Front. Biol. 2016, 11(2): 85–95
DOI 10.1007/s11515-016-1398-y

REVIEW

The epigenetics of CHARGE syndrome

Nina K. Latcheva[1,2], Rupa Ghosh[1], Daniel R. Marenda (✉)[1,3]

[1] *Department of Biology, Drexel University, Philadelphia, PA 19104, USA*
[2] *Program in Molecular and Cellular Biology and Genetics, Drexel University College of Medicine, Philadelphia, PA 19129, USA*
[3] *Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129, USA*

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2016

Abstract **In biology, we continue to appreciate the fact that the DNA sequence alone falls short when attempting to explain the intricate inheritance patterns for complex traits. This is particularly true for human disorders that appear to have simple genetic causes. The study of epigenetics, and the increased access to the epigenetic profiles of different tissues has begun to shed light on the genetic complexity of many basic biological processes, both physiological and pathological. Epigenetics refers to heritable changes in gene expression that are not due to alterations in the DNA sequence. Various mechanisms of epigenetic regulation exist, including DNA methylation and histone modification. The identification, and increased understanding of key players and mechanisms of epigenetic regulation have begun to provide significant insight into the underlying origins of various human genetic disorders. One such disorder is CHARGE syndrome (OMIM #214800), which is a leading cause of deaf-blindness worldwide. A majority of CHARGE syndrome cases are caused by haploinsufficiency for the *CHD7* gene, which encodes an ATP-dependent chromatin remodeling protein involved in the epigenetic regulation of gene expression. The CHD7 protein has been highly conserved throughout evolution, and research into the function of CHD7 homologs in multiple model systems has increased our understanding of this family of proteins, and epigenetic mechanisms in general. Here we provide a review of CHARGE syndrome, and discuss the epigenetic functions of CHD7 in humans and CHD7 homologs in model organisms.**

Keywords *Drosophila*, Kismet, CHD7, CHARGE syndrome, chromatin remodeling

Introduction

Interest in epigenetics has increased dramatically in recent years, beginning with the recognition of its importance in oncology, and moving into multiple fields ranging from immunology to neuroscience. The term epigenetics was first used by C. H. Waddington in 1942 to explain how genes may interact with their environment to produce a phenotype (Kouzarides, 2007). The definition has since been standardized to mean "*the study of mechanisms of temporal and spatial control of gene activity during the development of complex organisms*" (Allis et al., 2007). Thus we refer to an "*epigenetic trait as a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence*" (Kirmizis et al., 2007).

Received March 31, 2016; accepted April 15, 2016
Correspondence: Daniel R. Marenda
E-mail: dm562@drexel.edu

The importance of epigenetics became evident with genetic and epidemiological studies of monozygotic twins. Even though these twin pairs share identical DNA, they are often not completely phenotypically identical, and possess different susceptibilities to diseases (Fraga et al., 2005). In a large cohort study of monozygotic twins, it was found that while twin pairs are epigenetically indistinguishable early on, older monozygotic twins exhibited enormous differences in their content and distribution of 5-methylcytosine (5 mC) DNA and histone acetylation, two significant global markers of epigenetic gene expression (Fraga et al., 2005). These findings brought to light the influence of environmental factors on gene expression, as older monozygotic twins spent less time together and were more likely to have differing lifestyles, which could contribute to the epigenetic changes observed (Kaminsky et al., 2009).

With our growing understanding of epigenetics, it is becoming more apparent how important these changes are during developmental processes, and how alteration of key epigenetic players could lead to human disorders. An example of the importance of epigenetic regulation can be

demonstrated by CHARGE syndrome, a congenital developmental disorder that affects approximately 1 in 10000 individuals worldwide (Vissers et al., 2004; Blake and Prasad, 2006; Sanlaville and Verloes, 2007; Schnetz et al., 2009). CHARGE syndrome primarily affects the sensory organ systems, with vision, smell, and hearing most often affected (Blake et al., 2008; Zentner et al., 2010). Individuals with CHARGE syndrome can display a wide variety and severity of the symptoms of this disease however (Zentner et al., 2010). This heterogeneity of features among CHARGE individuals is consistent with misregulation of early developmental events orchestrated by epigenetic factors. In support of this, a 2004 study showed that mutations in the *CHD7* gene, which encodes an ATP-dependent chromatin remodeling protein, were associated with a majority of CHARGE syndrome cases (Vissers et al., 2004). In this review, we will provide an analysis of the function of CHD7 and its homologs, and how this analysis has helped us understand the underling mechanisms behind CHARGE syndrome characteristics.

Epigenetic mechanisms

In eukaryotes, DNA is packaged into chromatin via a complex of macromolecules. The fundamental unit of chromatin is the nucleosome, which is composed of an octamer of four core histones (H2A, H2B, H3, and H4) with 147 base pairs of DNA wrapped around this histone core (Kornberg and Lorch, 1999; Richmond and Davey, 2003). Nucleosomes can be spaced farther apart in less condensed chromatin structures referred to as euchromatin or they can be highly compacted into heterochromatin. Heterochromatin is usually transcriptionally inactive due to the lack of access of the transcriptional machinery to the DNA (Kouzarides, 2007). Currently there are two main systems that contribute to epigenetic regulation of genes: DNA methylation, and histone tail modifications. DNA methylation is the most widely studied of these and consists of the covalent addition of a methyl group at the 5-position of cytosines, usually resulting in gene silencing (Kouzarides, 2007).

The covalent modification of histones is another important mechanism of epigenetic control, and can result in either gene silencing or gene activation depending upon the relevant histone modification. The N-terminal tails of histones can be post-translationally modified with at least eight distinct types of reversible modifications, which are carried out by different enzymes. These modifications include: acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, deimination, and proline isomerization (Kouzarides, 2007). These histone modifications can have very different effects on chromatin structure and transcriptional activation depending on their properties and placement. Common features have been identified in the composition and enrichment of the modifications on actively transcribed genes. These common features are thought to be part of a simple and redundant histone code in which collections of modifications have similar roles in governing chromatin structure. For example, methylations of lysine residues 4 and 36 on histone 3 are generally associated with transcriptional activation, while methylation of lysine residues 9 and 27 on histone 3 are generally associated with gene silencing (Kouzarides, 2007; Torres-Padilla et al., 2007). These different modifications tend to have specific enrichment profiles throughout the genome and seem to be a conserved characteristic in eukaryotes from yeast to humans (Kouzarides 2007).

Three distinct groups of epigenetic enzymes are involved with translating the post-translational modifications of histones into the relevant changes in gene expression: writers, erasers, and readers. Epigenetic writers include histone acetyltransferases (HATs) and histone methyltransferases (HMTs) and can covalently attach acetyl or methyl groups to amino acids found in the N-terminal tails of histones. Erasers include histone deacetylases (HDACs) and lysine demethylases (KDMs) and function to remove acetyl groups and methyl groups (respectively) from histone tails (Torres-Padilla et al., 2007). Readers include chromo- and bromo-domain containing proteins, and are known to recognize and bind to specific epigenetic marks; however, much less is known about their function. Histone modifications serve to influence chromatin structure, either autonomously by affecting the contacts between DNA and the nucleosome, or non-autonomously by providing a mark that enables the recruitment of chromatin remodeling complexes (Kouzarides, 2007). Lysine acetylation is an example of an autonomous effect on gene expression, by changing the basic charge of the lysine residue on histones from positive to neutral. This facilitates the unraveling of DNA from the nucleosome due to the weakening electrostatic interactions with the negatively charged DNA backbone. The non-autonomous mechanism of action of histone modifications is to recruit non-histone proteins to carry out further structural alterations. Chromatin remodeling is thus not limited to the covalent post-translational modification of histones, but also includes alteration of nucleosome organization around target DNA (Aalfs and Kingston, 2000; Workman, 2006; Gangaraju and Bartholomew, 2007). Identification of the key chromatin remodeling proteins, and the complexes they participate in, will facilitate a better understanding of the mechanisms by which they exert their effects on downstream target genes. This will be crucial to the understanding of events from a neurodevelopmental perspective that leads to complex disorders such as CHARGE syndrome.

The current definition of epigenetics, as stated above, refers to a stably “heritable” phenotype. This part of the description has been called into question regarding histone modifications due to the lack of knowledge on the transmission of a heritable mark from generation to generation (Kouzarides, 2007). Evidence exists that epigenetic enzymes, such as

polycomb group repressors (PcG) and trithorax group activators (TrxG), can sustain a cellular memory of chromatin structure via interactions with cis-regulatory elements in the genome (Paro et al., 1998; Cavalli and Paro, 1999), and that this chromatin state is mitotically heritable during development and can also be passed down through meiosis. The mechanism of this heritability however has remained elusive until recently, when it was shown *in vivo* that HMTs are continuously positioned at their response elements on the new DNA strand during replication (Petruk et al., 2012). These enzymes dissociate from the single stranded DNA and instead associate with the replication machinery during the progression of the replication fork (Petruk et al., 2012). After replication, the histone modifying enzymes quickly re-associated with double stranded DNA and can re-establish the specific histone code on the un-modified histone tails (Petruk et al., 2012). This mechanism is thought to be similar in both mitosis and meiosis, and helps explain how histone modifications can persist trans-generationally, solidifying their epigenetic status.

CHARGE syndrome, CHD7 and its homologs

Two-thirds of CHARGE syndrome cases are due to haploinsufficiency of the *CHD7* gene (Souriau et al., 2005; Blake and Prasad, 2006; Lalani et al., 2006). CHARGE is an acronym that corresponds to some of the abnormalities seen in CHARGE patients: Coloboma, Heart defects, Atresia of the choanae, Retardation of growth and development, Genital hypoplasia, and Ear abnormalities (Vissers et al., 2004). However, CHARGE individuals can also display a number of other abnormalities including temporal bone anomalies, semicircular canal hypoplasia/dysplasia, facial nerve paralysis, and hypotonia (Zentner et al., 2010). As with many disorders, a number of model systems have been created in attempt to further understand the underlying pathogenesis of the disorder. These models have proven to be tremendously important in modeling CHARGE syndrome, and exhibit a number of phenotypes similar to what is observed in human CHARGE individuals.

Homozygous mutations of *Chd7* in mice, or high doses of morpholino-mediated knockdown of *Chd7* (*chd7-MO*) in zebrafish lead to lethality, whereas heterozygous mutations or low doses of *chd7-MO* lead to CHARGE-like phenotypes (Schnetz et al., 2009; Melicharek et al., 2010; Zentner et al., 2010; Zentner et al., 2010; Hurd et al., 2011; Kita et al., 2012; Balow et al., 2013). Similar dosage sensitivity with *Chd7-MO* was observed in the *Xenopus* system (Bajpai et al., 2010). Consistent with the results in higher eukaryotic systems, in *Drosophila melanogaster*, moderate loss-of-function in the *Chd7* homolog *kismet* (*kis*) led to CHARGE-like phenotypes in adult flies, while a stronger knockdown in *kis* led to late pupal lethality (Melicharek et al., 2010).

CHD7 structure, isoforms and localization

CHD7 is an ATP-dependent chromatin remodeling protein that belongs to subclass III of the family of chromodomain helicase DNA binding proteins (CHD) in mammals (Schnetz et al., 2010; Kita et al., 2012). The CHD7 locus gives rise to two isoforms: CHD7-L and CHD7-S, due to alternative splicing of exon 6 (Kita et al., 2012). CHD7-L encodes a protein of approximately 340 kDa which shows both nucleoplasmic and nucleolar localization (Zentner et al., 2010). The CHD7 protein exhibits ubiquitous expression during fetal development, with high expression levels in the brain, epithelia, and ganglia, showing preferential localization in tissues affected in CHARGE syndrome (Vissers et al., 2004; Bosman et al., 2005; Schnetz et al., 2009; Schnetz et al., 2010).

CHD7-L has two chromodomains, one ATPase-domain, two helicase domains, two BRK domains, and one SANT-SLIDE domain (Fig. 1) (Hurd et al., 2007; Schnetz et al., 2009; Kita et al., 2012; Balow et al., 2013). In CHARGE syndrome patients, *CHD7* mutations arise mostly *de novo*, however, rare familial cases of transmission have been reported and were found to be due to germline mosaicism (Hurd et al., 2007; Jongmans et al., 2008). Nonsense mutations and frameshift insertions or deletions account for the vast majority of pathogenic alterations at 78%, while splice site and missense mutations account for 19% (Zentner et al., 2010; Janssen et al., 2012). The remaining 3% of mutations in CHARGE syndrome patients are larger deletions, duplications, and translocations. Approximately 47% of the mutations in *CHD7* in CHARGE syndrome studied to date are associated with regions upstream of amino acid 1899, potentially leading to severely impaired CHD7 enzymatic function (Bouazoune and Kingston; 2012; Janssen et al., 2012). However, only about 30% of the reported mutations fall in regions which code for functional domains (Basson and van Ravenswaaij-Arts, 2015). Additionally, the majority of mutations are evenly distributed throughout the coding region, including at the C terminus, suggesting the presence of essential structures throughout the protein. Further, mutations that only subtlety affect protein–protein interactions and/or ATP-dependent chromatin remodeling activity may only lead to subtle alterations in target gene expression. Still, these mutations could have significant effects on organismal development depending on the tissue and temporal importance of CHD7-mediated gene expression (Schnetz et al., 2010; Bouazoune and Kingston, 2012).

The N-terminal region of the CHD7 protein contains the central "chromodomain-ATPase" and the SANT-SLIDE module, both of which are essential for efficient ATP-dependent chromatin remodeling activity (Fig. 1) (Bouazoune and Kingston, 2012). The function of the BRK domain remains unknown, however a mutation in the second BRK domain that introduced a pre-mature STOP codon led to the development of CHARGE syndrome, suggesting that this

domain is important for protein function (Allen et al., 2007). The SANT-SLIDE domain may function as the "histone tail presenter" or histone-tail binding module to stabilize histone tail conformation for further modification by remodeling enzymes with ATP-dependent catalytic activity (Boyer et al., 2004). CHD7-S is approximately 100 kDa and consists of only one chromodomain, lacking all the other domains of CHD7-L. CHD7-S also has a nucleolar localization, which is dependent on a nucleolar localization signal spanning amino acid residues between 641 and 660 (Kita et al., 2012). However, the function of CHD7-S is not yet well understood.

Kismet structure, isoforms and localization

Overall, both CHD7 and its *Drosophila* ortholog Kismet reveal a good structure-function correlation, suggesting evolutionarily conserved roles despite the ~670 million years of evolutionary distance between them (Fig. 1). The *kismet* gene also produces two transcripts of 8.5 kb and 17 kb in length that encode proteins with approximate molecular masses of 225 kDa (2151 amino acids) for Kis-S, and 574 kDa (5322 amino acids) for Kis-L (Daubresse et al., 1999; Therrien et al., 2000). These transcripts are uniformly distributed along the anterior-posterior axis throughout *Drosophila* development with the exception of the adult stage. During development, the transcripts begin to preferentially localize to the ventral nerve cord and brain in the late embryo, confirming published roles for this protein in nervous system development and function in the fly (Daubresse et al., 1999; Melicharek et al., 2008; Melicharek et al., 2010; Ghosh et al., 2014). The Kismet protein exhibits nuclear localization with ubiquitous expression in the *Drosophila* embryo (Daubresse et al., 1999). Similarly, in third instar larvae, the Kismet protein exhibits nuclear localization within the central nervous system, ventral nerve cord, and developing retina (Melicharek et al., 2010; Ghosh et al., 2014). Kismet was originally identified as a dominant suppressor of polycomb gene function (Daubresse et al., 1999). As a trithorax group protein, Kismet has been shown to positively regulate homeotic gene expression, and to act antagonistically to polycomb group proteins, which repress homeotic genes (Daubresse et al., 1999; Srinivasan et al., 2005; Srinivasan et al., 2008; Dorighi and Tamkun, 2013). An increase in the dosage of *kismet* has been shown to enhance homeotic transformations caused by *polycomb* mutations (Daubresse et al., 1999). Similarly, heterozygous *kismet* mutations counteract homeotic transformations mediated by heterozygous *polycomb* mutation (Daubresse et al., 1999). In later stages of development Kismet is thought to maintain heritable states of transcription of these genes, thereby controlling cell fate.

Kismet-L has two chromodomains (CD1 and CD2), and an SNF2-ATPase domain (Fig. 1). The ATPase domain is related to the Brahma (Brm) ATPase (44% identity), the SWI2/SNF2 chromatin remodeling complex, and the CHD family of chromatin remodelers (50% identity) (Daubresse et al., 1999; Srinivasan et al., 2005). Brm shares similarity with the ATPase subunits of the SWI2/SNF2 of the SWI/SNF complex, the RSC in yeast, and the BAF and PBAF chromatin remodeling complexes in humans (Daubresse et al., 1999; Srinivasan et al., 2005; Reisman et al., 2009).

Kis-L and Kis-S have a common C-terminal domain consisting of 2105 amino acids. The BRK domain is encoded by 41 amino acid residues within this common region. While the function of the BRK domain is still unknown, it is also found in human BRG1 and human BRM proteins, suggesting

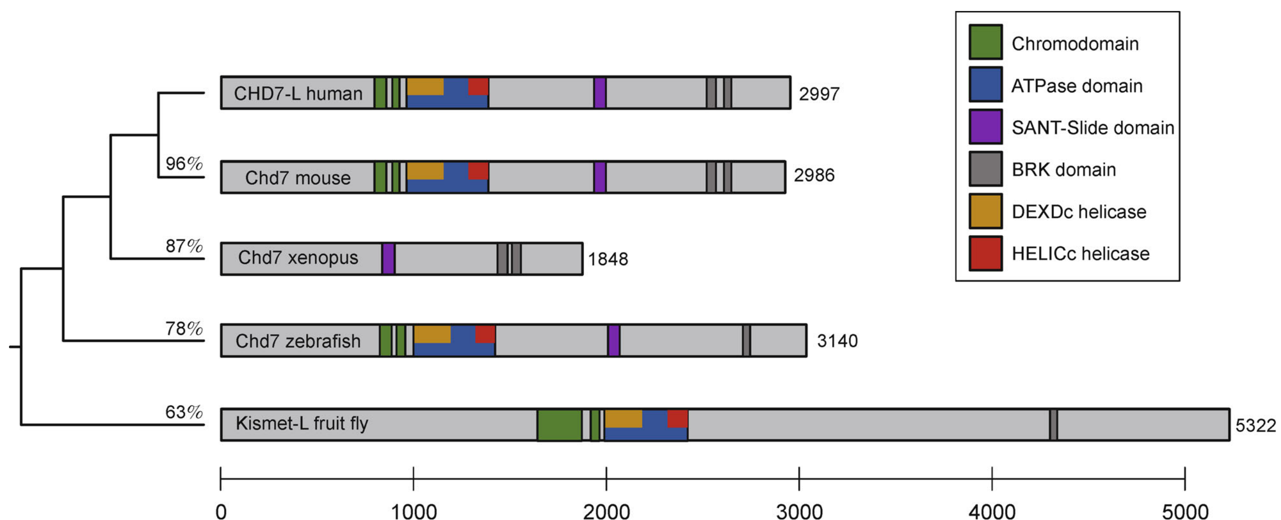

Figure 1 Homology between Chd7 proteins in model organisms. Schematic representation of conserved Chd7 proteins in model organisms used to study CHARGE syndrome. Percent value indicates homology to human CHD7. Green is chromodomain; blue is ATPase domain; purple is Switching-defective protein 3, Adaptor 2, Nuclear receptor co-repressor, Transcription factor (TF)IIIB (SANT)-Slide domain; dark gray is Brahma and Kismet (BRK) domain; yellow is DEAD-like helicase (DEXDc) domain; red is helicase superfamily C-terminal (HELICc) domain.

an evolutionary requirement for this domain (Allen et al., 2007). Overall, CHD7 and Kismet show similarities with respect to their larger isoforms and cellular localization. However, CHD7-S consists of only one chromodomain, and its structure is markedly different from that of Kis-S (Srinivasan et al., 2005; Kita et al., 2012). This may be suggestive of functional disparity between the two isoforms, although it remains to be tested.

CHD7 has a multitude of binding sites in the genome, preferentially located at distal enhancer sites and transcriptional start sites of target genes (Schnetz et al., 2009; Bajpai et al., 2010; Schnetz et al., 2010; Zentner et al., 2010; Engelen et al., 2011). Similarly, widespread localization of Kismet on salivary gland polytene chromosomes has also been shown (Srinivasan et al., 2005; Srinivasan et al., 2008). This is indicative of global scale regulation of downstream targets genes, although the functional consequences of many of these genes with respect to CHD7 or Kismet mediated regulation remains to be determined.

Function of CHD7 and Kismet in central nervous system development

The severe phenotypic variability in CHARGE syndrome and the broad extent of affected organ systems support the idea that developmental defects may arise as early as the formation of the neural tube (de Lonlay-Debeney et al., 1997; Tellier et al., 1998; Kosaki, 2011). Occasionally CHARGE syndrome patients display CNS anomalies such as midline defects (in 22% cases), anatomical asymmetry (in 18% of cases), and hindbrain abnormalities (in 14% of case) (Tellier et al., 1998; Sanlaville and Verloes, 2007; Janssen et al., 2012). Consistent with this, Kismet was shown to play important roles during the third instar larval, pupal, and the adult stages of CNS development (Therrien et al., 2000; Melicharek et al., 2008; Melicharek et al., 2010; Terriente-Felix et al., 2011), and is required for the proper development and migration of mushroom body neurons, dorsal cluster neurons, and photoreceptor axons (Melicharek et al., 2010). In particular, decreased Kismet function leads to defects in axonal pruning, guidance, extension of these neuronal populations, and midline axon crossing defects with variable frequency in their occurrence (Melicharek et al., 2010). Behaviors associated with proper functioning of these circuits such as locomotion, learning, and memory are also affected. Thus, there is a potential developmental basis for deficits in behavior.

Chd7 was found to be involved in maintaining the balance between neurogenesis and glial cell fates in the subventricular zone (SVZ), one of the two neurogenic regions in the adult mammalian brain (Micucci et al., 2014). Chd7 deficiencies resulted in a reduction of immature and mature interneurons while increasing oligodendrocyte and glial populations. Treatment with retinoic acid (RA) partially attenuated these differentiation defects, and Chd7 was found to directly bind to the RA receptor promoter (Micucci et al., 2014). Additionally, Chd7 is necessary for neuronal differentiation in both neurogenic regions of the adult mouse brain, the SVZ and the subgranular zone (SGZ) of the hippocampus (Feng et al., 2013; Feng and Liu, 2013; Kim and Roberts, 2013). Loss of *Chd7* led to neuronal differentiation defects in both regions as well as aberrant dendritic development of newborn neurons (Feng et al., 2013). *Sox4* and *Sox11*, which are essential for neuronal property determinations, were found to be direct targets of Chd7 regulation and their overexpression *in vitro* led to a rescue of the differentiation defects associated with loss of *Chd7* (Feng et al., 2013; Feng and Liu, 2013). Interestingly, the same study showed that physical exercise can rescue both of these defects in the SGZ, leading to the speculation that an alternate pathway can stimulate differentiation in the absence of Chd7 (Kim and Roberts, 2013).

Recently, Chd7 was shown to be necessary for the differentiation of oligodendrocyte precursor cells into mature oligodendrocytes (He et al., 2016). Additionally, Chd7 depletion prevented remyelination of axons in a demyelination injury model. Genome-wide base-resolution mapping of Chd7 targets found Chd7 present at enhancer sites for myelinogenic factors such as Myrf, Nkx2.2, Sip1 and Olig (He et al., 2016). These findings may help explain white matter defects observed in some CHARGE individuals (Gregory et al., 2013; He et al., 2016)

CHD7 in neural crest development

Chd7 has been shown to play an active role in neural crest cell development and function. In *Xenopus laevis* embryos, mutating the catalytically active ATPase domain of Chd7 (Chd7K998R) recapitulated phenotypes associated with CHARGE syndrome (Bajpai et al., 2010). Chd7 was also shown to affect migration of cephalic neural crest cells to pharengeal arches in *Xenopus,* potentially by misregulating essential transcription factors such as Twist and Slug, who's expression was significantly downregulated in *Chd7* depleted embryos (Bajpai et al., 2010). Chd7 was demonstrated to be required in human cells for the formation of migratory neural crest-like cells (hNCLC's) in vitro, but was not essential for the earlier events at the neural plate border territory. Disorganized, reduced, or absent cranial neural crest populations have also been reported in a zebrafish model of CHARGE syndrome (Patten et al., 2012).

In hNCLC's, CHD7 was shown to cooperate with PBAF (related to SWI/SNF) to regulate expression of genes (*TWIST*, *SOX9* and *SLUG*) involved in neural crest formation and migration (Bajpai et al., 2010; Balow et al., 2013). Specifically, CHD7 and BRG1 co-occupy the *SOX9* distal enhancer and genomic region upstream of *TWIST1* with a concomitant enrichment of H3K4Me1 marks. Thus, CHD7 and BRG1 may function together to influence cell migration and gene expression of neural crest specific genes. Others have independently confirmed this correlation between Chd7

and Brg1 in mouse embryonic stem cells as well (Schnetz et al., 2009; Schnetz et al., 2010).

Kismet and CHD7 in eye development

Approximately 79% of CHARGE syndrome patients exhibit anatomical defects of the eye such as coloboma of the retina and/or iris, microphthalmia and/or anophthalmia, leading to loss of vision (Tellier et al., 1998). Kismet proteins are ubiquitously expressed in the *Drosophila* retina, and have been shown to be an upstream regulator of *atonal* transcription (Melicharek et al., 2008). Atonal is required for proper formation of retinal founder R8 cells, and adult flies with reduced Kismet function exhibit a slightly rough and pronounced glassy eye phenotype. Additionally, Kismet regulates early events at and near the morphogenetic furrow, underscoring its importance in eye development (Melicharek et al., 2008).

In zebrafish, Chd7 is highly similar to human CHD7 with the exception that it lacks one BRK domain (Balow et al., 2013). The zebrafish *Chd7* transcript exhibits ubiquitous expression during early embryogenesis and high expression in the brain and eye 48 h post fertilization, and is required for normal eye development (Bajpai et al., 2010; Patten et al., 2012; Balow et al., 2013). Morpholino-mediated knockdown of zebrafish *Chd7* led to underdevelopment of the eye lens or missing anterior eye structures, phenotypically small eyes, and lack of the photoreceptor cell layer (Patten et al., 2012; Balow et al., 2013). Chd7 function is also required for proper retinal organization and lamination in zebrafish, as Chd7 depletion led to reduced expression of the retinal ganglion Zn-8 marker, representative of a role in differentiation (Patten et al., 2012). Similarly, in zebrafish embryos, expression of the catalytically inactive hCHD7 ATPaseK998R led to coloboma of the eye and other CHARGE syndrome phenotypes (Bajpai et al., 2010).

CHD7 in craniofacial development

A majority of CHARGE syndrome patients display cranial nerve abnormalities that manifest in the form of difficulty in breathing, swallowing, and uni- or bilateral facial palsy. Zebrafish and *Xenopus* with reduced Chd7 also display craniofacial cartilage defects (Bajpai et al., 2010; Balow et al., 2013). Several developmental abnormalities are associated with cranial motor neurons, facial branchiomotor neurons, and vagal motor neurons (Patten et al., 2012). Chd7 depleted *Xenopus* embryos exhibit decreases in facial width and eye distance suggesting the presence of midline defects as well (Bajpai et al., 2010).

CHD7 in ear development and olfaction

Chd7 gene-trap mice exhibit reduced growth, inner-ear defects, and circling and head-bobbing behaviors, which are consistent with vestibular defects associated with CHARGE syndrome (Hurd et al., 2007). In agreement with this, the Chd7-MO zebrafish displayed circling swimming behavior in response to tactile or auditory stimulus (Patten et al., 2012). The zebrafish also displayed asymmetry in either the size of otoliths, or the presence of just one otolith. Additionally, anatomical defects in size and morphology of semicircular canals were seen (Patten et al., 2012).

Chd7 was shown to be necessary for proliferation of inner ear neuroblasts in mice by regulating expression and patterning of proneural genes involved in inner ear formation and multiple pathways that lead to semicircular canal formation (Hurd et al., 2010; Hurd et al., 2011; Hurd et al., 2012). Mice displayed middle ear defects in the form of low to mid-frequency hearing loss. These stem from mild elevation in hearing threshold measured by auditory brainstem response (sensorineural), and flattened or absent DPOAE (distortion product otoacoustic emission) or absent conductive hearing loss (Hurd et al., 2012).

Chd7 also positively regulates olfactory neural stem cell proliferation and olfactory sensory neuron formation (Layman et al., 2009; Layman et al., 2011). Expression of Chd7 was found to be high in adult olfactory epithelial stem cells and lower in mature olfactory sensory neurons in mice, suggesting a role in stem cell maintenance (Layman et al., 2009). Additionally, Chd7 heterozygous gene trapped mice exhibited significant defects in olfactory stem cell proliferation indicating a link to neurogenesis (Layman et al., 2009). This finding was later supported when Chd7 deficient mice showed a decrease in the amount of mature interneurons in the olfactory bulb (Micucci et al., 2014). These defects were considered to be due to a decrease in neurogenesis in the subventricular zone which gives rise to neuroblasts that travel via the rostral migratory stream to their final destination in the olfactory bulb (Micucci et al., 2014). The results provide a mechanism of how Chd7 haploinsufficiency may cause olfactory dysfunction.

CHD7 and reproductive development

Genital hypoplasia is one of the five hallmark symptoms observed in CHARGE syndrome patients, with up to 65% being affected (Pinto et al., 2005; Jongmans et al., 2006). The hypoplasia is generally due to deficiencies in gonadotropins such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are usually released by the pituitary in response to gonadotropin-releasing hormone (GnRH), which is synthesized in the hypothalamus. CHD7 is required for the development and maintenance of GnRH neurons in order to maintain normal puberty and reproduction (Layman et al., 2011). Reduced CHD7 resulted in multiple effects including decreased GnRH neurons in the hypothalamus and cellular proliferation, decreased LH and FSH but

normal response to GnRH agonist and antagonist, and reduced expression of Otx2, GnRH1, and GnRHR in the pituitary (Layman et al., 2011).

CHD7 in Spinal Cord Development

A less common spinal abnormality in the form of scoliosis is observed in CHARGE syndrome patients. Studies in zebrafish have provided a basis for involvement of Chd7 in the development of this abnormality (Gao et al., 2007; Jacobs-McDaniels and Albertson, 2011). Chd7-MO injected zebrafish showed asymmetrical expression of somitogenic genes leading to irregular segmentation patterns (Jacobs-McDaniels and Albertson, 2011; Patten et al., 2012). Skeletal malformation due to improper somite boundary, and segmental vasculature defects were also seen. A CHD7 polymorphism has also been implicated in spinal deformities, which include: curvature of the long body axis, decrease in bone ossification, and mineralization of the spine and abnormally shaped vertebrae (Jacobs-McDaniels and Albertson, 2011; Patten et al., 2012). In *Drosophila*, Kismet is present in the ventral nerve cord (a structure similar to the vertebrate spinal cord) within the nuclei of motor neurons, and has been shown to be important for the proper function of the neuromuscular junction (Ghosh et al., 2014).

CHD7 in rRNA biogenesis: Is CHARGE syndrome a ribopathy?

Chd7 was shown to be a positive regulator of rRNA gene transcription in mouse embryonic stem cells and heterozygous *Chd7* mouse embryos (Zentner et al., 2010). As evidenced by ChIP and ChIP-seq data, Chd7 directly bound to multiple sites along rDNA in both mouse embryonic stem cells and a human colorectal cancer cell line DLD1-A2. Sites of CHD7 binding correlated with regions of hypomethylation in DLD1-A2 cells (Schnetz et al., 2009; Zentner et al., 2010). Chd7 showed a positive correlation with 45s pre-rRNA levels in DLD1-A2, mouse embryonic stem cells, and whole mouse embryos (Zentner et al., 2010). siRNA mediated knockdown of *CHD7* showed reductions in both protein synthesis and cell proliferation in DLD1-A2 cells (Zentner et al., 2010). Accordingly, Chd7 is involved either in the maintenance of the active state or transcriptional initiation of rRNA.

Further evidence to support the importance of rRNA in CHARGE phenotypes comes from zebrafish. The jumonji domain-containing histone demethylase Fbxl10/Kdm2bb is a known suppressor of r-RNA genes. *Fbxl10/Kdm2bb* knockdown within a Chd7 zebrafish knockdown animal completely rescued the gross morphology phenotypes normally associated with loss of Chd7 in this model (Balow et al., 2013). Further, *Fbxl10/Kdm2bb* knockdown also completely rescued the cell proliferation defects normally associated with decreased Chd7 in zebrafish (Balow et al., 2013). Therefore, it is feasible that Chd7 may act to remodel the chromatin structure at rDNA sequences to increase transcription. This would prevent repressive methylation by methyltransferases recruited by nucleolar remodeling complex (NoRc), which is involved in suppressing rDNA transcription (Santoro et al., 2002; Zentner et al., 2010). Further research involving NoRc and Chd7 are required to investigate this model.

In addition to rRNA, nucleolar genes may also be regulated by CHD7. Neural crest defects in CHARGE syndrome may be explained in the context of rRNA, as these cells require high levels of protein synthesis, undergo significant migration, and are the precursor cells which give rise to a number of tissues affected in CHARGE syndrome (Zentner et al., 2010a). Thus, reduction in 45S pre-rRNA levels can be strongly implicated in the pathogenesis of CHARGE syndrome (Zentner et al., 2010a, Zentner et al., 2010b). Consistent with these findings, CHD7-L and CHD7-S work synergistically to positively regulate rRNA levels and cell proliferation in HeLa cells (Kita et al., 2012). The nucleolar localization signal of CHD7-S was found to be essential for this regulation.

CHD7 in chromatin remodeling and chromatin occupancy

CHD7 is an ATP-dependent nucleosome remodeling factor (Bouazoune and Kingston, 2012). It is biochemically similar to hSWI/SNF, as both require energy from ATP hydrolysis to expose the nucleosomal DNA to cleavage. CHD7 also shares similarity with SNF2H for its ability to slide the nucleosome octamer to the center of the DNA fragment. It is similar to ISWI and CHD1 enzymes, as it requires DNA protruding from the nucleosome as a substrate for remodeling (Mueller-Planitz et al., 2013). CHD7 can remodel intact, as well as tail-less, histones, with remodeling activity being slower for tail-less histones (Bouazoune and Kingston, 2012).

CHD7 is highly distributed to regions distal to transcriptional start sites, and is also located at the transcriptional start sites themselves in human colorectal carcinoma cells (Schnetz et al., 2009). DNase-chip in these cells demonstrated a strong correlation between CHD7 binding and regions of open chromatin, and showed a 61% overlap with DNase sites that are thought to consist of regulatory elements such as enhancers and insulators (Schnetz et al., 2009). In SH-SY5Y and DLD1 cells, CHD7 binding showed a higher degree of correlation with the H3K4me1 and H3K4me2 histone modifications present at sites distal to transcription start sites, which are marks usually associated with enhancers (Schnetz et al., 2010). However, it also showed some correlation in binding to H3K4Me3 at transcriptional start sites, a mark associated with active genes, and H3K4Me2 at sites present and distal to the transcriptional start site (Schnetz et al., 2010; Balasubramanian et al., 2012). The chromodomains of CHD7 bound all methylated forms of H3K4 in vitro (Schnetz et al., 2009). This is in agreement with the

association of CHD7 with transcriptionally active genes. Based on these studies, we can conclude that CHD7 may function at either enhancer elements, or transcriptional start sites to mediate gene expression.

To examine the role of Chd7 in embryonic stem cell differentiation, Chd7 binding sites were determined in mouse embryonic stem cells and differentiated neural precursor (NP) cells (Schnetz et al., 2009; Schnetz et al., 2010). In these two types of cells, Chd7 binding sites correlated with all forms of H3K4 methylation, but with a higher degree of overlap with either mono- or di- methylation of H3K4 (Schnetz et al., 2009). Further, Chd7 concomitantly changed with the mono- and di- methylation marks from the undifferentiated to differentiated state (Schnetz et al., 2009). CHD7 binding sites also correlated with P300 protein, which is a strong predictor of enhancer activity (Schnetz et al., 2010). Taken together, these studies suggest that Chd7 not only occupied open regions of chromatin and showed a strong correlation to the epigenetic signatures H3K4me1 and H3K4me2 which are associated with enhancer regions, but also to P300 (Schnetz et al., 2010; Zentner et al., 2010).

Chd7 binding showed a strong correlation with the binding of the transcription factors Oct4, Sox2 and Nanog, which are considered to be embryonic stem cell master regulators (Schnetz et al., 2010). Chd7 may function in enhancer-mediated transcription of embryonic stem cell specific genes by forming a complex with these transcription factors to modulate mouse embryonic stem cell specific gene expression. While Chd7 may be part of a cell type specific complex in embryonic stem cell transcriptional machinery, it is dispensable for the stem cell self-renewal and pluripotent properties (Schnetz et al., 2010). This is intriguing, and demands further research in order to determine Chd7's cell, and tissue specific functions.

In HEK293T cells, CHD7-L and CHD7-S both co-immunoprecipitated with FLAG-SOX2, but had distinct roles in regulating gene expression (Kita et al., 2012). CHD7-L alone promotes SOX2 mediated transcription of the NOTCH signaling regulators *JAG1* and *RBPJ* and suppresses that of *FGF4* and *UTF1*, which are markers of embryonic stem cells (Schnetz et al., 2010; Engelen et al., 2011). CHD7-L and SOX2 may therefore be part of a complex that mediates positive effects on transcription of downstream genes that are critical to neural stem cell development (Kita et al., 2012). In contrast, CHD7-S might shuttle between the nucleolus and nucleoplasm, with a preference for nucleoplasm when SOX2 is abundantly expressed. CHD7-S interacts with SOX2 to mediate antagonistic effects to that of CHD7-L to regulate Notch signaling and embryonic stem cell markers (Kita et al., 2012). Thus, CHD7 isoforms have localization-specific effects.

Based on these studies, the proposed model for CHD7-mediated transcription is that CHD7 binds to distal enhancer regions and facilitates interaction with promoters by forming loops in the chromatin. This would bring the enhancer in the vicinity of the promoter regions to modulate gene transcription along with other cofactors (Zentner et al., 2010; Basson and van Ravenswaaij-Arts, 2015). The ATP-dependent chromatin remodeling activity of CHD7 may facilitate this by making target DNA regions more accessible to transcriptional co-activators.

Kismet in epigenetics and mechanisms of action on downstream target genes

Genome-wide distribution of Kismet has yet to be studied by ChIP-seq approaches; however, the current distribution profile was largely inferred from polytene chromosome staining. Kismet has widespread distribution on *Drosophila* salivary gland polytene chromosomes, which correlates with inter-band regions (regions with active transcription) (Srinivasan et al., 2008). Its occupancy also parallels that of RNA polymerase II (Srinivasan,et al., 2005). Overexpression of Kis-L does not lead to alterations to the polytene chromosomal structure, suggesting that Kismet is not responsible for maintaining higher order chromatin structure (Fasulo,et al., 2012).

While CHD7 binding to methylated histone tails was successfully demonstrated in vitro, the binding of the CD2 domain of Kismet with unmethylated or methylated histones could not be established *in vitro* (Srinivasan et al., 2008; Schnetz et al., 2009). Nevertheless, the possibility of full length Kis-L interacting with histone tail modifications has not been ruled out. The distribution of Kis-L on polytene chromosomes shows significant overlap with H3K4Me2 and H3K4Me3 marks (Srinivasan et al., 2008). Chromatin immunoprecipitation showed enrichment of Kis-L at the transcription start site of the *forkhead* gene, which is known to be a subject of trithorax group protein transcriptional regulation (Srinivasan et al., 2008). This was consistent with a previous finding where Kis-L was enriched around the promoter region of the *ultrabithorax* gene, another trithorax group protein target gene (Papp and Muller, 2006). There was no apparent correlation between Kis-L and H3K4Me3 distribution for *forkhead*, suggesting that H3K4 methylation does not significantly influence the recruitment of Kis-L to the chromatin (Srinivasan et al., 2008). These results are unlike those obtained for CHD7 and H3K4 methylation marks (Schnetz et al., 2009; Schnetz et al., 2010; Zentner et al., 2010). However, since these were not genome-wide studies of Kismet, a positive correlation between Kis-L and the H3K4 methylated marks associated with active gene transcription cannot yet be ruled out.

Discussion

The study of epigenetics is multifaceted and applies to a wide variety of cellular processes. It is of no surprise that misregulation of such a ubiquitous mechanism can have

such profound effects *in vivo*. Losing 50% of CHD7 function affects the development and function of many different tissues associated with the symptoms observed in CHARGE syndrome. Proper development requires just the right amount of CHD7 function as overexpression and complete protein null mutations both often lead to lethality in model organisms. Focusing our efforts on early events regulated by CHD7 will be key to understating the development and progression of CHARGE syndrome, as will further studies into the function and regulation of the CHD7 protein itself. Animal models of CHARGE have proven to be a vital tool for elucidating such questions. Use of these models will allow for a better understanding of what cellular processes, gene targets, and cellular contexts CHD7 is involved in. These models will also be critical for testing potential novel therapeutics for intervention in CHARGE syndrome. The fact that epigenetic mechanisms are transient and often reversible allows for the hopeful prospect of one day possessing the ability to modulate CHD7 dependent cellular processes in CHARGE individuals. Overall, the studies outlined here have allowed us to build a foundation of knowledge not only of CHARGE syndrome, but also of a better understanding of the relationship between our genes and our environment.

Acknowledgements

We would like to thank Edward A. Waddell and Dr. Faith Liebl for critical reading of the manuscript, the CHARGE syndrome Foundation, and members of the CHARGE syndrome Foundation scientific advisory board for their work on CHARGE. Work in the Marenda laboratory is supported by a grant from the National Science Foundation (IOS 1256114) to DRM.

Compliance with ethics guidelines

Nina Latcheva, Rupa Ghosh, and Daniel Marenda declare that they have no conflict of interest.

References

Aalfs J D, Kingston R E (2000). What does chromatin remodeling mean? Trends Biochem Sci, 25(11): 548–555

Allen M D, Religa T L, Freund S M, Bycroft M (2007). Solution structure of the BRK domains from CHD7. J Mol Biol, 371(5): 1135–1140

Allis C D, Berger S L, Cote J, Dent S, Jenuwien T, Kouzarides T, Pillus L, Reinberg D, Shi Y, Shiekhattar R, Shilatifard A, Workman J, Zhang Y (2007). New nomenclature for chromatin-modifying enzymes. Cell, 131(4): 633–636

Bajpai R, Chen D A, Rada-Iglesias A, Zhang J, Xiong Y, Helms J, Chang C P, Zhao Y, Swigut T, Wysocka J (2010). CHD7 cooperates with PBAF to control multipotent neural crest formation. Nature, 463(7283): 958–962

Balasubramanian D, Akhtar-Zaidi B, Song L, Bartels C F, Veigl M, Beard L, Myeroff L, Guda K, Lutterbaugh J, Willis J, Crawford G E, Markowitz S D, Scacheri P C (2012). H3K4me3 inversely correlates with DNA methylation at a large class of non-CpG-island-containing start sites. Genome Med, 4(5): 47

Balow S A, Pierce L X, Zentner G E, Conrad P A, Davis S, Sabaawy H E, McDermott B MJr, Scacheri P C (2013). Knockdown of fbxl10/kdm2bb rescues chd7 morphant phenotype in a zebrafish model of CHARGE syndrome. Dev Biol, 382(1): 57–69

Basson M A, van Ravenswaaij-Arts C (2015). Functional Insights into Chromatin remodelling from studies on CHARGE syndrome. Trends Genet, 31(10): 600–611

Blake K D, Hartshorne T S, Lawand C, Dailor A N, Thelin J W (2008). Cranial nerve manifestations in CHARGE syndrome. Am J Med Genet A, 146A(5): 585–592

Blake K D, Prasad C (2006). CHARGE syndrome. Orphanet J Rare Dis, 1(1): 34

Bosman E A, Penn A C, Ambrose J C, Kettleborough R, Stemple D L, Steel K P (2005). Multiple mutations in mouse Chd7 provide models for CHARGE syndrome. Hum Mol Genet, 14(22): 3463–3476

Bouazoune K, Kingston R E (2012). Chromatin remodeling by the CHD7 protein is impaired by mutations that cause human developmental disorders. Proc Natl Acad Sci USA, 109(47): 19238–19243

Boyer L A, Latek R R, Peterson C L (2004). The SANT domain: a unique histone-tail-binding module? Nat Rev Mol Cell Biol, 5(2): 158–163

Cavalli G, Paro R (1999). Epigenetic inheritance of active chromatin after removal of the main transactivator. Science, 286(5441): 955–958

Daubresse G, Deuring R, Moore L, Papoulas O, Zakrajsek I, Waldrip W R, Scott M P, Kennison J A, Tamkun J W (1999). The Drosophila kismet gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity. Development, 126(6): 1175–1187

de Lonlay-Debeney P, Cormier-Daire V, Amiel J, Abadie V, Odent S, Paupe A, Couderc S, Tellier A L, Bonnet D, Prieur M, Vekemans M, Munnich A, Lyonnet S (1997). Features of DiGeorge syndrome and CHARGE association in five patients. J Med Genet, 34(12): 986–989

Dorighi K M, Tamkun J W (2013). The trithorax group proteins Kismet and ASH1 promote H3K36 dimethylation to counteract Polycomb group repression in *Drosophila*. Development, 140(20): 4182–4192

Engelen E, Akinci U, Bryne J C, Hou J, Gontan C, Moen M, Szumska D, Kockx C, van Ijcken W, Dekkers D H, Demmers J, Rijkers E J, Bhattacharya S, Philipsen S, Pevny L H, Grosveld F G, Rottier R J, Lenhard B, Poot R A (2011). Sox2 cooperates with Chd7 to regulate genes that are mutated in human syndromes. Nat Genet, 43(6): 607–611

Fasulo B, Deuring R, Murawska M, Gause M, Dorighi K M, Schaaf C A, Dorsett D, Brehm A, Tamkun J W (2012). The *Drosophila* MI-2 chromatin-remodeling factor regulates higher-order chromatin structure and cohesin dynamics *in vivo*. PLoS Genet, 8(8): e1002878

Feng W, Khan M A, Bellvis P, Zhu Z, Bernhardt O, Herold-Mende C, Liu H K (2013). The chromatin remodeler CHD7 regulates adult neurogenesis via activation of SoxC transcription factors. Cell Stem Cell, 13(1): 62–72

Feng W, Liu H K (2013). Epigenetic regulation of neuronal fate

determination: the role of CHD7. Cell Cycle, 12(24): 3707–3708

Fraga M F, Ballestar E, Paz M F, Ropero S, Setien F, Ballestar M L, Heine-Suner D, Cigudosa J C, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector T D, Wu Y Z, Plass C, Esteller M (2005). Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci USA, 102(30): 10604–10609

Gangaraju V K, Bartholomew B (2007). Mechanisms of ATP dependent chromatin remodeling. Mutat Res, 618(1–2): 3–17

Gao X, Gordon D, Zhang D, Browne R, Helms C, Gillum J, Weber S, Devroy S, Swaney S, Dobbs M, Morcuende J, Sheffield V, Lovett M, Bowcock A, Herring J, Wise C (2007). CHD7 gene polymorphisms are associated with susceptibility to idiopathic scoliosis. Am J Hum Genet, 80(5): 957–965

Ghosh R, Vegesna S, Safi R, Bao H, Zhang B, Marenda D R, Liebl F L (2014). Kismet positively regulates glutamate receptor localization and synaptic transmission at the *Drosophila* neuromuscular junction. PLoS ONE, 9(11): e113494

Gregory L C, Gevers E F, Baker J, Kasia T, Chong K, Josifova D J, Caimari M, Bilan F, McCabe M J, Dattani M T (2013). Structural pituitary abnormalities associated with CHARGE syndrome. J Clin Endocrinol Metab, 98(4): E737–E743

He D, Marie C, Zhao C, Kim B, Wang J, Deng Y, Clavairoly A, Frah M, Wang H, He X, Hmidan H, Jones B V, Witte D, Zalc B, Zhou X, Choo D I, Martin D M, Parras C, Lu Q R (2016). Chd7 cooperates with Sox10 and regulates the onset of CNS myelination and remyelination. Nat Neurosci, doi: 10.1038/nn.4258

Hurd E A, Adams M E, Layman W S, Swiderski D L, Beyer L A, Halsey K E, Benson J M, Gong T W, Dolan D F, Raphael Y, Martin D M (2011). Mature middle and inner ears express Chd7 and exhibit distinctive pathologies in a mouse model of CHARGE syndrome. Hear Res, 282(1–2): 184–195

Hurd E A, Capers P L, Blauwkamp M N, Adams M E, Raphael Y, Poucher H K, Martin D M (2007). Loss of Chd7 function in gene-trapped reporter mice is embryonic lethal and associated with severe defects in multiple developing tissues. Mamm Genome, 18(2): 94–104

Hurd E A, Micucci J A, Reamer E N, Martin D M (2012). Delayed fusion and altered gene expression contribute to semicircular canal defects in Chd7 deficient mice. Mech Dev, 129(9–12): 308–323

Hurd E A, Poucher H K, Cheng K, Raphael Y, Martin D M (2010). The ATP-dependent chromatin remodeling enzyme CHD7 regulates pro-neural gene expression and neurogenesis in the inner ear. Development, 137(18): 3139–3150

Jacobs-McDaniels N L, Albertson R C (2011). Chd7 plays a critical role in controlling left-right symmetry during zebrafish somitogenesis. Dev Dyn, 240(10): 2272–2280

Janssen N, Bergman J E, Swertz M A, Tranebjaerg L, Lodahl M, Schoots J, Hofstra R M, van Ravenswaaij-Arts C M, Hoefsloot L H (2012). Mutation update on the CHD7 gene involved in CHARGE syndrome. Hum Mutat, 33(8): 1149–1160

Jongmans M C, Admiraal R J, van der Donk K P, Vissers L E, Baas A F, Kapusta L, van Hagen J M, Donnai D, de Ravel T J, Veltman J A, Geurts van Kessel A, De Vries B B, Brunner H G, Hoefsloot L H, van Ravenswaaij C M (2006). CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. J Med Genet, 43(4): 306–314

Jongmans M C, Hoefsloot L H, van der Donk K P, Admiraal R J, Magee A, van de Laar I, Hendriks Y, Verheij J B, Walpole I, Brunner H G, van Ravenswaaij C M (2008). Familial CHARGE syndrome and the CHD7 gene: a recurrent missense mutation, intrafamilial recurrence and variability. Am J Med Genet A, 146A (1): 43–50

Kaminsky Z A, Tang T, Wang S C, Ptak C, Oh G H, Wong A H, Feldcamp L A, Virtanen C, Halfvarson J, Tysk C, McRae A F, Visscher P M, Montgomery G W, Gottesman I I, Martin N G, Petronis A (2009). DNA methylation profiles in monozygotic and dizygotic twins. Nat Genet, 41(2): 240–245

Kim K H, Roberts C W (2013). CHD7 in charge of neurogenesis. Cell Stem Cell, 13(1): 1–2

Kirmizis A, Santos-Rosa H, Penkett C J, Singer M A, Vermeulen M, Mann M, Bahler J, Green R D, Kouzarides T (2007). Arginine methylation at histone H3R2 controls deposition of H3K4 trimethylation. Nature, 449(7164): 928–932

Kita Y, Nishiyama M, Nakayama K I (2012). Identification of CHD7S as a novel splicing variant of CHD7 with functions similar and antagonistic to those of the full-length CHD7L. Genes Cells, 17(7): 536–547

Kornberg R D, Lorch Y (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell, 98(3): 285–294

Kosaki K (2011). Role of rare cases in deciphering the mechanisms of congenital anomalies: CHARGE syndrome research. Congenit Anom (Kyoto), 51(1): 12–15

Kouzarides T (2007). Chromatin modifications and their function. Cell, 128(4): 693–705

Kouzarides T (2007). SnapShot: Histone-modifying enzymes. Cell, 131 (4): 822–822.e1

Kouzarides T (2007). SnapShot: Histone-modifying enzymes. Cell, 128 (4): 802

Lalani S R, Safiullah A M, Fernbach S D, Harutyunyan K G, Thaller C, Peterson L E, McPherson J D, Gibbs R A, White L D, Hefner M, Davenport S L, Graham J MJr, Bacino C A, Glass N L, Towbin J A, Craigen W J, Neish S R, Lin A E, Belmont J W (2006). Spectrum of CHD7 mutations in 110 individuals with CHARGE syndrome and genotype-phenotype correlation. Am J Hum Genet, 78 (2): 303–314

Layman W S, Hurd E A, Martin D M (2011). Reproductive dysfunction and decreased GnRH neurogenesis in a mouse model of CHARGE syndrome. Hum Mol Genet, 20(16): 3138–3150

Layman W S, McEwen D P, Beyer L A, Lalani S R, Fernbach S D, Oh E, Swaroop A, Hegg C C, Raphael Y, Martens J R, Martin D M (2009). Defects in neural stem cell proliferation and olfaction in Chd7 deficient mice indicate a mechanism for hyposmia in human CHARGE syndrome. Hum Mol Genet, 18(11): 1909–1923

Melicharek D, Shah A, DiStefano G, Gangemi A J, Orapallo A, Vrailas-Mortimer A D, Marenda D R (2008). Identification of novel regulators of atonal expression in the developing *Drosophila* retina. Genetics, 180(4): 2095–2110

Melicharek D J, Ramirez L C, Singh S, Thompson R, Marenda D R (2010). Kismet/CHD7 regulates axon morphology, memory and locomotion in a Drosophila model of CHARGE syndrome. Hum Mol Genet, 19(21): 4253–4264

Micucci J A, Layman W S, Hurd E A, Sperry E D, Frank S F, Durham

M A, Swiderski D L, Skidmore J M, Scacheri P C, Raphael Y, Martin D M (2014). CHD7 and retinoic acid signaling cooperate to regulate neural stem cell and inner ear development in mouse models of CHARGE syndrome. Hum Mol Genet, 23(2): 434–448

Mueller-Planitz F, Klinker H, Ludwigsen J, Becker P B (2013). The ATPase domain of ISWI is an autonomous nucleosome remodeling machine. Nat Struct Mol Biol, 20(1): 82–89

Papp B, Muller J (2006). Histone trimethylation and the maintenance of transcriptional ON and OFF states by trxG and PcG proteins. Genes Dev, 20(15): 2041–2054

Paro R, Strutt H, Cavalli G (1998). Heritable chromatin states induced by the Polycomb and trithorax group genes. Novartis Found Symp, 214: 51–61; discussion 61–56, 104–113

Patten S A, Jacobs-McDaniels N L, Zaouter C, Drapeau P, Albertson R C, Moldovan F (2012). Role of Chd7 in zebrafish: a model for CHARGE syndrome. PLoS ONE, 7(2): e31650

Petruk S, Sedkov Y, Johnston D M, Hodgson J W, Black K L, Kovermann S K, Beck S, Canaani E, Brock H W, Mazo A (2012). TrxG and PcG proteins but not methylated histones remain associated with DNA through replication. Cell, 150(5): 922–933

Pinto G, Abadie V, Mesnage R, Blustajn J, Cabrol S, Amiel J, Hertz-Pannier L, Bertrand A M, Lyonnet S, Rappaport R, Netchine I (2005). CHARGE syndrome includes hypogonadotropic hypogonadism and abnormal olfactory bulb development. J Clin Endocrinol Metab, 90(10): 5621–5626

Reisman D, Glaros S, Thompson E A (2009). The SWI/SNF complex and cancer. Oncogene, 28(14): 1653–1668

Richmond T J, Davey C A (2003). The structure of DNA in the nucleosome core. Nature, 423(6936): 145–150

Sanlaville D, Verloes A (2007). CHARGE syndrome: an update. Eur J Hum Genet, 15(4): 389–399

Santoro R, Li J, Grummt I (2002). The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. Nat Genet, 32(3): 393–396

Schnetz M P, Bartels C F, Shastri K, Balasubramanian D, Zentner G E, Balaji R, Zhang X, Song L, Wang Z, Laframboise T, Crawford G E, Scacheri P C (2009). Genomic distribution of CHD7 on chromatin tracks H3K4 methylation patterns. Genome Res, 19(4): 590–601

Schnetz M P, Handoko L, Akhtar-Zaidi B, Bartels C F, Pereira C F, Fisher A G, Adams D J, Flicek P, Crawford G E, Laframboise T, Tesar P, Wei C L, Scacheri P C (2010). CHD7 targets active gene enhancer elements to modulate ES cell-specific gene expression. PLoS Genet, 6(7): e1001023

Souriau J, Gimenes M, Blouin C, Benbrik I, Benbrik E, Churakowskyi A, Churakowskyi B (2005). CHARGE syndrome: developmental and behavioral data. Am J Med Genet A, 133A(3): 278–281

Srinivasan S, Armstrong J A, Deuring R, Dahlsveen I K, McNeill H, Tamkun J W (2005). The Drosophila trithorax group protein Kismet facilitates an early step in transcriptional elongation by RNA Polymerase II. Development, 132(7): 1623–1635

Srinivasan S, Dorighi K M, Tamkun J W (2008). Drosophila Kismet regulates histone H3 lysine 27 methylation and early elongation by RNA polymerase II. PLoS Genet, 4(10): e1000217

Tellier A L, Cormier-Daire V, Abadie V, Amiel J, Sigaudy S, Bonnet D, de Lonlay-Debeney P, Morrisseau-Durand M P, Hubert P, Michel J L, Jan D, Dollfus H, Baumann C, Labrune P, Lacombe D, Philip N, LeMerrer M, Briard M L, Munnich A, Lyonnet S (1998). CHARGE syndrome: report of 47 cases and review. Am J Med Genet, 76(5): 402–409

Terriente-Felix A, Molnar C, Gomez-Skarmeta J L, de Celis J F (2011). A conserved function of the chromatin ATPase Kismet in the regulation of hedgehog expression. Dev Biol, 350(2): 382–392

Therrien M, Morrison D K, Wong A M, Rubin G M (2000). A genetic screen for modifiers of a kinase suppressor of Ras-dependent rough eye phenotype in *Drosophila.* Genetics, 156(3): 1231–1242

Torres-Padilla M E, Parfitt D E, Kouzarides T, Zernicka-Goetz M (2007). Histone arginine methylation regulates pluripotency in the early mouse embryo. Nature, 445(7124): 214–218

Vissers L E, van Ravenswaaij C M, Admiraal R, Hurst J A, de Vries B B, Janssen I M, van der Vliet W A, Huys E H, de Jong P J, Hamel B C, Schoenmakers E F, Brunner H G, Veltman J A, van Kessel A G (2004). Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. Nat Genet, 36(9): 955–957

Workman J L (2006). Nucleosome displacement in transcription. Genes Dev, 20(15): 2009–2017

Zentner G E, Hurd E A, Schnetz M P, Handoko L, Wang C, Wang Z, Wei C, Tesar P J, Hatzoglou M, Martin D M, Scacheri P C (2010a). CHD7 functions in the nucleolus as a positive regulator of ribosomal RNA biogenesis. Hum Mol Genet, 19(18): 3491–3501

Zentner G E, Layman W S, Martin D M, Scacheri P C (2010b). Molecular and phenotypic aspects of CHD7 mutation in CHARGE syndrome. Am J Med Genet A, 152A(3): 674–686