Roles of HIF and 2-Oxoglutarate dependent enzymes in controlling gene expression in hypoxia

Julianty Frost¹, Mark Frost¹, Michael Batie¹, Hao Jiang², and Sonia Rocha*¹

¹ Department of Molecular Physiology and Cell Signalling, Institute of Systems, Molecular and Integrative Biology. University of Liverpool. L697ZB Liverpool. United Kingdom.

Juliante.Frost@liverpool.ac.uk; Mark.Frost@liverpool.ac.uk; Michael.Batie@liverpool.ac.uk

² Centre for Gene Regulation and Expression. School of Life Sciences. University of Dundee. DD15EH. Dundee. United Kingdom; h.y.jiang@dundee.ac.uk.

* Correspondence: srocha@liverpool.ac.uk; Tel.: +44 (0)151 794 9084

Simple Summary: Hypoxia — reduction in oxygen availability—plays key roles in both physiological and pathological processes. Given the importance of oxygen for cell and organism viability, mechanisms to sense and respond to hypoxia are in place. A variety of enzymes utilise molecular oxygen, but of particular importance to oxygen sensing are the 2-oxoglutarate (2-OG) dependent dioxygenases (2-OGDs). Of these, Prolyl-hydroxylases have long been recognised to control the levels and function of Hypoxia Inducible Factor (HIF), a master transcriptional regulator in hypoxia, via their hydroxylase activity. However, recent studies are revealing that dioxygenases are involved in almost all aspects of gene regulation, including chromatin organisation, transcription and translation.

Abstract: Hypoxia — reduction in oxygen availability—plays key roles in both physiological and pathological processes. Given the importance of oxygen for cell and organism viability, mechanisms to sense and respond to hypoxia are in place. A variety of enzymes utilise molecular oxygen, but of particular importance to oxygen sensing are the 2-oxoglutarate (2-OG) dependent dioxygenases (2-OGDs). Of these, Prolyl-hydroxylases have long been recognised to control the levels and function of Hypoxia Inducible Factor (HIF), a master transcriptional regulator in hypoxia, via their hydroxylase activity. However, recent studies are revealing that dioxygenases are involved in almost all aspects of gene regulation, including chromatin organisation, transcription and translation. We highlight the relevance of HIF and 2-OG dioxygenases in the control of gene expression in response to hypoxia and their relevance to human cancers.

Keywords: hypoxia; 2-OG dioxygenases; chromatin; transcription, translation; cancer

1. Introduction

The importance of oxygen for energy production in multicellular organisms has been appreciated since the identification of the mechanism of oxidative phosphorylation located in the mitochondria. Reductions in oxygen availability, or hypoxia, are therefore either danger signals or a cue for physiological processes such as development. Given the importance of oxygen, cells have perfected mechanisms to sense and respond to hypoxia, in order to minimise damage, preserve energy and, when possible, adapt to the new oxygen supply normality.
The main transcription factor activated under low oxygen conditions, called Hypoxia Inducible Factor (HIF) was identified in 1992 [1]. HIF is composed of a heterodimer of HIF-α (of which there are 3 isoforms, HIF-1α, HIF-2α (encoded by the EPAS1 gene) and HIF3-α) and HIF-1β (encoded by the gene ARNT) [2]. HIFs control many genes, most of which are crucial for cell survival and adaptation to low oxygen conditions [2]. Under pathological conditions, such as cancer and altitude sickness, induction of some of these genes by the HIF transcription factors has been linked to disease progression and treatment resistance [3]. In addition, HIF can also be induced by non-oxygen dependent mechanisms, such as inflammation [4]. This is particularly relevant for human cancers, where hypoxia and inflammation often co-occur [5].

The mechanism leading to the activation of HIF was unravelled in 2001 [6,7]. HIF-α, under normal oxygen conditions, is continually transcribed and translated, but rapidly degraded by the ubiquitin dependent proteasomal system (Figure 1). Ubiquitination is promoted by the E3-ligase composed of Von Hippel-Lindau Tumor Suppressor (VHL) Ring-Box 1 (RBX1), Cullin 2 (CUL2) and Elongin B/C (ELOCB/C) [3]. VHL affinity toward HIF-α is dramatically increased by the presence of a specific post-translational modification. This modification is a proline hydroxylation (Figure 1), mediated by Prolyl-Hydroxylases (PHDs). PHDs are part of the 2-oxoglutarate (2-OG) dependent dioxygenase (2-OGD) superfamily of enzymes, requiring oxygen, iron and 2-OG for activity.[8] Mammals possess three PHDs, PHD2 (gene name EGLN1), PHD3 (gene name EGLN3) and PHD1 (gene name EGLN2) [6]. Biochemical characterisation revealed that PHDs have low affinity for molecular oxygen. Low affinity for molecular oxygen signifies that when oxygen availability is reduced, these enzymes are quickly inhibited, leading to HIF stabilisation and activation of target genes. PHD inhibition in hypoxia has resulted in them being termed molecular oxygen sensors in the cell [9]. Subsequently, many more 2-OGDs have been identified, most of which act independently of HIF but play a role in coordinating the cellular response to hypoxia. These include Factor Inhibiting HIF1 (FIH), Jumonji C (JmjC)-domain containing demethylases (JmjC demethylases) (which demethylate both histones and non-histone proteins), Ten-Eleven Translocation (TET) enzymes (mediators of DNA-demethylation), and RNA-demethylases (Box 1). Given the function of some of these enzymes, it is conceivable that hypoxia could influence all aspects of gene expression, from chromatin [G] structure and epigenetics [G] to RNA biology, translation and protein turnover (Figure 2). This perfectly equips the cell when faced with hypoxia. Under such conditions, the cell must make a coordinated effort to allow for restoration of oxygen homeostasis, while reducing energy expenditure if it is to survive.

Genetic models in several model organisms has helped identify the key roles of HIFs as well as 2-OGDs in development and disease (Sup. Table 1). Furthermore, genomic techniques such as chromatin immunoprecipitation followed by sequencing (ChIP-seq) RNA sequencing (RNA-seq), and Chromatin capture have more recently been used to understand how cells responds to changes in oxygen, but also in response to 2-OGD inhibition.
Figure 1. Regulation of HIF levels and activity in normoxia and hypoxia. Under normal oxygen conditions, A, normoxia, HIF-α is constantly hydroxylated by PHDs and FIH. PHD-mediated hydroxylation increases binding affinity with the tumour suppressor VHL, which promotes ubiquitination and degradation by the proteosome. As oxygen levels decrease, in mild hypoxia B, PHDs are inhibited, HIF-α is stabilised, though still hydroxylated by FIH, binds to HIF-1β and is able to induce transcription of certain target genes. With further reduction in oxygen levels, in severe hypoxia C, FIH is also inhibited and HIF is able to become fully active by the recruitment of co-activators such as p300.

In this review, we highlight the importance of oxygen sensing in coordinating an efficient response to hypoxia. We discuss the relevance of HIF transcription factors, and roles of 2-OGDs in controlling almost all aspects of gene expression from chromatin structure, to transcription, translation and post-translational modifications.

Box1. 2-OGDs and their reported affinities for oxygen from in vitro assays
## 2-OGD Type

| Enzyme       | $O_2 K_M$ (µM) | Potential $O_2$ sensor (Yes/No) | Substrate     | Effect on gene expression in hypoxia |
|--------------|----------------|---------------------------------|---------------|-------------------------------------|
| Hydroxylases |                |                                 |               |                                     |
| PHD1         | 230            | Y                               | Multiple      | Y                                   |
| PHD2         | 240*           | Y                               | Multiple      | Y                                   |
| PHD3         | 230            | Y                               | Multiple      | Y                                   |
| 4-PHα1       | 40             |                                 | Collagen      |                                     |
| PAHX         | 93             | Y                               | Isovaleryl CoA|                                     |
| CDO1         | 76             | N                               | Taurine       |                                     |
| FIH          | 110*           | Y/N                             | Multiple      | Y                                   |
| Hydroxylases (known as DNA demethylases) | | | | |
| TET1         | 30             | N                               | DNA           | Y                                   |
| TET2         | 30             | N                               | DNA           | Y                                   |
| JmjC demethylases | | | | |
| KDM4A        | 173            | Y                               | Histone H3    | Y                                   |
| KDM4A        | 57             | N                               | Histone H3    | Y                                   |
| KDM4C        | 158            | Y                               | Histone H3    | Y                                   |
| KDM4E        | 197            | Y                               | Histone H3    | Y                                   |
| KDM5A        | 90             | Y                               | Histone H3    | Y                                   |
| KDM5B        | 40             | N                               | Histone H3    | Y                                   |
| KDM5C        | 35             | N                               | Histone H3    | Y                                   |
| KDM5D        | 25             | N                               | Histone H3    | Y                                   |
| KDM6A        | 180            | Y                               | Histone H3    | Y                                   |
| KDM6B        | 20             | N                               | Histone H3    | Y                                   |

* Median $K_M$ from multiple studies

### [H1] Effects of hypoxia on gene transcription

It is now appreciated that the cellular and organism response to hypoxia involves profound changes to gene expression, with vast changes in gene transcription being detected in all systems studied.
Figure 2. Hypoxia via the regulation of 2-OGDs has the potential of controlling all aspects of gene expression and protein function. Action of JmjC-histone demethylases and TETs (mediators of DNA demethylation) will impact on chromatin and DNA regulation. RNA demethylases, and several hydroxylases acts on mRNA processing, and fate. Hydroxylases also control rates of translation and ribosome activity, while JmjC-demethylases and hydroxylases can control protein function directly or indirectly by controlling other PTMs.

*Hypoxia-induced changes to transcription are largely mediated by HIF*

As mentioned earlier HIFs are the master regulators of gene transcriptional changes in hypoxia. HIFs are known to bind to Hypoxia Responsive Elements (HREs) (5-(A/G)CGTG-3) of DNA [8]. Since the identification of the Erythropoietin gene (*EPO*) as the first hypoxia responsive HIF target gene, we now know HIF controls a wide range targets, influencing numerous biological processes,
including angiogenesis, glycolysis, cell death and cell cycle progression (reviewed in[2,10]). There are
now thousands of identified hypoxia responsive genes and over 100 validated HIF target genes, with
over a thousand putative targets, and all classes of RNAs can be induced by low oxygen (reviewed
in[11]). The discovery of hypoxia responsive genes and HIF targets has been driven greatly by
transcript profiling and genome occupancy technologies, including microarrays, RNA-seq and ChIP-
seq (reviewed in [12-14]). These and analyses of publicly available transcriptional datasets[15,16] have
shed light on cell type differences in hypoxia responsive genes and HIF targets on a genome wide
scale, as well as identifying hypoxic signatures conserved across multiple cell types. Why different cell
types have different transcriptional responses to hypoxia and HIF target genes is an important
question for the field, with chromatin structure organisation thought to be a major factor in conferring
specificity. Further to HIF isoform expression and activity, evidence points towards pre-established
chromatin accessibility and local chromatin environment, including RNA pol II availability, pre-existing
promoter enhancer interactions at HREs, and HRE DNA methylation status, as cell-type specificity
determinants of hypoxia transcriptional responses (reviewed in [12-14,17]).

Whilst most HIF binding sites are at proximal promoters, binding to distal intergenic regions
also occurs and HIF can regulate transcription of long genomic intervals, interacting at promoter-
enhancer loops [18-20]. Although there is no doubt HIF is the main transcription factor controlling
hypoxia-induced transcriptional changes, there is the involvement of other transcription factors,
including Nuclear Factor-κB (NF-κB), Tumor Protein p53 (p53), MYC Proto-Oncogene (MYC) and
Activator Protein 1 (AP1), which function in the regulation of the hypoxia response via HIF-dependent
and independent pathways (reviewed in [21]). In addition, HIF mostly acts as an activator of
transcription, and thus most of the observed hypoxia-induced gene silencing is either independent
of HIF, or via indirect mechanisms, including through the actions of chromatin remodeller complexes,
co-repressor complexes or induction of other transcription factors (reviewed in [22]).

Functions encoded by HIF-induced genes

HIF induced genes are involved in a variety of different pathways, acting at different stages of
the response to hypoxia (Figure 3). For example, genes involved in restoration of oxygen homeostasis
including angiogenesis and red blood cell production, involved in metabolic shift (metabolism and
epigenetics), preservation of energy (cell cycle, apoptosis, autophagy, epigenetics) and adaptation to
the hypoxic environment (angiogenesis, migration, epigenetics, metabolism).
Figure 3. HIF induced targets control a variety of important pathways in the cell. Direct HIF-dependent target genes have been involved in controlling restoration of oxygen homeostasis, angiogenesis, metabolism, epigenetics, survival and death pathways and cell cycle progression. Examples of such genes are included.

Although we now have access to a vast number of transcriptomic studies of cells in hypoxia, very few studies have investigated the proteomic changes under such an important stress, using techniques ranging from the “old-fashioned” two-dimensional electrophoresis (2-DE) coupled with MALDI-TOF-TOF-MS [23], Sharma, 2013 #656;Hoang, 2001 #293), to the more accurate and robust quantitative multiplexed proteomics workflow [24-27], which usually combines isobaric labelling, two-dimensional liquid chromatography (2D-LC) and high resolution MS. The few studies available have shown that hypoxia exposure of different cells and tissues possesses broad effects at the whole proteome level, including changes to the protein expression of annexin family [23], glycolytic and
antioxidant enzymes, transcription factors, heat shock proteins, S100 family proteins, and also other proteins involved in TCA-cycle, metabolism, and immune response. A proteomic study has revealed novel non-HIF hypoxia regulators, including the chromatin organizer protein Heterochromatin Protein 1 Binding Protein 3 (HP1BP3), which mediates chromatin condensation. The use of multi-omics techniques (transcriptomics, proteomics and metabolomics) and analysis using integrated bioinformatics identified consistent changes to proteins and metabolites in heart tissues under antenatal hypoxia. These proteins and metabolites are involved in energy metabolism, oxidative stress and inflammation-related pathways, required for the reprogramming of the mitochondrion.

Analysis of secreted proteins (secretome) show hypoxic conditions usually selectively increase their expression with relevance to angiogenesis, inflammation, extracellular matrix and signalling processes. In prostate cancer cells adapting to hypoxia during proliferation, the secretome controls hypoxia-dependent intercellular signalling, resulting in higher protein content (primarily in epithelial adherens junction pathway) higher metalloprotease activity and increased levels of diverse signalling molecules Transforming Growth Factor Beta 2 (TGF-β2), (Tumour Necrosis Factor) (TNF), Interleukin 6 (IL6), Tumor Susceptibility 101 (TSG101), AKT Serine/Threonine Kinase 1 (AKT), Integrin Linked Kinase (ILK), and Catenin Beta1 (CTNNB1) compared to exosomes at normal oxygen tensions. The results suggested that under hypoxia, with loading unique proteins in exosomes, cancer cells could enhance invasiveness and create/change the microenvironment for aggressiveness.

**Preferred translation of hypoxia target**

Despite hypoxia decreasing global translation due inhibition of translation initiation (please see below), cells still require translation of hypoxia-inducible genes to promote cell survival and restore oxygen homeostasis. The first discovered mechanism of selective translation was internal ribosomal entry sites (IRES) in the 5' UTR which facilitates ribosome assembly within the mRNA bypassing the requirement of eIF4 5' cap initiation. These sequences are present in hypoxia inducible genes such as Vascular Endothelial Growth Factors (VEGFs), Fibroblast Growth Factors (FGFs), Platelet derived Growth Factors (PDGFs), Epidermal Growth Factor Receptor (EGFR) and HIF1A. Preferential translation of these target genes allow adaptation to low oxygen through stimulating broad programmes of gene transcription for angiogenesis, cell survival, and HIF-dependent gene changes.

Another mechanism relies on the presence of upstream open reading frames (uORFs) which are short sequences in mRNA 5' UTRs including an upstream AUG (uAUG) start site. In normoxia when Eukaryotic Translation Initiation Factor (eIF) 2A (eIF2α) activity is high, translation will initiate at the first 5‘ uORF, preventing translation from the genuine UAG start site. In hypoxia, when eIF2α phosphorylation is high, and activity is low, the ribosome will identify the uAUG flanking sequence to bypass the uORF site, so translation mostly shifts to the downstream genuine ORF, resulting in an increase in translation of the gene. This mechanism has been identified for the EPO gene in response to hypoxia.

Some mRNAs contain an RNA HRE (rHRE), a sequence which can recruit an alternative initiation complex for selective cap-dependent translation. This complex involves HIF-2α, one of...
the HIF family of transcription factors stabilised in hypoxia through PHD inhibition, RNA Binding Motif Protein 4 RBM4, and the eIF4E homologue eIF4E2, which together in hypoxia bind to rHREs and initiate translation of genes such as EGFR, PDGR Receptor Alpha (PDGFRα), and Insulin Like Growth Factor 1 Receptor (IGF1R). Finally, a potentially new and exciting mechanism involves an epigenetic transcriptomic mark in RNA, methylation of 6 adenosine (m6A). mRNA m6A modification at the 5'UTR can recruit m6A binding proteins such as YTH N6-Methyladenosine RNA Binding Protein 2 (YTHDF2) in heat shock stress which upregulates translation through binding eIF3 and the 40S subunit [45], and such a mechanism may prove true in other stress responses such a hypoxia. The RNA demethylating enzymes FTO Alpha-Ketoglutarate Dependent Dioxygenase (FTO) and AlkB Homolog 5, RNA Demethylase (ALKBH5), are 2-OGDs, and although their ability to sense oxygen has yet to be investigated, their inhibition in hypoxia could result in an increase in global RNA methylation, which would help to regulate translation and RNA fate in hypoxia.

Chromatin regulation in hypoxia

Central to the hypoxia response is the activation of a dynamic transcriptional programme. HIF transcription factors are the primary mediators of hypoxia induced gene transcriptional changes (reviewed in[8]). Further to HIF stabilisation and activation under low oxygen tensions, the chromatin landscape also plays a complex role in co-ordinating hypoxia inducible changes to gene transcription. Most aspects of chromatin regulation are altered in response to low oxygen, including histone methylation and acetylation, DNA methylation, actions of chromatin remodeler complexes and non-coding RNAs, histone eviction and incorporation of histone variants, and chromatin accessibility (reviewed in [11-13]). However, this is still a vastly unexplored aspect of the hypoxia response. As mentioned above, in addition to PHDs, TETs (mediators of DNA demethylation), and JmjC demethylases (histone and non-histone protein demethylases), are also 2-OGDs. Recent studies demonstrate the potential of TETs and JmjC demethylases to function as molecular oxygen sensors, directly linking oxygen sensing to transcriptional control via epigenetics in cells. Below we summarise DNA and histones methylation changes in hypoxia, with a focus on oxygen sensing mechanisms via TETs and JmjC demethylases. (Figure 4).
Figure 4. Chromatin oxygen sensing via JmjC histone demethylases and TETs. JmjC histone demethylases and TETs (mediators DNA demethylation) are 2-OGDs. Reduced activity of these enzymes in hypoxia, due to their oxygen sensitivity, can alter the chromatin landscape and mediate hypoxia induced transcription changes. Reduced activity of KDM5A in hypoxia increases H3K4me3 at the promoters of a subset hypoxia induced genes, facilitating their transcriptional activation. Reduced
activity of KDM6A in hypoxia increases H3K27me3 at the promoters of a subset of hypoxia-repressed genes and represses their transcription. Reduced TET1/2 activity in hypoxia also represses gene transcription via DNA hypermethylation at gene promoters.

**JmjC histone demethylases and chromatin regulation in hypoxia**

Histone methylation is a recognised mechanism controlling chromatin structure and is associated with regulation of gene transcription, with some marks clearly leading to open chromatin, while other are firmly associated with closed conformation [46]. The family of enzymes predominantly responsible for histone demethylation are JmjC demethylases, which are part of the JmjC-domain containing group of 2-OGDs which includes demethylases and hydroxylases (JmjC 2-OGDs). *In vitro* studies demonstrate the varied oxygen sensitivities of these enzymes, some are potentially direct molecular oxygen sensors (Box 1). Oxygen affinities, oxygen availability and protein expression levels will likely dictate JmjC demethylase activities in hypoxia. Several groups have reported increases in total levels of histone methylation modifications in response to hypoxia across a range of human and mouse cell types and human tumours using immunoblotting, immunohistochemistry, immunofluorescence and quantitative proteomics ([47], reviewed in [12]). ChIP-sequencing approaches have revealed site-specific, hypoxia induced changes in Histone (H)3 Lysine (K)4 trimethylation (me3) [10,48], H3K36me3 [10] and H3K27me3 [48,49], which correlate with changes in gene expression. There is now evidence, through the use of *in vitro* and in cell histone demethylation assays, in coordination with mutagenesis analysis, gene expression analysis and histone methylation analysis, that Lysine Demethylase (KDM) 6A (KDM6A) [49] and potentially KDM5A[10], which demethylate H3K27me3 and H3K4me3 respectively, are inhibited by reduced oxygen levels in hypoxia. These result in increased histone methylation modifications which coordinate hypoxia inducible gene transcriptional changes (Figure 4) and hypoxia induced cellular responses. Specifically, KDM6A inhibition in hypoxia triggers hypermethylation of H3K27me3 at a subset of hypoxia repressed gene promoters, reducing their expression. Conversely, potential KDM5A inhibition in hypoxia, triggers H3K4me3 hypermethylation at a subset of hypoxia inducible gene promoters, this precedes increases in their expression in hypoxia and is required for their full transcriptional activation in hypoxia. In cell and *in vitro* H3K9me3 demethylation assays have also revealed that the demethylase activity of KDM4A is highly sensitive to oxygen concentrations over physiologically relevant ranges, thus KDM4A can also be classed as an oxygen sensor [50]. Interestingly, KDM4A has been shown to positively regulate HIF-1α levels via H3K9me3 demethylation at the *HIF1A* gene locus, this effect is observed in mild hypoxia (2% oxygen), but impaired at severe hypoxia (>0.1 oxygen) [51]. This may provide a mechanism of increasing HIF-1α levels in conditions of hypoxia, were this is still residual PHD activity. Future work should investigate if the oxygen sensitive H3K9me3 demethylase activity of KDM4A is linked to control of gene expression and chromatin regulation in hypoxia. Importantly, some JmjC demethylases remain active at low oxygen concentrations and function in hypoxia through histone their demethylase activity. KDM4C [52] and KDM3A [53,54]display HIF coactivator activity in hypoxia via demethylation of H3K9 at HIF target gene promoters, facilitating transcriptional activation at the genes. Furthermore, many JmjC histone demethylases are HIF target genes that are upregulated in hypoxia (reviewed in [55]), this is thought in part to be a compensatory mechanism to counteract reduced demethylase activity acting as a hypoxia feedback loop similar to what is seen with
transcriptional upregulation of PHD2/3 by HIF. Thus, there is complex crosstalk between histone methylation, gene expression and hypoxia, mediated in part through JmjC demethylases. However, further characterisation of the oxygen sensitives of JmjC demethylases is needed.

**TET mediated DNA demethylation functions in hypoxia**

TETs, of which there are 3 variants, Ten Eleven Ten translocation Methylcytosine Dioxygenase 1 (TET1), TET2 and TET3, are 2-ODGs which function as hydroxylases, mediating mammalian DNA demethylation through catalysing the oxidation of 5methylcytosine (5mc) to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine. These TET oxidised derivatives of 5mc can then be demethylated by mechanisms of active and passive demethylation (reviewed in [56]). DNA methylation can repress gene transcription, consequently tumour hypoxia results in aberrant DNA methylation profiles promoting tumour suppressor gene silencing (hypermethylation) [57] and oncogene activation (hypomethylation) [58]. Recently, using *in vitro* biochemical binding assays, *in vivo* studies on HIF binding and DNA methylation status in human cancer cell lines, and in silico structural modelling D’Anna and colleagues find that DNA methylation at HREs impairs HIF binding, and HRE DNA methylation status is a key factor in determining cell type specific transcriptional responses to hypoxia[59]. There is heterogeneity regarding the effects of hypoxia, both in cell models and in tumours, on global DNA methylation levels and TET activity (reviewed in [11,60]). As such, whether TETs are impaired or functionally active in hypoxia, and the consequences this has for gene transcription appear highly context dependent. Researchers have shown TET activity in hypoxia, and HIF dependent TET upregulation and coactivator functions, have been demonstrated at hypoxia inducible genes (reviewed in [11,60]). TET activity in low oxygen environments is supported by *in vitro* oxygen affinities of TET1 and TET2 (Box 1) and is supported by the known roles of TETs in the bone marrow and during development where oxygen tensions are low [61,62]. Conversely, Thienpont et al. showed that severe hypoxia (0.5% oxygen) in human and murine cells and tumour hypoxia in multiple human tumours causes DNA hypermethylation at gene promoters correlating with gene silencing at a subset of hypoxia repressed genes and gene silencing linked to hypoxia associated tumour progression. DNA hypermethylation was attributed to oxygen dependent reduction in TET1 and TET2 activity in hypoxia, with a 50% reduction in activity observed at 0.3% oxygen for TET1 and 0.5% for TET2 *in vitro*. Thus, TET1 and TET2 may be characterised as tumour oxygen sensors, and depending on the context of oxygen deprivation, may remain active in hypoxia environments or display inhibition. However, more work is needed to establish the oxygen dependence of TET activity in cells and *in vivo* and the physiological contexts in which TETs can sense changes in oxygen availability as well as the consequences this has for DNA methylation, gene transcription and cellular responses. Indeed, the seemingly contradictory roles for TETs in hypoxia from studies to date may be dependent on the different cell models used and timing/severity of hypoxic stimulus.

While there is growing evidence for a dynamic role of chromatin/epigenetics in sensing and responding to hypoxia to facilitate transcriptional changes, via dependent and independent HIF mechanisms, efforts to elucidate molecular mechanisms underpinning such changes and the extent to which chromatin/epigenetic changes are required for coordinating hypoxia/HIF transcriptional effects are ongoing. The discoveries of oxygen sensing by TETs and JmjCs provide an exciting link
between oxygen availability and chromatin regulation and future work on oxygen sensing by chromatin will be essential in better hypoxia driven processes.

Effects of hypoxia on protein levels

Protein levels and function are key aspects to achieve the correct hypoxia response. Although transcription is important, mechanisms controlling protein levels and function supersede any change in transcriptional output. In hypoxia, mechanisms exist that control translation but also post-translation aspects of protein function.

Translation is globally repressed in response to hypoxia

In addition to the regulation of gene transcription in hypoxia, gene expression is also controlled through regulation of translation (Figure 5). The cellular response to hypoxia includes a reduction in the energy demands of the cell due to limited ATP production through oxidative phosphorylation. This adaptation results in a reversible global decrease in energy-expensive protein synthesis (reviewed in [2]). This inhibition of translation is a highly regulated response to low oxygen levels preceding ATP depletion (reviewed in [2]).
Figure 5. **Hypoxia induces a global inhibition of protein translation.** Global translation is mostly inhibited at initiation through mTOR inhibition of eIF4 and PERK inhibition of eIF2α, which then inhibit cap-dependent translation of mRNA. mTOR is inhibited through hypoxia-induced DDIT4-dependent release of TSC2 from 14-3-3 binding proteins resulting in the TSC1/2 dimer inhibiting mTOR. Elongation is also regulated through mTOR, as well as AMPK through its inhibition of RPS6KB1, for in hypoxia eEF2K is not inhibited, which allows its phosphorylation and inhibition of eEF2. PHD2 can also hydroxylate eEF2K, which in normoxia causes its disassociation from calmodulin, decreasing its autophosphorylation. Termination is regulated by JMJD4-mediated hydroxylation of eRF1 which is required for termination. Selective translation of genes in hypoxia is regulated by UTR sequences such as IRES and uORFs, which allow increased translation specifically in hypoxia. RNA binding proteins can bind to various parts of the mRNA and result in different regulatory outcomes. Hydroxylation of splicing regulatory (SR) proteins results in differential splicing or exon choice, such as skipping the
first exon with the hydroxylation of SRSF11. The ribosome can also be hydroxylated by RIOX1 and RIOX2, though it is not yet clear what role these modifications have.

Global inhibition of protein expression is largely regulated at the point of translation initiation through two pathways. Firstly, Mechanistic Target Of Rapamycin Kinase (mTOR) is inhibited by DNA Damage Inducible Transcript 4 (DDIT4) (a HIF target gene). DDIT4-dependent release of TSC Complex Subunit 2 (TSC2) from 14-3-3 binding proteins leads to mTOR inhibition. This allows the formation of an active TSC1-TSC2 dimer inhibiting the phosphorylation of Ribosomal Protein S6 Kinase B1 (RPS6KB1) by the protein complex, mTOR, which in turn inhibits the phosphorylation of Ribosomal Protein S6 (RPS6), part of the 40S ribosomal subunit required for initiation [63]. mTOR inhibition also causes hypophosphorylation of Eukaryotic Translation Initiation Factor (eIF) 4E Binding Protein 1 (eIF4EBP1) allowing sequestering of eIF4E, decreasing 5’cap-dependent initiation [64,65]. Secondly, PKR-like Endoplasmic Reticulum Kinase (PERK) (gene name EIF2AK3) is phosphorylated and activated, subsequently phosphorylating eIF2α at S51 causing effective inactivation [66]. Phospho-eIF2α prevents binding with eIF2B for exchange of GDP for GTP, therefore remaining in an inactive state and preventing subsequent rounds of translation from the mRNA [67]. eIF2α normally recruits the initiator aminoaacylated tRNA to the 40S ribosome, thus limiting global initiation of translation. This second mechanism is independent of HIF and as of yet has not been linked to any 2-OGD.

Inhibition of translation is also regulated at the stage of polypeptide elongation. Elongation is inhibited by phosphorylation of Eukaryotic Elongation Factor 2 (eEF2) at T56 by eEF2 Kinase (eEF2K) [68]. This process has been shown to be dependent on mTOR and 5’-AMP-activated protein kinase catalytic subunit alpha-1 (AMPK) (gene name PRKAA1)[69,70] Interestingly, eEF2 kinase (eEF2K) is also regulated by hydroxylation by PHD2 at P98 in an oxygen-dependent manner [71]. In hypoxia, when PHD2 inhibited, eEF2K activity is induced.

In addition to PHD2 dependent hydroxylation, there are several other hydroxylation reactions involved in the regulation of translation, catalysed by other 2-OGDs. Hydroxylation is important for the biosynthesis of tRNAPhe with position 37 requiring a hypermodified nucleoside Wybutosine (yW), which can be hydroxylated to form hydroxywybutosine (OHyW), by the JmjC hydroxylase, TRNA-YW Synthesizing Protein 5 (TYW5), which maintains translational fidelity. It is currently unknown whether TYW5 is responsive to the levels of oxygen, but its transcription is decreased in hypoxia [72] linking hypoxia to a decreased accuracy of translation, which globally decreases the successful translation of proteins [73].

The rate and accuracy of translation are positively regulated by hydroxylation of the central translation machinery. JmjC hydroxylases hydroxylate histidyl residues in ribosomal proteins, with Ribosomal Oxygenase 2 (RIOX2) 2 and RIOX1 hydroxylating Ribosomal Protein L (RPL) 27a (RPL27A) and (RPL8), respectively. The hydroxylation occurs at residues close to the peptidyl transfer centre, thereby increasing translation efficiency [74]. RIOX1 and RIOX2 transcription is reduced in hypoxia [72,74]. Furthermore, RPL8 hydroxylation is also reduced in hypoxia [74]. However, it is not yet known whether these enzymes are inhibited by low oxygen levels, or lower hydroxylation is solely due to lower transcription. Additionally, hydroxylation of 40S Ribosomal Protein S23 (RPS23) by the 2-OGD, 2-Oxoglutarate And Iron Dependent Oxygenase Domain Containing 1 (OGFOD1), is required for efficient translation [75]. OGFOD1 transcription is also decreased in hypoxia, but the enzyme remains...
mostly active even in acute hypoxia [76], suggesting this mechanism is not through direct 2-OGD oxygen sensing.

Efficient decoding of the mRNA during translation requires the JmjC hydroxylase, AlkB Homolog 8, TRNA Methyltransferase, ALKBH8, which hydroxylates tRNA at the wobble position [77,78]. This 2-OGD has yet to be linked to hypoxia, though it would be interesting to investigate its oxygen sensitivity. Finally, lysyl hydroxylation of eukaryotic release factor 1 (eRF1) by the JmjC hydroxylase Jumonji Domain Containing 4 (JMJD4) is required for proper termination of translation [79], although its activity is not significantly inhibited in hypoxia.

**Utilising proteomics for the identification of non-histone protein PTMs**

Proteomics approaches revealed hypoxia induces changes to many post translational modifications (PTMs) on non-histone proteins, such as proline hydroxylation[80,81](regulating protein levels and interactions), phosphorylation [82,83], SUMOylation [84], acetylation[85], glycosylation[86], nitration[87]and nitrosylation[88](all of which regulate protein functions in different ways).

As one of the most widely studied PTMs, the phosphorylation on some transcriptional factors and regulators has been found to be changed under various hypoxic conditions, CAMP Responsive Element Binding Protein 1CREB1, NFKB Inhibitor Alpha (NFKBIA), a regulator of NF-κB, and HIF (reviewed in [89]). More recently, through the analysis of phospho-proteomics in renal clear cell carcinoma cells under VHL-independent hypoxic responses, up-regulation of known biomarkers of RCC and signalling adaptor were found. Meanwhile, such hypoxic responses decreased the phosphorylation on intracellular Carbonic Anhydrase 2 (CA2), which might be an unusual way to control the CA2 expression and enhance the activity of the NFκB pathway, resulting in loss of VHL [82].

In recent years, non-HIF targets have been identified to be hydroxylated on prolines by PHDs (reviewed in [13]), resulting in their degradation and/or changes to downstream activity including Centrosomal Protein 192 (CEP192)[90] and Forkhead Box O3 (FOXO3)[91] by PHD1, Actin Beta (ACTB) by PHD3[92], and AKT Serine/Threonine Kinase 1 (AKT1) by PHD2[93]. Interestingly, a new study indicated that prolyl-hydroxylation could be crucial for GMGC kinase activation [94]. This could imply an intricate interchange between these two types of PTMs, suggesting yet another role for oxygen-dependent signalling in the cell.

Other common PTMs have also been found to responding hypoxia in their own ways. The deSUMOylation of Transcription Factor AP-2 Alpha (TFAP2A), which is known to interact with HIF-1, could enhance the transcriptional activity of HIF-1 under hypoxic conditions [84]. Hypoxia could increase the NAD+-sensitive Sirtuin 3 (SIRT3) activity, that deacetylates key metabolic enzymes and significantly changes the acetylation pattern within the mitochondria. This results in reduced mitochondrial oxidative capacity to match the lowered oxygen availability [85]. In cancer cells, HIF-1α and Glucose transporter 1 (GLUT1) (gene name SLCA1) are critical for O-linked GlcNAc Transferase-mediated regulation of metabolic stress. Reducing O-GlcNAcylation levels increases alpha-ketoglutarate, HIF-1α hydroxylation, and interaction with VHL, resulting in HIF-1α degradation [95]. Some glycosylation also takes part in driving the cell migration and invasion under hypoxia (Reviewed in [86]).
Thus, unbiased proteomic studies on novel PTMs sites [80,96], system-wide analysis of PHDs substrates other than HIF-α [81] and crosstalk of PTMs on PHDs targets in response to hypoxia are now emerging.

**Other potential roles of JmjC 2-OGDs in the hypoxia response**

Further to known and potential oxygen sensing roles of JmjC 2-ODGs (demethylases and hydroxylases) in regulation of chromatin and translation discussed earlier, there are other functions of JmjC 2-ODGs which may influence the hypoxia response (Figure 6). One of the most prominent such enzymes is the dual function of the JmjC 2-OGD, JMJD6, Arginine Demethylase And Lysine Hydroxylase, which has unique activity as both an arginine demethylase and lysine hydroxylase [97,98]. JMJD6 expression is increased in hypoxic conditions in the placenta, and can downregulate HIF-1α [99], though it has been found to operate in diverse pathways. JMJD6 can promote the formation of stress granules through demethylation and de-repression of G3BP Stress Granule Assembly Factor 1 (G3BP1), resulting in the cytoplasmic sequestering of stalled mRNA-ribosome complexes to reversibly prevent mRNA degradation [100,101]. This would allow a fast re-start of protein synthesis when oxygen homeostasis is restored. JMJD6 also regulates mRNA splicing through hydroxylating the splicing regulatory (SR) proteins LUC7 Like 2, Pre-MRNA Splicing Factor (LUC7L2, U2 Small Nuclear RNA Auxiliary Factor 2 (U2AF2)) [102], and Serine And Arginine Rich Splicing Factor 11 (SRSF11) [98]. The SR proteins are involved in exon definition and alternative splicing, with SRSF11 hydroxylation resulting in skipping of the most 5′ exon, and hydroxylation of U2AF65 possibly enacting pre-mRNA looping in order to present to the splicing machinery different cis splice enhancer or silencer sequences [103]. However, this only occurs for selected mRNAs and is not a global effect [103]. Nevertheless, this mechanism would allow selection of alternate splice variants as a response to hypoxia. JMJD6 can also interact with both Bromodomain Containing 4 (BRD4) and the positive Transcription Factor Elongation Factor b (P-TEFb) complex [104], eventually resulting in the release of paused DNA polymerase II and resumption of mRNA synthesis at specifically regulated genes [103]. This implies that hypoxia could use this mechanism to stall transcription of genes that are not required for the stress response to hypoxia and would allow a re-start of gene expression when oxygen levels are restored.

Another JmjC hydroxylase, KMD8, which can hydroxylate arginine residues in both RCC1 Domain Containing 1 (RCCD1) and RPS6 [105]. Although not necessarily dependent on its hydroxylation activity, KDM8 is required for cell proliferation and chromosomal stability [106], and can negatively regulate p53 affecting gene expression and control cell cycle and proliferation [107,108]. Also recently, a biochemical function has been assigned to JMJD7 as a lysyl hydroxylase, which targets Developmentally Regulated GTP Binding Protein 1 (DRG1) and DRG2, which are part of the Translation Factor (TRAFAC) family of GTPases, and could affect their binding with messenger, or ribosomal RNA, though this requires further investigation [109].

The JmjC demethylase, KDM2A represses NF-κB activity via demethylation of RELA, providing a possible link to hypoxia and inflammation crosstalk. [110]. It is more than likely that other JmjC demethylases interact and directly demethylate additional transcription factors which may coordinate transcriptional responses to hypoxia. However, unbiased analysis is required to fully assess this aspect of hypoxia induced gene regulation.
Relevance to human biology and health

Although we currently do not know the importance of all of the 2-OGDs present in the genome, several of the key players in the hypoxia response have important functions and relevance to human biology and health. This is exemplified by the phenotypes observed in null mice, or by the presence of disease associated mutations in humans.

**Figure 6.** Other potential roles of JmjC 2-OGDs dioxygenases in the hypoxia response. JmjC-demethylases have additional functions in the cell, involving control RNA splicing, transcription elongation, translation and RNA fate.

**PHD/HIF/VHL axis**

Given the cellular functions mentioned above, it is no surprise that genetic mutations of most dioxygenases and HIFs have been implicated with human diseases. Mutations in HIF-2α and PHD2 have been found in patients with vascular pathologies, such as erythrocytosis, polycythemia, and
pheochromocytoma (Table 1). As HIF mediates hypoxia adaptation responses, including the regulation of erythropoiesis and vasculogenesis, it is not surprising that mutations within the PHD/HIF/VHL axis are associated with vascular pathologies. The crucial role of HIFs in vascular pathologies is strongly demonstrated by genetic studies of mice, as highlighted in Supplementary Table 1. Knockout mice of HIF-1α or HIF-2α are embryonic lethal with vascular defects (Table 1, Supplementary Table 1), whereas the deletion of PHD2 that activates HIF signalling results in embryonic lethality in mice due to placental and heart defects (Table 1, Supplementary Table 1). VHL mutations also result in highly vascularized tumours, including pheochromocytomas, renal cell cancer carcinoma, retinal and central nervous system hemangioblastomas (Table 1). Hundreds of VHL mutations have been identified in VHL syndrome patients (listed in the Human Gene Mutation Database [111]). The homozygous VHL mutation R200W, which prevents efficient HIF-α degradation in normoxia, is found in all individuals with Chuvash Polycythemia (CP) [112]. CP is characterized by congenital erythrocytosis, and patients have been associated with pulmonary hypertension, thrombosis, vertebral hemangiomas, cerebral vascular events and other vascular abnormalities [113,114], displaying the role of VHL in HIF-dependent regulation of vasculogenesis and erythropoiesis.

Table 1 | Available mice and human mutations phenotypes for HIF and dioxygenases.

| Gene (mouse/human) | Homozygote phenotype in mouse | Human phenotype |
|--------------------|-----------------------------|----------------|
| **HIFs**           |                             |                |
| Hif1a/HIF1A (HIF-1α) | Embryonic lethal with cardiovascular malformations, cephalic vascularisation and neural tube defects [115-117]*. | Schizophrenia [118]*. Maximal oxygen consumption [119]*. Renal cell carcinoma [120]*. |
| Epas1/EPAS1 (HIF-2α) | Embryonic lethal with bradycardia due to defective catecholamine homeostasis [121]*, vascular remodelling defects [122]*, cardiac failure and neonatal lethal with respiratory failure [123]. | Congenital heart disorder [124]*. Autism spectrum disorder [125]*. Pheochromocytoma/ paraganglioma-polycythaemia [126,127] [128-130] [131] [132]*/somatostatinoma [133]*. Erythrocytosis and polycythaemia with paraganglioma [128-130]*. Erythrocytosis [134-139]*. Pulmonary arterial hypertension [140]*. |
| Hif3a/HIF3A (HIF-3α) | Mice deficient of an alternative spliced protein of HIF-3α, NEPAS, are viable and develop enlarged right ventricular owing to | NR |


| 2-OGDs- hydroxylases |  |
|-----------------------|------------------|
| **Egl2/EGLN2 (PHD1)** | Viable [142]*.  |
| **Egl1/EGLN1 (PHD2)** | Embryonic lethal with severe cardiac and placental defects [142]*.  |
| **Egl3/EGLN3 (PHD3)** | Viable [142]* with developmental defect of sympathoadrenal system [161]*.  |
| **P4ha1/P4HA1** | Embryonic lethal with delayed development and defective collagen IV assembly, resulting in base membrane rupture [162]*.  |
| **P4ha2/P4HA2** | Viable and fertile with no obvious phenotypic abnormalities [164]*.  |
| **Phyh/PHYH (PAHX)** | Viable without distinct developmental abnormalities [166]*.  |
| **Hif1an/HIF1AN (FIH)** | Abnormal energy metabolism with reduced body weight, elevated metabolic rate and hyperventilation [175]*.  |

2-OGDs - hydroxylases (mediators of DNA demethylation)

| **Tet1/TET1** | Knockout of *TET1* via 5' coding sequence results in partial embryonic lethal in mice [177-179]*, with surviving female mice displaying decreased fertility and reduced ovary size due to meiotic abnormality [177,178]*. Whereas,  |

NR

NR
mice knockout via deletion of the catalytic domain of *TET1* are viable and fertile [179-181]*, with slightly reduced body size [180]*, as well as impaired spatial learning and short-term memory [182]*.

| Tet2/TET2 | Disordered hematopoiesis and eventually develop myeloid malignancies [183-185], and T- and B-cell malignancies [184]*. | Myelodysplastic/myeloproliferative disease [186]*. Prostate cancer [187]*. Myeloproliferative neoplasms [188]*. |
| Tet3/TET3 | Neonatal lethality [178,189]*. | Intellectual disability, developmental delay, autistic traits, hypotonia, growth abnormalities, facial dysmorphism and movement disorders [190]*. |

### 2-OGDs – hydroxylases (RNA demethylases)

| Fto/FTO | Abnormal brain and cardiac development [191]*. | Developmental delay and dysmorphic facial features (Çağlayan (2016) J Hum Genet 61, 395). Growth retardation and multiple malformations (Boissel (2009) Am J Hum Genet 85, 106). Developmental delay and growth retardation (Daoud (2016) J Med Genet 53, 200). Growth retardation and multiple malformations (Rohena (2016) Am J Med Genet 170, 1023). Obesity (Song (2008) Obesity (Silver Spring) 16, 2472); (Yang (2012) Nature 490, 267). Type II diabetes (Frayling (2007) Science 316, 889). Metabolic syndrome including obesity, hypertension, dyslipidemia, and defective glucose tolerance Hotta (2011) J Hum Genet 56, 647). |

### 2-ODGs – JmjC demethylases and hydroxylases

| JmjD4/JMJD4 | Viable and fertile with normal physiology [192]*. | NR |
| JmjD6/JMJD6 | Perinatal lethal with growth retardation and exhibit severe tissue and organ differentiation defects, including brain, lung, liver, kidney, intestine, heart and thymus development at different stages of | NR |
| Gene | Description | Notes |
|------|-------------|-------|
| Kdm2a/KDM2A | Embryonic lethal with severe growth retardation and defective neural tube closure [197]*. | NR |
| Kdm2b/KDM2B | KDM2B-1-deletion mice display moderate penetrance of neural tube defects, leading to exencephaly and death at birth [198]. Whereas, mice deficient of both KDM2B-1 and KDM2B-2 isoforms are embryonic lethal with full penetrant developmental defects, including abnormal somitogenesis, reduced size, defective neural tube and heart [199-201]*; especially a more severe developmental in female embryos [200]*. Furthermore, KDM2B-2-deleted mice also display similar developmental abnormalities and increased lethality, particularly in females [200]*. | NR |
| Kdm3a/KDM3A | Develop obesity, abnormal fat metabolism [202,203]*, reduced energy expenditure, and display metabolic syndrome, including, high plasma cholesterol, insulin, triglyceride, and leptin levels [203]*. Male infertility [206]*. | |
| Gene        | Description                                                                 | Phenotypes                                                                 |
|-------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Kdm3b/KDM3B | Postnatal growth restriction and female mice were infertile due to decreased ovulation, prolonged estrous cycles, reduced fertilisation and uterine decidual response. Male knockout mice have impaired reproductive function, sperm development and maturation. Knockout mice exhibit myelodysplastic syndrome and defective hematopoiesis including leukocytosis, moderate anemia, and granulocytosis. | Schizophrenia [209]*. Intellectual disability [210]*. Wilms tumour and hyperpigmentation [211]*. Hepatoblastoma, autism, intellectual disability, and abnormal pigmentation [211]*. Acute myeloid leukemia, mild intellectual disability, congenital hypothyroidism and congenital hip dysplasia [211]*. Hodgkin lymphoma, feeding difficulties, intellectual disability, umbilical and inguinal hernia [212]*. Intellectual disability, facial dysmorphism and short stature [212]*. |
| Jmjd1c/JMJD1C | Males gradually develop infertility with decreasing testes size due to progressive loss of germ cells. | Congenital heart disease in patients with 22q11.2 deletion syndrome [213]*. Rett syndrome [214]*[215]*. Autism spectrum disorder [214]*. Intellectual disability [214]*. Intracranial germ cell tumour [146]*. |
| Kdm4a/KDM4A | Viable [216]*. | NR |
| Kdm4b/KDM4B | Viable [217]*. Viable with lower birth rate. Early weaning results in death. Susceptible to obesity with impaired energy expenditure, adaptive thermogenesis and adipose tissue lipolysis. | NR |
| Gene       | Viable/Fertile | Upper aerodigestive tract cancer [221]*. Age at menarche [222]*. | Intellectual disability, dyslexia, global developmental delay, facial dysmorphism, aggressive behaviour, hypospadias [230]*. |
|------------|---------------|------------------------------------------------------------------|------------------------------------------------------------------|
| Kdm4c/KDM4C| Viable and fertile [219]*. However, another reported that it leads to embryonic lethally [220]*. | Upper aerodigestive tract cancer [221]*. Age at menarche [222]*. | Intellectual disability, dyslexia, global developmental delay, facial dysmorphism, aggressive behaviour, hypospadias [230]*. |
| Kdm4d/KDM4B| Viable and fertile without gross abnormalities [223]*. | NR | Intellectual disability, dyslexia, global developmental delay, facial dysmorphism, aggressive behaviour, hypospadias [230]*. |
| Kdm5a/KDM5A| Viable [224,225]*. Mice displayed mild behavioural and haematological abnormalities [224]*. | Intellectual disability [226]*. Congenital heart disease (Zaidi (2013) Nature 498, 220). |
| Kdm5b/KDM5B| Embryonic lethal [227,228]*. Neonatal lethal due to failure to establish respiratory function, defective neural system and homeotic skeletal transformations [229]*. | Intellectual disability, dyslexia, global developmental delay, facial dysmorphism, aggressive behaviour, hypospadias [230]*. | Intellectual disability, dyslexia, global developmental delay, facial dysmorphism, aggressive behaviour, hypospadias [230]*. |
| Kdm5c/KDM5C| Hemizygous KDM5Cnull male mice are embryonic lethal due to defective neurulation and cardiogenesis [231]*. Male hemizygous knockout mice (Kdm5c<−/−) Viable with adaptive and cognitive abnormalities, including increased aggression, impaired social behaviour, limited learning, fear memory deficits, defective dendritic spines [232,233]* and significant reduced body weight [233]*. | X-linked intellectual disability [234]*[235]*[236]*[237]*[238]*[239]*[240]*[241]*[242] [243]*[244]*[245]*[246]*. Autism spectrum disorder [247]*. | X-linked intellectual disability [234]*[235]*[236]*[237]*[238]*[239]*[240]*[241]*[242] [243]*[244]*[245]*[246]*. Autism spectrum disorder [247]*. |
| Kdm5d/KDM5D| A large scale screening using CRISPR/Cas9-mediated genome | NR | |
| Kdm6a/KDM6A | Embryonic lethal with cardiac development defects and neural tube closure. While female knockout mice died mid-gestational, some hemizygous KDM6A-null male mice survive into adulthood [231,249-252]* and are fertile [251,252], with reduced lifespan and smaller in size [251]*. Female embryonic lethal, abnormal/truncated posterior bodies, anaemic (hematopoiesis), severe heart development defect and neural tube closure. Male died around birth due to neuron tube closure defect and inability to breath [253]*. | Kabuki syndrome [254]*[255]*[256,257]* syndrome [258]*[259]*[260]*[261]*[262]*[263]*. Biliary atresia with Kabuki syndrome-like features [264]*. Renal cancer [265]*. |
| Kdm6b/KDM6B | Embryonic [266] and perinatal lethal with respiratory failure [267-269]*, detail reviewed here [270]*. Reduced proliferation and hypertrophy of chondrocytes, as well as delayed endochondral ossification in mice [271]*. Delayed osteoblast proliferation and hypertrophy. | Intellectual disability [226]*. Intellectual disability, brachydactyly and dysmorphism [273]*. |
| Gene/Protein | Description | Phenotype |
|-------------|-------------|-----------|
| Uty/UTY | Hemizygous male mice are viable [249]*. | NR |
| Kdm7a/KDM7A | A large-scale genome-wide tissue phenotype screen revealed that abnormal hair follicles, sebaceous gland, tail and hair follicle bulge morphology in KDM7A knockout mice [274]* | |
| Phf8/PHF8 | Impaired learning and memory, hippocampal long-term potentiation [275]*. | X-linked mental retardation with cleft lip/palate [276]*[277]*[278]*. Autism and Asperger syndrome [279]*. Autism spectrum disorder, intellectual disability, cleft palate and Aarskog syndrome [280]*. Intellectual disability [239]*. |
| Kdm8/KDM8 | Embryonic lethal with delayed development in multiple organs [281]* and growth retardation [282]*. | NR |

**2-OGDs - hydroxylases**

Similar to PHDs, prolyl-4-hydroxylases P4HA1 and P4HA2 are hypoxia-inducible, but P4HA1/2 prolyl hydroxylation is required for different processes, that is collagen fiber formation. Consistent to their roles in collagen synthesis, P4HA1 and P4HA2 mutations are found in patients with collagen-related extracellular matrix disorders (Table 1; Supplementary Table 1). Furthermore, homozygous deletion of P4HA1 is embryonic lethal with base membrane rupture due to defective collagen IV assembly (Table 1). PAHX is another hydroxylase, but of phytanoyl-CoA; essential for breaking down phytanic fatty acid. Mutations in PAHX is well associated with Refsum disease, a rare inherited neurological disorder caused by neurotoxic phytanic acid as these mutations result in an enzymatically inactive protein, thus leading to phytanic acid accumulation.

The roles of TET1–3 in development are demonstrated in knockout mouse models (reviewed in [283]). TET1-null mice present several defects but these depend on the mode of genetic deletion. TET3 deletion results in neonatal lethality, highlighting TET3 role in development. TET3 mutations have been found in patients with intellectual disability and/or delayed global development (Table 1; Supplementary Table 1). Although somatic alterations of TET2 have been found in several cancers, these mutations are majorly associated with myelodysplastic syndromes (Table 1; Supplementary Table 1). In addition to the listed mutations in Supplementary Table 1, a study reported TET2 somatic mutations in 46 patients with myelodysplastic syndromes, myeloproliferative disorder, secondary
acute myeloid leukemia, or chronic myelomonocytic leukemia [284]. Most of these mutations are predicted to lead to partial or total loss of function due to protein truncation.

2-OGDs - JmjC demethylases

Many of the JmjC- demethylase genes have been associated with human diseases. In particular, several of them are mutated in patients with neurodevelopmental disorders, midline defects and cancers (Table 1; Supplementary Table 1). Although KDM3A is found to be mutated in infertile males [206], its role in infertility is not clear. Mutations in KDM3B are frequently implicated with intellectual disability, but also found in cancers including myeloid leukemias (Table 1). Similarly, JMJD1C mutations have been identified in individuals with autism spectrum disorder and intellectual disability. JMJD1C is also associated with congenital heart disease manifestation in 22q11.2 deletion syndrome patients. Amongst KDM4s, there are only two reports – single nucleotide substitutions of KDM4C in upper aerodigestive tract cancer and age of menarche. Mutations of KDM5B, KDM5C and KDM6B have been associated with neurodevelopment and a global developmental delay (Table 1; Supplementary Table 1). In particular, KDM5C is well recognised as an X-linked intellectual disability gene that is highly expressed in neural tissue. Mutations in PHD Finger Protein 8 (PHF8) are also associated with X-linked mental retardation and often accompanied by cleft lip/palate or autism. The phenotypes of KDM5C or PHF8 mutations in humans are reflected by the deletion of these genes in mice (Table 1). On the other hand, KDM6A mutations are frequently found in individuals with Kabuki syndrome (KS), a genetic disease with developmental delay and congenital anomalies, (Table 1) highlighting the role of KDM6A in development. In addition to mutation listed in Supplementary Table 1, others have reported gross deletions, gross duplications, or chromosomal rearrangement in patients with KS or KS-like clinical manifestations [256,258,285-287]. However, whether the phenotypes observed are due to loss of demethylase activity solely is currently unknown.

Overall, the presence and connections of HIFs or dioxygenase mutations in human disorders and the knockout studies demonstrate the essential roles of these genes.

Conclusion and future perspectives

As our understanding of the cellular response to hypoxia advances, new aspects continue to unravel. The role of oxygen has surfaced as far broader than just an acceptor molecule in oxidative phosphorylation in the mitochondria. Through acting as a co-factor for diverse and functionally important enzymes, oxygen is mechanistically identified as a potent signalling molecule in cells. The emerging focus of the field includes new aspects of chromatin regulation, RNA biology and broad regulation of protein post-translational modifications directly controlled by oxygen levels. This advanced understanding in conjunction with development of novel therapeutic chemicals targeting dioxygenases should provide not only exciting new biological insights, but also better treatments for patients suffering from a range of diseases. One area of technological advancement that will greatly progress the field is the adaptation of novel and unbiased quantitative techniques for measuring chromatin structure, transcriptional output, proteomic changes and cellular behaviour. These approaches may provide resolution to some of the persisting major questions pertaining mechanisms controlling gene expression in response to hypoxia.
References

1. Semenza, G.L.; Wang, G.L. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 1992, 12, 5447-5454.

2. Rocha, S. Gene regulation under low oxygen: Holding your breath for transcription. *Trends Biochem Sci* 2007, 32, 389-397.

3. Kliwe, F.; Engelhardt, M.; Aref, R.; Schuller, H.J. Promoter recruitment of corepressors sin3 and cyc8 by activator proteins of the yeast *Saccharomyces cerevisiae*. *Curr Genet* 2017, 63, 739-750.

4. D'Ignazio, L.; Batie, M.; Rocha, S. Hypoxia and inflammation in cancer, focus on hif and nf-kappab. *Biomedicines* 2017, 5.

5. Biddlestone, J.; Bandarra, D.; Rocha, S. The role of hypoxia in inflammatory disease (review). *Int J Mol Med* 2015, 35, 859-869.

6. Epstein, A.C.; Gleadle, J.M.; McNeill, L.A.; Hewitson, K.S.; O'Rourke, J.; Mole, D.R.; Mukherji, M.; Metzen, E.; Wilson, M.I.; Dhanda, A., et al. C. *E. coli* egl-9 and mammalian homologs define a family of dioxygenases that regulate hif by prolyl hydroxylation. *Cell* 2001, 107, 43-54.

7. Ivan, M.; Kondo, K.; Yang, H.; Kim, W.; Valiando, J.; Ohh, M.; Salic, A.; Asara, J.M.; Lane, W.S.; Kaelin, W.G., Jr. Hifalpha targeted for vhl-mediated destruction by proline hydroxylation: Implications for o2 sensing. *Science* 2001, 292, 464-468.

8. Kaelin, W.G., Jr.; Ratcliffe, P.J. Oxygen sensing by metazoans: The central role of the hif hydroxylase pathway. *Mol Cell* 2008, 30, 393-402.

9. Ehrismann, D.; Flashman, E.; Genn, D.N.; Mathioudakis, N.; Hewitson, K.S.; Ratcliffe, P.J.; Schofield, C.J. Studies on the activity of the hypoxia-inducible-factor hydroxylases using an oxygen consumption assay. *Biochem J* 2007, 401, 227-234.

10. Batie, M.; Frost, J.; Frost, M.; Wilson, J.W.; Schofield, P.; Rocha, S. Hypoxia induces rapid changes to histone methylation and reprograms chromatin. *Science* 2019, 363, 1222-1226.

11. Choudhry, H.; Harris, A.L. Advances in hypoxia-inducible factor biology. *Cell Metab* 2018, 27, 281-298.

12. Batie, M.; Rocha, S. Gene transcription and chromatin regulation in hypoxia. *Biochem Soc Trans* 2020.

13. Wilson, J.W.; Shakir, D.; Batie, M.; Frost, M.; Rocha, S. Oxygen-sensing mechanisms in cells. *Febs J* 2020.

14. Schodel, J.; Ratcliffe, P.J. Mechanisms of hypoxia signalling: New implications for nephrology. *Nat Rev Nephrol* 2019, 15, 641-659.

15. Ortiz-Barahona, A.; Villar, D.; Pescador, N.; Amigo, J.; del Peso, L. Genome-wide identification of hypoxia-inducible factor binding sites and target genes by a probabilistic model integrating transcription-profiling data and in silico binding site prediction. *Nucleic Acids Res* 2010, 38, 2332-2345.

16. Benita, Y.; Kikuchi, H.; Smith, A.D.; Zhang, M.Q.; Chung, D.C.; Xavier, R.J. An integrative genomics approach identifies hypoxia-inducible factor-1 (hif-1)-target genes that form the core response to hypoxia. *Nucleic Acids Res* 2009, 37, 4587-4602.
17. Dengler, V.L.; Galbraith, M.; Espinosa, J.M. Transcriptional regulation by hypoxia inducible factors. *Crit Rev Biochem Mol Biol* **2014**, *49*, 1-15.
18. Schodel, J.; Oikonomopoulos, S.; Ragoussis, J.; Pugh, C.W.; Ratcliffe, P.J.; Mole, D.R. High-resolution genome-wide mapping of hif-binding sites by chip-seq. *Blood* **2011**, *117*, e207-217.
19. Platt, J.L.; Salama, R.; Smythies, J.; Choudhry, H.; Davies, J.O.; Hughes, J.R.; Ratcliffe, P.J.; Mole, D.R. Capture-c reveals preformed chromatin interactions between hif-binding sites and distant promoters. *EMBO Rep* **2016**, *17*, 1410-1421.
20. Orlando, I.M.C.; Lafleur, V.N.; Storti, F.; Spielmann, P.; Crowther, L.; Santambrogio, S.; Schodel, J.; Hoogewijs, D.; Mole, D.R.; Wenger, R.H. Distal and proximal hypoxia response elements cooperate to regulate organ-specific erythropoietin gene expression. *Haematologica* **2019**.
21. Kenneth, N.S.; Rocha, S. Regulation of gene expression by hypoxia. *Biochem J* **2008**, *414*, 19-29.
22. Batie, M.; Del Peso, L.; Rocha, S. Hypoxia and chromatin: A focus on transcriptional repression mechanisms. *Biomedicines* **2018**, *6*.
23. Hu, R.; Jin, H.; Zhou, S.; Yang, P.; Li, X. Proteomic analysis of hypoxia-induced responses in the syncytialization of human placental cell line bewo. *Placenta* **2007**, *28*, 399-407.
24. Li, Q.; Luo, T.; Lu, W.; Yi, X.; Zhao, Z.; Liu, J. Proteomic analysis of human periodontal ligament cells under hypoxia. *Proteome Sci* **2019**, *17*, 3.
25. Dou, L.; Yan, Q.; Liang, P.; Zhou, P.; Zhang, Y.; Ji, P. Itraq-based proteomic analysis exploring the influence of hypoxia on the proteome of dental pulp stem cells under 3d culture. *Proteomics* **2018**, *18*.
26. Dutta, B.; Ren, Y.; Hao, P.; Sim, K.H.; Cheow, E.; Adav, S.; Tam, J.P.; Sze, S.K. Profiling of the chromatin-associated proteome identifies hp1bp3 as a novel regulator of cell cycle progression. *Mol Cell Proteomics* **2014**, *13*, 2183-2197.
27. Gao, Y.; Dasgupta, C.; Huang, L.; Song, R.; Zhang, Z.; Zhang, L. Multi-omics integration reveals short and long-term effects of gestational hypoxia on the heart development. *Cells* **2019**, *8*.
28. Sharma, N.K.; Sethy, N.K.; Bhargava, K. Comparative proteome analysis reveals differential regulation of glycolytic and antioxidant enzymes in cortex and hippocampus exposed to short-term hypobaric hypoxia. *J Proteomics* **2013**, *79*, 277-298.
29. Hoang, V.M.; Foulk, R.; Clauer, K.; Burlingame, A.; Gibson, B.W.; Fisher, S.J. Functional proteomics: Examining the effects of hypoxia on the cytotrophoblast protein repertoire. *Biochemistry* **2001**, *40*, 4077-4086.
30. Shakib, K.; Norman, J.T.; Fine, L.G.; Brown, L.R.; Godovac-Zimmermann, J. Proteomics profiling of nuclear proteins for kidney fibroblasts suggests hypoxia, meiosis, and cancer may meet in the nucleus. *Proteomics* **2005**, *5*, 2819-2838.
31. Vodisch, M.; Scherlach, K.; Winkler, R.; Hertweck, C.; Braun, H.P.; Roth, M.; Haas, H.; Werner, E.R.; Brakhage, A.A.; Kniemeyer, O. Analysis of the aspergillus fumigatus proteome reveals metabolic changes and the activation of the pseurotin a biosynthesis gene cluster in response to hypoxia. *J Proteome Res* **2011**, *10*, 2508-2524.
32. Janker, L.; Mayer, R.L.; Bileck, A.; Kreutz, D.; Mader, J.C.; Utpatel, K.; Heudobler, D.; Agis, H.; Gerner, C.; Slany, A. Metabolic, anti-apoptotic and immune evasion strategies of primary human myeloma cells indicate adaptations to hypoxia. *Mol Cell Proteomics* 2019, 18, 936-953.

33. Chen, J.T.; Liu, C.C.; Yu, J.S.; Li, H.H.; Lai, M.C. Integrated omics profiling identifies hypoxia-regulated genes in hct116 colon cancer cells. *J Proteomics* 2018, 188, 139-151.

34. Cosme, J.; Guo, H.; Hadipour-Lakmehsari, S.; Emili, A.; Gramolini, A.O. Hypoxia-induced changes in the fibroblast secretome, exosome, and whole-cell proteome using cultured, cardiac-derived cells isolated from neonatal mice. *J Proteome Res* 2017, 16, 2836-2847.

35. Ramteke, A.; Ting, H.; Agarwal, C.; Mateen, S.; Somasagara, R.; Hussain, A.; Graner, M.; Frederick, B.; Agarwal, R.; Deep, G. Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules. *Mol Carcinog* 2015, 54, 554-565.

36. Holcik, M.; Sonenberg, N. Translational control in stress and apoptosis. *Nat Rev Mol Cell Biol* 2005, 6, 318-327.

37. Stein, I.; Itin, A.; Einat, P.; Skaliter, R.; Grossman, Z.; Keshet, E. Translation of vascular endothelial growth factor mrna by internal ribosome entry: Implications for translation under hypoxia. *Mol Cell Biol* 1998, 18, 3112-3119.

38. Vagner, S.; Gensac, M.C.; Maret, A.; Bayard, F.; Amalric, F.; Prats, H.; Prats, A.C. Alternative translation of human fibroblast growth factor 2 mrna occurs by internal entry of ribosomes. *Mol Cell Biol* 1995, 15, 35-44.

39. Bernstein, J.; Sella, O.; Le, S.Y.; Elroy-Stein, O. Pdgif2/c-sis mrna leader contains a differentiation-linked internal ribosomal entry site (d-ires). *J Biol Chem* 1997, 272, 9356-9362.

40. Webb, T.E.; Hughes, A.; Smalley, D.S.; Spriggs, K.A. An internal ribosome entry site in the 5' untranslated region of epidermal growth factor receptor allows hypoxic expression. *Oncogenesis* 2015, 4, e134.

41. Lang, K.J.; Kappel, A.; Goodall, G.J. Hypoxia-inducible factor-1alpha mrna contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. *Mol Biol Cell* 2002, 13, 1792-1801.

42. Schepens, B.; Tinton, S.A.; Bruynooghe, Y.; Beyaert, R.; Cornelis, S. The polypyrimidine tract-binding protein stimulates hif-1alpha ires-mediated translation during hypoxia. *Nucleic Acids Res* 2005, 33, 6884-6894.

43. Barbosa, C.; Romao, L. Translation of the human erythropoietin transcript is regulated by an upstream open reading frame in response to hypoxia. *RNA* 2014, 20, 594-608.

44. Uiniacke, J.; Holtermann, C.E.; Lachance, G.; Franovic, A.; Jacob, M.D.; Fabian, M.R.; Payette, J.; Holcik, M.; Pause, A.; Lee, S. An oxygen-regulated switch in the protein synthesis machinery. *Nature* 2012, 486, 126-129.

45. Meyer, K.D.; Patil, D.P.; Zhou, J.; Zinoviev, A.; Skabkin, M.A.; Elemento, O.; Pestova, T.V.; Qian, S.B.; Jaffrey, S.R. 5' utr m(6)a promotes cap-independent translation. *Cell* 2015, 163, 999-1010.

46. Kouzarides, T. Chromatin modifications and their function. *Cell* 2007, 128, 693-705.
47. Hsu, K.F.; Wilkins, S.E.; Hopkinson, R.J.; Sekirnik, R.; Flashman, E.; Kawamura, A.; McCullagh, J.S.O.; Walport, L.J.; Schofield, C.J. Hypoxia and hypoxia mimetics differentially modulate histone post-translational modifications. *Epigenetics* 2020, 1-14.

48. Prickaerts, P.; Adriaens, M.E.; Beucken, T.V.; Koch, E.; Dubois, L.; Dahlmans, V.E.; Gits, C.; Evelo, C.T.; Chan-Seng-Yue, M.; Wouters, B.G., et al. Hypoxia increases genome-wide bivalent epigenetic marking by specific gain of h3k27me3. *Epigenetics Chromatin* 2016, 9, 46.

49. Chakraborty, A.A.; Laukka, T.; Myllärykoski, M.; Ringel, A.E.; Booker, M.A.; Tolstorukov, M.Y.; Meng, Y.J.; Meier, S.R.; Jennings, R.B.; Creech, A.L., et al. Histone demethylase kdm6a directly senses oxygen to control chromatin and cell fate. *Science* 2019, 363, 1217-1222.

50. Hancock, R.L.; Masson, N.; Dunne, K.; Flashman, E.; Kawamura, A. The activity of jmjc histone lysine demethylase kdm4a is highly sensitive to oxygen concentrations. *ACS Chem Biol* 2017, 12, 1011-1019.

51. Dobrynin, G.; McAllister, T.E.; Leszczynska, K.B.; Ramachandran, S.; Krieg, A.J.; Kawamura, A.; Hammond, E.M. Kdm4a regulates hif-1 levels through h3k9me3. *Sci Rep* 2017, 7, 11094.

52. Luo, W.; Chang, R.; Zhong, J.; Pandey, A.; Semenza, G.L. Histone demethylase jmjd2c is a coactivator for hypoxia-inducible factor 1 that is required for breast cancer progression. *Proc Natl Acad Sci U S A* 2012, 109, E3367-3376.

53. Krieg, A.J.; Rankin, E.B.; Chan, D.; Razorenova, O.; Fernandez, S.; Giaccia, A.J. Regulation of the histone demethylase jmjd1a by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. *Molecular and cellular biology* 2010, 30, 344-353.

54. Wan, W.; Peng, K.; Li, M.; Qin, L.; Tong, Z.; Yan, J.; Shen, B.; Yu, C. Histone demethylase jmjd1a promotes urinary bladder cancer progression by enhancing glycolysis through coactivation of hypoxia inducible factor 1alpha. *Oncogene* 2017, 36, 3868-3877.

55. Shmakova, A.; Batie, M.; Druker, J.; Rocha, S. Chromatin and oxygen sensing in the context of jmjc histone demethylases. *Biochem J* 2014, 462, 385-395.

56. Wu, X.; Zhang, Y. Tet-mediated active DNA demethylation: Mechanism, function and beyond. *Nat Rev Genet* 2017, 18, 517-534.

57. Thienpont, B.; Steinbacher, J.; Zhao, H.; D’Anna, F.; Kuchnio, A.; Ploumakis, A.; Ghesquiere, B.; Van Dyck, L.; Boeckx, B.; Schoonjans, L., et al. Tumour hypoxia causes DNA hypermethylation by reducing tet activity. *Nature* 2016, 537, 63-68.

58. Shahrazad, S.; Bertrand, K.; Minhas, K.; Coomber, B.L. Induction of DNA hypomethylation by tumor hypoxia. *Epigenetics* 2007, 2, 119-125.

59. D’Anna, F.; Van Dyck, L.; Xiong, J.; Zhao, H.; Berrens, R.V.; Qian, J.; Bieniasz-Krzywiec, P.; Chandra, V.; Schoonjans, L.; Matthews, J., et al. DNA methylation repels binding of hypoxia-inducible transcription factors to maintain tumor immunotolerance. *Genome Biol* 2020, 21, 182.

60. Camuzi, D.; de Amorim, I.S.S.; Ribeiro Pinto, L.F.; Oliveira Trivilin, L.; Mencalha, A.L.; Soares Lima, S.C. Regulation is in the air: The relationship between hypoxia and epigenetics in cancer. *Cells* 2019, 8.

61. Ito, S.; D’Alessio, A.C.; Taranova, O.V.; Hong, K.; Sowers, L.C.; Zhang, Y. Role of tet proteins in 5mc to 5shm conversion, es-cell self-renewal and inner cell mass specification. *Nature* 2010, 466, 1129-1133.
62. Tahiliani, M.; Koh, K.P.; Shen, Y.; Pastor, W.A.; Bandukwala, H.; Brudno, Y.; Agarwal, S.; Iyer, L.M.; Liu, D.R.; Aravind, L., et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by mll partner tet1. *Science* 2009, 324, 930-935.

63. Brugarolas, J.; Lei, K.; Hurley, R.L.; Manning, B.D.; Reiling, J.H.; Hafen, E.; Witters, L.A.; Ellisen, L.W.; Kaelin, W.G., Jr. Regulation of mtor function in response to hypoxia by redd1 and the tsc1/tsc2 tumor suppressor complex. *Genes Dev* 2004, 18, 2893-2904.

64. Connolly, E.; Braunstein, S.; Formen ti, S.; Schneider, R.J. Hypoxia inhibits protein synthesis through a 4e-bp1 and elongation factor 2 kinase pathway controlled by mtor and uncoupled in breast cancer cells. *Mol Cell Biol* 2006, 26, 3955-3965.

65. Sonenberg, N.; Hinnebusch, A.G. Regulation of translation initiation in eukaryotes: Mechanisms and biological targets. *Cell* 2009, 136, 731-745.

66. Koumenis, C.; Naczk, C.; Koritzinsky, M.; Rastani, S.; Diehl, A.; Sonenberg, N.; Koromilas, A.; Wouters, B.G. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase perk and phosphorylation of the translation initiation factor eif2alpha. *Mol Cell Biol* 2002, 22, 7405-7416.

67. Donnelly, N.; Gorman, A.M.; Gupta, S.; Samali, A. The eif2alpha kinases: Their structures and functions. *Cell Mol Life Sci* 2013, 70, 3493-3511.

68. Ryazanov, A.G.; Davydova, E.K. Mechanism of elongation factor 2 (ef-2) inactivation upon phosphorylation. Phosphorylated ef-2 is unable to catalyze translocation. *FEBS Lett* 1989, 251, 187-190.

69. Browne, G.J.; Proud, C.G. A novel mtor-regulated phosphorylation site in elongation factor 2 kinase modulates the activity of the kinase and its binding to calmodulin. *Mol Cell Biol* 2004, 24, 2986-2997.

70. Liu, L.; Cash, T.P.; Jones, R.G.; Keith, B.; Thompson, C.B.; Simon, M.C. Hypoxia-induced energy stress regulates mrna translation and cell growth. *Mol Cell* 2006, 21, 521-531.

71. Moore, C.E.; Mikolajek, H.; Regufe da Mota, S.; Wang, X.; Kenney, J.W.; Werner, J.M.; Proud, C.G. Elongation factor 2 kinase is regulated by proline hydroxylation and protects cells during hypoxia. *Mol Cell Biol* 2015, 35, 1788-1804.

72. Pollard, P.J.; Loenarz, C.; Mole, D.R.; McDonough, M.A.; Gleadle, J.M.; Schofield, C.J.; Ratcliffe, P.J. Regulation of jumonji-domain-containing histone demethylases by hypoxia-inducible factor (hif)-1alpha. *Biochem J* 2008, 416, 387-394.

73. Kato, M.; Araiso, Y.; Noma, A.; Nagao, A.; Suzuki, T.; Ishitani, R.; Nureki, O. Crystal structure of a novel jmjc-domain-containing protein, tyw5, involved in trna modification. *Nucleic Acids Res* 2011, 39, 1576-1585.

74. Ge, W.; Wolf, A.; Feng, T.; Ho, C.H.; Sekirnik, R.; Zayer, A.; Granatino, N.; Cockman, M.E.; Loenarz, C.; Loik, N.D., et al. Oxygenase-catalyzed ribosome hydroxylation occurs in prokaryotes and humans. *Nat Chem Biol* 2012, 8, 960-962.

75. Loenarz, C.; Sekirnik, R.; Thalhammer, A.; Ge, W.; Spivakovsky, E.; Mackeen, M.M.; McDonough, M.A.; Cockman, M.E.; Kessler, B.M.; Ratcliffe, P.J., et al. Hydroxylation of the eukaryotic ribosomal decoding center affects translational accuracy. *Proc Natl Acad Sci U S A* 2014, 111, 4019-4024.

76. Singleton, R.S.; Liu-Yi, P.; Formenti, F.; Ge, W.; Sekirnik, R.; Fischer, R.; Adam, J.; Pollard, P.J.; Wolf, A.; Thalhammer, A., et al. Ogfod1 catalyzes prolyl hydroxylation of rps23 and is
involved in translation control and stress granule formation. *Proc Natl Acad Sci U S A* 2014, 111, 4031-4036.

77. Fu, D.; Brophy, J.A.; Chan, C.T.; Atmore, K.A.; Begley, U.; Paules, R.S.; Dedon, P.C.; Begley, T.J.; Samson, L.D. Human alkb homolog abh8 is a trna methyltransferase required for wobble uridine modification and DNA damage survival. *Mol Cell Biol* 2010, 30, 2449-2459.

78. Noma, A.; Ishitani, R.; Kato, M.; Nagao, A.; Nureki, O.; Suzuki, T. Expanding role of the jumonji c domain as an rna hydroxylase. *J Biol Chem* 2010, 285, 34503-34507.

79. Feng, T.; Yamamoto, A.; Wilkins, S.E.; Sokolova, E.; Yates, L.A.; Munzel, M.; Singh, P.; Hopkinson, R.J.; Fischer, R.; Cockman, M.E., et al. Optimal translational termination requires c4 lysyl hydroxylation of erf1. *Mol Cell* 2014, 53, 645-654.

80. Zhou, T.; Erber, L.; Liu, B.; Gao, Y.; Ruan, H.B.; Chen, Y. Proteomic analysis reveals diverse proline hydroxylation-mediated oxygen-sensing cellular pathways in cancer cells. *Oncotarget* 2016, 7, 79154-79169.

81. Cockman, M.E.; Webb, J.D.; Kramer, H.B.; Kessler, B.M.; Ratcliffe, P.J. Proteomics-based identification of novel factor inhibiting hypoxia-inducible factor (fih) substrates indicates widespread asparaginyl hydroxylation of ankyrin repeat domain-containing proteins. *Mol Cell Proteomics* 2009, 8, 535-546.

82. Malec, V.; Coulson, J.M.; Urbe, S.; Clague, M.J. Combined analyses of the vhl and hypoxia signaling axes in an isogenic pairing of renal clear cell carcinoma cells. *J Proteome Res* 2015, 14, 5263-5272.

83. Silverman-Gavrila, L.B.; Lu, T.Z.; Prashad, R.C.; Nejatbakhsh, N.; Charlton, M.P.; Feng, Z.P. Neural phosphoproteomics of a chronic hypoxia model--lymnaea stagnalis. *Neuroscience* 2009, 161, 621-634.

84. Chachami, G.; Stankovic-Valentin, N.; Karagiota, A.; Basagianni, A.; Plessmann, U.; Urlaub, H.; Melchior, F.; Simos, G. Hypoxia-induced changes in sumo conjugation affect transcriptional regulation under low oxygen. *Mol Cell Proteomics* 2019, 18, 1197-1209.

85. Dhillon, R.S.; Richards, J.G. Hypoxia induces selective modifications to the acetylome in the brain of zebrafish (danio rerio). *Comp Biochem Physiol B Biochem Mol Biol* 2018, 224, 79-87.

86. Arriagada, C.; Silva, P.; Torres, V.A. Role of glycosylation in hypoxia-driven cell migration and invasion. *Cell Adh Migr* 2019, 13, 13-22.

87. Peinado, M.A.; Hernandez, R.; Peragon, J.; Ovelleiro, D.; Pedrosa, J.A.; Blanco, S. Proteomic characterization of nitrated cell targets after hypobaric hypoxia and reoxygenation in rat brain. *J Proteomics* 2014, 109, 309-321.

88. Chen, S.C.; Huang, B.; Liu, Y.C.; Shyu, K.G.; Lin, P.Y.; Wang, D.L. Acute hypoxia enhances proteins’ s-nitrosylation in endothelial cells. *Biochem Biophys Res Commun* 2008, 377, 1274-1278.

89. Kumar, G.K.; Prabhakar, N.R. Post-translational modification of proteins during intermittent hypoxia. *Respir Physiol Neurobiol* 2008, 164, 272-276.

90. Moser, S.C.; Bensaddek, D.; Ortmann, B.; Maure, J.F.; Mudie, S.; Blow, J.J.; Lamond, A.I.; Swedlow, J.R.; Rocha, S. Phd1 links cell-cycle progression to oxygen sensing through hydroxylation of the centrosomal protein cep192. *Dev Cell* 2013, 26, 381-392.
91. Zheng, X.; Zhai, B.; Koivunen, P.; Shin, S.J.; Lu, G.; Liu, J.; Geisen, C.; Chakraborty, A.A.; Moslehi, J.J.; Smalley, D.M., et al. Prolyl hydroxylation by egln2 destabilizes foxo3a by blocking its interaction with the usp9x deubiquitinase. *Genes Dev* **2014**, *28*, 1429-1444.

92. Luo, W.; Lin, B.; Wang, Y.; Zhong, J.; O’Meally, R.; Cole, R.N.; Pandey, A.; Levchenko, A.; Semenza, G.L. Phd3-mediated prolyl hydroxylation of nonmuscle actin impairs polymerization and cell motility. *Mol Biol Cell* **2014**, *25*, 2788-2796.

93. Guo, J.; Chakraborty, A.A.; Liu, P.; Gan, W.; Zheng, X.; Inuzuka, H.; Wang, B.; Zhang, L.; Yuan, M., et al. Pvh1 suppresses kinase activity of akt in a proline-hydroxylation-dependent manner. *Science* **2016**, *353*, 929-932.

94. Lee, S.B.; Ko, A.; Oh, Y.T.; Shi, P.; D’Angelo, F.; Frangaj, B.; Koller, A.; Chen, E.I.; Cardozo, T.; Iavarone, A., et al. Proline hydroxylation primes protein kinases for autophosphorylation and activation. *Mol Cell* **2020**, *79*, 376-389.e378.

95. Ferrer, C.M.; Lynch, T.P.; Sodi, V.L.; Falcone, J.N.; Schwab, L.P.; Peacock, D.L.; Vocadlo, D.J.; Seagroves, T.N.; Reginato, M.J. O-glcnacylation regulates cancer metabolism and survival stress signaling via regulation of the hif-1 pathway. *Mol Cell* **2014**, *54*, 820-831.

96. Arsenault, P.R.; Heaton-Johnson, K.J.; Li, L.S.; Song, D.; Ferreira, V.S.; Patel, N.; Master, S.R.; Lee, F.S. Identification of prolyl hydroxylation modifications in mammalian cell proteins. *Proteomics* **2015**, *15*, 1259-1267.

97. Chang, B.; Chen, Y.; Zhao, Y.; Bruick, R.K. Jmjd6 is a histone arginine demethylase. *Science* **2007**, *318*, 444-447.

98. Islam, M.S.; McDonough, M.A.; Chowdhury, R.; Gault, J.; Khan, A.; Pires, E.; Schofield, C.J. Biochemical and structural investigations clarify the substrate selectivity of the 2-oxoglutarate oxygenase jmjd6. *J Biol Chem* **2019**, *294*, 11637-11652.

99. Alahari, S.; Post, M.; Caniggia, I. Jumonji domain containing protein 6: A novel oxygen sensor in the human placenta. *Endocrinology* **2015**, *156*, 3012-3025.

100. Tsai, W.C.; Reineke, L.C.; Jain, A.; Jung, S.Y.; Lloyd, R.E. Histone arginine demethylase jmjd6 is linked to stress granule assembly through demethylation of the stress granule-nucleating protein g3bp1. *J Biol Chem* **2017**, *292*, 18886-18896.

101. Tsai, W.C.; Gayatri, S.; Reineke, L.C.; Sbardella, G.; Bedford, M.T.; Lloyd, R.E. Arginine demethylation of g3bp1 promotes stress granule assembly. *J Biol Chem* **2016**, *291*, 22671-22685.

102. Webb, C.J.; Wolf, A.; Gromak, N.; Dreger, M.; Kramer, H.; Kessler, B.; Nielsen, M.L.; Schmitz, C.; Butler, D.S.; Yates, J.R., 3rd, et al. Jmjd6 catalyses lysyl-hydroxylation of u2af65, a protein associated with rna splicing. *Science 2009*, *325*, 90-93.

103. Kwok, J.; O’Shea, M.; Hume, D.A.; Lengeling, A. Jmjd6, a jmjc dioxygenase with many interaction partners and pleiotropic functions. *Front Genet* **2017**, *8*, 32.

104. Liu, W.; Ma, Q.; Wong, K.; Li, W.; Ohgi, K.; Zhang, J.; Aggarwal, A.; Rosenfeld, M.G. Brd4 and jmjd6-associated anti-pause enhancers in regulation of transcriptional pause release. *Cell 2013*, *155*, 1581-1595.

105. Wilkins, S.E.; Islam, M.S.; Gannon, J.M.; Markolovic, S.; Hopkinson, R.J.; Ge, W.; Schofield, C.J.; Chowdhury, R. Jmjd5 is a human arginyl c-3 hydroxylase. *Nat Commun* **2018**, *9*, 1180.

106. Marcon, E.; Ni, Z.; Pu, S.; Turinsky, A.L.; Trimble, S.S.; Olsen, J.B.; Silverman-Gavrila, R.; Silverman-Gavrila, L.; Phanse, S.; Guo, H., et al. Human-chromatin-related protein
interactions identify a demethylase complex required for chromosome segregation. Cell Rep 2014, 8, 297-310.

107. Huang, X.; Zhang, S.; Qi, H.; Wang, Z.; Chen, H.W.; Shao, J.; Shen, J. Jmjd5 interacts with p53 and negatively regulates p53 function in control of cell cycle and proliferation. Biochim Biophys Acta 2015, 1853, 2286-2295.

108. Ishimura, A.; Terashima, M.; Tange, S.; Suzuki, T. Jmjd5 functions as a regulator of p53 signaling during mouse embryogenesis. Cell Tissue Res 2016, 363, 723-733.

109. Markolovic, S.; Zhuang, Q.; Wilkens, S.E.; Eaton, C.D.; Abboud, M.I.; Katz, M.J.; McNeil, H.E.; Lesniak, R.K.; Hall, C.; Struwe, W.B., et al. The jumonji-c oxygenase jmjd7 catalyzes (3s)-lysyl hydroxylation of trafac gtpases. Nat Chem Biol 2018, 14, 688-695.

110. Lu, T.; Jackson, M.W.; Wang, B.; Yang, M.; Chance, M.R.; Miyagi, M.; Gudkov, A.V.; Stark, G.R. Regulation of nf-kappab by nsd1/fbxl11-dependent reversible lysine methylation of p65. Proc Natl Acad Sci U S A 2010, 107, 46-51.

111. Stenson, P.D.; Mort, M.; Ball, E.V.; Shaw, K.; Phillips, A.; Cooper, D.N. The human gene mutation database: Building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. Hum Genet 2014, 133, 1-9.

112. Ang, S.O.; Chen, H.; Hirota, K.; Gordeuk, V.R.; Jelinek, J.; Guan, Y.; Liu, E.; Sergueeva, A.I.; Miasnikova, G.Y.; Mole, D., et al. Disruption of oxygen homeostasis underlies congenital chuvash polycythemia. Nat Genet 2002, 32, 614-621.

113. Gordeuk, V.R.; Sergueeva, A.I.; Miasnikova, G.Y.; Okhotin, D.; Voloshin, Y.; Choyke, P.L.; Butman, J.A.; Jedlickova, K.; Prchal, J.T.; Polyakova, L.A. Congenital disorder of oxygen sensing: Association of the homozygous chuvash polycythemia vhl mutation with thrombosis and vascular abnormalities but not tumors. Blood 2004, 103, 3924-3932.

114. Smith, T.G.; Brooks, J.T.; Balanos, G.M.; Lappin, T.R.; Layton, D.M.; Leedham, D.L.; Liu, C.; Maxwell, P.H.; McMullin, M.F.; McNamara, C.J., et al. Mutation of von hippel-lindau tumour suppressor and human cardiopulmonary physiology. PLoS Med 2006, 3, e290.

115. Iyer, N.V.; Kotch, L.E.; Agani, F.; Leung, S.W.; Laughner, E.; Wenger, R.H.; Gassmann, M.; Gearhart, J.D.; Lawler, A.M.; Yu, A.Y., et al. Cellular and developmental control of o2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 1998, 12, 149-162.

116. Ryan, H.E.; Lo, J.; Johnson, R.S. Hif-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J 1998, 17, 3005-3015.

117. Compernolle, V.; Brusselmans, K.; Franco, D.; Moorman, A.; Dewerchin, M.; Collen, D.; Carmeliet, P. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1alpha. Cardiovasc Res 2003, 60, 569-579.

118. Gulsuner, S.; Walsh, T.; Watts, A.C.; Lee, M.K.; Thornton, A.M.; Casadei, S.; Rippey, C.; Shahin, H.; Consortium on the Genetics of, S.; Group, P.S., et al. Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. Cell 2013, 154, 518-529.

119. Prior, S.J.; Hagberg, J.M.; Phares, D.A.; Brown, M.D.; Fairfull, L.; Ferrell, R.E.; Roth, S.M. Sequence variation in hypoxia-inducible factor 1alpha (hif1a): Association with maximal oxygen consumption. Physiol Genomics 2003, 15, 20-26.
120. Ollerenshaw, M.; Page, T.; Hammonds, J.; Demaine, A. Polymorphisms in the hypoxia inducible factor-1alpha gene (hif1a) are associated with the renal cell carcinoma phenotype. *Cancer Genet Cytogenet* 2004, 153, 122-126.

121. Tian, H.; Hammer, R.E.; Matsumoto, A.M.; Russell, D.W.; McKnight, S.L. The hypoxia-responsive transcription factor epas1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev* 1998, 12, 3320-3324.

122. Peng, J.; Zhang, L.; Drysdale, L.; Fong, G.H. The transcription factor epas-1/hypoxia-inducible factor 2alpha plays an important role in vascular remodeling. *Proc Natl Acad Sci U S A* 2000, 97, 8386-8391.

123. Compernolle, V.; Brusselmans, K.; Acker, T.; Hoet, P.; Tjwa, M.; Beck, H.; Plaisance, S.; Dor, Y.; Keshet, E.; Lupu, F., et al. Loss of hif-2alpha and inhibition of vegf impair fetal lung maturation, whereas treatment with vegf prevents fatal respiratory distress in premature mice. *Nat Med* 2002, 8, 702-710.

124. Pan, H.; Chen, Q.; Qi, S.; Li, T.; Liu, B.; Liu, S.; Ma, X.; Wang, B. Mutations in epas1 in congenital heart disease in tibetans. *Biosci Rep* 2018, 38.

125. An, J.Y.; Cristino, A.S.; Zhao, Q.; Edson, J.; Williams, S.M.; Ravine, D.; Wray, J.; Marshall, V.M.; Hunt, A.; Whitehouse, A.J., et al. Towards a molecular characterization of autism spectrum disorders: An exome sequencing and systems approach. *Transl Psychiatry* 2014, 4, e394.

126. Lorenzo, F.R.; Yang, C.; Ng Tang Fui, M.; Vankayalapati, H.; Zhuang, Z.; Huynh, T.; Grossmann, M.; Pacak, K.; Prchal, J.T. A novel epas1/hif2a germline mutation in a congenital polycythemia with paraganglioma. *J Mol Med (Berl)* 2013, 91, 507-512.

127. Welander, J.; Andreasson, A.; Brauckhoff, M.; Backdahl, M.; Larsson, C.; Gimm, O.; Soderkvist, P. Frequent epas1/hif2alpha exons 9 and 12 mutations in non-familial pheochromocytoma. *Endocr Relat Cancer* 2014, 21, 495-504.

128. Yang, C.; Hong, C.S.; Prchal, J.T.; Balint, M.T.; Pacak, K.; Zhuang, Z. Somatic mosaicism of epas1 mutations in the syndrome of paraganglioma and somatostatinoma associated with polycythemia. *Hum Genome Var* 2015, 2, 15053.

129. Comino-Mendez, I.; de Cubas, A.A.; Bernal, C.; Alvarez-Escola, C.; Sanchez-Malo, C.; Ramirez-Tortosa, C.L.; Pedrinaci, S.; Rapizzi, E.; Ercolino, T.; Bernini, G., et al. Tumoral epas1 (hif2a) mutations explain sporadic pheochromocytoma and paraganglioma in the absence of erythrocytosis. *Hum Mol Genet* 2013, 22, 2169-2176.

130. Zhuang, Z.; Yang, C.; Lorenzo, F.; Merino, M.; Fojo, T.; Kebebew, E.; Popovic, V.; Stratakis, C.A.; Prchal, J.T.; Pacak, K. Somatic hif2a gain-of-function mutations in paraganglioma with polycythemia. *N Engl J Med* 2012, 367, 922-930.

131. Toledo, R.A.; Qin, Y.; Srikantan, S.; Morales, N.P.; Li, Q.; Deng, Y.; Kim, S.W.; Pereira, M.A.; Toledo, S.P.; Su, X., et al. In vivo and in vitro oncogenic effects of hif2a mutations in pheochromocytomas and paragangliomas. *Endocr Relat Cancer* 2013, 20, 349-359.

132. Buffet, A.; Smati, S.; Mansuy, L.; Menara, M.; Lebras, M.; Heymann, M.F.; Simian, C.; Favier, J.; Murat, A.; Cariou, B., et al. Mosaicism in hif2a-related polycythemia-paraganglioma syndrome. *J Clin Endocrinol Metab* 2014, 99, E369-373.
133. Xiang, K.; Ouzhuluobu; Peng, Y.; Yang, Z.; Zhang, X.; Cui, C.; Zhang, H.; Li, M.; Zhang, Y.; Bianba, et al. Identification of a tibetan-specific mutation in the hypoxic gene egln1 and its contribution to high-altitude adaptation. *Mol Biol Evol* 2013, 30, 1889-1898.

134. Perrotta, S.; Stiehl, D.P.; Punzo, F.; Scianguetta, S.; Borriello, A.; Bencivenga, D.; Casale, M.; Nobili, B.; Fasoli, S.; Balduzzi, A., et al. Congenital erythrocytosis associated with gain-of-function hif2a gene mutations and erythropoietin levels in the normal range. *Haematologica* 2013, 98, 1624-1632.

135. Furlow, P.W.; Percy, M.J.; Sutherland, S.; Bierl, C.; McMullin, M.F.; Master, S.R.; Lappin, T.R.; Lee, F.S. Erythrocytosis-associated hif-2alpha mutations demonstrate a critical role for residues c-terminal to the hydroxylacceptor proline. *J Biol Chem* 2009, 284, 9050-9058.

136. Percy, M.J.; Chung, Y.J.; Harrison, C.; Mercieca, J.; Hoffbrand, A.V.; Dinardo, C.L.; Santos, P.C.; Fonseca, G.H.; Gualandro, S.F.; Pereira, A.C., et al. Two new mutations in the hif2a gene associated with erythrocytosis. *Am J Hematol* 2012, 87, 439-442.

137. Martini, M.; Teofili, L.; Cenci, T.; Giona, F.; Torti, L.; Rea, M.; Foa, R.; Leone, G.; Larocca, L.M. A novel heterozygous hif2am535i mutation reinforces the role of oxygen sensing pathway disturbances in the pathogenesis of familial erythrocytosis. *Haematologica* 2008, 93, 1068-1071.

138. Percy, M.J.; Beer, P.A.; Campbell, G.; Dekker, A.W.; Green, A.R.; Oscier, D.; Rainey, M.G.; van Wijk, R.; Sutherland, S.; Bierlings, M.; Lee, F.S. Erythrocytosis associated with a novel missense mutation in the hif2a gene. *Blood* 2008, 112, 5400-5402.

139. Gale, D.P.; Harten, S.K.; Reid, C.D.; Tuddenham, E.G.; Maxwell, P.H. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating hif2 alpha mutation. *Blood* 2008, 112, 919-921.

140. Yamashita, T.; Ohneda, O.; Nagano, M.; Iemitsu, M.; Makino, Y.; Tanaka, H.; Miyauchi, T.; Goto, K.; Ohneda, K.; Fujii-Kuriyama, Y., et al. Abnormal heart development and lung remodeling in mice lacking the hypoxia-inducible factor-related basic helix-loop-helix pas protein nepas. *Mol Cell Biol* 2008, 28, 1285-1297.

141. Takeda, K.; Ho, V.C.; Takeda, H.; Duan, L.J.; Nagy, A.; Fong, G.H. Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. *Mol Cell Biol* 2006, 26, 8336-8346.

142. Zhu, Z.; Gao, X.; He, Y.; Zhao, H.; Yu, Q.; Jiang, D.; Zhang, P.; Ma, X.; Huang, H.; Dong, D., et al. An insertion/deletion polymorphism within rert-lncrna modulates hepatocellular carcinoma risk. *Cancer Res* 2012, 72, 6163-6172.

143. Che, J.; Jiang, D.; Zheng, Y.; Zhu, B.; Zhang, P.; Lu, D.; Zhang, J.; Xiao, J.; Wang, J.; Gao, Y., et al. Polymorphism in phd1 gene and risk of non-small cell lung cancer in a chinese population. *Tumour Biol* 2014, 35, 8921-8925.

144. Zhu, J.; Luo, J.Z.; Li, C.B. Correlations of an insertion/deletion polymorphism (rs10680577) in the rert-lncrna with the susceptibility, clinicopathological features, and prognosis of lung cancer. *Biochem Genet* 2019, 57, 147-158.
146. Wang, L.; Yamaguchi, S.; Burstein, M.D.; Terashima, K.; Chang, K.; Ng, H.K.; Nakamura, H.; He, Z.; Doddapaneni, H.; Lewis, L., et al. Novel somatic and germline mutations in intracranial germ cell tumours. *Nature* 2014, *511*, 241-245.

147. Li, C.; Feng, L.; Niu, L.; Teng Li, T.; Zhang, B.; Wan, H.; Zhu, Z.; Liu, H.; Wang, K.; Fu, H., et al. An insertion/deletion polymorphism within the promoter of egln2 is associated with susceptibility to colorectal cancer. *Int J Biol Markers* 2017, *32*, e274-e277.

148. Yang, C.; Zhuang, Z.; Fliedner, S.M.; Shankavaram, U.; Sun, M.G.; Bullova, P.; Zhu, R.; Elkahloun, A.G.; Kourlas, P.J.; Merino, M., et al. Germ-line phd1 and phd2 mutations detected in patients with pheochromocytoma/paraganglioma-polycythemia. *J Mol Med (Berl)* 2015, *93*, 93-104.

149. Ladroue, C.; Hoogewijs, D.; Gad, S.; Carcenac, R.; Storti, F.; Barrois, M.; Gimenez-Roqueplo, A.P.; Leporrier, M.; Casadevall, N.; Hermine, O., et al. Distinct deregulation of the hypoxia inducible factor by phd2 mutants identified in germline DNA of patients with polycythemia. *Haematologica* 2012, *97*, 9-14.

150. Albiero, E.; Ruggeri, M.; Fortuna, S.; Bernardi, M.; Finotto, S.; Madeo, D.; Rodeghiero, F. Analysis of the oxygen sensing pathway genes in familial chronic myeloproliferative neoplasms and identification of a novel egln1 germ-line mutation. *Br J Haematol* 2011, *153*, 405-408.

151. Al-Sheikh, M.; Moradkhani, K.; Lopez, M.; Wajcman, H.; Prehu, C. Disturbance in the hif-1alpha pathway associated with erythrocytosis: Further evidences brought by frameshift and nonsense mutations in the prolyl hydroxylase domain protein 2 (phd2) gene. *Blood Cells Mol Dis* 2008, *40*, 160-165.

152. Bento, C.; Percy, M.J.; Gardie, B.; Maia, T.M.; van Wijk, R.; Perrotta, S.; Della Ragione, F.; Almeida, H.; Rossi, C.; Girodon, F., et al. Genetic basis of congenital erythrocytosis: Mutation update and online databases. *Hum Mutat* 2014, *35*, 15-26.

153. Jang, J.H.; Seo, J.Y.; Jang, J.; Jung, C.W.; Lee, K.O.; Kim, S.H.; Kim, H.J. Hereditary gene mutations in korean patients with isolated erythrocytosis. *Ann Hematol* 2014, *93*, 931-935.

154. Percy, M.J.; Zhao, Q.; Flores, A.; Harrison, C.; Lappin, T.R.; Maxwell, P.H.; McMullin, M.F.; Lee, F.S. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. *Proc Natl Acad Sci U S A* 2006, *103*, 654-659.

155. Bento, C.; Almeida, H.; Maia, T.M.; Relvas, L.; Oliveira, A.C.; Rossi, C.; Girodon, F.; Fernandez-Lago, C.; Aguado-Diaz, A.; Fraga, C., et al. Molecular study of congenital erythrocytosis in 70 unrelated patients revealed a potential causal mutation in less than half of the cases (where is/are the missing gene(s)?). *Eur J Haematol* 2013, *91*, 361-368.

156. Wilson, R.; Syed, N.; Shah, P. Erythrocytosis due to phd2 mutations: A review of clinical presentation, diagnosis, and genetics. *Case Rep Hematol* 2016, *2016*, 6373706.

157. Percy, M.J.; Furlow, P.W.; Beer, P.A.; Lappin, T.R.; McMullin, M.F.; Lee, F.S. A novel erythrocytosis-associated phd2 mutation suggests the location of a hif binding groove. *Blood* 2007, *110*, 2193-2196.

158. Ladroue, C.; Carcenac, R.; Leporrier, M.; Gad, S.; Le Hello, C.; Galateau-Salle, F.; Feunteun, J.; Pouyss segur, J.; Richard, S.; Gardie, B. Phd2 mutation and congenital erythrocytosis with paraganglioma. *N Engl J Med* 2008, *359*, 2685-2692.
159. Albiero, E.; Ruggeri, M.; Fortuna, S.; Finotto, S.; Bernardi, M.; Madeo, D.; Rodeghiero, F.
    Isolated erythrocytosis: Study of 67 patients and identification of three novel germ-line
    mutations in the prolyl hydroxylase domain protein 2 (phd2) gene. *Haematologica* 2012, 97,
    123-127.

160. Talbot, N.P.; Smith, T.G.; Balanos, G.M.; Dorrington, K.L.; Maxwell, P.H.; Robbins, P.A.
    Cardiopulmonary phenotype associated with human phd2 mutation. *Physiol Rep* 2017, 5.

161. Aragones, J.; Schneider, M.; Van Geyte, K.; Frais, P.; Dresselaers, T.; Mazzone, M.; Dirx, R.;
    Zachigna, S.; Lemieux, H.; Jeoung, N.H., et al. Deficiency or inhibition of oxygen sensor
    phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat Genet* 2008, 40,
    170-180.

162. Holster, T.; Pakkanen, O.; Soininen, R.; Sormunen, R.; Nokelainen, M.; Kivirikko, K.I.;
    Myllyharju, J. Loss of assembly of the main basement membrane collagen, type iv, but not
    fibril-forming collagens and embryonic death in collagen prolyl 4-hydroxylase i null mice. *J
    Biol Chem* 2007, 282, 2512-2519.

163. Zou, Y.; Donkervoort, S.; Salo, A.M.; Foley, A.R.; Barnes, A.M.; Hu, Y.; Makareeva, E.; Leach,
    M.E.; Mohassel, P.; Dastgir, J., et al. P4ha1 mutations cause a unique congenital disorder of
    connective tissue involving tendon, bone, muscle and the eye. *Hum Mol Genet* 2017, 26,
    2207-2217.

164. Aro, E.; Salo, A.M.; Khatri, R.; Finnila, M.; Miinalainen, I.; Sormunen, R.; Pakkanen, O.; Holster,
    T.; Soininen, R., et al. Severe extracellular matrix abnormalities and chondrodysplasia in mice lacking collagen prolyl 4-hydroxylase isoenzyme ii in combination with a reduced amount of isoenzyme i. *J Biol Chem* 2015, 290, 16964-16978.

165. Guo, H.; Tong, P.; Liu, Y.; Xia, L.; Wang, T.; Tian, Q.; Li, Y.; Hu, Y.; Zheng, Y.; Jin, X., et al.
    Mutations of p4ha2 encoding prolyl 4-hydroxylase 2 are associated with nonsyndromic high
    myopia. *Genet Med* 2015, 17, 300-306.

166. Ferdinandusse, S.; Zomer, A.W.; Komen, J.C.; van den Brink, C.E.; Thanos, M.; Hamers, F.P.;
    Wanders, R.J.; van der Saag, P.T.; Poll-The, B.T.; Brites, P. Ataxia with loss of purkinje cells in a
    mouse model for refsum disease. *Proc Natl Acad Sci U S A* 2008, 105, 17712-17717.

167. Jansen, G.A.; Hogenhout, E.M.; Ferdinandusse, S.; Waterham, H.R.; Ofman, R.; Jakobs, C.;
    Skjeldal, O.H.; Wanders, R.J. Human phytanoyl-coa hydroxylase: Resolution of the gene
    structure and the molecular basis of refsum’s disease. *Hum Mol Genet* 2000, 9, 1195-1200.

168. Chahal, A.; Khan, M.; Pai, S.G.; Barbosa, E.; Singh, I. Restoration of phytanic acid oxidation in
    refsum disease fibroblasts from patients with mutations in the phytanoyl-coa hydroxylase
    gene. *FEBS Lett* 1998, 429, 119-122.

169. Jansen, G.A.; Waterham, H.R.; Wanders, R.J. Molecular basis of refsum disease: Sequence
    variations in phytanoyl-coa hydroxylase (phyh) and the pts2 receptor (pex7). *Hum Mutat*
    2004, 23, 209-218.

170. Jansen, G.A.; Ferdinandusse, S.; Skjeldal, O.H.; Stokke, O.; de Groot, C.J.; Jakobs, C.; Wanders,
    R.J. Molecular basis of refsum disease: Identification of new mutations in the phytanoyl-coa
    hydroxylase cdna. *J Inherit Metab Dis* 1998, 21, 288-291.

171. Zhao, L.; Wang, F.; Wang, H.; Li, Y.; Alexander, S.; Wang, K.; Willoughby, C.E.; Zaneveld, J.E.;
    Jiang, L.; Soens, Z.T., et al. Next-generation sequencing-based molecular diagnosis of 82
    retinitis pigmentosa probands from northern ireland. *Hum Genet* 2015, 134, 217-230.
172. Mihalik, S.J.; Morrell, J.C.; Kim, D.; Sacksteder, K.A.; Watkins, P.A.; Gould, S.J. Identification of pahx, a refsum disease gene. *Nat Genet* 1997, 17, 185-189.

173. Kohlschutter, A.; Santer, R.; Lukacs, Z.; Altenburg, C.; Kemper, M.J.; Ruther, K. A child with night blindness: Preventing serious symptoms of refsum disease. *J Child Neurol* 2012, 27, 654-656.

174. Aylward, A.; Cai, Y.; Lee, A.; Blue, E.; Rabinowitz, D.; Haddad, J., Jr.; University of Washington Center for Mendelian, G. Using whole exome sequencing to identify candidate genes with rare variants in nonsyndromic cleft lip and palate. *Genet Epidemiol* 2016, 40, 432-441.

175. Zhang, N.; Fu, Z.; Linke, S.; Chicher, J.; Gorman, J.J.; Visk, D.; Haddad, G.G.; Poellinger, L.; Peet, D.J.; Powell, F., *et al.* The asparaginyl hydroxylase factor inhibiting hif-1alpha is an essential regulator of metabolism. *Cell Metab* 2010, 11, 364-378.

176. Webb, E.L.; Rudd, M.F.; Sellick, G.S.; El Galta, R.; Bethke, L.; Wood, W.; Fletcher, O.; Penegar, S.; Withey, L.; Qureshi, M., *et al.* Search for low penetrance alleles for colorectal cancer through a scan of 1467 non-synonymous snps in 2575 cases and 2707 controls with validation by kin-cohort analysis of 14 704 first-degree relatives. *Hum Mol Genet* 2006, 15, 3263-3271.

177. Yamaguchi, S.; Hong, K.; Liu, R.; Shen, L.; Inoue, A.; Diep, D.; Zhang, K.; Zhang, Y. Tet1 controls meiosis by regulating meiotic gene expression. *Nature* 2012, 492, 443-447.

178. Kang, J.; Lienhard, M.; Pastor, W.A.; Chawla, A.; Novotny, M.; Tsagaratou, A.; Lasken, R.S.; Thompson, E.C.; Surani, M.A.; Koralov, S.B., *et al.* Simultaneous deletion of the methylcytosine oxidases tet1 and tet3 increases transcriptome variability in early embryogenesis. *Proc Natl Acad Sci U S A* 2015, 112, E4236-4245.

179. Khoueiry, R.; Sohni, A.; Thienpont, B.; Luo, X.; Velde, J.V.; Bartoccetti, M.; Boeckx, B.; Zwijsen, A.; Rao, A.; Lambrechts, D., *et al.* Lineage-specific functions of tet1 in the postimplantation mouse embryo. *Nat Genet* 2017, 49, 1061-1072.

180. Dawlaty, M.M.; Ganz, K.; Powell, B.E.; Hu, Y.C.; Markoulaki, S.; Cheng, A.W.; Gao, Q.; Kim, J.; Choi, S.W.; Page, D.C., *et al.* Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell* 2011, 9, 166-175.

181. Liu, J.; Yue, Y.; Han, D.; Wang, X.; Fu, Y.; Zhang, L.; Jia, G.; Yu, M.; Lu, Z.; Deng, X., *et al.* A mettl3-mettl14 complex mediates mammalian nuclear rna n6-adenosine methylation. *Nat Chem Biol* 2014, 10, 93-95.

182. Zhang, R.R.; Cui, Q.Y.; Murai, K.; Lim, Y.C.; Smith, Z.D.; Jin, S.; Ye, P.; Rosa, L.; Lee, Y.K.; Wu, H.P., *et al.* Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell* 2013, 13, 237-245.

183. Li, Z.; Cai, X.; Cai, C.L.; Wang, J.; Zhang, W.; Petersen, B.E.; Yang, F.C.; Xu, M. Deletion of tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood* 2011, 118, 4509-4518.

184. Pan, F.; Wingo, T.S.; Zhao, Z.; Gao, R.; Makishima, H.; Qu, G.; Lin, L.; Yu, M.; Ortega, J.R.; Wang, J., *et al.* Tet2 loss leads to hypermutagenicity in haematopoietic stem/progenitor cells. *Nat Commun* 2017, 8, 15102.

185. Zhao, Z.; Chen, L.; Dawlaty, M.M.; Pan, F.; Weeks, O.; Zhou, Y.; Cao, Z.; Shi, H.; Wang, J.; Lin, L., *et al.* Combined loss of tet1 and tet2 promotes b cell, but not myeloid malignancies, in mice. *Cell Rep* 2015, 13, 1692-1704.
186. Ismael, O.; Shimada, A.; Hama, A.; Elshazley, M.; Muramatsu, H.; Goto, A.; Sakaguchi, H.; Tanaka, M.; Takahashi, Y.; Yinyan, X., et al. De novo childhood myelodysplastic/myeloproliferative disease with unique molecular characteristics. *Br J Haematol* 2012, 158, 129-137.

187. Nickerson, M.L.; Im, K.M.; Misner, K.J.; Tan, W.; Lou, H.; Gold, B.; Wells, D.W.; Bravo, H.C.; Fredrikson, K.M.; Harkins, T.T., et al. Somatic alterations contributing to metastasis of a castration-resistant prostate cancer. *Hum Mutat* 2013, 34, 1231-1241.

188. Schaub, F.X.; Looser, R.; Li, S.; Hao-Shen, H.; Lehmann, T.; Tichelli, A.; Skoda, R.C. Clonal analysis of tet2 and jak2 mutations suggests that tet2 can be a late event in the progression of myeloproliferative neoplasms. *Blood* 2010, 115, 2003-2007.

189. Gu, T.P.; Guo, F.; Yang, H.; Wu, H.P.; Xu, G.F.; Liu, W.; Xie, Z.G.; Shi, L.; He, X.; Jin, S.G., et al. The role of tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. *Nature* 2011, 477, 606-610.

190. Beck, D.B.; Petracovici, A.; He, C.; Moore, H.W.; Louie, R.J.; Ansar, M.; Douzgou, S.; Sithambaram, S.; Cottrell, T.; Santos-Cortez, R.L.P., et al. Delineation of a human mendelian disorder of the DNA demethylation machinery: Tet3 deficiency. *Am J Hum Genet* 2020, 106, 234-245.

191. Boissel, S.; Reish, O.; Proulx, K.; Kawagoe-Takaki, H.; Sedgwick, B.; Yeo, G.S.; Meyre, D.; Golzio, C.; Molinari, F.; Kadhom, N., et al. Loss-of-function mutation in the dioxygenase-encoding fto gene causes severe growth retardation and multiple malformations. *Am J Hum Genet* 2009, 85, 106-111.

192. Yoo, H.; Son, D.; Lee, Y.J.; Hong, K. Mouse jmjd4 is dispensable for embryogenesis. *Mol Reprod Dev* 2016, 83, 588-593.

193. Li, M.O.; Sarkisian, M.R.; Mehal, W.Z.; Rakic, P.; Flavell, R.A. Phosphatidylserine receptor is required for clearance of apoptotic cells. *Science* 2003, 302, 1560-1563.

194. Kunisaki, Y.; Masuko, S.; Noda, M.; Inayoshi, A.; Sanui, T.; Harada, M.; Sasazuki, T.; Fukui, Y. Defective fetal liver erythropoiesis and t lymphopoiesis in mice lacking the phosphatidylserine receptor. *Blood* 2004, 103, 3362-3364.

195. Bose, J.; Gruber, A.D.; Helming, L.; Schiebe, S.; Wegener, I.; Hafner, M.; Beales, M.; Kontgen, F.; Lengeling, A. The phosphatidylserine receptor has essential functions during embryogenesis but not in apoptotic cell removal. *J Biol* 2004, 3, 15.

196. Schneider, J.E.; Bose, J.; Bamforth, S.D.; Gruber, A.D.; Broadbent, C.; Clarke, K.; Neubauer, S.; Lengeling, A.; Bhattacharya, S. Identification of cardiac malformations in mice lacking pttdsr using a novel high-throughput magnetic resonance imaging technique. *BMC Dev Biol* 2004, 4, 16.

197. Kawakami, E.; Tokunaga, A.; Ozawa, M.; Sakamoto, R.; Yoshida, N. The histone demethylase fbxl11/kdm2a plays an essential role in embryonic development by repressing cell-cycle regulators. *Mech Dev* 2015, 135, 31-42.

198. Fukuda, T.; Tokunaga, A.; Sakamoto, R.; Yoshida, N. Fbxl10/kdm2b deficiency accelerates neural progenitor cell death and leads to exencephaly. *Mol Cell Neurosci* 2011, 46, 614-624.

199. Boulard, M.; Edwards, J.R.; Bestor, T.H. Fbxl10 protects polycomb-bound genes from hypermethylation. *Nat Genet* 2015, 47, 479-485.
200. Boulard, M.; Edwards, J.R.; Bestor, T.H. Abnormal x chromosome inactivation and sex-specific gene dysregulation after ablation of fbxl10. *Epigenetics Chromatin* 2016, 9, 22.

201. Andricovich, J.; Kai, Y.; Peng, W.; Foudi, A.; Tzatsos, A. Histone demethylase kdm2b regulates lineage commitment in normal and malignant hematopoiesis. *J Clin Invest* 2016, 126, 905-920.

202. Tateishi, K.; Okada, Y.; Kallin, E.M.; Zhang, Y. Role of jhdm2a in regulating metabolic gene expression and obesity resistance. *Nature* 2009, 458, 757-761.

203. Inagaki, T.; Tachibana, M.; Magoori, K.; Kudo, H.; Tanaka, T.; Okamura, M.; Naito, M.; Kodama, T.; Shinkai, Y.; Sakai, J. Obesity and metabolic syndrome in histone demethylase jhdm2a-deficient mice. *Genes Cells* 2009, 14, 991-1001.

204. Liu, Z.; Zhou, S.; Liao, L.; Chen, X.; Meistrich, M.; Xu, J. Jmjd1a demethylase-regulated histone modification is essential for camp-response element modulator-regulated gene expression and spermatogenesis. *Cell Biol Chem* 2010, 285, 2758-2770.

205. Qin, L.; Xu, Y.; Yu, X.; Toneff, M.J.; Li, D.; Liao, L.; Martinez, J.D.; Li, Y.; Xu, J. The histone demethylase kdm3a is required for normal epithelial proliferation, ductal elongation and tumor growth in the mouse mammary gland. *Oncotarget* 2017, 8, 84761-84775.

206. Hojati, Z.; Soleimanpour, E.; Javadirad, S.M.; Nasr-Esfahani, M.H. Identification of two novel mutations in kdm3a regulatory gene in iranian infertile males. *Iran Biomed J* 2019, 23, 220-227.

207. Liu, Z.; Chen, X.; Zhou, S.; Liao, L.; Jiang, R.; Xu, J. The histone h3k9 demethylase kdm3b is required for somatic growth and female reproductive function. *Int J Biol Sci* 2015, 11, 494-507.

208. Li, S.; Ali, S.; Duan, X.; Liu, S.; Du, J.; Liu, C.; Dai, H.; Zhou, M.; Zhou, L.; Yang, L., et al. Jmjd1b demethylates h4r3me2s and h3k9me2 to facilitate gene expression for development of hematopoietic stem and progenitor cells. *Cell Rep* 2018, 23, 389-403.

209. Guipponi, M.; Santoni, F.A.; Setola, V.; Gehrig, C.; Rotharmel, M.; Cuenca, M.; Guillin, O.; Dikeos, D.; Georgantopoulos, G.; Papadimitriou, G., et al. Exome sequencing in 53 sporadic cases of schizophrenia identifies 18 putative candidate genes. *PLoS One* 2014, 9, e112745.

210. Deciphering Developmental Disorders, S. Prevalence and architecture of de novo mutations in developmental disorders. *Nature* 2017, 542, 433-438.

211. Mahamdallie, S.; Yost, S.; Poyastro-Pearson, E.; Holt, E.; Zachariou, A.; Seal, S.; Elliott, A.; Clarke, M.; Warren-Perry, M.; Hanks, S., et al. Identification of new wilms tumour predisposition genes: An exome sequencing study. *Lancet Child Adolesc Health* 2019, 3, 322-331.

212. Diets, I.J.; van der Donk, R.; Baltrunaite, K.; Waanders, E.; Reijnders, M.R.F.; Dingemans, A.J.M.; Pfundt, R.; Vulto-van Silfhout, A.T.; Wiel, L.; Gilissen, C., et al. De novo and inherited pathogenic variants in kdm3b cause intellectual disability, short stature, and facial dysmorphism. *Am J Hum Genet* 2019, 104, 758-766.

213. Guo, T.; Chung, J.H.; Wang, T.; McDonald-McGinn, D.M.; Kates, W.R.; Hawula, W.; Coleman, K.; Zackai, E.; Emanuel, B.S.; Morrow, B.E. Histone modifier genes alter conotruncal heart phenotypes in 22q11.2 deletion syndrome. *Am J Hum Genet* 2015, 97, 869-877.
214. Saez, M.A.; Fernandez-Rodriguez, J.; Moutinho, C.; Sanchez-Mut, J.V.; Gomez, A.; Vidal, E.; Petazzi, P.; Szczesna, K.; Lopez-Serra, P.; Lucariello, M., et al. Mutations in jmjd1c are involved in rett syndrome and intellectual disability. *Genet Med* 2016, 18, 378-385.

215. Neale, B.M.; Kou, Y.; Liu, L.; Ma’ayan, A.; Samocha, K.E.; Sabo, A.; Lin, C.F.; Stevens, C.; Wang, L.S.; Makarov, V., et al. Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature* 2012, 485, 242-245.

216. Zhang, Q.; Chen, H.Z.; Wang, L.; Liu, D.P.; Hill, J.A.; Liu, Z.P. The histone trimethyllysine demethylase jmjd2a promotes cardiac hypertrophy in response to hypertrophic stimuli in mice. *J Clin Invest* 2011, 121, 2447-2456.

217. Kawazu, M.; Saso, K.; Tong, K.I.; McQuire, T.; Goto, K.; Son, D.O.; Wakeham, A.; Miyagishi, M.; Mak, T.W.; Okada, H. Histone demethylase jmjd2b functions as a co-factor of estrogen receptor in breast cancer proliferation and mammary gland development. *PLoS One* 2011, 6, e17830.

218. Cheng, Y.; Yuan, Q.; Vergnes, L.; Rong, X.; Youn, J.Y.; Li, J.; Yu, Y.; Liu, W.; Cai, H.; Lin, J.D., et al. Kdm4b protects against obesity and metabolic dysfunction. *Proc Natl Acad Sci U S A* 2018, 115, E5566-E5575.

219. Pedersen, M.T.; Agger, K.; Laugesen, A.; Johansen, J.V.; Cloos, P.A.; Christensen, J.; Helin, K. The demethylase jmjd2c localizes to h3k4me3-positive transcription start sites and is dispensable for embryonic development. *Mol Cell Biol* 2014, 34, 1031-1045.

220. Ozaki, Y.; Fujiiwara, K.; Ikeda, M.; Ozaki, T.; Terui, T.; Soma, M.; Inazawa, J.; Nagase, H. The oncogenic role of gasc1 in chemically induced mouse skin cancer. *Mamm Genome* 2015, 26, 591-597.

221. Canova, C.; Hashibe, M.; Simonato, L.; Nelis, M.; Metspalu, A.; Lagiou, P.; Trichopoulos, D.; Ahrens, W.; Pigeot, I.; Merletti, F., et al. Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 European countries: The arcage project. *Cancer Res* 2009, 69, 2956-2965.

222. Perry, J.R.; Day, F.; Elks, C.E.; Sulem, P.; Thompson, D.J.; Ferreira, T.; He, C.; Chasman, D.I.; Esko, T.; Thorleifsson, G., et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature* 2014, 514, 92-97.

223. Iwamori, N.; Zhao, M.; Meistrich, M.L.; Matzuk, M.M. The testis-enriched histone demethylase, kdm4d, regulates methylation of histone h3 lysine 9 during spermatogenesis in the mouse but is dispensable for fertility. *Biol Reprod* 2011, 84, 1225-1234.

224. Klose, R.J.; Yan, Q.; Tothova, Z.; Yamane, K.; Erdjument-Bromage, H.; Tempst, P.; Gilliland, D.G.; Zhang, Y.; Kaelin, W.G., Jr. The retinoblastoma binding protein rbp2 is an h3k4 demethylase. *Cell* 2007, 128, 889-900.

225. Lin, W.; Cao, J.; Liu, J.; Beshiri, M.L.; Fujiwara, Y.; Francis, J.; Cherniack, A.D.; Geisen, C.; Blair, L.P.; Zou, M.R., et al. Loss of the retinoblastoma binding protein 2 (rbp2) histone demethylase suppresses tumorigenesis in mice lacking rb1 or men1. *Proc Natl Acad Sci U S A* 2011, 108, 13379-13386.

226. Najmabadi, H.; Hu, H.; Garshasbi, M.; Zemojtel, T.; Abedini, S.S.; Chen, W.; Hosseini, M.; Behjati, F.; Haas, S.; Jamali, P., et al. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature* 2011, 478, 57-63.
227. Catchpole, S.; Spencer-Dene, B.; Hall, D.; Santangelo, S.; Rosewell, I.; Guenatri, M.; Beatson, R.; Scibetta, A.G.; Burchell, J.M.; Taylor-Papadimitriou, J. Plu-1/jarid1b/kdm5b is required for embryonic survival and contributes to cell proliferation in the mammary gland and in er+ breast cancer cells. *Int J Oncol* 2011, 38, 1267-1277.

228. Zou, M.R.; Cao, J.; Liu, Z.; Huh, S.J.; Polyak, K.; Yan, Q. Histone demethylase jumonji at-rich interactive domain 1b (jarid1b) controls mammary gland development by regulating key developmental and lineage specification genes. *J Biol Chem* 2014, 289, 17620-17633.

229. Albert, M.; Schmitz, S.U.; Kooistra, S.M.; Malatesta, M.; Morales Torres, C.; Rekling, J.C.; Johansen, J.V.; Abarrategui, I.; Helin, K. The histone demethylase jarid1b ensures faithful mouse development by protecting developmental genes from aberrant h3k4me3. *PLoS Genet* 2013, 9, e1003461.

230. Faundes, V.; Newman, W.G.; Bernardini, L.; Canham, N.; Clayton-Smith, J.; Dallapiccola, B.; Davies, S.J.; Demos, M.K.; Goldman, A.; Gill, H., et al. Histone lysine methylases and demethylases in the landscape of human developmental disorders. *Am J Hum Genet* 2018, 102, 175-187.

231. Cox, B.J.; Vollmer, M.; Tamplin, O.; Lu, M.; Biechele, S.; Gertsenstein, M.; van Campenhout, C.; Floss, T.; Kuhn, R.; Wurst, W., et al. Phenotypic annotation of the mouse x chromosome. *Genome Res* 2010, 20, 1154-1164.

232. Iwase, S.; Brookes, E.; Agarwal, S.; Badeaux, A.I.; Ito, H.; Vallianatos, C.N.; Tomassy, G.S.; Kasza, T.; Lin, G.; Thompson, A., et al. A mouse model of x-linked intellectual disability associated with impaired removal of histone methylation. *Cell Rep* 2016, 14, 1000-1009.

233. Vallianatos, C.N.; Raines, B.; Porter, R.S.; Bonefas, K.M.; Wu, M.C.; Garay, P.M.; Collette, K.M.; Seo, Y.A.; Dou, Y.; Keegan, C.E., et al. Mutually suppressive roles of kmt2a and kdm5c in behaviour, neuronal structure, and histone h3k4 methylation. *Commun Biol* 2020, 3, 278.

234. Ounap, K.; Puusepp-Benazzouz, H.; Peters, M.; Vaher, U.; Rein, R.; Proos, A.; Field, M.; Reimand, T. A novel c.2t > c mutation of the kdm5c/jarid1c gene in one large family with x-linked intellectual disability. *Eur J Med Genet* 2012, 55, 178-184.

235. Grozeva, D.; Carss, K.; Spasic-Boskovic, O.; Tejada, M.I.; Gecz, J.; Shaw, M.; Corbett, M.; Haan, E.; Thompson, E.; Friend, K., et al. Targeted next-generation sequencing analysis of 1,000 individuals with intellectual disability. *Hum Mutat* 2015, 36, 1197-1204.

236. Grafordatskaya, D.; Chung, B.H.; Butcher, D.T.; Turinsky, A.L.; Goodman, S.J.; Choufani, S.; Chen, Y.A.; Lou, Y.; Zhao, C.; Rajendram, R., et al. Multilocus loss of DNA methylation in individuals with mutations in the histone h3 lysine 4 demethylase kdm5c. *BMC Med Genomics* 2013, 6, 1.

237. Redin, C.; Gerard, B.; Lauer, J.; Herenger, Y.; Muller, J.; Quartier, A.; Masurel-Paulet, A.; Willems, M.; Lesca, G.; El-Chehadeh, S., et al. Efficient strategy for the molecular diagnosis of intellectual disability using targeted high-throughput sequencing. *J Med Genet* 2014, 51, 724-736.

238. Santos-Reboucas, C.B.; Fintelman-Rodrigues, N.; Jensen, L.R.; Kuss, A.W.; Ribeiro, M.G.; Campos, M., Jr.; Santos, J.M.; Pimentel, M.M. A novel nonsense mutation in kdm5c/jarid1c gene causing intellectual disability, short stature and speech delay. *Neurosci Lett* 2011, 498, 67-71.
239. Hu, H.; Haas, S.A.; Chelly, J.; Van Esch, H.; Raynaud, M.; de Brouwer, A.P.; Weinert, S.; Froyen, G.; Frints, S.G.; Laumonnier, F., et al. X-exome sequencing of 406 unresolved families identifies seven novel intellectual disability genes. *Mol Psychiatry* **2016**, *21*, 133-148.

240. Brookes, E.; Laurent, B.; Ounap, K.; Carroll, R.; Moeschler, J.B.; Field, M.; Schwartz, C.E.; Ge, J.; Shi, Y. Mutations in the intellectual disability gene kdm5c reduce protein stability and demethylase activity. *Hum Mol Genet* **2015**, *24*, 2861-2872.

241. Abidi, F.E.; Holloway, L.; Moore, C.A.; Weaver, D.D.; Simensen, R.J.; Stevenson, R.E.; Rogers, R.C.; Schwartz, C.E. Mutations in jarid1c are associated with X-linked mental retardation, short stature and hyperreflexia. *J Med Genet* **2008**, *45*, 787-793.

242. Tzschach, A.; Lenzner, S.; Moser, B.; Reinhardt, R.; Chelly, J.; Fryns, J.P.; Kleefstra, T.; Raynaud, M.; Turner, G.; Ropers, H.H., et al. Novel jarid1c/smcx mutations in patients with X-linked mental retardation. *Hum Mutat* **2006**, *27*, 389.

243. Jensen, L.R.; Amende, M.; Gurok, U.; Moser, B.; Gimmel, V.; Tzschach, A.; Janecke, A.R.; Tariverdian, G.; Chelly, J.; Fryns, J.P., et al. Mutations in the jarid1c gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. *Am J Hum Genet* **2005**, *76*, 227-236.

244. Santos, C.; Rodriguez-Revenga, L.; Madrigal, I.; Badenas, C.; Pineda, M.; Mila, M. A novel mutation in jarid1c gene associated with mental retardation. *Eur J Hum Genet* **2006**, *14*, 583-586.

245. Rujirabanjerd, S.; Nelson, J.; Tarpey, P.S.; Hackett, A.; Edkins, S.; Raymond, F.L.; Schwartz, C.E.; Turner, G.; Iwase, S.; Shi, Y., et al. Identification and characterization of two novel jarid1c mutations: Suggestion of an emerging genotype-phenotype correlation. *Eur J Hum Genet* **2010**, *18*, 330-335.

246. Vissers, L.E.; de Ligt, J.; Gilissen, C.; Janssen, I.; Steehouwer, M.; de Vries, P.; van Lier, B.; Arts, P.; Wieskamp, N.; del Rosario, M., et al. A de novo paradigm for mental retardation. *Nat Genet* **2010**, *42*, 1109-1112.

247. Adegbola, A.; Gao, H.; Sommer, S.; Browning, M. A novel mutation in jarid1c/smcx in a patient with autism spectrum disorder (asd). *Am J Med Genet A* **2008**, *146*, 505-511.

248. Lu, Y.; Oura, S.; Matsumura, T.; Oji, A.; Sakurai, N.; Fujihara, Y.; Shimada, K.; Miyata, H.; Tobita, T.; Noda, T., et al. Crispr/cas9-mediated genome editing reveals 30 testis-enriched genes dispensable for male fertility in mice. *Biol Reprod* **2019**, *101*, 501-511.

249. Shpargel, K.B.; Sengoku, T.; Yokoyama, S.; Magnuson, T. Utx and uty demonstrate histone demethylase-independent function in mouse embryonic development. *PLoS Genet* **2012**, *8*, e1002964.

250. Lee, S.; Lee, J.W.; Lee, S.K. Utx, a histone h3-lysine 27 demethylase, acts as a critical switch to activate the cardiac developmental program. *Dev Cell* **2012**, *22*, 25-37.

251. Welstead, G.G.; Creighton, M.P.; Bilodeau, S.; Cheng, A.W.; Markoulaki, S.; Young, R.A.; Jaenisch, R. X-linked h3k27me3 demethylase utx is required for embryonic development in a sex-specific manner. *Proc Natl Acad Sci U S A* **2012**, *109*, 13004-13009.

252. Thieme, S.; Gyarfás, T.; Richter, C.; Ozhan, G.; Fu, J.; Alexopoulou, D.; Muders, M.H.; Michalk, I.; Jakob, C.; Dahl, A., et al. The histone demethylase utx regulates stem cell migration and hematopoiesis. *Blood* **2013**, *121*, 2462-2473.
253. Wang, C.; Lee, J.E.; Cho, Y.W.; Xiao, Y.; Jin, Q.; Liu, C.; Ge, K. Utx regulates mesoderm differentiation of embryonic stem cells independent of h3k27 demethylase activity. *Proc Natl Acad Sci U S A* 2012, 109, 15324-15329.

254. Bogershausen, N.; Gatinois, V.; Riehmer, V.; Kayserili, H.; Becker, J.; Thoenes, M.; Simsek-Kiper, P.O.; Barat-Houari, M.; Elcioglu, N.H.; Wieczorek, D., et al. Mutation update for kabuki syndrome genes kmt2d and kdm6a and further delineation of x-linked kabuki syndrome subtype 2. *Hum Mutat* 2016, 37, 847-864.

255. Guo, Z.; Liu, F.; Li, H.J. Novel kdm6a splice-site mutation in kabuki syndrome with congenital hydrocephalus: A case report. *BMC Med Genet* 2018, 19, 206.

256. Banka, S.; Lederer, D.; Benoit, V.; Jenkins, E.; Howard, E.; Bunstone, S.; Kerr, B.; Mc Kee, S.; Lloyd, I.C.; Shears, D., et al. Novel kdm6a (utx) mutations and a clinical and molecular review of the x-linked kabuki syndrome (ks2). *Clin Genet* 2015, 87, 252-258.

257. Micale, L.; Augello, B.; Maffeo, C.; Selicorni, A.; Zucchetti, F.; Fusco, C.; De Nittis, P.; Pellico, M.T.; Mandriani, B.; Fischetto, R., et al. Molecular analysis, pathogenic mechanisms, and readthrough therapy on a large cohort of kabuki syndrome patients. *Hum Mutat* 2014, 35, 841-850.

258. Van Laarhoven, P.M.; Neitzel, L.R.; Quintana, A.M.; Geiger, E.A.; Zackai, E.H.; Clouthier, D.E.; Artinger, K.B.; Ming, J.E.; Shaikh, T.H. Kabuki syndrome genes kmt2d and kdm6a: Functional analyses demonstrate critical roles in craniofacial, heart and brain development. *Hum Mol Genet* 2015, 24, 4443-4453.

259. Miyake, N.; Koshimizu, E.; Okamoto, N.; Mizuno, S.; Ogata, T.; Nagai, T.; Kosho, T.; Ohashi, H.; Kato, M.; Sasaki, G., et al. Mll2 and kdm6a mutations in patients with kabuki syndrome. *Am J Med Genet A* 2013, 161A, 2234-2243.

260. Miyake, N., Mizuno, S., Okamoto, N., Ohashi, H., Shiina, M., Ogata, K., Tsurusaki, Y., Nakashima, M., Saitsu, H., Niikawa, N., et al. Kdm6a point mutations cause kabuki syndrome. *Hum Mutat* 2013, 34, 108-110.

261. Lederer, D.; Shears, D.; Benoit, V.; Verellen-Dumoulin, C.; Maystadt, I. A three generation x-linked family with kabuki syndrome phenotype and a frameshift mutation in kdm6a. *Am J Med Genet A* 2014, 164A, 1289-1292.

262. Frans, G.; Meyts, I.; Devriendt, K.; Liston, A.; Vermeulen, F.; Bossuyt, X. Mild humoral immunodeficiency in a patient with x-linked kabuki syndrome. *Am J Med Genet A* 2016, 170, 801-803.

263. Cheon, C.K.; Sohn, Y.B.; Ko, J.M.; Lee, Y.J.; Song, J.S.; Moon, J.W.; Yang, B.K.; Ha, I.S.; Bae, E.J.; Jin, H.S., et al. Identification of kmt2d and kdm6a mutations by exome sequencing in korean patients with kabuki syndrome. *J Hum Genet* 2014, 59, 321-325.

264. Masui, D.; Fukahori, S.; Mizuochi, T.; Watanabe, Y.; Fukui, K.; Ishi, S.; Saikusa, N.; Hashizume, N.; Higashidate, N.; Sakamoto, S., et al. Cystic biliary atresia with paucity of bile ducts and gene mutation in kdm6a: A case report. *Surg Case Rep* 2019, 5, 132.

265. van Haaften, G.; Dalgliesh, G.L.; Davies, H.; Chen, L.; Bignell, G.; Greenman, C.; Edkins, S.; Hardy, C.; O’Meara, S.; Teague, J., et al. Somatic mutations of the histone h3k27 demethylase gene utx in human cancer. *Nat Genet* 2009, 41, 521-523.
266. Ohtani, K.; Zhao, C.; Dobreva, G.; Manavski, Y.; Kluge, B.; Braun, T.; Rieger, M.A.; Zeiher, A.M.; Dimmeler, S. Jmjd3 controls mesodermal and cardiovascular differentiation of embryonic stem cells. Circ Res 2013, 113, 856-862.

267. Burgold, T.; Voituron, N.; Caganova, M.; Tripathi, P.P.; Menuet, C.; Tusi, B.K.; Sprefico, F.; Bevengut, M.; Gestreau, C.; Buontempo, S., et al. The h3k27 demethylase jmjd3 is required for maintenance of the embryonic respiratory neuronal network, neonatal breathing, and survival. Cell Rep 2012, 2, 1244-1258.

268. Satoh, T.; Takeuchi, O.; Vandenbon, A.; Yasuda, K.; Tanaka, Y.; Kumagai, Y.; Miyake, T.; Matsushita, K.; Okazaki, T.; Saijoh, T., et al. The jmjd3-irf4 axis regulates m2 macrophage polarization and host responses against helminth infection. Nat Immunol 2010, 11, 936-944.

269. Li, Q.; Wang, H.Y.; Chepelev, I.; Zhu, Q.; Wei, G.; Zhao, K.; Wang, R.F. Stage-dependent and locus-specific role of histone demethylase jumonji d3 (jmjd3) in the embryonic stages of lung development. PLoS Genet 2014, 10, e1004524.

270. Burchfield, J.S.; Li, Q.; Wang, H.Y.; Wang, R.F. Jmjd3 as an epigenetic regulator in development and disease. Int J Biochem Cell Biol 2015, 67, 148-157.

271. Zhang, F.; Xu, L.; Xu, L.; Xu, Q.; Karsenty, G.; Chen, C.D. Histone demethylase jmjd3 is required for osteoblast differentiation in mice. Sci Rep 2015, 5, 13418.

272. Zhang, F.; Xu, L.; Xu, L.; Xu, Q.; Li, D.; Yang, Y.; Karsenty, G.; Chen, C.D. Jmjd3 promotes chondrocyte proliferation and hypertrophy during endochondral bone formation in mice. J Mol Cell Biol 2015, 7, 23-34.

273. Yavarna, T.; Al-Dewik, N.; Al-Mureikhi, M.; Ali, R.; Al-Mesaiifri, F.; Mahmoud, L.; Shahbeck, N.; Lakhani, S.; AlMulla, M.; Nawaz, Z., et al. High diagnostic yield of clinical exome sequencing in middle eastern patients with mendelian disorders. Hum Genet 2015, 134, 967-980.

274. Laumonnier, F.; Holbert, S.; Ronce, N.; Faravelli, F.; Schwartz, C.E.; Lespinasse, J.; Van Esch, H.; Lacombe, D.; Goizet, C., et al. Mutations in phf8 are associated with x linked mental retardation and cleft lip/cleft palate. J Med Genet 2005, 42, 780-786.

275. Chen, X.; Wang, S.; Zhou, Y.; Han, Y.; Li, S.; Xu, Q.; Xu, L.; Zhu, Z.; Deng, Y.; Yu, L., et al. Phf8 histone demethylase deficiency causes cognitive impairments through the mtor pathway. Nat Commun 2018, 9, 114.

276. Abidi, F.; Miano, M.; Murray, J.; Schwartz, C. A novel mutation in the phf8 gene is associated with x-linked mental retardation with cleft lip/cleft palate. Clin Genet 2007, 72, 19-22.

277. Koivisto, A.M.; Ala-Mello, S.; Lemmela, S.; Komu, H.A.; Rautio, J.; Jarvela, I. Screening of mutations in the phf8 gene and identification of a novel mutation in a finnish family with xlmr and cleft lip/cleft palate. Clin Genet 2007, 72, 145-149.

278. Nava, C.; Lamari, F.; Heron, D.; Mignot, C.; Rastetter, A.; Keren, B.; Cohen, D.; Faudet, A.; Bouteiller, D.; Gilleron, M., et al. Analysis of the chromosome x exome in patients with autism spectrum disorders identified novel candidate genes, including tmlhe. Transl Psychiatry 2012, 2, e179.
Exploration of fam120c as a positional candidate gene for autism. *Am J Med Genet A* 2014, 164A, 3035-3041.

281. Oh, S.; Janknecht, R. Histone demethylase jmjd5 is essential for embryonic development. *Biochem Biophys Res Commun* 2012, 420, 61-65.

282. Ishimura, A.; Minehata, K.; Terashima, M.; Kondoh, G.; Haru, T.; Suzuki, T. Jmjd5, an h3k36me2 histone demethylase, modulates embryonic cell proliferation through the regulation of cdkn1a expression. *Development* 2012, 139, 749-759.

283. Rasmussen, K.D.; Helin, K. Role of tet enzymes in DNA methylation, development, and cancer. *Genes Dev* 2016, 30, 733-750.

284. Delhommeau, F.; Dupont, S.; Della Valle, V.; James, C.; Trannoy, S.; Masse, A.; Kosmider, O.; Le Couedic, J.P.; Robert, F.; Alberdi, A., et al. Mutation in tet2 in myeloid cancers. *N Engl J Med* 2009, 360, 2289-2301.

285. Lederer, D.; Grisart, B.; Digilio, M.C.; Benoit, V.; Crespin, M.; Ghariani, S.C.; Maystadt, I.; Dallapiccola, B.; Verellen-Dumoulin, C. Deletion of kdm6a, a histone demethylase interacting with mll2, in three patients with kabuki syndrome. *Am J Hum Genet* 2012, 90, 119-124.

286. Yang, P.; Tan, H.; Xia, Y.; Yu, Q.; Wei, X.; Guo, R.; Peng, Y.; Chen, C.; Li, H.; Mei, L., et al. De novo exonic deletion of kdm6a in a chinese girl with kabuki syndrome: A case report and brief literature review. *Am J Med Genet A* 2016, 170, 1613-1621.

287. Lindgren, A.M.; Hoyos, T.; Talkowski, M.E.; Hanscom, C.; Blumenthal, I.; Chiang, C.; Ernst, C.; Pereira, S.; Ordulu, Z.; Clericuzio, C., et al. Haploinsufficiency of kdm6a is associated with severe psychomotor retardation, global growth restriction, seizures and cleft palate. *Hum Genet* 2013, 132, 537-552.

**Competing Interests**

The authors declare no competing interests.

**Acknowledgements**

This work was supported by the Wellcome Trust (206293/Z/17/Z) and the University of Liverpool.

**Related Links**

Supplementary Table 1 and references.

**Display items**

Tables: 1
Figures: 6
Boxes: 1

**Glossary:**

Chromatin is a complex of DNA and proteins that forms chromosomes within the nucleus of eukaryotic cells.
Epigenetics- Reversible modifications to chromatin, typically referring to DNA and histones, which can alter gene expression.

IRES- Internal ribosome entry site (IRES) elements are RNA regions that recruit the translation machinery internally.