression across zebrafish, mice, and humans
Key words:
Cardiac development, Kabuki Syndrome, epigenetics, cardiac neural crest cells, RNA sequencing, zebrafish, mice, human

Introduction

Kabuki syndrome is a rare congenital abnormality with characteristic facial features superficially similar to the makeup used by actors in traditional Japanese theatre where the name arises. The craniofacial malformation results from defects in the eyelid tears, arched and broad eyebrows, depressed nasal tip, and prominent or cupped ears (Adam et al., 2019). These patients also present with cardiac issues as well as growth defects such as short stature and microcephy. Approximately 28-80 % of Kabuki Syndrome patients are afflicted with congenital heart defects (Digilio et al., 2017). Common heart defects include atrial and ventricular septal defects, bicuspid aortic valves, aortic coarctation, double outlet right ventricle, transposition of great arteries, infundibular pulmonary stenosis, dysplastic mitral valve, Tetralogy of Fallot, etc. (Cocciadiferro et al., 2018; Digilio et al., 2017; Shangguan et al., 2019; Yap et al., 2020).

The combination of defects observed in Kabuki Syndrome are similar to those observed in embryos with deficiencies in neural crest cells (NCCs), a multipotent population of cells that give rise to craniofacial cartilage and bone, cardiac outflow track as well as multiple other derivatives. NCCs form early in development as part of the central nervous system, and then migrate long distances to begin a differentiation program. Because they undergo multiple morphogenetic and cellular processes, NCCs rely on a precise gene and epigenetic regulatory network for their development. Several epigenetic processes are important for development including DNA methylation (Serra-Juhe et al., 2015), histone modifications (Zaidi et al., 2013; Q. J. Zhang & Liu, 2015), chromatin remodeling (Bruneau, 2010; Delgado-Olguin, Takeuchi, & Bruneau, 2006), and microRNA (Toni, Hailu, & Sucharov, 2020) and contribute to the etiology of developmental disorders. Because of the ability to transcriptionally regulate many genes at the same time, defects in epigenetic regulators help to explain why patients with the same genotype and syndrome often have large variations in phenotype, penetrance, and severity. Indeed, mutations in chromatin
remodeler Chd7 and histone acetyltransferase Kat6a/b are associated with birth defects leading to Charge and Ohdo syndromes, respectively (Vissers et al., 2004) (Campeau et al., 2012).

*KMT2D*, also known as MLL2 methylates histone 3 at lysine 4 (H3K4) which is associated with transcriptional repression, and *KDM6A*, demethylates histone 3 at lysine 27 (H3K27), mostly associated with transcriptional activation (Ali, Hom, Blakeslee, Ikenouye, & Kutateladze, 2014; Hong et al., 2007; Koutsoumpa et al., 2019; Lan et al., 2007), both genes are frequently mutated in patients with Kabuki Syndrome (Cocciadiferro et al., 2018; Gazova, Lengeling, & Summers, 2019; Luperchio, Applegate, Bodamer, & Bjornsson, 2019; Shangguan et al., 2019; Tekendo-Ngongang, Kruszka, Martinez, & Muenke, 2019; Yap et al., 2020) (Ang et al., 2016; Lee, Lee, & Lee, 2012; Schwenty-Lara, Nurnberger, & Borchers, 2019; Serrano, Demarest, Tone-Pah-Hote, Tristani-Firouzi, & Yost, 2019). *KMT2D* or lysine-specific methyltransferase 2D, is also known as *MLL2* or myeloid/lymphoid or mixed-lineage leukemia 2. It belongs to a family of 7 SET1-like histone methyltransferases (Ali et al., 2014). KMT2D contains a SET domain for histone methylation, PHD and coiled-coil domains, and zinc finger domains (Ali et al., 2014). The PHD domain of zebrafish *kmt2d*, which shares 86.1% identity with human *KMT2D* (Figure 1A).

Recent studies have significantly contributed to our understanding of cardiac development and function as regulated by *KMT2D* (Ang et al., 2016; Schwenty-Lara, Nurnberger, et al., 2019; Serrano et al., 2019), and *KDM6A* (Lee et al., 2012). The PHD domain of zebrafish *kmt2d*, which shares 86.1% identity with human *KMT2D*, was targeted by CRISPR-mediated gene disruption in a recent study on *kmt2d* in heart development (Serrano et al., 2019). The study generated 3 alleles with premature stop codons that cause a truncation of the PHD domain (Serrano et al., 2019) and categorized phenotypes, including heart edema and microcephaly, and a shorter body axis (Serrano et al., 2019). They report a robust Notch signaling hyperactivation in endocardial and endothelial cells that affect vasculogenesis and angiogenesis, and a rescue of cardiovascular phenotype by pharmacological inhibition of Notch signaling (Serrano et al., 2019). Another recent study on *kmt2d* showed morpholino-based knockdown of *kmt2d* in *Xenopus* causes heart defects (Schwenty-Lara, Nurnberger, et al., 2019), and impairs NCC development (Schwenty-Lara, Nehl, & Borchers, 2019). In mouse studies, development of limbs, palate, central nervous system, and heart are impaired upon mutating *Kmt2d*, with hypoplasia in frontonasal bone, fully penetrant cleft palate, mandible hypoplasia, deficits in palatal shelf elevation and cranial base ossification.
(Fahrner et al., 2019; Shpargel, Mangini, Xie, Ge, & Magnuson, 2020). Further, disruption of Kmt2d show disorganized interventricular septum, hypoplasia of compact myocardium indicating decreased cardiomyocyte proliferation and failure of outflow tract septation, suggesting a role of Kmt2d in cardiac development (Ang et al., 2016).

KDM6A (and kdm6l in zebrafish) or Lysine Demethylase 6A, is also known as UTX or Ubiquitously transcribed tetratricopeptide repeat, X chromosome. UTX, UTY and JMJD3 comprise a subfamily of proteins containing JmjC-domain (Jumonji C) and zinc fingers, and they are evolutionarily conserved from Caenorhabditis elegans to human (Klose, Kallin, & Zhang, 2006; Lan et al., 2007; Shi & Whetstine, 2007). The JmjC-domain is associated with histone demethylation (Accari & Fisher, 2015), while the tetratricopeptide repeats at N-terminal regions of UTX and UTY are predicted protein interaction motifs (Lan et al., 2007). It is important to note that KDM6a or UTX is an X-linked gene, with 2 copies in females (XX) and 1 copy in males (XY), where the Y chromosome has the homolog, UTY (Itoh et al., 2019). Studies indicate that UTY and UTX diverged from a common ancestor, yet the functional role of UTY in histone demethylation needs further investigation, because some studies show that it has a lower histone demethylation activity than UTX (Faralli et al., 2016; Hong et al., 2007; Itoh et al., 2019; Lan et al., 2007; Shpargel, Starmer, Wang, Ge, & Magnuson, 2017; Shpargel, Starmer, Yee, Pohlers, & Magnuson, 2014; Walport et al., 2014).

To determine the effect of patient mutations on the transcriptome, we performed RNA-seq analysis on human patient lymphoblastoid cells (LBCs). We determined that 1995 genes were differentially regulated for KMT2D, and 1917 for KDM6A, as compared to control LBCs. We then compared common gene targets of KMT2D and KDM6A from published zebrafish, mice, with our human RNA-seq datasets in order to prioritize downstream genes for further study. Our results reveal that some of the genes which are involved in cardiogenesis, cardiac function, vasculature, neurogenesis, and overall development, are regulated by KMT2D and KDM6A across zebrafish, mice, and humans. Our in vivo analysis in CRISPR-mutated zebrafish indicate that kmt2d and kdm6a regulate genes involved in early embryonic, cardiac, and NCC development. Our results support the idea that mutations in zebrafish KMT2D and KDM6A have defects similar to Kabuki Syndrome patients, supporting previous studies in the field, identifies targets in human patients LBCs, and presents a pan-species perspective of the roles of KMT2D and KDM6A in development.
Materials and Methods

Human Cell Samples

Patient-derived lymphoblastoid cell lines (LCLs) used in this study were previously generated from KS patients with mutations in KMT2D/MLL2 and KDM6A (Van Laarhoven et al. 2015). Control LCLs from age and demography-matched subjects, were obtained from the Coriell Institute for Medical Research https://www.coriell.org/

RNAseq of human Kabuki Syndrome patients and comparison across taxa

Human LCLs from KMT2D and KDM6A patients and controls, as mentioned above, were grown to a count of $10^6$ and pelleted. RNA was extracted by Zymo Research Direct-zol™ RNA MiniPrep kit (Catalog #R2050). Two cDNA libraries for each genotype (e.g. wildtype, KMT2D mutant, KDM6A mutant) were prepared from separate RNA MiniPreps using the Nugen mRNA kit for Illumina sequencing. Sequencing was performed on an Illumina NovaSEQ6000 (150bp paired-end reads) at the Genomics and Microarray Shared Resource Core Facility at University Of Colorado Denver Cancer Center. Libraries were sequenced to an average depth of 42 million reads. Reads were aligned to the human genome (hg38) using STAR Aligner (v2.7.3a) (Dobin et al., 2013), and gene counts were computed from STAR using quant mode (Dobin et al., 2013). Differential expression was performed in R using Deseq2 (Love, Huber, & Anders, 2014).

We compared our human RNAseq data to previously published datasets of KMT2D and KDM6A mutants from mice and zebrafish (Ang et al., 2016; Fahrner et al., 2019; Itoh et al., 2019; Lei & Jiao, 2018; Serrano et al., 2019; Shpargel et al., 2017). RNAseq datasets were downloaded from the NCBI Gene Expression Omnibus. Datasets included GSE129365 (Kmt2d...
mouse chondrocytes, (Fahrner et al., 2019)), GSE103849 (Kdm6a mouse neural crest cells, (Shpargel et al., 2017), GSE121703 and GSE128615 (mouse Cd4 cells, (Itoh et al., 2019)), GSE110392 (mouse neural stem cells, (Lei & Jiao, 2018)). In addition an RNAseq dataset of whole embryo zebrafish Kmt2d mutants was provided by Serrano et al. (Serrano et al., 2019).

Where necessary, downloaded RNAseq datasets were re-analyzed using DESeq2 for differential expression.

To identify potential core genes regulated by Kmt2d and Kdm6a we found the intersection set of genes differentially expressed at a nominal p-value of 0.05 in each dataset for all Kmt2d and Kdm6a RNAseq datasets respectively. To do this, we used BioMart as implemented in R to convert non-human gene symbols into orthologous human ensemble identifiers, while allowing multiple mappings to account for gene duplications. Intersection sets were computed from these human ensemble identifiers.

Statistical Analysis

Data shown are the means ± SEM from the number of samples or experiments indicated in the figure legends. All assays were repeated at least three times with independent samples. $P$ values were determined with Student’s $t$ tests using GraphPad Prism.

Results

Comparative analyses of the transcriptome of KS patients reveal common targets of $KMT2D$ and $KDM6A$

To translate our results into the context of human birth defects, we investigated the transcriptome of 2 human Kabuki Syndrome patients carrying mutations in $KMT2D$ and $KDM6A$, by RNA sequencing of their lymphoblastoid cells (LBCs). The patient with $KMT2D$ mutation has a transition c.4288T>C (p.C1430R) (Figure 1A) and the patient with $KDM6A$ mutation has a microdeletion in chromosome X resulting in a deletion of $KDM6A$ (Figure 1A). Both mutations
are predicted to result in a loss of function mutation. As controls, we used LBCs from age and ethnicity matched individuals. To ascertain differential gene expression, generated reads from RNA-seq were mapped to the human genome using STAR aligner (Dobin et al., 2013) and differential expression was performed in R with DESeq2. Differentially expressed genes (nominal p value <= 0.05) from KMT2D and KDM6A showed 1995 and 1917 differentially expressed genes as compared to control LBCs. Gene Ontology analysis suggests that many of these genes regulate pathways as presented in Table 1, consistent with both source and timing of cells that were sequenced.

We asked whether gene expression changes observed in our human RNA-seq datasets of KMT2D and KDM6A mutations are similar to those observed in datasets from the experimental model systems mouse and zebrafish. To do this, we compared RNA sequencing datasets for Kmt2d-mutant mice and zebrafish generated by other researchers (see methods) to our human dataset. For mice, the RNA sequencing dataset is derived from embryonic hearts (Ang et al., 2016), and the zebrafish data are derived from whole embryos (Serrano et al., 2019). Likewise, our KDM6A human RNA seq datasets were compared to three distinct RNA seq datasets of mice that are mutant for Kdm6a. The mice datasets are derived from NCCs (Shpargel et al., 2017), neural stem cells (Lei & Jiao, 2018), and CD4+ cells (Itoh et al., 2019). For KDM6A, the comparison is only between humans and mice, since zebrafish RNA sequencing datasets for kdm6a mutation are not available.

For each, we asked what genes are differentially expressed in all three datasets. A comparison between KMT2D datasets from 3 species revealed 76 orthologous genes differentially expressed in all three datasets (Figure 1B). Heat map analysis shows that 30 of the 76 common targets are either upregulated or downregulated in a similar pattern across the 3 species, while 46 do not share a similarity in expression patterns among the 3 species (Figure 1C). The dissimilarity is likely due to differences in cell type and age. For KDM6A, 7 common targets were found between human and the 3 mice datasets (Figure 1A). The variation in the number of common targets between KMT2D and KDM6A datasets could partly be due to the differences in the roles and targets of KMT2D and KDM6A, as well as functional redundancy of other family members across species (Akerberg, Henner, Stewart, & Stankunas, 2017; Crump & Milne, 2019; Fellous, Earley, & Silvestre, 2019; Nottke, Colaiacovo, & Shi, 2009).
Despite the dissimilarity among datasets, genes identified in these analyses may represent a set of genes commonly regulated by KMT2D and KDM6A across tissue types and taxa. We identified several genes based on known roles in regulating development with special focus on genes that affect development of craniofacial, cardiac, or NCC development including KMT2D datasets are SOX12, SLC22A23, FOXP4, NR2F6, and SIX3. The genes for KDM6A datasets are ID3, PRDM1, and ANXA4.

<table>
<thead>
<tr>
<th>Genes common to KMT2D datasets</th>
<th>Table 1: Characteristics relevant to cardiac and overall development</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX12</td>
<td>Expressed mice outflow tract 12.5-16.5 dpc (days post coitum), Sox4+/−/11+/−/12+/− mice have common trunk or truncus arteriosus, a CHD with single outflow tract (Bhattaram et al., 2010; Hoser et al., 2008; Paul, Harvey, Wegner, &amp; Sock, 2014), expressed broadly throughout neural plate and migrating NCCs (Uy, Simoes-Costa, Koo, Sauka-Spengler, &amp; Bronner, 2015)</td>
</tr>
<tr>
<td>SLC22A23</td>
<td>Expressed in heart and integral to mouse heart development expressed in heart, integral in mouse heart development (Aberg et al., 2012; Christoforou et al., 2008; Ekizoglu, Seven, Ulutin, Guliyev, &amp; Buyru, 2018)</td>
</tr>
<tr>
<td>FOXP4</td>
<td>Mutants develop two hearts having proper chamber formation, and normal trabecular and compact myocardial development, with the same heart rate but asynchronous, lethal at E12.5 (Li, Zhou, Lu, &amp; Morrisey, 2004; Y. Zhang et al., 2010)</td>
</tr>
<tr>
<td>NR2F6</td>
<td>Important for normal cardiac morphogenesis, including the development of coronary vasculature, left ventricular compact zone, and heart valves PMID: (Crispino et al., 2001; Hermann-Kleiter &amp; Baier, 2014; Huggins, Bacani, Boltax, Aikawa, &amp; Leiden, 2001)</td>
</tr>
<tr>
<td>SIX3</td>
<td>Early brain development (Inbal, Kim, Shin, &amp; Solnica-Krezel, 2007), mutation implicated in holoprosencephaly, coloboma, cleft lip/palate (Lacbawan et al., 2009)</td>
</tr>
</tbody>
</table>
**Table 2: Characteristics relevant to cardiac and overall development**

<table>
<thead>
<tr>
<th>Genes common to KDM6A datasets</th>
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</thead>
<tbody>
<tr>
<td><strong>ID3</strong></td>
<td>Knockout in various combinations with ID1/2/4 causes ventricular septation defects, outflow tract atresia, missing heart tube-forming region, decreased heart size and function (Y. Chen et al., 2004; Cunningham et al., 2017; Fraidenraich et al., 2004; Hu, Xin, Hu, Sun, &amp; Zhao, 2019)</td>
</tr>
<tr>
<td><strong>PRDM1</strong></td>
<td>Functions in mesoderm of second heart field, where it interacts with Tbx1, during outflow tract morphogenesis in mouse embryo (Vincent et al., 2014)</td>
</tr>
<tr>
<td><strong>ANXA4</strong></td>
<td>Expressed in cardiac myocytes and upregulated in human failing hearts (Lewin et al., 2009; Matteo &amp; Moravec, 2000)</td>
</tr>
</tbody>
</table>

Comparison of the KMT2D and KDM6A datasets between human patients generated by us, with those of mice and zebrafish generated by other groups, show variations in the expression patterns of the some genes that are common across different species and cell types. The observation indicates there may be a tight spatial and temporal orchestration of the roles of KMT2D and KDM6A in gene regulation.

**Discussion**

In animal models, mutations or knockdowns in Kabuki Syndrome genes show similar NCC related phenotypes. kmt2d mutations in zebrafish cause defects in cardiovascular development, angiogenesis and aortic arch formation, as well as cardiac hypoplasticity (Serrano et al., 2019) while Kmt2d associates with genes in cardiomyocytes, and myocardial deletion of Kmt2d reduces cardiac gene expression during murine heart development (Ang et al., 2016). In Xenopus, morpholino-based knockdown of kmt2d causes cardiac hypoplasticity, reduced expression of early cardiac developmental genes tbx20, isl1, nкс2.5 (Schwenty-Lara, Nurnberger, et al., 2019), and impaired formation and migration of NCCs by reducing the expression of early NCC-specific
markers like *pax3, foxd3, twist*, and *slug* (Schwenty-Lara, Nehl, et al., 2019). Interestingly we did not observe these same defects in single zebrafish Kabuki Syndrome mutants. It is likely that genetic compensation between closely related paralogs are able to compensate for the loss of function of a single gene. Future studies will test this by the creation of double and triple KS mutant zebrafish.

Less is known about *kdm6a* and its zebrafish paralog *kdm6al*. In mouse embryonic stem cells, *KDM6A* or *Utx* promotes the differentiation (ESCs) into a cardiac lineage, and serves as a co-activator of core cardiac transcription factors like *Srf, Nkx2.5, Tbx5*, and *Gata4* (Lee et al., 2012). *KDM6A* or *Utx* associates with cardiac-specific genes like *Anf* and *Baf60c*, to help recruit Brg1, an ATP-dependent chromatin remodeler that activates transcription of core cardiac transcription factors (Hang et al., 2010; Lee et al., 2012; Lickert et al., 2004). A neural crest-specific conditional deletion of *KDM6A* or *Utx* allele with a *Wnt1-Cre* transgene causes CHDs like patent ductus arteriosus and craniofacial defects including frontonasal hypoplasia, facial depression, and eyelid tears (Shpargel et al., 2017). In a percentage of the embryos a cleft palate is observed. Interestingly the phenotype is more severe in females likely due to the remaining copy of UTY on the Y chromosome. Because UTY has a non-functional methyltransferase, it is also possible that the ability of UTY to rescue UTX in males is methyltransferase independent (Shpargel et al., 2017). Consistent with the mouse data, zebrafish *kdm6a* and *kdm6al* mutants have minor reductions in gene expression, craniofacial cartilage and cardiac morphology as well as a reduction in histone modifications. Whether this function is methyltransferase dependent in zebrafish remains to be determined.

We present a comparison between the transcriptome of zebrafish, mice, and humans with mutations in *KMT2D*, and a comparison between transcriptomes of humans and three distinct cell populations of mice with mutations in *KDM6A*. Although our analyses involves transcriptomic datasets from different species, cell types, mutations, and developmental stages, we identify some common targets of KMT2D and KDM6A that transcend these biological differences. A subset of genes involved in cardiac development and physiology, vasculature, and overall development, appeared as common targets across all datasets for each mutation. Specifically, our analysis revealed 76 genes as common targets of *KMT2D* upon comparing our human datasets with the zebrafish and mouse datasets. 30 out of 76 genes show a similar expression pattern across the 3
species, while 46 genes do not. The dissimilar expression patterns of the 46 genes can partly be explained by the fact that the transcriptomes of the 3 species are derived from different cell populations derived during different stages. Hence, epigenetic regulation by KMT2D is tightly orchestrated spatially and temporally. On the other hand, a similar expression pattern in 3 species for the 30 genes by KMT2D indicates that its role is partly conserved. For KDM6A, we identified 7 common targets upon comparing human RNA-seq datasets with 3 mice datasets obtained from neural crest, neural stem, and CD4+ cells. Our in vivo analysis of kmt2d and kdm6a mutations in zebrafish corroborate the findings of previous studies investigating these two genes in cardiac development and disease (Ang et al., 2016; Lee et al., 2012; Schwenty-Lara, Nurnberger, et al., 2019; Serrano et al., 2019). The caveat with this type of transcriptomic analyses comparison is that the RNA-seq datasets are obtained from different sources and cell types across three species of humans, mice, and zebrafish, harboring different mutations in KMT2D and KDM6A. Despite the non-uniformity, there are some shared gene regulatory networks, and thus can serve as a resource to identify core or shared biological signatures of KMT2D and KDM6A. Although, a more direct comparison between similar mutations and cell-types are warranted in future studies, this analyses identifies the common targets of KMT2D and KDM6A across different species, cell types, and mutations.

The importance of histone modifications in cardiac and craniofacial development and function has been the focus of many recent studies (Stein et al., 2011). In mice, H3K27me3 is important for postnatal cardiac homeostasis (Delgado-Olguin et al., 2012), and the Polycomb complex regulates heart development (He et al., 2012) (Lee et al., 2012; Monroe et al., 2019). In zebrafish, H3K27me3 has recently been shown to silence structural genes in proliferating cardiomyocytes during wound invasion and regeneration of injured hearts (Ben-Yair et al., 2019). ChIP-sequencing studies on primary neonatal murine cardiomyocytes revealed that H3K4me3 is enriched, while H3K27me3 is reduced, at promoters of cardiac-specific genes like Mef2c, Gata4, and Tbx5 (Z. Liu et al., 2016). This study further showed that early re-patterning of H3K4me3 and H3K27me3 occurs during reprogramming of induced cardiomyocytes (iCMs), which are used for modeling cardiac diseases and regeneration (Z. Liu et al., 2016). Cardiac reprogramming and epigenetics have been focused in other publications (Engel & Ardehali, 2018; Ieda et al., 2010; L. Liu, I. Lei, H. Karatas, et al., 2016; Liu, Lei, & Wang, 2016; Monroe et al., 2019). In craniofacial
development, we have identified Kat2a and Kat2b as being important in regulating cartilage and bone development in both zebrafish and mice.

To conclude, several features of the roles of KMT2D and KDM6A remain to be deciphered, especially other factors that cross-talk with H3K4me3/K27me3 which are linked to craniofacial defects (Shpargel et al., 2017; Vallianatos & Iwase, 2015; Wilderman, VanOudenhove, Kron, Noonan, & Cotney, 2018) and CHDs such as KDM1A/LSD1, KDM4A, KDM4C, EZH2 (Agger et al., 2007; Ahmed & Streit, 2018; L. Chen et al., 2012; Lee et al., 2012; Nicholson et al., 2013; Rosales, Carulla, Garcia, Vargas, & Lizcano, 2016; Willaredt, Gorgas, Gardner, & Tucker, 2012; Wu et al., 2015; Yang et al., 2019; Q. J. Zhang et al., 2011). We take a small step towards informing future studies which will be using various species to study the regulation of development and disease by KMT2D and KDM6A. Taken together, a comprehensive understanding of epigenetic regulation of heart and craniofacial development and disease, in combination with large next-generation sequencing datasets from patients that are continuously being generated and animal models will contribute to the emerging field of personalized medicine to translate epigenetics-based research from the bench to the bedside.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest

References


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doi:10.1186/s13059-014-0550-8


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**Figure Legends**

**Figure 1**: *KMT2D* shares common targets across humans, mice and zebrafish.

Table: Mutation information of patients.

A. Venn diagrams showing overlap between gene lists derived from RNA-sequencing (seq) analysis of *KMT2D* mutant datasets from humans, mice, and zebrafish as labeled.

B. Heat maps showing 76 common targets among human, mice, and zebrafish. Genes selected for RT-qPCR in Figure 1 C. are highlighted in green boxes.

**Figure 2**: *KDM6A* shares common targets across humans and various cell types of mice, which are similarly regulated in zebrafish.
A. Venn diagrams showing overlap between gene lists derived from RNA-seq analysis of KDM6A mutant datasets from humans and three distinct cell types of mice, as labeled.

B. Heat maps showing 7 common targets among human and 3 cell types of mice. Abbreviations: Ms – mouse, NCC – neural crest cells, NSC – neural stem cells, Cd4 – Cd4 T lymphocytes.

Genes selected for RT-qPCR in Figure 2 C. are highlighted in green boxes.

<table>
<thead>
<tr>
<th>Table</th>
<th>Gene Affected</th>
<th>Sex</th>
<th>Status</th>
<th>Effect at cDNA Level</th>
<th>Effect at Protein Level</th>
<th>Chromosomal Location (hg19)</th>
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<tr>
<td></td>
<td>KMT2D</td>
<td>♂</td>
<td>Proband</td>
<td>c.4288T&gt;C</td>
<td>p.C1430R</td>
<td>chr12:47726789(hg18)</td>
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<tr>
<td></td>
<td>KDM6A</td>
<td>♀</td>
<td>Proband</td>
<td>Microdeletion, Gene deleted</td>
<td>Gene deleted</td>
<td>chrX:43620636-46881568</td>
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</tbody>
</table>
Sen et al Figure 2