Mouse Models for the Dissection of CHD7 Functions in Eye Development and the Molecular Basis for Ocular Defects in CHARGE Syndrome

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PURPOSE. CHARGE syndrome (Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital and/or urinary tract abnormalities, and Ear abnormalities and deafness) is the second-leading cause of deaf-blindness after Usher syndrome. Heterozygous mutations in CHD7 cause CHARGE syndrome in 70% to 90% of patients. We tested the hypothesis that tissue-specific mutant mice provide models for molecularly dissecting CHD7 functions during eye development.

METHODS. The conditional *Chd7^{fllox}* allele was mated together with tissue-specific Cre transgenes. Immunohistochemistry was used to determine the normal CHD7 pattern in the early eye primordia and to assess *Chd7* mutants for expression of region-specific protein markers.

RESULTS. CHD7 is present in the neural ectoderm and surface ectoderm of the eye. Deletion from neural and surface ectoderm results in severely dysmorphic eyes generally lacking recognizable optic cup structures and small lenses. Deletion from the neural ectoderm results in similar defects. Deletion from the surface ectoderm results in eyes with smaller lenses. Lens tissue and the major subdivisions of the neural ectoderm are present following conditional deletion of *Chd7* from the neural ectoderm. Closure of the optic fissure depends on the *Chd7* gene dose within the neural ectoderm.

CONCLUSIONS. Eye development requires CHD7 in multiple embryonic tissues. Lens development requires CHD7 in the surface ectoderm, whereas optic cup and stalk morphogenesis require CHD7 in the neural ectoderm. CHD7 is not absolutely required for specification of the major subdivisions within the neural ectoderm. As in humans, normal eye development in mice is sensitive to *Chd7* haploinsufficiency. These data indicate the *Chd7* mutant mice are models for determining the molecular etiology of ocular defects in CHARGE syndrome.

Keywords: coloboma, epigenetics, CHD7

The mature eye is a complex organ that forms from a synthesis of multiple embryonic stem cell lineages, including neural ectoderm of the early central nervous system and neighboring surface ectoderm. During the early period of eye development, the neural ectoderm evaginates outward from either side of the developing diencephalon to form the optic vesicles.¹ Evagination of the optic vesicles continues until they contact the head surface ectoderm, where they induce formation of a lens placode. Subsequently, coordinated invagination of the lens placode and optic vesicle results in formation of the lens vesicle and the double layered optic cup, the outer layer of which is fated to become the pigmented epithelium of the eye while the inner layer will form the retina proper. During this phase, essential signaling between the two layers activates expression of tissue-specific transcription factors. Gross genetic or environmental insults acting during these early events result in microphthalmia or anophthalmia. Invagination of the optic vesicle also results in formation of a transient gap, termed the embryonic or choroidal fissure, along

the ventral side of the optic cup. Normally, this embryonic fissure subsequently closes; failure to close results in coloboma or persistent gap in the retina and/or iris. The cellular and molecular mechanisms regulating closure of the optic fissure are poorly understood.

CHARGE syndrome (Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital and/or urinary tract abnormalities, and Ear abnormalities and deafness) results in eye defects including coloboma with or without microphthalmia.^{2,3} Vision loss due to ocular phenotypes can also be a major contributor to neurocognitive deficits in affected individuals. CHARGE syndrome affects approximately 1:10,000 live births worldwide, making it the second leading genetic cause of deaf-blindness after Usher syndrome.^{4,5} At present, care options are limited to trying to maintain existing visual function; unfortunately, no approaches for correcting vision loss apart from glasses are currently available.

Heterozygous mutations in *CHD7* are the causative event in as many as 85% of CHARGE patients who have been molecularly evaluated.⁶ *CHD7* encodes a chromatin-remodeling protein that binds to thousands of enhancers and transcription start sites throughout the mammalian genome.^{7,8} The CHD7 protein recognizes no consensus DNA binding site itself; instead, it is thought to form cell type-specific complexes with other chromatin remodeling proteins, histone methyltransferases, and transcription factors to regulate expression of critical downstream target genes.^{3,9,10} Identification of the essential genes regulated by CHD7 in each affected organ will provide important insights into the pathways that are disrupted in CHARGE syndrome and may suggest potential therapeutic modalities.

The lack of suitable animal models has been a significant impediment to making advances in our understanding of the normal molecular functions in eye development and how reductions in CHD7 levels leads to coloboma and associated ocular defects in CHARGE syndrome. Heterozygous *Chd7*[±] mice model some aspects of CHARGE syndrome, such as keratoconjunctivitis sicca or dry eye.¹¹ However, it has not been reported whether coloboma and other ocular features of CHARGE are present in heterozygous mice, at least in the relatively small cohort that has been tested thus far.¹¹ Additionally, global null mutants are of limited use in understanding CHD7 function in eye development because they are embryonic lethal by e11.5.^{11,12} For these reasons, as well as the expression of CHD7 in multiple interacting tissue layers during the critical stages of early eye development (see below), analysis of tissue-specific conditional mutants will be critical for identifying both normal sites of CHD7 function during eye development and the underlying molecular etiology of ocular defects in CHARGE patients. In this study, we report the expression pattern of CHD7 during the early period of eye morphogenesis when the embryonic fissure normally closes and use conditional knockout approaches to assess the tissue specific requirements for *Chd7* during early eye development. In the process, we identify important new mouse models that will be highly useful for the analysis of CHD7 functions in normal eye development and the molecular etiology of ocular defects in CHARGE syndrome.

MATERIALS AND METHODS

Mouse Strains and Husbandry

All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals and the Statement for Use of Animals in Ophthalmic and Vision Research. All procedures using mice were preapproved by the Committee on the Use and Care of Animals at the University of Michigan, an Association for Assessment and Accreditation of Laboratory Animal Care accredited institution. Generation of the null *Chd7*^{Gt} allele, the conditional *Chd7*^{fllox} allele, and the *Foxg1-Cre*, *Le-Cre*, and *Rx-Cre* mouse strains has been described previously.^{11–16} Mice were mated to generate timed pregnancies. The relevant crosses are *Foxg1-Cre;Chd7*^{+/fllox} X *Chd7*^{fllox/fllox}, *Le-Cre;Chd7*^{+/fllox} X *Chd7*^{fllox/fllox}, and *Rx-Cre;Chd7*^{+/fllox} X *Chd7*^{fllox/fllox}. The resulting embryos were genotyped for *Cre*, *Chd7*⁺, or *Chd7*^{fllox} using PCR-based methods¹⁵ and processed for histology as previously described.¹⁷

Embryo Processing and Histology

All embryos were fixed in 4% paraformaldehyde diluted in PBS, washed in PBS, dehydrated through graded alcohols, and

processed into Paraplast Plus (McCormick Scientific, St. Louis, MO, USA) for paraffin sectioning. Mounted paraffin sections for morphologic analysis were dewaxed, rehydrated, and stained with hematoxylin and eosin (H&E).

Immunohistochemistry

Paraffin sections were immunostained as previously described.¹⁷ Primary antibodies against CHD7 (Abcam, Cambridge, MA, USA), β -crystallin (gift from Samuel Zigler, New York, NY, USA), PAX2 (Covance, Princeton, NJ, USA), PAX6 (Covance), VSX2 (Exalpha, Shirley, MA, USA), and neuronal β -tubulin (Covance) were used.

RESULTS

CHD7 Expression During Early Mammalian Eye Morphogenesis

We began our assessment of CHD7 function in mammalian eye development by using immunohistochemistry to determine the normal *Chd7* expression pattern between embryonic days 9.5 and 12.5 (e9.5–12.5), which corresponds with the critical period during which the embryonic fissure forms and then closes in mice. CHD7 protein is already present in the surface ectoderm and neural ectoderm primordia of the eye by e9.5 (Fig. 1A). CHD7 persists in the surface ectoderm through the periods of lens placode induction, formation of the lens vesicle, and early lens fiber development at e12.5 (Figs. 1B–D). In the neural ectoderm, CHD7 protein is present in the optic vesicle by e9.5 (Fig. 1A). Expression continues in the optic cup and optic stalk, as well as subsequently in the outer layer of the optic cup that gives rise to the pigmented epithelium and the inner layer of the optic cup that represents the primordium to the retina (Figs. 1B–D). CHD7 was not observed in the periocular mesenchyme during these stages. Collectively, the results demonstrate that CHD7 is widely expressed in the surface and neural ectoderm layers during early mammalian eye morphogenesis.

Conditional Deletion of *Chd7* From Both Surface and Neural Ectoderm Severely Disrupts Early Eye Morphogenesis

Global deletion of *Chd7* leads to lethality by e11.5, precluding the ability to assess gene function during eye development.¹² Therefore, we began our analysis of *Chd7* function in eye development by analyzing mice that were homozygous for the conditional *Chd7*^{fllox} allele and also carried the *Foxg1-Cre* transgene.^{12,14} In these mice, *Chd7* is deleted from both the surface and neural ectoderm contributing to the eye primordia¹⁵ (data not shown). As expected, the optic vesicle in control mice is present and approaching contact with the surface ectoderm by e9.5 (Fig. 1E). By e10.5, the optic vesicle in control eyes is transformed into the optic cup and stalk, whereas the surface ectoderm has formed the lens pit (Fig. 1F). Subsequently, the inner layer of the optic cup expands, giving rise to the early retina, and the lens vesicle detaches from the overlying surface ectoderm and primary lens fibers emerge to fill the inner cavity by e12.5 (Figs. 1G, 1H).

In *Foxg1-Cre;Chd7*^{fllox/fllox} mutant littermates lacking gene function in both neural and surface ectoderm, the optic vesicle is present and appears histologically normal at e9.5 (Fig. 1I). However, strikingly, subsequent eye morphogenesis is severely blocked, as no progression toward formation of optic cup or lens structures is apparent (Figs. 1J–L). This is true even as late as e12.5, indicating that the phenotype does not simply result from a developmental delay. Collectively, these data indicate

FIGURE 1. CHD7 expression in surface and/or neural ectoderm is essential for early eye morphogenesis. CHD7 protein expression at the indicated stages was detected by immunohistochemistry (A–D). The *Foxg1-Cre* transgene was used to ablate *Chd7* in both the surface and neural ectoderm, and eye morphogenesis in control (E–H) and conditional knockout embryos (CKO) (I–L) at the indicated stages was assessed following staining by H&E. CHD7 is expressed in both surface and neural ectoderm at all stages examined. Conditional ablation of *Chd7* in both surface and neural ectoderm severely disrupts early eye morphogenesis, in extreme cases resulting in the total absence of any optic cup or lens structures. All images are taken from coronal sections and are oriented with dorsal at the top. ov, optic vesicle; se, surface ectoderm; oc, optic cup; lv, lens vesicle; r, retina; l, lens.

that early eye morphogenesis, including formation of the optic cup and lens vesicle, requires *Chd7* function in the surface ectoderm, the neural ectoderm, or both.

Major Requirement for *Chd7* During Early Eye Morphogenesis Is in the Neural Ectoderm

We next used further, more refined tissue-specific knockout strategies to individually assess the potential requirements for *Chd7* function in the surface versus neural ectoderm. First, we analyzed surface ectoderm-specific knockout embryos (*Chd7^{seko/seko}*) generated by mating the conditional *Chd7^{fllox}* allele together with the *Le-Cre* transgene, which is specifically expressed in the ocular surface ectoderm beginning prior to lens induction.¹³ In contrast to mutants lacking gene function in both the surface and neural ectoderm, *Chd7^{seko/seko}* mutants have well-formed eye structures at e12.5 that appear similar to control littermates (Figs. 2A–C). The optic cup and stalk are present and, within the optic cup, the emerging retinal and pigmented epithelial layers appear normal (Figs. 2B, 2C). Lenses in *Chd7^{seko/seko}* mutants at e12.5 appear largely normal, albeit potentially reduced in size, with each separated from the overlying surface ectoderm and filled with primary lens fibers.

We next analyzed neural ectoderm-specific knockout embryos (*Chd7^{neko/neko}*) generated by mating the conditional *Chd7^{fllox}* allele together with the *Rx-Cre* transgene, which is specifically expressed in the eye field and other neural ectodermal tissues prior to emergence of the optic vesicle.¹⁶ Cre-mediated excision from the neural ectoderm of the eye primordia in *Chd7^{neko/neko}* mice is highly efficient (Supplementary Fig. S1C). In addition, reduction in CHD7 staining intensity is generally apparent in the neural ectoderm eye primordia of *Chd7^{+/neko}* littermates as well (Supplementary Fig. S1B). Eye morphogenesis in *Chd7^{neko/neko}* mutant embryos at e12.5 is severely disrupted, resulting in phenotypes ranging from highly dysmorphic optic cups in more moderately affected eyes (Fig. 2E) to the complete absence of discernable optic cup structures in more severely affected eyes (Fig. 2F). Pigmented epithelial cells are generally identifiable and, in eyes where some optic structure is present, the inner layer is thickened and resembles the emerging retina in eyes of control littermates. Lens structures are either present but very small or not apparent (Figs. 2E, 2F). Thus, eyes of *Chd7^{neko/neko}* mutants generally phenocopy those of the *Foxg1-Cre;Chd7^{fllox/fllox}* embryos reported above at a comparable stage. Collectively, these results are consistent with the idea that the major requirement for *Chd7* during early eye morphogenesis occurs in the neural ectoderm.

FIGURE 2. Tissue-specific ablation of *Chd7* from surface versus neural ectoderm differentially affects early eye morphogenesis. The *Le-Cre* and *Rx-Cre* transgenes were used to ablate *Chd7* specifically from the surface ectoderm (seko) (A–C) and neural ectoderm (neko) (D–F), respectively. Representative sections from e12.5 control and knockout embryos were imaged following staining by H&E. Tissue-specific ablation of *Chd7* from the surface ectoderm resulted in moderate (B) to significant (C) reduction in lens size but normal-appearing development of the neural ectoderm, including the optic cup. Tissue-specific ablation of *Chd7* from the neural ectoderm resulted in significant (E) to severe (F) dysmorphogenesis of the optic cup and significant alterations in lens development. All images are taken from coronal sections and are oriented with dorsal at the top.

Closure of the Embryonic Fissure Is Sensitive to *Chd7* Gene Dose in the Neural Ectoderm

Heterozygous *Chd7* mice have been reported to model some aspects of CHARGE syndrome, but to date, no systematic assessment of these mice for coloboma has been reported, despite that fact that this phenotype is a cardinal ocular feature in most patients. We therefore assessed our mutant *Chd7* mice for evidence of coloboma. We found that global

Chd7^{+/^{Gt} mice exhibit coloboma with full penetrance, although the extent of involvement within in the retina and iris appeared to vary significantly (Figs. 3B, 3B'). We were unable to examine homozygous global knockout animals because they die by e11.5, prior to closure of the embryonic fissure.^{11,18} We then examined tissue-specific knockout eyes to determine where CHD7 is required in order for the embryonic fissure to close properly during development. Neither *Chd7*^{+/^{seko} nor *Chd7*^{seko/^{seko} mice exhibit coloboma,}}}

FIGURE 3. Heterozygous and homozygous neural ectoderm-specific *Chd7* knockout embryos exhibit coloboma. The state of embryonic fissure closure in e12.5 control and neural ectoderm specific *Chd7* knockout embryos was analyzed in whole mount (A–C) and in corresponding sagittal sections (A'–C'). Coloboma is uniformly present in both heterozygous and homozygous *Chd7* neural ectoderm-specific mutants, and the defect is significantly more severe in homozygous embryos. Embryos and sections are oriented with anterior at the top.

FIGURE 4. CHD7 function in neural ectoderm is not required to specify lens tissue in the surface ectoderm. Immunohistochemistry against β -crystallin was used to assay for the presence of lens tissue in e12.5 control (A) and *Chd7^{neko/neko}* embryos. Lens tissue was present in all *Chd7^{neko/neko}* eyes examined. In most cases, small but distinct lenses are present (B) but, in the most severely affected eyes, lens tissue is limited to small patches of β -crystallin positive cells in the surface ectoderm (C, C'). All images are taken from coronal sections and are oriented with dorsal at the top.

indicating that closure of the fissure does not require intact CHD7 function in the surface ectoderm (data not shown). In contrast, both *Chd7^{+/-neko}* and *Chd7^{neko/neko}* mice show clear, full-depth colobomas at e12.5 (Fig. 3). This phenotype is completely penetrant, and the severity of the coloboma is consistently greater in the conditional homozygotes than the conditional heterozygotes. To determine whether the observed phenotype at e12.5 is likely to represent a persistent coloboma or simply a delay in closure of the embryonic fissure, we examined *Chd7^{+/-Gt}* embryos at e15.5 and found that coloboma was present in all eyes (six of six). Collectively, we conclude that closure of the embryonic fissure is sensitive to CHD7 levels in the neural ectoderm and that both the global and neural ectoderm specific mutants will be important models in the future for discovering the molecular etiology of coloboma in CHARGE syndrome.

Chd7 Function in the Neural Ectoderm Is Not Required to Specify Lens in the Overlying Surface Ectoderm

Lens and optic cup formation are the result of coordinated morphogenetic movements that require reciprocal inductive signaling events between the neural ectoderm of the optic vesicle and the adjacent surface ectoderm.¹⁹ Disruption of signaling in either direction between the two layers can result in a block of lens and optic cup morphogenesis. For example, loss of BMP4 expression in the optic vesicle results in the absence of lens induction and optic cup formation, a phenotype that resembles key features of the *Chd7^{neko/neko}* mutant eyes.^{20,21} Therefore, we molecularly assessed whether lens induction is intact in *Chd7^{neko/neko}* eyes by examining expression of β -crystallin, a marker of committed lens fibers (Fig. 4A). We found that a small β -crystallin-expressing lens that is distinct from the overlying surface ectoderm is present in the eyes of all but the most severely affected *Chd7^{neko/neko}* eyes (Fig. 4B). Beta-crystallin-positive lens fibers are identifiable within the surface ectoderm even in the most severely affected eyes that completely lack evidence of a distinct lens

structure and an optic cup (Figs. 4C, 4C'). From these data, we infer that an absence of lens induction and a resulting disruption in signaling from the surface ectoderm to the optic vesicle are unlikely to be a major cause of the defects to optic cup morphogenesis in *Chd7^{neko/neko}* eyes.

Cell-Autonomous Function of CHD7 Is Not Required for Early Regionalization Within the Ocular Neural Ectoderm

The emergence of the distal optic cup and the proximal optic stalk from the neural ectoderm optic vesicle is followed by specification of the retinal precursor and pigmented epithelium layers within the optic cup, each of which are accompanied by the activation of region-specific gene expression patterns. The extreme morphologic changes observed in mutants made it difficult to readily identify analogous structures in *Chd7^{neko/neko}* mutant eyes based solely on histology. We therefore used analysis of specific markers to assess whether each subregion is specified within the neural ectoderm.

Although initially present more broadly within the neural ectoderm, expression of the paired homeodomain protein PAX2 becomes limited to the more proximal tissue that gives rise to the optic stalk and future optic nerve head within the optic cup by e12.5 (Fig. 5A). The highly related paired homeodomain protein, PAX6, has the reciprocal expression pattern within the neural ectoderm and is present more distally, throughout both layers of the optic cup but is excluded from the optic stalk (Fig. 5D).¹⁹ PAX6 is also expressed within the lens and ocular surface ectoderm (Fig. 5D). Both PAX2 (Figs. 5B, 5C) and PAX6 (Figs. 5E, 5F) are expressed in approximately the expected locations within the neural ectoderm of *Chd7^{neko/neko}* mutant eyes at e12.5, indicating that the proximal and distal regions of the neural ectoderm of the eye primordia are specified in *Chd7^{neko/neko}* mutant eyes despite their high degree of overall disorganization. This is true even in the most severely affected eyes (Fig. 5F). Collectively, these data suggest that cell-autonomous function of CHD7 is not required for initial distal-proximal patterning of the neural ectoderm.

As the optic cup forms, cells of the inner layer become specified to a retinal fate. To determine whether committed retinal cells are present in *Chd7^{neko/neko}* mutant eyes, we assessed expression of the homeodomain transcription factor VSX2 (CHX10), the earliest known molecular marker of presumptive retinal tissue (Fig. 5G).²² VSX2 is expressed in even the most severely affected mutants, indicating that cell-autonomous functions of CHD7 are not required for specification of neural ectoderm to a retinal fate (Figs. 5H, 5I). Cell signaling from the surrounding periocular mesenchyme specifies cells of the outer layer of the optic cup to a pigmented epithelial rather than a retinal fate.²³ The presence of a pigmented epithelial layer is readily identifiable in *Chd7^{neko/neko}* eyes at e12.5 (Figs. 3D, 3D'). Expression of the transcription factor MITF in even the most severely affected *Chd7^{neko/neko}* eyes confirms the presence of committed pigmented epithelia (Figs. 5K, 5L). We conclude that cell-autonomous function of CHD7 is not a requirement for specification of retinal or pigmented epithelial cell fates within the optic cup during early eye development.

Finally, we assessed whether cells of the *Chd7^{neko/neko}* mutant optic cup were capable of generating neurons by immunostaining for expression of pan-neuronal β -tubulin (TUJ1). Positively staining neurons populate the central retina of control eyes by e12.5 (Fig. 5J). These represent retinal ganglion cells, the first neurons to be born in the developing

FIGURE 5. Cell autonomous function of CHD7 is not required to specify major subdivisions within the neural ectoderm during early eye development. Immunohistochemistry was used on sections from e12.5 control and *Chd7^{neko/neko}* embryos to test for specification of appropriate regions of neural ectoderm as optic stalk (PAX2), optic cup (PAX6), retina (VSX2), pigmented epithelium (MITF), and neurons (TUJ1). All four markers are expressed in mutant eyes. All images are taken from coronal sections and are oriented with dorsal at the top.

retina. Although the extent of staining varies greatly, all mutant eyes examined contained neurons, as indicated by the presence of TUJ1⁺ cells (Figs. 5K, 5L). This includes even the most severely malformed examples (Fig. 5L). We conclude that CHD7 function is not required for specification of neurons from neural ectoderm of the eye primordia.

DISCUSSION

Coloboma is the major ophthalmic feature leading to vision loss in CHARGE syndrome and usually involves both the retina and choroid. Unlike in the ear, where significant progress in

understanding CHD7 function has been made due to the availability of effective models of CHARGE-related defects, the ability to make advances into understanding the mechanisms by which CHD7 functions in eye development has been limited because animal models expressing the coloboma phenotype have not been identified.^{11,24} Our current work is significant because we now report fully penetrant coloboma in two *Chd7* mouse models and show using a neural ectoderm specific knockout approach that closure of the embryonic fissure is highly sensitive to *Chd7* gene dosage. Further, we establish that CHD7 is cell-autonomously required in the neural ectoderm primordia to the eye for normal eye morphogenesis, including

formation of the optic cup and closure of the optic fissure. CHD7 function in the surface ectoderm is essential for normal lens size but does not appear to have a non-cell-autonomous effect on development of ocular structures derived from the neural ectoderm.

Our demonstration of ocular coloboma in two distinct models represents a significant departure from previously reported *Cbd7* mouse models, which lacked evidence of coloboma.^{11,24} These differences likely result from the effects of genetic background, similar to the strain-dependent effects on expressivity and severity of other CHARGE features in mice that have been reported.^{12,24} We are maintaining *Cbd7*^{Gt/+} mice on a C57BL/6J background, which is significantly different from those previously reported to that do not support coloboma.^{12,24} In the future, it will be important to rigorously establish the effects of genetic background on expressivity and severity of the ocular phenotypes because the subsequent mapping and identification of modifiers would provide a significant opportunity to identify critical genes that function together with *Cbd7* during early eye morphogenesis and optic fissure closure.

Our ability to generate conditional tissue-specific *Cbd7* mutants also allowed us to gain important insights into the relative contributions of CHD7 function in neural versus surface ectoderm during early eye morphogenesis. The phenotypes of the *Rx-Cre* mutants indicate a major requirement for CHD7 function in the neural ectoderm, where loss of *Cbd7* resulted in phenotypes ranging from highly dysmorphic optic cups in more moderately affected eyes to the complete absence of discernable optic cup structures in more severely affected eyes. The *Rx-Cre* mutants also demonstrated that closure of the embryonic fissure is highly sensitive to *Cbd7* gene dose in the neural ectoderm. In contrast, the phenotype of the *Le-Cre* knockout mice indicates that CHD7 activity in the surface ectoderm is dispensable for morphogenesis of the optic cup from the neural ectoderm and closure of the embryonic fissure, as well as many events during lens development, including induction of the lens placode in response to signaling from the adjacent neural ectoderm, formation of the optic vesicle, and differentiation of lens fibers. Rather, the role of CHD7 function may be limited to regulation of lens size.

Our current work provides important insights into the tissue specific requirements for *Cbd7* during early eye development but the specific downstream genes and genetic pathways that are regulated by CHD7 protein during eye development remain unknown. Elsewhere in the central nervous system, in the ear, and in the heart, modulation or inhibition of BMP signaling is an important mechanism by which CHD7 controls developmental processes.^{18,25,26} These observations may provide important clues into potential CHD7-regulated pathways during eye development as alterations in BMP signaling result in both disruption to early eye morphogenesis and coloboma.^{20,27} Therefore, although it is beyond the scope of the current work, the new models that we have identified should be assessed for potential impacts on BMP signaling.

We focused on the requirements for CHD7 during early eye morphogenesis. However, *Cbd7* expression in the developing eye continues as the different neuronal lineages of the mature retina are specified and differentiate (data not shown). Recent results have highlighted a critical role for CHD7 during neuronal differentiation in the inner ear,¹⁵ in the nasal placode,²⁸ in cerebellar development,²⁹ and in neural stem cells in the subventricular zone^{30,31} and hippocampus.³² Collectively, these observations raise the possibility that continued CHD7 function may also be critical for retinal neurogenesis. The combination of our conditional *Cbd7* allele with available retinal neuron specific Cre transgenes will be

critical for assessing the potential continuing requirement(s) for CHD7 during retinal development.

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