Published in final edited form as:
Gene Expr Patterns. 2013 December ; 13(8): . doi:10.1016/j.gep.2013.05.006.

Expression of *CXCL12* and *CXCL14* during eye development in Chick and Mouse

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Abstract

Vertebrate eye development is a complex multistep process coordinated by signals from the lens, optic cup and periocular mesenchyme. Although chemokines are increasingly being recognized as key players in cell migration, proliferation, and differentiation during embryonic development, their potential role during eye development has not been examined. In this study, we demonstrate by section *in situ* hybridization that *CXCL12* and *CXCL14* are expressed during ocular development. *CXCL12* is expressed in the periocular mesenchyme, ocular blood vessels, retina, and eyelid mesenchyme, and its expression pattern is conserved between chick and mouse in most tissues. Expression of *CXCL14* is localized in the ocular ectoderm, limbal epithelium, scleral papillae, eyelid mesenchyme, corneal keratocytes, hair follicles, and retina, and it was only conserved in the upper eyelid ectoderm of chick and mouse. The unique and non-overlapping patterns of *CXCL12* and *CXCL14* expression in ocular tissues suggest that these two chemokines may interact and have important functions in cell proliferation, differentiation and migration during eye development.

Keywords

Cornea; limbal epithelium; eyelid; nictitating membrane; scleral ossicles; retina

1. INTRODUCTION

Chemokines are a large family of small-secreted chemoattractant cytokines that function in many physiological and pathological processes. Although initially identified as inducers of leukocyte migration during inflammatory response (Wong and Fish, 2003; Kiefer and Siekmann, 2011), recent studies have shown that chemokines are involved in tumor development, neurodegenerative diseases, angiogenesis, and embryogenesis (Rostene et al., 2007; Olesnicky et. al., 2009; Banisadr et al., 2011; Kiefer and Siekmann, 2011). Despite increasing evidence of the involvement of CXCL12 (stromal cell-derived fator-1, SDF-1) and CXCL14 (BRAK, Scyba, or MIP-2γ) in non-immune processes such as cell migration, proliferation, and differentiation, very little is known about their involvement in eye development.

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CXCL12 signaling mediated by the receptors CXCR4 and/or CXCR7 plays an important role during hematopoiesis, angiogenesis, and in the migration of germ cells, neural crest cells, neural precursors, and limb progenitor cells (Lazarini et al., 2003; Stebler et al., 2004; Yusuf et al, 2006; Theveneau et al., 2010; Belmadani et al., 2009). During embryonic development, *CXCL12* is expressed in the mesenchyme of the head, limbs, sclerotome, and in regions surrounding major blood vessels, gonads, and mesonephros (Vasyutina et al., 2005; Rehimi et al., 2008; Garcia-Andres and Torres, 2010). Mutations of CXCL12 or CXCR4 in mice are lethal between embryonic day (E)15 and birth, and the embryos have defective neurogenesis, cardiovascular development, and impaired hematopoiesis (Nagasawa et al., 1996; Ma et al., 1998; Zou et al., 1998). Similar defects are observed in CXCR7 deficient mice, although hematopoiesis was normal (Sierro et al., 2007).

Until recent, very little was known about the involvement of CXCL14 in embryonic development and the receptor(s) that mediate its function remain unknown. In adults, *CXCL14* is constitutively expressed in normal epithelial tissues such as the breast, kidney, brain, and lungs (Hromas et al., 1999; Meuter and Moser, 2008). During early embryogenesis in chick and mouse, *CXCL14* is expressed in the ectoderm, central nervous system, paraxial mesoderm, limbs, and in the trigeminal and dorsal root ganglia (Park et al., 2012; Gordon et al., 2011; Garcia-Andres and Torres, 2010). Other patterns of *CXCL14* expression are observed in Xenopus and zebrafish embryos during development of the cement gland, otic vesicle, dorsal retina, and in migratory cranial neural crest cells and neuromasts of the lateral line (Park et al., 2009; Long et al., 2000). Although CXCL14 is a chemoattractant for immune cells (Kurth et al., 2001; Shellenberger et al., 2004; Tanegashima et al., 2010), CXCL14 deficient mice do not show severe defects in the immune system (Meuter et al., 2007). However, these mice show altered feeding behavior and glucose metabolism that is associated with improved insulin sensitivity (Nara et al., 2007; Tanegashima et al., 2010). CXCL14 inhibits endothelial cell migration *in vitro* and prevents neovascularization of the cornea in micropocket assays involving various angiogenic factors (Shellenberger et al., 2004). Recent studies have reported that CXCL14 plays a role in differentiation of Langerhans cells in the skin and Schwann cells (Schaerly et al., 2005; Barbaria et al., 2009; Hara and Tanegashima, 2012). Also CXCL14 is upregulated through calcium–calmodulin signaling in a cell density-dependent manner, accompanied by the upregulation of several keratinocyte differentiation markers of epithelial cells (Ikoma et al., 2012).

Vertebrate eye development is a multistep process that involves interactions between cells and tissues from different embryonic origins that result in the formation of a functional organ. At the rudimentary stage, the eye is comprised of the lens vesicle and overlaying ectoderm, the optic cup, and the periocular mesenchyme comprised of neural crest cells and cranial mesoderm. The following events occur during subsequent development of the eye: (1) The lens vesicle forms the lens epithelium and crystalline cells (Cvekl and Mitton, 2010; Ogino et al., 2012); (2) The periocular mesenchyme combines with the overlying ectoderm to form the cornea and the eyelids (Johnston et al., 1979; Hay, 1980; Creuzet et al., 2005; Lwigale et al., 2005); (3) Angioblasts in the periocular region undergo vasculogenesis to form the complex network of limbal blood vessels (Kwiatkowski et al, 2013); (4) The optic cup differentiates into the retinal pigment epithelium and the neuroretina (Venters et al., 2011; Fuhrmann, 2010); and (5) The trigeminal, ciliary, and oculomotor nerves project into the eye to provide sensory and sympathetic innervation (Narayanan and Narayanan, 1978). Signaling between the ocular tissues is essential for cell migration, proliferation, and differentiation that enable the proper formation of a functional eye. Although CXCL12 and CXCL14 have been associated with such cellular events, these chemokines have not been studied during eye development. To determine the potential roles of CXCL12 and CXCL14 during eye development, we studied their expression patterns during the formation of ocular

tissues. Due to differences between avian (Johnston et al., 1979) and murine (Pei and Rhodin, 1970) eye development, we compared the expression of *CXCL12* and *CXCL14* between chick and mouse embryos. Our results show that *CXCL12* and *CXCL14* are expressed in complementary patterns in most tissues during ocular development. Expression of *CXCL12* is conserved between chick and mouse in most ocular tissues, whereas *CXCL14* expression is only conserved in the upper eyelid ectoderm. The expression patterns of *CXCL12* and *CXCL14* indicate their potential interaction and involvement in ocular development.

2. RESULTS AND DISCUSSION

To analyze the expression of *CXCL12* and *CXCL14*, we performed *in situ* hybridization on sections through the eyes of chick and mice embryos at different stages of development. For chick we examined embryos between E3-E17. Since our expression data for *CXCL12* and *CXCL14* in E3 chick eyes confirms previously observed patterns at this stage (Garcia-Andres and Torres, 2010), we show data starting at E5. Because eye development in mice is a continuous process with cell proliferation and changes in gene expression persisting into late postnatal stages (Zieske, 2004), we analyzed embryos between E11, postnatal day (P)0, and in some cases, young adults.

2.1. Expression of *CXCL12* and *CXCL14* during development of the cornea and iris

During eye development, periocular neural crest cells migrate between the lens vesicle and the ectoderm to form the cornea. In chick this is a well-coordinated process involving an initial wave of neural crest cell migration from the periocular mesenchyme to the region between the lens and ectoderm (presumptive cornea epithelium) to form the inner most layer of the cornea (the cornea endothelium). This is followed by a second wave of neural crest cell migration between the endothelium and ectoderm to form the cornea stroma (Johnston et al., 1979; Hay, 1980; Creuzet et al., 2005; Lwigale et al., 2005). In mouse, neural crest cells migrate as a single mass between the lens vesicle and ectoderm, then the cells adjacent to the lens differentiate into the cornea endothelium while the rest form the stroma (Pei and Rhodin, 1970; Gage et. al., 2005). Shortly after cornea formation, the periocular neural crest cells and neuroepithelium at the tip of the optic cup coalesce to form the iris.

Expression of *CXCL12* in the chick eye is first detected in the periocular mesenchyme at E3 (data not shown and Garcia-Andres and Torres, 2010). By E5, expression of *CXCL12* persists in the periocular mesenchyme, and it is also expressed in the newly formed cornea endothelium (Fig. 1A, arrowhead), and blood vessels (Fig. 1A, A', asterisk). At E7 and E12, expression of *CXCL12* in the anterior eye is restricted to the periocular region in cells located adjacent to the retinal pigment epithelium, and it remains strong in the iris stroma and blood vessels (Fig. 1B and C). Similarly, in the mouse eye, *CXCL12* expression is initially robust in the periocular mesenchyme at E11.5 (Fig. 1G'), but it becomes restricted to the cells adjacent to the retinal pigment epithelium, iris stroma (Fig. 1G–I, arrows) and ocular blood vessels (Fig. 1H, asterisk). Since CXCL12 is involved in migration of neural crest cells (Olesnicky Killian et al., 2009; Kasemeier-Kulesa et al., 2010; Theveneau *et al.*, 2010) and other embryonic cell types (Doitsidou et al., 2002; Vasyutina et al., 2005; Chen et al., 2007; Li and Ransohoff, 2008), its expression by periocular neural crest cells suggests that it may play a similar role during cornea development. Expression of *CXCL12* in the forming ocular blood vessels is consistent with its proangiogenic role during vascular development (Yamaguchi et al., 2003; Hiasa et al., 2004). Since *CXCL12* is expressed in the limb during myogenesis (Garcia-Andres and Torres, 2010; Hunger et al., 2012), its presence in the iris stroma indicates a possible role during neural crest cell differentiation into iris muscles.

In contrast to *CXCL12* expression, *CXCL14* is not expressed in the anterior region of the chick eye by E5 (Fig. 1D). However, by E7 *CXCL14* expression is prominent in the cornea stroma (Fig. 1E and E') and coincides with neural crest differentiation into stromal keratocytes (Hay et al., 1979; Hay, 1980; Funderburgh et al., 1986). *CXCL14* is also expressed at low levels in the lens epithelium (Fig. 1E, arrowhead). During subsequent development, expression of *CXCL14* is restricted to the keratocytes in anterior region of the corneal stroma at E12 (Fig. 1F). By E15, *CXCL14* expression is absent in the stroma (data not shown), but it becomes expressed by cells on the surface and tip of the iris, where it persists through E17 (Fig. 1F'). Unlike most of the cranial and limb ectoderm where intense expression of *CXCL14* is observed (Garcia-Andres and Torres, 2010; Gordon et al., 2011), it is not expressed in the ectoderm of the presumptive cornea epithelium in chick (Fig. 1D–F).

In the mouse, *CXCL14* is initially expressed at low levels in the periocular mesenchyme, optic cup, and lens at E14.5 (Fig. 1J). Expression of *CXCL14* in the eyelid epithelium is strong at this time and during subsequent development (discussed in detail later). At E16.5 and P0, expression of *CXCL14* is restricted to the cornea epithelium, optic cup, neuroepithelium of the iris, and in the lens epithelium (Fig. 1K and 1L). Expression of *CXCL14* in the mouse cornea coincides with the differentiation of the cornea epithelium as indicated by the expression of the epithelial keratin markers, K12 and K14 (Kurpakus et al., 1994; Tanifuji-Terai et al., 2006). Although expression of *CXCL14* in the cornea is not conserved between chick and mouse, its presence in the chick corneal stroma and mouse corneal epithelium may indicate a possible role during keratocyte and epithelial cell differentiation, respectively.

2.2. Expression of *CXCL14* in the anterior ocular epithelium during development

At E7 and during subsequent development of the chick eye, expression of *CXCL12* is limited to the blood vessels and periocular mesenchyme adjacent to the retinal pigment epithelium (Fig. 1B and data not shown). The ectoderm spanning the anterior eye region of the chick does not express *CXCL14* during early stages of development (Fig. 1D–1F and data not shown). However, by E7 expression of *CXCL14* is vivid in the region of the ectoderm (Fig. 2A and 2A') corresponding to the location of the scleral papillae (Coulombre and Coulombre, 1962). Scleral papillae are thickenings in the conjunctival epithelium (Franz-Odendaal and Vickaryous, 2006), that induce the underlying neural crest mesenchyme to form skeletogenic condensations known as scleral ossicles (Coulombre and Coulombre, 1962; Pinto and Hall, 1991). Expression of *CXCL14* in the conjunctival epithelium coincides with the time when the mesenchyme becomes competent to induce the formation of scleral papillae in the overlying conjunctival epithelium (Wedlock et al., 1969; Hall, 1981; Duench and Franz-Odendaal, 2012). Section through a scleral papilla at more advanced stage of formation shows expression of *CXCL14* in the thickened ectoderm, but it is absent in the superficial cells above the thickest central region (Fig. 2B, asterisk). *CXCL14* is expressed at low levels as the scleral papillae increase in size to form a visible outgrowth above the epithelium (Fig. 2C, asterisk). By E11, expression of *CXCL14* is absent in this region of the conjunctival epithelium and coincides with the degeneration of the scleral papillae (Murray, 1943; Franz-Odendaal, 2008). Our results show *CXCL14* expression as a novel early marker for scleral papillae and suggest that it may play a role in establishing the transient placodal phase of the conjunctival epithelium.

By E12, expression of *CXCL14* is strong in another group of conjunctival epithelial cells located in the limbus region adjacent to the cornea epithelium (Fig. 2D and 2D'). By E17, cells expressing *CXCL14* are located in the basal cell layer of limbal epithelium near the margin of the cornea epithelium (Fig. 2E and 2E'). Unlike chick, expression of *CXCL14* in the mouse is maintained at modest levels in the cornea and conjunctival epithelium during

development (Fig. 1K and 1L). In adult mouse corneas, *CXCL14* is expressed in the basal and wing layers of the epithelium (Fig. 2F and 2G). In the limbal epithelium, *CXCL14* expression is maintained at a slightly higher level in comparison to the corneal epithelium (Fig. 2H). In adult eyes, the basal layer of the limbal epithelium contains a reservoir of stem cells that replenish the cornea epithelium (Tseng, 1989; Schlötzer-Schrehardt et al., 2005), but their origin and when they are established is not known. Based on our results, it is possible that *CXCL14* plays a role during the formation of the limbal stem cells. Similarly, previous studies have shown that *CXCL14* is expressed in regions where stem cells reside in other tissues including the dentate gyrus of the hippocampus (Banisadr et al., 2011), skin epithelial cells (Schaerli et al., 2005; Frick et al., 2011), and at the base of the intestinal crypts (Meuter and Moser, 2008).

2.3 Expression of *CXCL12* and *CXCL14* in the eyelids

The conjunctival epithelium also covers the inner eyelid and the nictitating membrane. The nictitating membrane is a transparent tissue that protects and lubricates the anterior surface of the eye. It exists in reptiles, birds, amphibians, and some mammals, but absent in primates and mice (Stibbe, 1928). The eyelids first appear as mesenchyme filled bumps in the ectoderm at about E7 in chick (Hamburger and Hamilton, 1951) and between E13 and E14 in mouse (Pei and Rhodin, 1970). In chick, the nictitating membrane also appears at about E7 as an additional mesenchyme filled bump adjacent to the eyelid.

Expression of *CXCL12* is not detected in the forming chick eyelid at E7 (Fig. 3A). By E8, *CXCL12* expression is prominent in the mesenchyme of the eyelid and low in the nictitating membrane (Fig. 3B). At E12, expression of *CXCL12* persists in the mid-region of the eyelid mesenchyme (Fig. 3C) that later differentiates into the eyelid muscles (Noden, 1986; Creuzet et al., 2005). At this time only a few cells express *CXCL12* in the mid-region of the nictitating membrane (data not shown). *CXCL12* is also expressed in the mesenchyme surrounding the feather buds on the eyelids (Fig. 3C').

CXCL14 is strongly expressed in the eyelid ectoderm at E7 (Fig. 3D). This pattern of *CXCL14* expression persists in the ectoderm of the eyelid and nictitating membrane at E8 and E12 (Fig. 3E and 3F). Expression of *CXCL14* is also observed in a few cells in the mid-region of the feather buds and in the surrounding surface ectoderm (Fig. 3F').

In the mouse, *CXCL12* is strongly expressed in the mesenchyme located in the mid-region of the eyelid by E14.5 and its intensity decreases towards the edges (Fig. 3G). This pattern of *CXCL12* expression persists in the eyelid mesenchyme at E16.5 (Fig. 3H) and during later stages of development (data not shown). In contrast, at E14.5 *CXCL14* is expressed in the ectoderm of the outer eyelid, but absent in the inner eyelid. *CXCL14* is also expressed in the mesenchyme at the anterior region of the eyelid at E14.5 (Fig. 3J), and it persists at low levels in this region at E16.5 (Fig. 3K).

Given that feather buds form at the posterior region of the eyelids in chick but hair follicles are at a relatively early stage of development in the mouse eyelids between E11.5-E16.5, we examined the expression patterns of *CXCL12* and *CXCL14* in the hair follicles of the whisker pad at E16.5. Although *CXCL12* is expressed at low levels in the developing hair follicles (Fig. 3I, dotted lines), it is strong in the surrounding mesenchyme. In contrast, *CXCL14* is strongly expressed in the hair follicles (Fig. 3L). Its expression is continuous with the basal layer of the skin and encompasses the outer root sheath, hair bulb (Fig. 3L, arrow) and bulge (Fig. 3L, arrowhead) regions of the hair follicle (Fig. 3L). Expression of *CXCL14* in the bulge region of the follicle where hair stem cells reside (Spradling et al., 2001; Wang et al., 2012) further indicates its potential role in stem cell development.

Our results show that *CXCL12* and *CXCL14* are expressed in complementary patterns during eyelid development. Expression of *CXCL12* in the eyelids is conserved between the chick and mouse at early stages of development but it is restricted in the posterior eyelid mesenchyme of the mouse by E16.5. Therefore CXCL12 may play similar role that occurs at different time in chick and mouse eyelid development. Expression of *CXCL14* in the eyelids also appears to be conserved between chick and mouse, with the exception of the inner eyelid epithelium in mouse where it is absent.

2.4 Expression of *CXCL12* and *CXCL14* in the retina

Following the formation of the optic cup, the outer layer becomes the retinal pigmented epithelium (RPE) and an inner layer gives rise to the neural retina (Pei and Rhodin, 1970, Agathocleous and Harris, 2009). After successive proliferation and differentiation, the neural retina is divided into three cellular layers: the outer nuclear layer (ONL) that contains rod and cone photoreceptors; the inner nuclear layer (INL) consisting of horizontal, amacrine, Muller, and bipolar cells; and the ganglion cell layer (GCL) located near the inner surface and comprised of retinal ganglion cells (Chow and Lang, 2001; Bassett and Wallace, 2012).

Expression of *CXCL12* in the posterior eye region of chick (Fig. 4A and 4B) and mouse (Fig. 4E and 4F) is restricted to the connective tissue and choroidal blood vessels adjacent to the RPE. A few cells in the anterior most region of the chick INL express *CXCL12* at E12 (Fig. 4B) but no expression of *CXCL12* was observed in the mouse neuroretina by P0 (Fig. 4F). Interestingly, a different pattern of *CXCL12* expression was observed in the internal limiting membrane (ILM) of the human retina at 2 weeks of gestation and believed to function in vessel assembly (Hasegawa et al., 2008). In addition to its function in angiogenesis, CXCL12 may also contribute to retina development since it promotes the survival of embryonic retinal ganglion cells that express *CXCR4* receptor (Chalasani et al., 2003).

Expression of *CXCL14* is sparse and diffuse in the chick neural retina at E7 (Fig. 4C). By E12, its expression is restricted to the INL in regions where amacrine and bipolar cells reside, and by a few cells in the GCL layers (Fig. 4C). In the mouse, *CXCL14* is broadly expressed in the neural retina at E16.5 (Fig. 4G). By P0, vivid expression of *CXCL14* is maintained in the neuroretina (Fig. 4H), but it is absent in the developing inner plexiform layer (IPL). All cell layers are formed in young adult mouse retinas and *CXCL14* is expressed in the inner boundary of the INL populated by amacrine cells (Doh et al., 2010; Bassett and Wallace, 2012), and in the GCL (Fig H, inset). Our results show that retina expression of *CXCL14* is conserved between chick and mouse. The asymmetric distribution of *CXCL14* in developing and adult retinas suggests a potential role in positioning, differentiation and/or maintenance of retinal cells in specific layers.

3. CONCLUSIONS

Our results revealed novel expression patterns of *CXCL12* and *CXCL14* and suggest that these two chemokines may play essential roles during ocular development in chick and mouse. Expression of *CXCL12* in the ocular mesenchyme and blood vessels was conserved between chick and mouse suggesting similar function during neural crest cell differentiation into muscles of the eyelid and iris, and during ocular vasculogenesis in both species. Chick-specific expression of *CXCL12* in the amacrine cells of the retina indicates potential role in cell proliferation or differentiation. In contrast, *CXCL14* was expressed in the ocular ectoderm and corresponded with the formation of the scleral papillae and hair follicles, and it was localized in the basal layer of the limbal epithelium where stem cells reside. The observed expression patterns indicate potential involvement of *CXCL14* in cell

differentiation and proliferation in the ocular epithelium. *CXCL14* expression in the eyelid mesenchyme and cornea suggests a possible function in neural crest differentiation into muscles or stromal keratocytes. CXCL14 may play an anti-angiogenic role (Shellenberger et al., 2004) by preventing *CXCL12*-expressing angioblasts and ocular blood vessels from entering the developing cornea. Complementary expression of *CXCL12* and *CXCL14* in the anterior eye and hair follicles suggests that interaction of these genes might be essential during development. However, further studies to elucidate the functional role(s) of CXCL12 and CXCL14 during ocular development are required.

4. EXPERIMENTAL PROCEDURES

4.1. Embryos

All animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Rice University. Fertilized White Leghorn chicken eggs (*Gallus gallus domesticus*) were obtained from a commercial supplier. Eggs were incubated at 38°C under humidified conditions. Chick embryos were collected between embryonic day (E)3 and E17. Wild-type C57/B6 mouse embryos were collected between E11.5 and E16.5, with the first appearance of a virginal plug considered as E0.5. Mouse eyes were also collected at postnatal day (P)0 and from adults. All tissues were collected in Ringer's solution, fixed overnight in modified Carnoy's fixative (60% ethanol, 30% formadehyde, and 10% glacial acetic acid) at 4°C. Tissues were dehydrated in ethanol series, cleared in Histosol and embedded in paraffin, then sectioned between 10 and 12 μm.

4.2. Synthesis of mRNA riboprobes

Fragments for *CXCL14 and CXCL12* were amplified from chick and mouse embryo cDNA using PCR. Primers were used as follows: *Gallus gallus* CXCL14, 5'GATTCTCTAACGTACGGAAGC (forward) and 5'CCAGCATCACTCATGTACCTCT(reverse); *Gallus gallus* CXCL12, 5'TGGCTCTGCTCGCCTTTGC (forward) and 5'AAGTATCTGTGCTGGGGTCC (reverse); *Mus musculus* CXCL14, 5' ACTGCGAGGAGAAGATGGTTAT (forward) and 5' GTAGAAGATGCTTCTGAGGCATC (reverse); *Mus musculus* CXCL12, 5' GCCGCACTTTCACTCTCG (forward) and 5' GGTCATGCTAAGGTTTGCCA (reverse). The PCR products (750bp and 870bp for *CXCL14*, and 495bp and 500bp for *CXCL12*) were cloned into pCR®II-TOPO® vector with dual promoters (Invitrogen) following manufacture's protocol, and the gene inserts were confirmed by sequencing. Plasmids were linearized with EcoRV and or BamH1 and labeled riboprobes were generated using T7 or SP6 polymerases.

4.3. *In situ* hybridization

In situ hybridization was performed on sections as described previously (Etchevers et al., 2001). Sense probes were used as control, and showed no specific signal.

4.4. Microscopy and imaging

Images of stained sections were acquired using a Zeiss microscope and Axio Vision program (Carl Zeiss AG, Oberkochen, Germany) at 5X, 10X and 20X objectives.

Acknowledgments

We thank members of the Lwigale lab for discussions of the raw data and editing the early version of the manuscript. This work was supported by NIH grant EY022158 to PYL.

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Highlights

- *CXCL12* is predominantly expressed in ocular mesenchyme-derived tissues, blood vessels, and retina, and it is mostly conserved between chick and mouse.
- *CXCL14* is expressed in the ocular ectoderm, limbal epithelium, scleral papillae, eyelid mesenchyme, corneal keratocytes, hair follicles, and retina.
- Expression of *CXCL14* is only conserved in the outer eyelid ectoderm of chick and mouse.
- Non-overlapping expression profiles of *CXCL12* and *CXCL14* are consistent with their potential interaction and functions in cell proliferation, differentiation, and migration during ocular development.

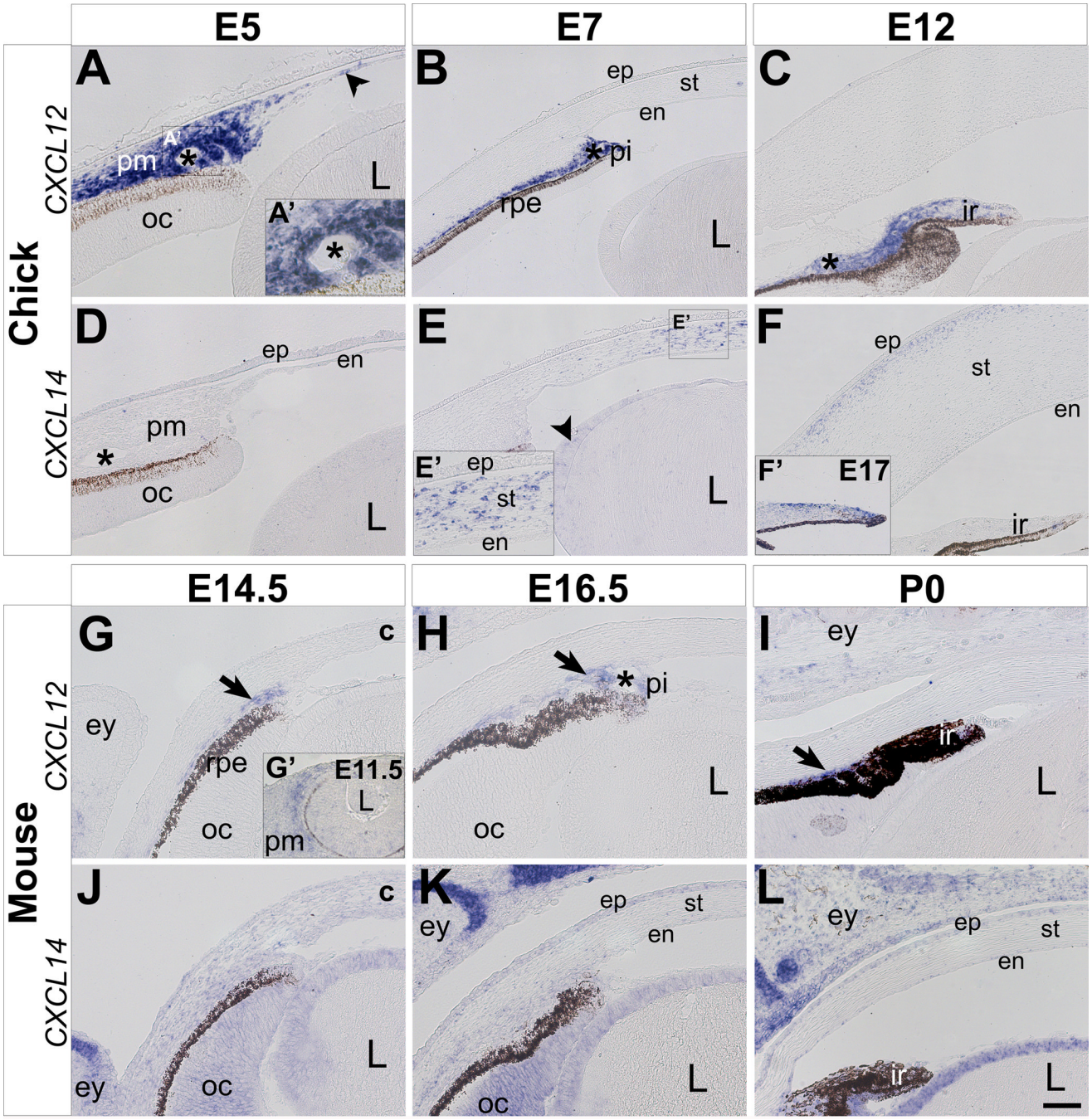

Figure 1. Expression of *CXCL12* and *CXCL14* during development of the cornea and iris in chick and mouse

In situ hybridization was performed on paraffin sections through the anterior eye of chick and mouse embryos. **(A–F)** During chick eye development, *CXCL12* is strongly expressed in the periocular mesenchyme and its derivatives between E5 and E12 (A–C). *CXCL14* expression is not detected in the periocular region and presumptive cornea at E5 (D), but it is later expressed in the cornea, lens, and iris between E7-E17 (E–F). **(G–L)** During mouse eye development, *CXCL12* is expressed in the periocular mesenchyme and its derivatives between E11.5 and P0 (G–I). *CXCL14* is initially expressed at low levels at E14.5 (J), and then it becomes restricted to the cornea epithelium, optic cup, and lens epithelium between

E16.5 and P0 (K–L). Asterisks in A–D and H indicate ocular blood vessels; c, cornea; en, cornea endothelium; ep, cornea epithelium; ey, eyelid; ir, iris; L, lens; oc, optic cup; pi, presumptive iris; pm, periocular mesenchyme; rpe, retinal pigmented epithelium; st, cornea stroma. Scale bar represents 100 μm in B, C, E, and F and 50 μm in A, D, G–L.

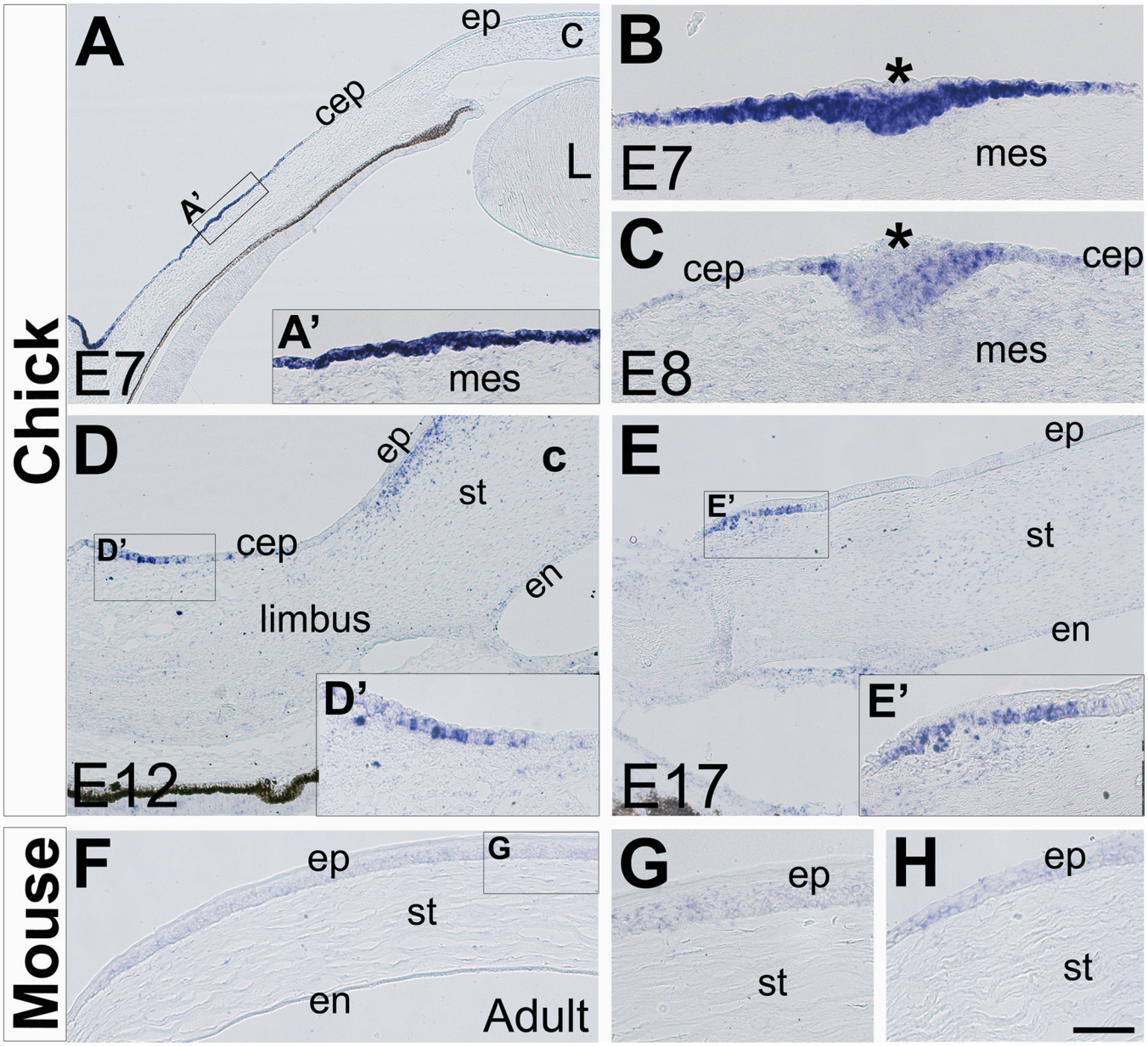

Figure 2. Expression of *CXCL14* in the developing chick conjunctival epithelium and adult mouse cornea epithelium

(A–E) Expression of *CXCL14* in the chick conjunctival epithelium (A–C) during formation of the scleral papillae between E7 and E8 chick eye development, and (D–E) in the limbal epithelium at E12 and E17. (**F–G**) Expression of *CXCL14* in the adult mouse cornea epithelium in the central (G) and peripheral (H) regions. Asterisks in B and E indicate superficial cells of the scleral papillae; c, cornea; cep, conjunctival epithelium; en, cornea endothelium; ep, cornea epithelium; L; lens; mes, mesenchyme; st, cornea stroma. Scale bar represents 200 μm in A, 100 μm in D, E and F, and 50 μm in B, C, G and H.

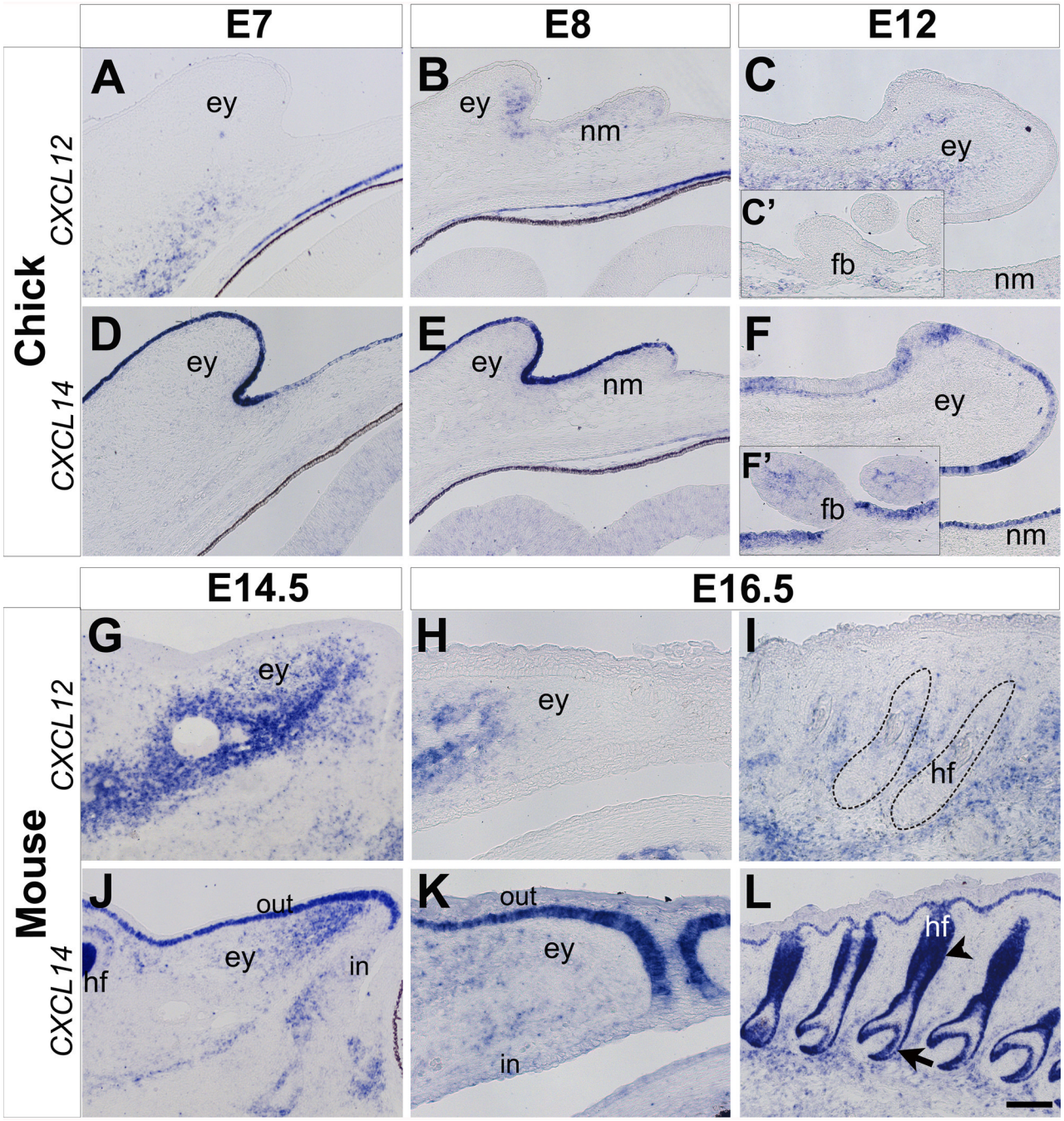

Figure 3. Expression of *CXCL12* and *CXCL14* during eyelid development in chick and mouse

(A–F) Expression of *CXCL12* (A–C) and *CXCL14* (D–F) during chick eyelid development. *CXCL12* is initially not expressed in the eyelid at E7 (A), but it becomes prominent between E8 and E12 (B–C). Expression of *CXCL12* is absent in the feather buds (C'). Expression of *CXCL14* is robust in the ectoderm of the eyelids and nictitating membrane between E7-E12 (D–F), but not continuous in the feather buds where it is expressed in the mesenchyme (F'). (**G–L**) Expression of *CXCL12* (G–I) and *CXCL14* (J–L) during eyelid and hair follicle development in the mouse. *CXCL12* is strongly expressed in the eyelid mesenchyme between E14.5- E16.5 (G and H). *CXCL12* is also expressed in the mesenchyme

surrounding the hair follicles (I, dotted outline). *CXCL14* is expressed in the eyelid ectoderm and mesenchyme between E14.5 and E16.5 (J and K), and in the hair follicles (L). ey, eyelid; nm, nictitating membrane; fb, feather buds; hf, hair follicle; in, inner eyelid epithelium; out, outer eyelid epithelium. Scale bar represents 100 μm in B, C, E, F, I, and L, and 50 μm in A, D, G, H, J, and K.

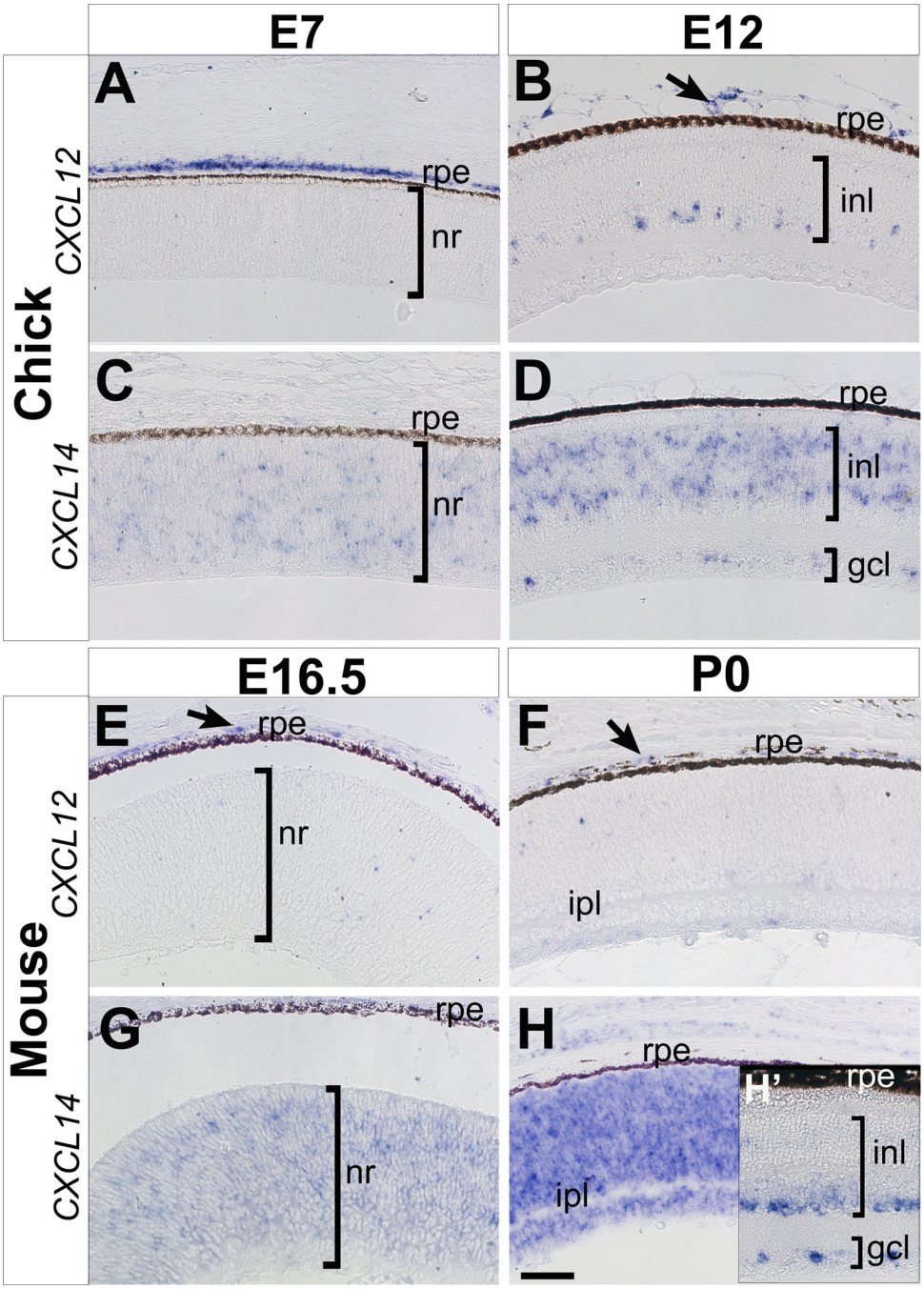

Figure 4. Expression of *CXCL12* and *CXCL14* during retina development in chick and mouse

(A–D) Expression of *CXCL12* (A and B) and *CXCL14* (C and D) in the chick retina at E7 and E12. **(E–F)** Expression of *CXCL12* (E and F) and *CXCL14* (G and H) in the mouse retina at E16.5, P0, and young adult (H'). gcl, ganglion cell layer; inl, inner nuclear layer; ipl, inner plexiform layer; nr, neural retina; rpe, retinal pigmented epithelium. Scale bar represents 50 μm.