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# Characterisation of stem and proliferating cells on the retina and lens of loach *Misgurnus anguillicaudatus*

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## Abstract

The eye of the fish has a lifelong persistent neurogenesis unlike eye of mammals, so it's highly interesting to study retinal neurogenesis and its genetic control to give complete knowledge about the cause of this property in fish in comparison to mammals. We performed fluorescent *in situ* hybridisation for loach *Misgurnus anguillicaudatus bmi1*, *msi1* and *sox2* genes, which are used as an indicator of the sites of multipotent stem cells. Proliferating cell nuclear antigen (PCNA), bromodeoxyuridine (BRDU) and KI67 markers were used as indicators of proliferating cells and glial fibrillary acidic protein (GFAP) immunofluorescence was used for detection of the glial property of cells, as well as, immunohistochemistry detected the role of peroxisome proliferator-activated receptor (PPAR)α and γ in retinal neurogenesis. Our results determined that the lens and the retina of loach *M. anguillicaudatus* contain proliferative and pluripotent stem cells that have both glial and neuroepithelial properties, which add new cells continuously throughout life even without injury-induced proliferation. The PPARα has an essential function in providing energy supply for retinal neurogenesis more than PPARγ.

**KEYWORDS**
cell proliferation, eye, loach, *Misgurnus anguillicaudatus*, PPAR, stem cells

## 1 | INTRODUCTION

Loach (Misgurnus anguillicaudatus) is a Chinese fish and from ideal and common species in aquaculture (Gao *et al*., 2014). There is a characteristic property of persistent neurogenesis in fishes as they can restore healthy and functional tissue again after injury caused by the trauma to the brain (Kizil *et al*., 2012) or the eye (Goldman, 2014; Lenkowski & Raymond, 2014). The eye of mammals cannot restore the function of the damaged parts (Bédard & Parent, 2004; Curtis *et al*., 2007). In fishes, there is lifelong growth in different tissues due to the presence of multipotent undifferentiated stem cells that control the continuous replacement of new cells. The retina of fish is formed from; nerve fibre layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), visual layer (VL) and pigment epithelium layer (PEL; Nagarjuna & Mohan, 2016). The ganglion cell layer composed of the cell bodies of the ganglion cells. The inner nuclear layer composed of the cell bodies of bipolar, amacrine and horizontal cells. The outer nuclear layer contains the nerve cell bodies of the visual layer (photoreceptor layer). The photoreceptors act as the light-sensitive compartment of the retina. The inner plexiform layer is composed of dendrites of ganglion cells and the axons of amacrine and bipolar cells, while the outer plexiform layer is composed of axons of photoreceptors, axons and dendrites of horizontal cells and dendrites of the bipolar cells (Germain *et al*., 2010). The ciliary marginal zone (CMZ) that exists between ciliary epithelium and retina play a vital role in retinal neurogenesis in adult amphibians and fish (Hitchcock *et al*., 2004; Wehman *et al*., 2005). Also, the Müller cells, which are glial in kind and located in the inner nuclear layer, can generate new neurons from the

pigmented epithelium Müller (Karl & Reh, 2010; Raymond *et al*., 2006; Than-Trong & Bally-Cuif, 2015; Todd *et al*., 2016; Wohl *et al*., 2012). The Müller glia cells can generate all cell subtypes which form the retinal cell (Raymond *et al*., 2006). In adult zebrafish *Danio rerio* (Hamilton 1822), the Müller glia cells after injury of retina rapidly proliferate and differentiate into neurogenic precursors that capable to regenerate new neurons and retain vision (Goldman, 2014). The rod precursors originate from the rapid proliferation of Müller glia cells of the INL, in the larval stage (Raymond & Rivlin, 1987) and adult fish eye (Otteson *et al*., 2001).

There are several genes controlling the differentiation of neural stem cells into neurogenic precursors and *sox2* is considered the primary regulator of this process (Maucksch *et al*., 2013). The *sox2* is a transcription factor usually expressed during development and in the adult brain (Ferrero *et al*., 2014). Expression of *sox2* regulates self-renewal of embryonic neural stem cells for further differentiation into neurogenic precursors (Rizzino, 2013). The Musashi 1 (*msi1*) is expressed all over the retina with unique expression in the inner nuclear layer (Kaneko & Chiba, 2009; Nickerson *et al*., 2011) and has a vital role in controlling differentiation of photoreceptor cells as it represses splicing of photoreceptor cells (Staff, 2016). The *bmi1* gene control retinal proliferation *via* regulation of cell cycle proteins involved in the apoptosis process in the retina, especially in photoreceptor cells (Zencak *et al*., 2013).

This study characterised the adult neurogenesis in the eye of loach *Misgurnus anguillicaudatus* (Cantor 1842) by determining the functional role of *bmi1*, *msi1* and *sox2* genes in the loach retina. Also, we determined the sites of proliferative and stem cells in some developmental stages in the eye of loach with the aid of fluorescent *in situ* hybridisation technique. We made histological observations of the eye in loach and immunohistochemistry of peroxisome proliferator-activated receptor (PPAR)α and γ. The functional role of *bmi1*, *msi1* and *sox2* genes, as well as, the role of PPARα and γ in retinal neurogenesis, was determined in the eye of loach; the sites of proliferating cells were also detected. This study is important for characterisation of the differences and similarities between loach and other fishes in sites of persistent neurogenesis. Also, it is important for the detection of the effect of age development from 1 month to 1 year in retinal neurogenesis.

## 2 | MATERIALS AND METHODS

This study follows the rules in the Guide for the Use and Care of Laboratory Animals of Huazhong Agricultural University.

### 2.1 | Fish sampling

Twenty loach were taken from aquaculture department located in the fishery college of Huazhong Agricultural University. Body mass ($M_T$), total lengths ($L_T$) and ages of the loaches are shown in (Table 1).

Ice was used to stop fish movement before the fish was sacrificed by decapitation with a sharp scalpel. The eyes were collected at some stages of development and preserved in 4% paraformaldehyde at 4°C overnight, then dehydrated with serial grades of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections (5 μm) were collected on positive-charge slides and kept at 4°C until used. We used five loaches at each age and *c*. 10 sections were obtained from each loach.

### 2.2 | Histology

The following stains were used for observation of tissue: Haematoxylin and eosin stain was for general histological observations and Holzer's stains for staining of glial cells. The staining procedures were according to Jensen (2008), then slides were examined by a Zeiss light microscope for taking photos (www.zeiss.com). The glial cells were counted. We collected the data from different microscopic fields ($n$ = 3), of Holzer's stained slides ($n$ = 4), with using objective lens x40, for each slide at a different age. The data for glial cells counting are shown in (Table 1).

### 2.3 | Statistical analysis

The data were analysed with generalised linear models (GLM) procedure of SAS (www.sas.com). Results were expressed as least square means (LSM ± SE) and Duncan's multiple range test (DMRT) were made to compare the differences between means. All data were evaluated for conformity to a normal distribution with the Kolmogorov–Smirnov (K-S) test. $Y_{ij} = \mu + A_i + e_{ij}$ statistical model was used to analyse phenotype parameters ($L_T$, $M_T$ and total number of glial cells), in where, $Y_{ij}$ is observation; $\mu$ is the parameters mean; $A_i$ is the effect for age per months (1st, 3rd,7th and 12th) and $e_{ij}$ is experimental error assumed to be randomly distributed ($\sigma 2 = 0$). The statistical analysis results are shown in (Table 1).

**TABLE 1** Least-squre mean (± SE; $n$ = 5) of age on body mass, length and total number of glial cells counted in the retina of *Misgurnus anguillicaudatus*

| | Age per month (LSM ± SE) | | | | | |
|---|---|---|---|---|---|---|
| | 1st | 3rd | 7th | 12th | *F* | *P* |
| Total length (mm) | $20.74 \pm 0.73^{d}$ | $55.44 \pm 0.73^{c}$ | $77.04 \pm 0.73^{b}$ | $132.86 \pm 0.73^{a}$ | 40.35 | < 0.001 |
| Total body mass (gm) | $0.059 \pm 0.08^{d}$ | $0.901 \pm 0.08^{c}$ | $2.301 \pm 0.08^{b}$ | $13.528 \pm 0.08^{a}$ | 57.23 | < 0.001 |
| Total number of glial cells (in eye sections at x40) | $65.00 \pm 1.96^{d}$ | $79.80 \pm 1.96^{c}$ | $95.01 \pm 1.96^{b}$ | $124.20 \pm 1.96^{a}$ | 166.42 | < 0.001 |

*Note*: Different superscript letters indicated significantly differences between means.

### 2.4 | Fluorescent *in situ* hybridisation (FISH)

The eye sections were cleared from wax by xylene, dehydrated, washed in diethyl pyrocarbonate (DEPC) treated water and in DEPC-PBS (phosphate buffer saline) for performing FISH according to Shi *et al*. (2012) with some modifications. The eye sections were then kept in 4% paraformaldehyde solution for 2 min and washed in PBS before the eye sections were incubated at 37°C for 5 min with 0.25% anhydride acetic acid to triethanolamine (0.1 M TEA, PH 8.0) prepared fresh for acetylation, as the half-life of acetic anhydride is very short. Then acetic acid was added to reach a concentration of 0.5% and incubated for another 5 min at 37°C. The eye sections were permeabilised by 1 μg $ml^{-1}$ proteinase K in TE buffer (Tris, EDTA buffer) for 7 min at 37°C and washed with glycine + PBS.

The eye sections were kept in a pre-hybridisation buffer (1x Denhardt's, salmon sperm DNA (0.5 mg $ml^{-1}$), 50% deionised formamide, 2 × stretch–shortening cycles (SSC), 0.5 mg $ml^{-1}$ from yeast tRNA and 0.02% sodium dodecyl sulphate (SDS); pH 7.0) at 65°C for 1 h. For the preparation of 50x Denhardt's solution, we used 10 g polyvinylpyrrolidone, 10 g bovine serum albumin (BSA) and 10 g Ficoll 400 in 1 l of sterile distilled $H_2O$ then filtered and stored at 4°C and warmed before use. The oligonucleotide antisense probes were added over the eye sections in the slides in combination with the hybridisation buffer (1x Denhardt's, 50% de-ionised formamide, yeast tRNA (0.5 mg $ml^{-1}$), 2 × SSC, salmon sperm DNA (0.5 mg $ml^{-1}$) and 10% dextran sulphate) overnight (14–16 h) at 60°C for performing triple fluorescent *in situ* hybridisation technique, which must be performed in the dark to prevent fading of fluorescence. Fluorescein isothiocyanate (FITC) labelled the *bmi1* probe, AMCA labelled the *msi1* probe and CY3 labelled the *sox2* probe. Each probe was examined singly before doing a triple FISH to confirm our results. The probe sequence determined according to Moffitt *et al*. (2016) by using the data obtained from three genes cloning and sequencing in loach fish.

Then the eye sections were washed in 2 × SSC, 1 × SSC and DEPC-treated PBS. The coverslip mounted by anti-fading medium (nine parts of glycerol and one part of PBS, pH 8.5) for examination by confocal microscope for taking photos. A control sense probe for each gene was used to confirm the results obtained by the antisense probes.

### 2.5 | Bromodeoxyuridine labelling

The bromodeoxyuridine solution (BrdU; Solarbio; www.solarbio.net) was injected intraperitoneally (10 mg $ml^{-1}$) in loach, which were collected at different ages and injected by BrdU solution as 50 μl $g^{-1}$ body mass. The fish kept after injection in water for 24 h then the eye samples were collected and processed as previously described in fish sampling. After dewaxing, the eye sections were reacted with 1–2 M HCl for 30 min at 37°C for DNA hydrolysis. They were neutralised by incubating them in 0.1 M sodium borate buffer pH 8.5 at room temperature for 10 min before being triple washed in PBS and left in 0.3% $H_2O_2$ in PBS (pH 7.4) for 30 min at room temperature in the dark to get rid of endogenous peroxidase activity, to prevent non-specific binding of antibodies. A further three washes with phosphate buffer saline (PBS) were made; bovine serum was used as a blocking agent. Eye sections were kept in an incubator with the rat monoclonal (BU1/75 (ICR1)) to BrdU (FITC) (ab74545) anti-BrdU antibodies and with rabbit polyclonal anti-KI67 antibodies (ab15580; Applied Biosystems; www.appliedbiosystems.com) at 4°C overnight. Antibodies dilution was 1/400. Then the sections were incubated with goat anti-rabbit immunoglobulin (IgG) antibodies labelled with CY3 for KI67 in the dark for 1 h at room temperature. Then after washing by PBS in a dark chamber, coverslip mounted with an anti-fading medium and photos taken *via* a confocal microscope.

### 2.6 | Immunohistochemistry

The eye sections were cleared with xylene, dehydrated with alcohol and given three rapid washes in PBS. Eye sections were kept in 0.3% $H_2O_2$ in PBS (pH 7.4) at room temperature for 30 min, then washed by PBS and blocked with blocking serum at room temperature for 1 h, then kept at 4°C overnight in an incubator with the following antibodies: rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA) antibody (ab19166; Applied Biosystems), rabbit polyclonal anti- peroxisome proliferator-activated receptor α (PPARα) alpha antibody (ab61182; Applied Biosystems), rabbit polyclonal anti-PPARγ antibody (ab66343; Applied Biosystems) and goat polyclonal anti- glial fibrillary acidic protein (GFAP) antibody (ab53554; Applied Biosystems); antibody dilution was 1/200. Slides washed by PBS before being incubated at room temperature for 1 h with secondary goat anti-rabbit IgG antibodies labelled with appropriate secondary dye. In case of PPARα and PPARγ immunohistochemistry staining biotinylated secondary antibodies with streptavidin-biotin-horseradish peroxidase complex were kept with slides at room temperature for 30 min. For PCNA the secondary goat anti-rabbit IgG antibodies labelled with CY3 (ab97057) were used. For GFAP we used polyclonal donkey anti-goat IgG labelled with FITC (ab6881). The secondary antibodies dilution was 1/200. Then were washed by PBS then coverslip was mounted with an anti-fading medium, then was observed with a light microscope (for PPARs) and confocal microscope (for PCNA and GFAP) for taking photos.

## 3 | RESULTS

### 3.1 | General histological observations of the eye of loach

The retina of loach fish is formed from seven layers that were, from inside to outside, ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, photoreceptor layer (layer of rods and cones) and pigment epithelium layer (Figure 1a,b). There were several glia cells found mainly in ganglion cell layer at the age of 1 month (Figure 1c), while at the age of 1 year the glial cells their number increased and found mainly in inner nuclear layer (Figure 1d).

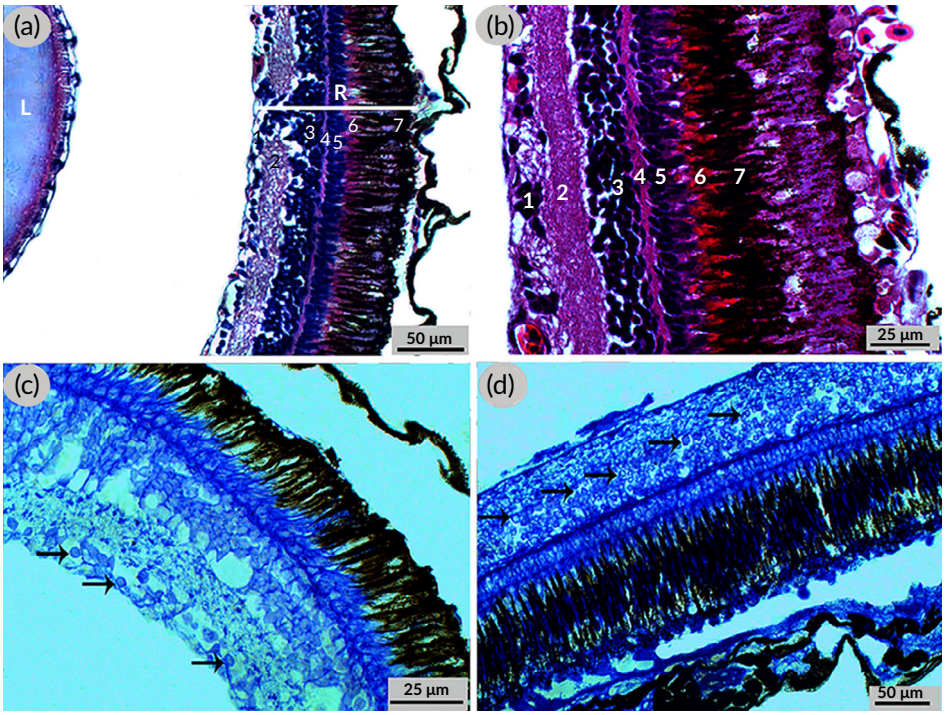


**FIGURE 1** General histological observations of the eye of *Misgurnus anguillicaudatus*, showing (a) the lens (L) and retina (R) at age 1 month stained with haematoxylin and eosin (H&E); (b) the seven layers of retina at age 1 month stained with H&E (1, ganglion cell layer; 2, inner plexiform layer; 3, inner nuclear layer; 4, outer plexiform layer; 5, outer nuclear layer; 6, photoreceptor layer or visual laye; 7, pigment epithelium); (c) retina at age 1 month stained with Holzer's crystal violet stain (→, glial cells); (d) at age 1 year stained with Holzer's crystal violet stain

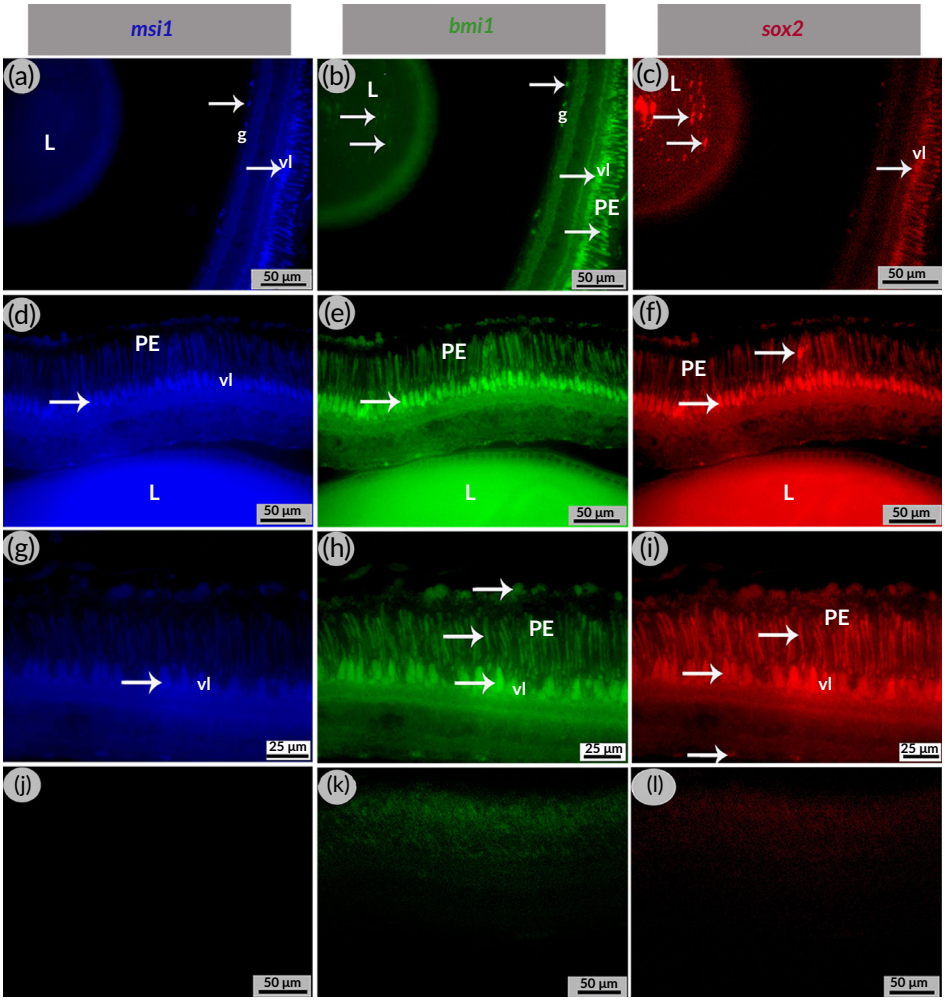


**FIGURE 2** The localisation in the retina of *Misgurnus anguillicaudatus* of (a), (d), (g), (j) *bmi1*, (b), (e), (h), (k) *msi1* and (c), (f), (i), (l) *sox2* genes (→) by using fluorescent *in situ* hybridisation: (a), (b), (c) 1 month old; (d), (e), (f) age of 7 months; (g), (h), (i) age of 1 year. The visual layer and some few pigment epithelial cells of retina, as well as the lens were the obvious sites for genes expressions. (j), (k), (l) Negative control sense probe. L, Lens; g, ganglion cell layer; vl, visual layer or photoreceptor layer; PE, pigment epithelium. The total number of fish used was $n$ = 15

### 3.2 | Gene expression analysis by fluorescent *in situ* hybridisation for *msi1*, *bmi1 and sox2* genes

At the age of 1 month, the *msi1* gene was detected in the outside borders of the lens, in ganglion cell layer, outer and inner plexiform layers, inner nuclear layer and was high in photoreceptor layer (Figure 2a). The *bmi1* gene was like *msi1* gene in sites of expression except in slight appearance in the middle of the lens (Figure 2b). The *sox2* gene was also present in the same locations as *msi1* and *bmi1* genes except it showed the highest for expression in the middle of the lens (Figure 2c).

At the age of 7 months, the *msi1* gene was high in the lens and all across the retina, particularly the layer of rods and cones (Figure 2d). The *bmi1* gene resembled *msi1* gene (Figure 2e). The *sox2* gene beside it was present in the same sites as *msi1* and *bmi1* genes it was found in high expression in some cells in the middle of the pigment epithelium layer (Figure 2f). At the age of 1 year, the *msi1* gene was found in all layers of the retina but especially the photoreceptor layer (Figure 2g), the *bmi1* and *sox2* genes were also in the same sites as the *msi1* gene but they were higher in expression in the pigment epithelium (Figure 2h,i).

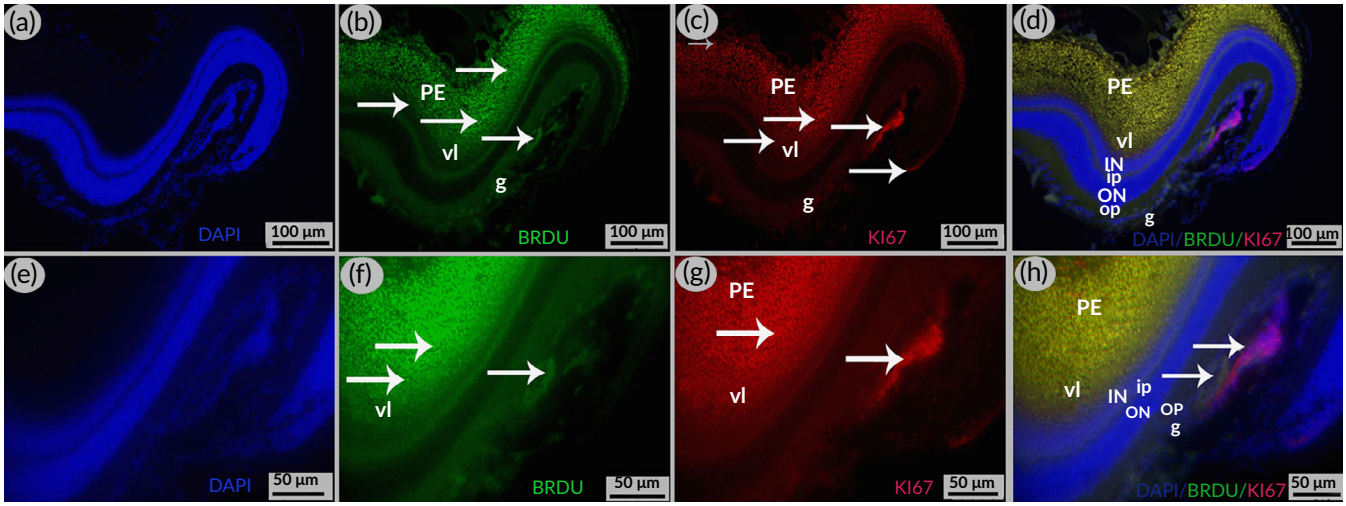


**FIGURE 3** Double immunofluorescence of *brdu* plus *ki67* in the retina of *Misgurnus anguillicaudatus* at age of 3 months (⟶). The pigment epithelium layer and the ganglion cell layer showed some proliferative cells. PE, Pigment epithelium layer; vl, photoreceptor layer; IN, inner nuclear layer; ip, inner plexiform layer; ON, outer nuclear layer; OP, outer plexiform layer; g, ganglion cell layer. The total number of fish used was *n* = 5

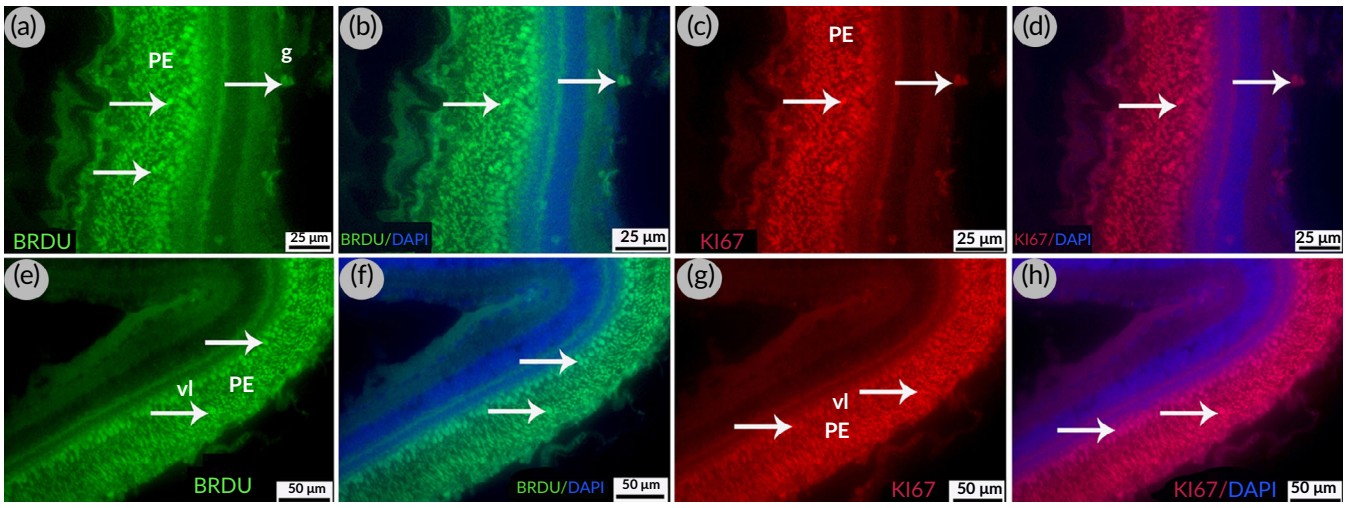


**FIGURE 4** Double Immunofluorescence of *brdu* plus *ki67* in the retina of *Misgurnus anguillicaudatus* (⟶): (a), (b) *brdu* labelled cells at 7 months and (c), (d) *ki67* labelled cells at 7 months; (e), (f) *brdu* labelled cells at 1 year and (g), (h) *ki67* labelled cells at 1 year. The pigment epithelium showed positive reaction at both ages as well as a few positively reacted cells at the ganglion cell layer at the age of 7 months only. PE, Pigment epithelium layer; g, ganglion cell layer; vl, photoreceptor layer. The total number of fish used was *n* = 10

Proliferating mitotically active S-phase cells in the eye were observed by labelling *via* intraperitoneal injection of 5-Bromo-2′-deoxyuridine (BrdU). The Ki67 was also used with BrdU for observation of proliferative cells. Both BrdU and Ki67 were few in number in the ganglion cell layer, inner and outer plexiform layers and high in photoreceptor and pigment epithelium layer at the age of 3 months (Figure 3). At the age of 7 months, the ganglion cell and pigment epithelium layers contain BrdU and ki67 labelled cells (Figure 4a–d), while at the age of 1 year they were found in photoreceptor and pigment epithelium layers (Figure 4e–h).

### 3.3 | Immunohistochemistry of GFAP, PCNA, PPARα and PPARγ

The positive cells for GFAP did not span the retina, they were mainly observed in the ganglion cell layer and at the periphery of the pigment epithelium layer (Figure 5a–c). The positive cells for PCNA were detected in the same regions, which indicates that most proliferating cells in the lens and retina are GFAP positive (Figure 5d–f). The positive reaction for PPARα was higher than PPARγ in the eye of loach. It was observed that at the age of 1 month, both kinds of PPARs were

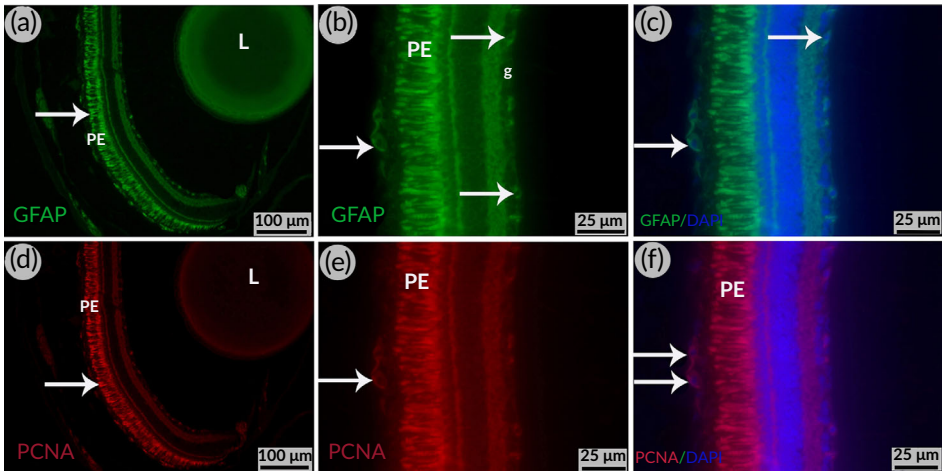


**FIGURE 5** Immunofluorescence for glial fibrillary acidic protein (GFAP) at the age of 1 month in the eye of *Misgurnus anguillicaudatus*: (a) glial cells in the periphery of lens (➞), (b) ganglion cell layer (➞), (c) inner and outer plexiform layers, photoreceptor layer and pigment epithelium layer (➞). Immunofluorescence for proliferating cell nuclear antigen (PCNA) in (d) glial cells in the periphery of lens (➞), (e) ganglion cell layer (➞), (f) inner and outer plexiform layers, photoreceptor layer and pigment epithelium layer (➞). L, Lens; PE, pigment epithelium layer; g, ganglion cell layer. The total number of fish used was *n* = 5

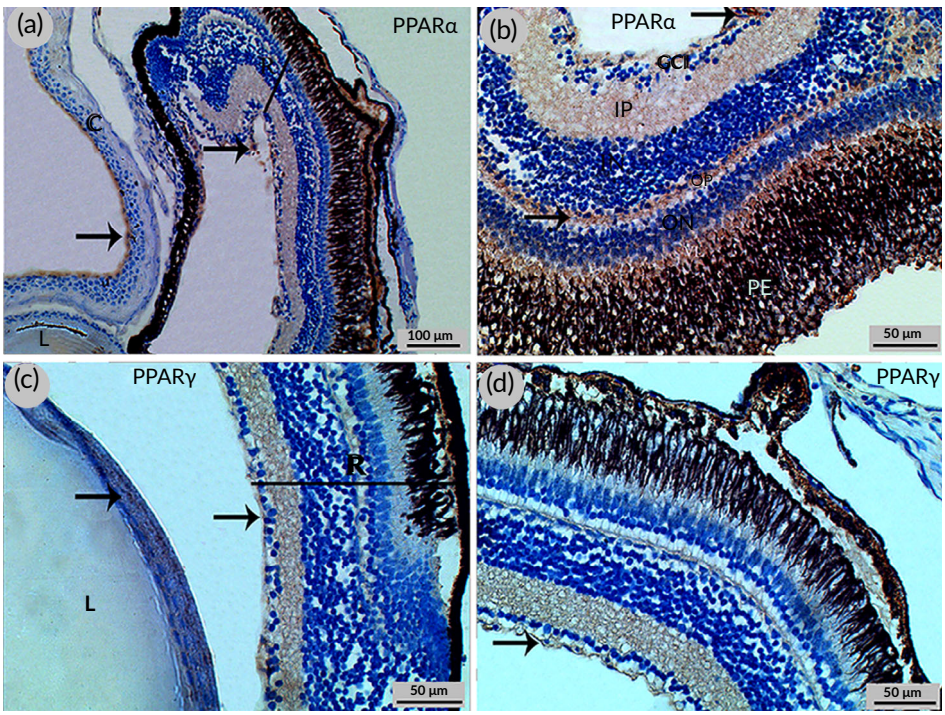


**FIGURE 6** The immunohistochemistry of (a), (b) peroxisome proliferator-activated receptor (PPAR)α and (c), (d) PPARγ in the eye (➞) of *Misgurnus anguillicaudatus*. The main sites for PPARα expression were ganglion cell layer, inner plexiform, outer plexiform and visual layers. R, Retina; C, cornea; L, lens (L); GCL, ganglion cell layer; IP, inner plexiform IN, inner nuclear; OP, outer plexiform; ON, outer nuclear; vl, photoreceptor layer; PE, pigment epithelium layer (PE). The total number of fish used was *n* = 5

low and became high at the age of 3 months then begins to decline in adult fish. The PPARα reacts positively with the cuboidal epithelium of cornea, lens and retina. The layers of the retina that showed a positive reaction with PPARα were ganglion cell layer, inner and outer plexiform layers, photoreceptors and pigment epithelium layer (Figure 6a,b). The PPARγ was observed in the ganglionic cell layer and inner plexiform layer but was very low in other parts of the eye (Figure 6c,d).

## 4 | DISCUSSION

In this study, we give some insight into the genetic control of retinal neurogenesis in the eyes of fish, to characterise the evidence of lens and retinal neurogenesis in loach. There was a high expression of *sox2* in the lens and retinal layers, which might suggest that *sox2* plays a major role in neurogenesis during the development of eyes in loach. Raymond and Rivlin (1987) also determined that the retina of fishes continuously grows throughout the lifetime.

The *sox2* expression was high in the centre of the lens more than *msi1* and *bmi1* at the age of 1 month which indicates the presence of pluripotent stem cells with neuroepithelial properties in that site as it reacts negatively with GFAP. Also, the photoreceptor layer, pigment epithelium and ganglion cell layer of the retina contain pluripotent stem cells (*sox2* positive) which have both proliferative and glial properties (positively react with PCNA, BrdU and GFAP). The glial Müller cells were previously described to be able to differentiate and act as multipotent retinal stem cells, which give rise to all retinal cell types including photoreceptors (Raymond *et al*., 2006; Wohl *et al*., 2012; Lenkowski & Raymond, 2014). It was found that *soxc* genes have a partially-overlapping expression pattern in the lens placode and neural retina of the developing fish eye (Cizelsky *et al*., 2013; Pillai-Kastoori *et al*., 2014). The *soxc* genes have a significant role in retinal neurogenesis and development of lens in developing eye of fish (Pillaikastoori *et al*., 2015). In the retina of adult mammals, they have little expression pattern (Usui *et al*., 2013). However, Jiang *et al*. (2013) mentioned that *sox4* and *sox11* knockout in mice led to the loss of ganglion cells of the retina. While in zebrafish their knockdown in the developing retina reduced only the number of mature rod photoreceptors (Pillai-Kastoori *et al*., 2014). As well as, Both *sox11a* and *sox11b* were expressed strongly in ganglion cell layer of the retina in zebrafish and their knockdown in retinal explants inhibited regrowth of radial glial cells axons (Mu *et al*., 2017). Also, Ma *et al*. (2009) added that *sox2* has a significant role in pigment epithelium of retina as it can initiate their differentiation into retinal neurons. The *bmi1* plays a very important role in controlling photoreceptor death during regeneration of the retina (Zencak *et al*., 2013). While *msi1* is mainly expressed in photoreceptors and pigment epithelium cells, it controls the transdifferentiation of retinal pigment epithelium so play an essential role in retinal regeneration (Kaneko & Chiba, 2009).

Our result indicated that expression of *sox2*, *bmi1* and *msi1* genes in the lens of loach increased with age as it was present in the free borders of the lens at the age of 1 month then begin to appear in the centre of the lens before spreading to involve the whole lens structure. Also, the *sox2*, *bmi1* and *msi1* genes play a vital role in the differentiation of photoreceptor cells during retina development and adult stage. The immunohistochemistry of proliferating cell nuclear antigen (PCNA) and BrdU can be used to detect active sites of proliferating cells in S-phase in the fish retina during development and regeneration (Bejarano-Escobarabcaad, 2009; Bernardos *et al*., 2007; Candal *et al*., 2005; Jin *et al*., 2012; Julian *et al*., 1998). The BrdU- labelling, ki67 and PCNA characterised the proliferative cells in loach retina. We found that proliferative cells were high in ganglion cell layer, pigment epithelium and few in photoreceptor layer till the age of 7 months while with the increase of age, it was found they increased in photoreceptor layer and decreased in ganglion cell layer. All of these proliferative cells have glial properties as they react positively with GFAP. We agree with Bernardos *et al*. (2007); Stenkamp (2011) as they mentioned that the Müller cells in adult fishes continuously proliferate to generate progenitors of rod photoreceptors that give rise to new rods photoreceptors. Also, the Müller glial cells function as constitutive progenitors for regeneration of retina in fishes, rodents and chicks, after the damage to part of the retina or under the influence of growth factor (Gallina *et al*., 2014; Raymond *et al*., 2006; Todd *et al*., 2016). In mammals, the Müller glial cells have a limited property for regenerating neurons in the damaged retina (Gallina *et al*., 2014).

PPARs have many functions, including degeneration of lipids for energy supply, oxidative stress response and regulation of immune function (Herzlich *et al*., 2008; Rosen & Spiegelman, 2001). From our findings, we conclude that PPARα has an essential role in the metabolic demands of the retina including the energy needed for cell proliferation in the postembryonic developing retina in loach. The lens, ganglion cell layer, inner plexiform layer, outer plexiform layer, photoreceptor layer and the pigment epithelium layer showed signs of proliferation reacting positively with PPARα. While PPARγ gives a low positive reaction in these sites except for the outer plexiform layer of the retina, which provides a moderate positive reaction. Although, there are many studies applied to determine the role of PPARs in the eye of mammals, little is known about their role in fishes. Zhu *et al*. (2013) established a significant role of PPARγ in neuroprotection of ganglion cells in the mammalian retina after the optic nerve has been crushed.

From the previously obtained results, we can conclude that *sox2*, *bmi1* and *msi1* genes expressions give some insights into the control mechanism of retinal neurogenesis in a short developmental stage of 1 month to 1 year the eye of fishes. The loach eye contains both proliferative and pluripotent neural stem cells that continuously add new cells during retina and lens development even without injury-induced proliferation. Also, the PPARα has a vital role in metabolic demand in the retina and lens of loach.

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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