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ORIGINAL ARTICLE

Knockout of *lws1* in zebrafish (*Danio rerio*) reveals its role in regulating feeding and vision-guided behavior

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Abstract

Long-wave sensitive (LWS) is a G protein-coupled receptor expressed in the retina, and zebrafish is a better model organism for studying vision, but the role of LWS1 in vision-guided behavior of larvae fish has rarely been reported. In this study, we found that zebrafish *lws1* and *lws2* are tandemly replicated genes, both with six exons, with *lws1* being more evolutionarily conserved. The presence of Y277F in the amino acid sequence of *lws2* may have contributed to the shift of λ_{max} to green light. We established a *lws1* knockout zebrafish model using CRISPR/Cas9 technology. *Lws1*$^{-/-}$ larvae showed significantly higher levels of feeding and appetite gene (*agrp*) expression than WT, and significantly lower levels of anorexia gene (*pomc*, *cart*) expression. In addition, green light gene compensation was observed in *lws1*$^{-/-}$ larvae with significantly increased expression levels of *rh2-1*. The light-dark movement test showed that *lws1*$^{-/-}$ larvae were more active under light-dark transitions or vibrational stimuli, and the expression of phototransduction-related genes was significantly up-regulated. This study reveals the important role of *lws1* gene in the regulation of vision-guided behavior in larvae.

Keywords *lws1* · Feeding · Behavior · Phylogeny · Zebrafish larvae

Introduction

Organisms use their sensory systems to obtain information from the environment and then integrate that information to produce relevant behavior (Privat et al. 2019). In visual system signalling, evolutionary and functional studies of optogenetic genes are often used to infer fundamental properties of the visual system (Horth 2007). Vertebrate visual pigmentation consists of opsins and light-sensitive chromogenic groups (11-cis-retinal or 11-cis-3,4-dehydroretinal), which confer different spectral sensitivities on cone receptors and play important roles in colour vision and signal transduction (Nathans 1990). There are five main subfamilies of opsins, one rod opsin (RH1, $\lambda_{max} \approx 478–510$ nm) that perceives dark or low light, and four cones opsins that perceive bright or strong light: shortwave-sensitive opsin 1 (SWS1, $\lambda_{max} \approx 360–440$ nm), shortwave-sensitive opsin 2 (SWS2, $\lambda_{max} \approx 400–430$ nm), medium-wave-sensitive opsin (RH2, $\lambda_{max} \approx 450–530$ nm), and long wavelength sensitive opsin (LWS, $\lambda_{max} \approx 510–560$ nm) (Chinen et al. 2003; Terakita 2005). Opsins play an important role in physiological functions such as feeding, growth, behavior and mate choice in the organism. (Escobar-Camacho et al. 2017; Musilova et al. 2021).

During biological evolution, genome-wide or tandem duplication and sequence divergence of opsins have facilitated the expansion of opsin family members in many species (Lin et al. 2017, Hofmann and Carleton 2009). When replicated subgenes are identical, functional redundancy occurs and one of them usually becomes a pseudogene during the evolutionary process (Zhang 2003), or a rare evolutionary equilibrium occurs in which the genes diverge functionally to allow for the stable conservation of both subgenes (Nowak et al. 1997). There are five important amino

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acid sites in opsin, the mutation site S180A, H197Y, Y277F, T285A, A308S (based on the human LWS retinoid number) is a major predictor of LWS/RH2/MWS opsin (with vitamin A1 as the chromor) λ_{max} in the range of 512 ~ 560 nm (Yokoyama and Radlwimmer 2001). Many studies have shown that the number of *lws* copies, protein expression and functional differentiation between subtypes varies by species (Musilova et al. 2021), and there is controversy as to whether or not fish copies of the *lws* co-facilitate visual function.

Humans having two reciprocal *lws* genes, the absence of one of which causes red-green color blindness (Cole 2002). There was no *lws* expression in the early evolutionary history of finch seabream (*Lepidosteus platystomus*) (Stieb et al. 2023). *Lws1* and *lws*2 in medaka (*Oryzias latipes*) were 98.9% similar and had basically identical absorbance maxima (Matsumoto et al. 2006). The medaka *lws1/lws2* single mutant has no behavioral impairment in response to red light sensitivity, indicating functional redundancy across paralogous homologous genes (Harada et al. 2019). Both guppy (*Poecilia reticulata*) and bluefin killifish (*Lucania goodei*) have four *lws* genes with different λ_{max}, and the four homologous *lws* genes of guppy have undergone evolutionary diversification (neofunctionalisation) (Kawamura et al. 2016), the four *lws* genes of Bluefin Killifish exhibited significant differences in expression levels (Chang et al. 2021).

Visual retinoids bind to chromatophores to absorb light, initiating a visual transduction cascade response to perceive different colors and intensities of light, which guides the organism to produce light behaviors (Hardie and Juusola 2015; Macias-Muñoz et al. 2019). Instinctive visually guided behaviors are essential for the survival of larvae fish (Brandt et al. 1987; Fuiman et al. 2006; Muto and Kawakami 2013). Zebrafish is a model organism in the field of vision research with a wide range of visually guided behaviors, such as photodynamic responses, phototropic behaviors and feeding behaviors (Portugues et al. 2013; Orger and de Polavieja 2017), but there are limited reports on the evolutionary development of the zebrafish *lws1* and *lws2* genes and the effect of the *lws1* gene on visually guided behaviors in larvae fish. It is with this aim in mind that the present experiment was conducted.

In this study, we found that the *lws1* in zebrafish is more conserved in evolution. According to the "five-site rule", the presence of S180A and Y277F in the amino acid sequence of *lws2* may cause λ_{max} to shift towards green light We generated a *lws1* knockout zebrafish model (*lws1*$^{-/-}$) using CRISPR/Cas9 technology. We found that *rh2-1* expression was significantly elevated in *lws1*$^{-/-}$ larvae, suggesting possible photocompensativeness. Enhanced visually guided behaviors (feeding and light sensitivity) in *lws1*$^{-/-}$ larvae may be associated with the up-regulation of phototransduction-related genes. In addition, we observed that *lws1*$^{-/-}$ larvae were also significantly more sensitive to vibratory stimuli. These findings suggest that *lws1* knockout not only increases visually guided behaviors and upregulates phototransduction-related genes, but may also play an important role in regulating environmental sensitivity.

Results

Phylogenetic analysis of the zebrafish *lws1* and *lws2* genes

The evolutionary tree shows that the *LWS* is divided into two branches, with mammals clustered into a separate branch and zebrafish *lws1* more closely related to the carp family (Fig. 1). Zebrafish (*Danio rerio*), medaka, turbot (*Scophthalmus maximus*), grass carp (*Ctenopharyngodon Idella*), yellow perch (*Perca flavescens*) and asian seabass (*Lates calcarifer*) all have *lws1* and *lws2* located adjacent to each other on the same chromosome, with strong conservation of neighboring genes, whereas human, cattle, nile rat, western clawed frog and brown-marbled grouper all have only one *lws* gene (Fig. 2). Compared to the *lws2* gene, the zebrafish *lws1* gene has higher amino acid sequence similarity to other species. The amino acid sequence similarity between zebrafish *lws1* and *lws2* genes was 91.6%. The similarity of zebrafish *lws1* to mammals ranged from 75.6–79% and with fish from 77.6–94.4%, while the similarity of *lws2* with mammals ranged from 75-76.7% and with fish from 76.4–91.3%. (Fig. S1). Sequence comparison revealed that amino acid at position 277 of *lws2* was mutated from Y to F. According to the law of loci, the λ_{max} of LWS2 will probably be shifted towards green light, lower than that of *lws1* opsin (Fig. 3). Both zebrafish *lws1* and *lws*2 genes have six exons and five introns, with the first intron of *lws1* being longer and the 5' flanking region shorter than that of *lws*2 (Fig. S2).

Establishment of *lws1* mutant zebrafish

We employed a dual-target knockout technique, placing both targets on exon 1, which altered the base sequence of *lws1*, thereby blocking protein translation (Fig. 4A). The *lws1*-pure mutant was missing 11 bp of bases as compared to WT (Fig. 4B), and all of these deletions led in the premature termination of LWS1 protein translation with just 13 amino acids (Fig. 4C). In addition, we designed a pair of full-length primers to differentiate zebrafish *lws1* expression differences in *lws1*$^{-/-}$ mutant and WT larvae (Fig. S3). Next, we sequenced the total length of *lws1* gene in *lws1*$^{-/-}$ and

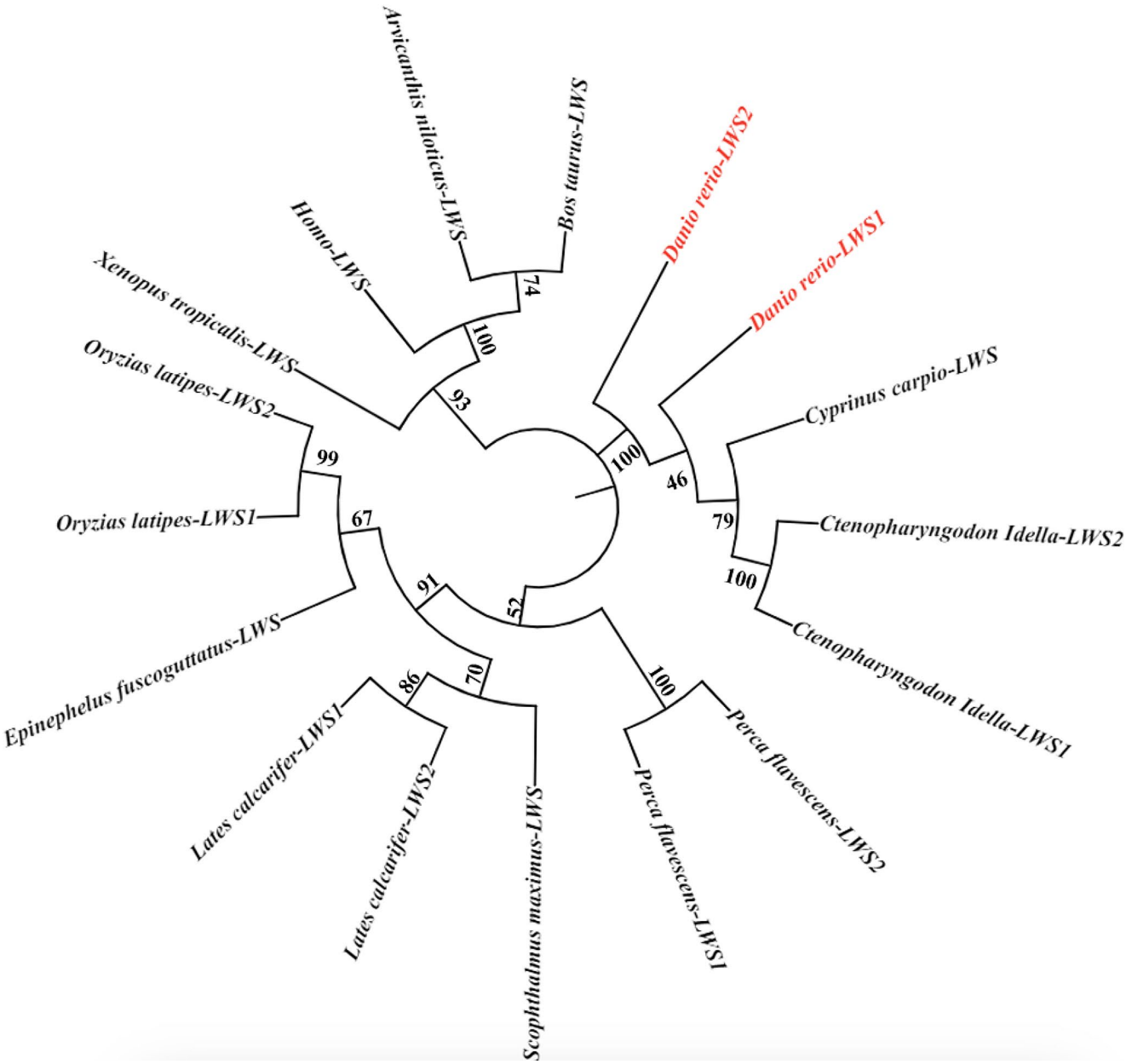

Fig. 1 Phylogenetic evolutionary tree of *lws1* and *lws2* genes in selected mammals and fish. The maximum likelihood technique was used to infer evolutionary tree analysis based on the LG+G model, and the evolutionary tree reliability scores were generated by repeating the study 500 times. The number shown at each branch node indicates the bootstrap value (%). Red emphasizes the LWS1 and LWS2 in zebrafish. Sequences are from the following species: *Danio rerio* (zebrafish), *Oryzias latipes* (Medaka), *Scophthalmus maximus* (Turbot), *Ctenopharyngodon Idella*(Grass carp), *Lates calcarifer* (Asian seabass), *Perca flavescens* (Yellow perch), *Cyprinus carpio* (Common carp), *Epinephelus fuscoguttatus* (Hybrid grouper), *Homo sapiens* (Human), *Bos taurus* (Cattle), *Arvicanthis niloticus* (Nile rat), *Xenopus tropicalis* (Western Clawed Frog). Information on species is shown in Table S1

WT larvae, which showed that the *lws1* mRNA level of the *lws1*$^{-/-}$ mutant was missing by 11 bp (Fig. S4).

Transcriptional expression of phototransduction genes and opsin genes

Real-time fluorescence quantitative PCR was used to compare the relative mRNA expression of opsin genes and phototransduction genes in WT and *lws1*$^{-/-}$larvae. The *lws1*$^{-/-}$ larvae had higher expression of the *lws2* gene, significantly higher expression of *rh2-1* and *rho* than in the WT, and significantly lower expression of *rh2-2* than in the WT ($p < 0.05$) (Fig. 5A). To investigate whether the *lws1* gene influences the phototransduction pathway, the results showed that the expression of all phototransduction

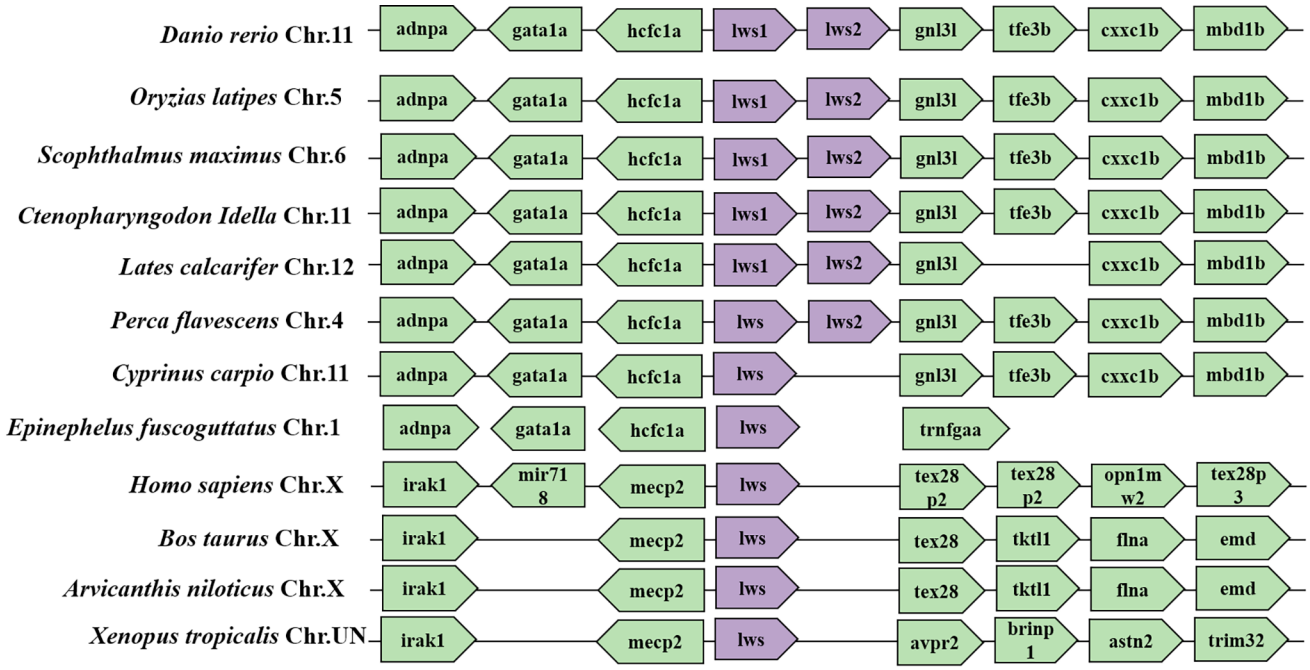

Fig. 2 Phylogenetic analysis of neighboring families. Comparison of orthologs and flanking genes of *lws1* and *lws2* loci in selected mammals and fishes. Target genes are marked with purple, and conserved neighborhood genes are marked with green

pathway genes was significantly higher in the $lws1^{-/-}$ larvae than in the WT ($p < 0.05$) (Fig. 5B).

Feeding tests and transcript levels of appetite-related genes

$Lws1^{-/-}$ zebrafish ingested significantly more paramecia than the WT (Fig. 6A, $p < 0.05$), the *agrp* gene had significantly greater relative expression than WT (Fig. 6B, $p < 0.05$), and the relative expression of the *npy*, *pomc*, and *cart* genes displayed significantly lower than the WT (Fig. 6C, $p < 0.05$).

Behavioral testing for alternating light and dark cycles

To confirm whether the $lws1^{-/-}$ mutation affects visually guided behavior in zebrafish, in this paper, we chose the cumulative distance moved per minute to describe the movement trend of zebrafish larvae during a 30 min behavioral test time and examined the movement of zebrafish during three alternating light-dark cycles. The results showed that the cumulative distance travelled by $lws1^{-/-}$ and WT larvae was small in darkness, and that the cumulative distance travelled in light increased sharply and then gradually returned to the level of the dark period (Fig. 7A). The cumulative distance moved by $lws1^{-/-}$ and WT larvae was significantly higher in the light period than the dark period (Fig. 7B, $p < 0.05$). The total travel distance of $lws1^{-/-}$ larvae within 30 min was significantly lower than that of WT (Fig. 7C, $p < 0.05$).

Behavioral response of zebrafish larvae during light-dark transitions

This experiment's photoperiod consisted of three light-on and two light-off periods, and we tracked the movements of $lws1^{-/-}$ and WT larvae for 5 s before and after light transitions (light-on or light-off). The results showed that after the lights were turned on or off, the distance traveled by zebrafish increased and then gradually slowed down. $Lws1^{-/-}$ and WT larvae became active at the instant of light on, but both were not significant (Fig. 8A). However, the cumulative distance travelled by $lws1^{-/-}$ larvae was significantly higher than that of WT when the light was switched off twice (Fig. 8B, $p < 0.05$).

Behavioral responses of zebrafish larvae to vibratory stimuli

$Lws1^{-/-}$ and WT larvae were treated to three vibrating stimuli during darkness and light, respectively, and moved aggressively at initially, before gradually returning to stillness. During darkness and light, $lws1^{-/-}$ larvae were substantially more sensitive (cumulative distance travelled) to vibratory stimuli than WT larvae (Fig. 9, $p < 0.05$).

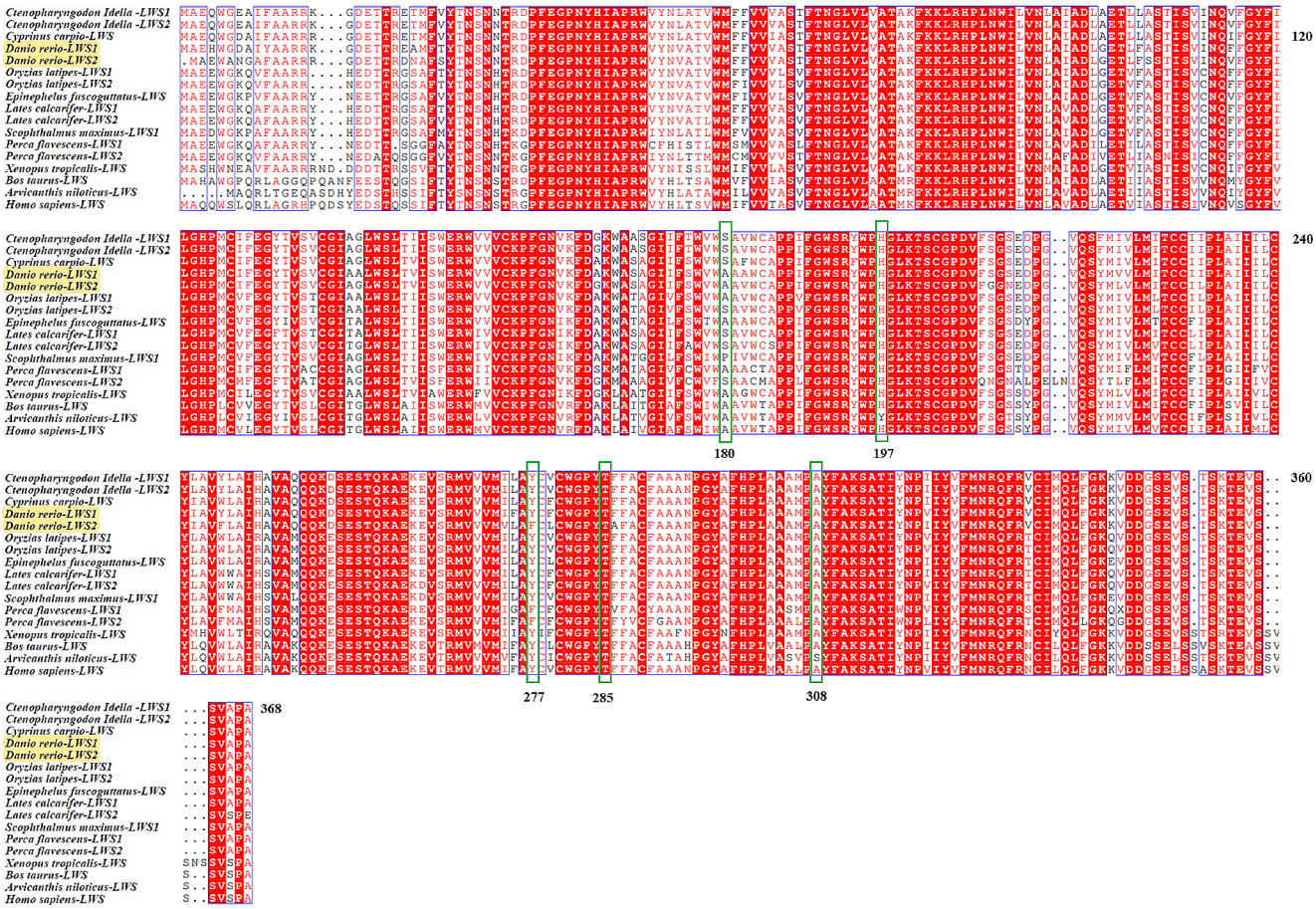

Fig. 3 ***Lws1*** **and** ***lws2*** **gene sequence alignment.** The amino acid sequences of several representative vertebrates *lws1* and *lws2* are aligned, and the red shade represents completely conserved residues. The green boxes represent five important loci in the visual genes, and the mutant loci S180A, H197Y, Y277F, T285A, and A308S (based on the human LWS retinoid number) are the main predictors of λ_{max} in the 512 ~ 560 nm range for LWS/RH2/MWS optical proteins (with vitamin A1 as the chromor)

Materials and methods

Lws gene sequence analysis in vertebrates

Species information for the vertebrates used in this experiment was obtained from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/genbank) or Ensembl (http://asia.ensembl.org/index.html), and species included: zebrafish, medaka, turbot, grass carp, asian seabass, yellow perch, common carp, hybrid grouper, human, cattle, nile rat, western clawed frog.

The names and accession numbers of the *lws* gene for all species are shown in (Table S1). Multiple sequence alignment was performed on all gene sequences using the Clustal W tool in the MEGA X software with alignment and manual adjustments (Kumar et al. 2018). The maximum likelihood technique was used to infer evolutionary tree analysis based on the LG + G model (Lv et al. 2019), and the evolutionary tree reliability scores were generated by repeating the study 500 times. Homology analysis between genomes was performed by the online software Genomicus (http://www.dyogen.ens.fr/genomicus) (Dupré and Tostivint 2014). Zebrafish *lws1* and *lws2* gene structures were analyzed by Gene Structure Display Server 2.0(http://gsds.gao-lab.org/?(Hu et al 2015).

Construction of *lws1* mutant zebrafish by CRISPR/Cas9 technology

Lws1 mutant zebrafish were constructed by CRISPR/Cas9 technology. Sequencing results for single-guide RNAs (sgRNAs) and PCR primers are shown in Supplementary Table S2. sgRNA were cloned into the pMD-18T vector and synthesized using the TranscriptAid T7 High Yield Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) for synthesis. Compounds of sgRNA (50 ng/μL) and Cas9 protein (New England Biolabs, Ipswich, MA, USA) were co-injected into wild-type embryos at the first or second cell stage of development. F_0 generation zebrafish were crossed with wild type to obtain F_1 generation zebrafish.

Fig. 4 Generation and characterization of *lws1* and *lws2* mutant zebrafish. (A, B) Design of dual targets for *lws1* based on CRISPR/Cas9 technology. Exons are indicated by yellow boxes, and single guide RNAs (sgRNAs) are labelled in exons in green and red. sgRNA sequences are highlighted in green and red boxes, and -11 bp deletions are indicated by sequencing validation. (C) Illustration of the structure of the deduced proteins of the WT and *lws1* mutants, with numbers representing the number of amino acids coding for the protein

Individuals with *lws1* heterozygous mutations were detected using the T7 Nucleic Acid Endonuclease 1 Assay (Vazyme, Nanjing, China) according to the manufacturer's instructions, followed by sequencing (Fig. S3-4). F_1 heterozygous individuals were crossed to produce F_2 pure individuals, and all experiments were performed with F_3 pure individuals. *Lws1* mutant zebrafish named *lws1*$^{-/-}$ zebrafish.

Feeding experiments with zebrafish larvae

Lipophilic tracer 4-(4-(di-decylamino) styryl)-N-methyl-pyridinium iodide (4-Di-10-ASP; Invitrogen, Carlsbad, CA, USA) was used for fluorescent labeling of paramecia cultured (Shimada et al. 2012) in our laboratory. The paramecia were suspended in 1 mL of distilled water, and then 25 mg/mL of 4-Di-10-ASP was added. After 1 h of staining, the paramecia were washed by centrifugation to remove residual 4Di-10-ASP. At 6 days post hatching (6 dph), the *lws1*$^{-/-}$ and WT larvae were fed with 4-Di-10-ASP labeled paramecia for 30 min. The intra-abdominal fluorescence signal was measured with a fluorescein filterset using the Victor2 fluorescent plate reader (PerkinElmer, Boston, MA, USA) in well scan mode. Each point's total was specified as being paramecia to the amounts of paramecia provided to each zebrafish (Shimada et al. 2012). After WT and *lws1*$^{-/-}$ zebrafish swallowed non-fluorescent paramecia for 30 min, samples were collected and analyzed for appetite gene expression.

Behavioral testing experiments

WT and *lws1*$^{-/-}$ larvae were placed in 24-well plates (wells 15.74 mm), 1 tail/well, and 12 parallel samples were set up for WT and *lws1*$^{-/-}$ larvae, and after waiting for the larvae to acclimate for 12 h, the data were processed by a behavioral monitor, the DanioVision TM (Noldus, The Netherlands), and by the accompanying EthoVision XT 14 video tracking software to process the behavioral data of larvae (Jiang et al. 2018). The behavioral test procedure of the instrument started with 5 min of dark adaptation, followed by three cycles of 5 min of darkness and 5 min of light, and one rapid and strong vibratory stimulus was given 1 min before every light transition, the surroundings were kept quiet, and the room temperature was controlled at 28 ± 0.5 °C. The distance and speed of the movement of the fish were recorded every 0.1 s, and the experiment was repeated two times.

RNA isolation and quantitative RT-PCR

Using a multi-function microplate reader, the amount of RNA was measured (Biotek, USA). Following the manufacturer's instructions, 1 µg of total RNA was reverse-transcribed using the HiScript Q RT SuperMix for qPCR

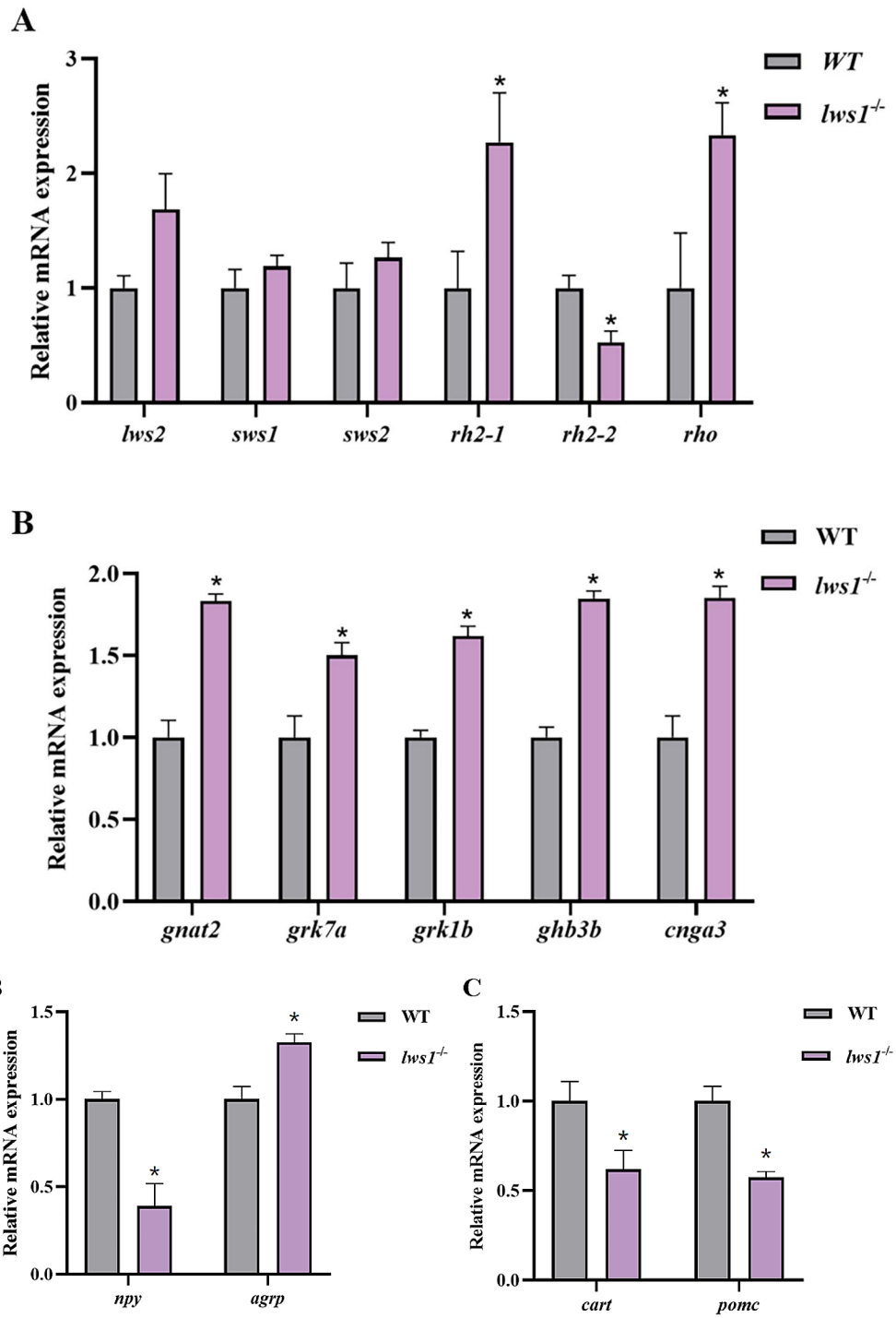

Fig. 5 Transcriptional expression of *lws1*$^{-/-}$ and WT larvae opsin genes (A) and phototransduction genes (B). The data is expressed as: mean ± standard error (mean ± SEM, n=24), with a marker (*) reflecting statistically significant differences ($p < 0.05$)

Fig. 6 Feeding and appetite gene expression in *LWS1*$^{-/-}$ and WT larvae. (A) Relative levels of ingested paramecia in the digestive tract over a 30-minute period. (B) Expression of orexigenic genes. (C) Expression of anorexigenic genes. The data is expressed as: mean ± standard error (mean ± SEM, n=24), with a marker (*) reflecting statistically significant differences ($p < 0.05$)

(+gDNA wiper) kit from Vazyme, Nanjing, China. The PCR products were detected using 1.2% agarose gel electrophoresis, which was carried out for 20 min at 120 V. The Gel Imaging System was then used to take pictures of the gel under ultraviolet light. In the following investigations, real-time PCR was used to analyse the level of gene expression. Table S3 contained details regarding relative gene primers. *β-actin* was a housekeeping gene in organisms (Lu et al. 2023). The AceQ® qPCR SYBR Green-Master Mix kit was used for the qPCR (Vazyme). The Ct ($2^{-\Delta\Delta Ct}$) value

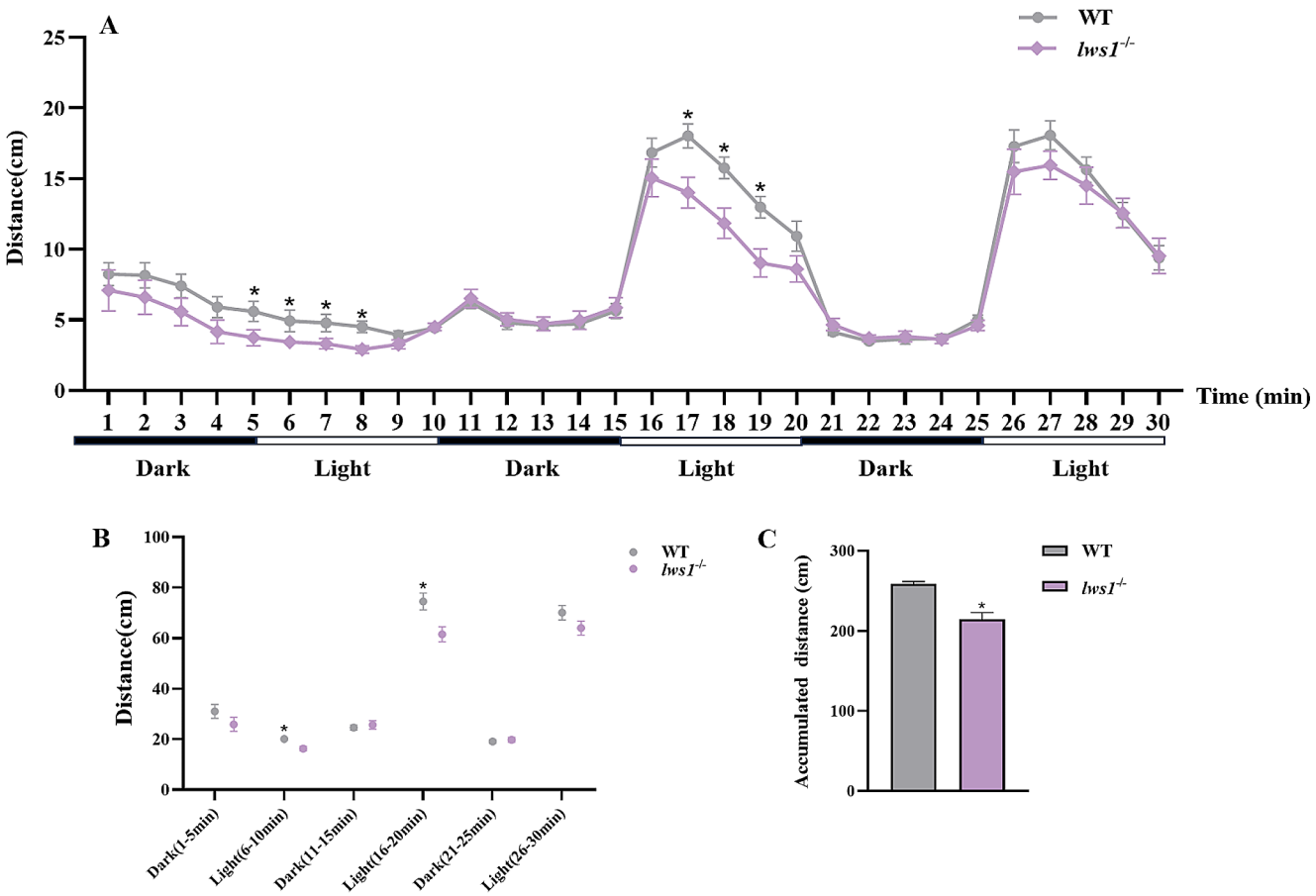

Fig. 7 Photoperiodic movements of*lws1*$^{-/-}$ and WT larvae. (A) Cumulative movement of *lws1*$^{-/-}$ and WT larvae per minute of the light cycle. (B) Cumulative distance travelled by *lws1*$^{-/-}$and WT larvae during the light or dark cycle (5 min). (C) Cumulative distance travelled over a 30 min period. The data is expressed as: mean ± standard error (mean ± SEM, n=24), with a marker (*) reflecting statistically significant differences ($p < 0.05$)

method was used to calculate the relative gene expression (Livak and Schmittgen 2001).

Data analysis

Statistical analysis was performed with SPSS 25.0 software. All data were presented as mean ± S.E.M (standard error of the mean). Differences between the three groups were analysed using one-way ANOVA and Duncan's multiple range test, and comparisons between the two groups were made using Student's T-test, with $p < 0.05$ considered statistically significant.

Discussion

The phylogeny and function of *lws* gene have been more comprehensively studied in many species, but the function played by the *lws1* gene in the phototransduction behavior of zebrafish larvae has been rarely reported. By comparing *lws* gene sequences from different species, we can see the duplication and selection events that *lws* gene go through during evolution. *Lws* is strongly conserved in fish, with distinct homologies occurring in mammals and toads. The zebrafish *lws1* gene has more homologous similarity to other species, and *lws2* appears as Y277F in the "five-site rule" (Yokoyama and Radlwimmer 2001), with its λ_{max} potentially shifted towards green light. The mRNA expression of the green light gene (*rh2-1*) was significantly increased when *lws1* was knocked down, suggesting that the *lws1* gene may be involved in the green light-sensitive response, and that when the *lws1* gene was knocked down, the expression of *rh2-1* was greatly increased due to light compensatory. Previous studies have found that osteoglossus lineages have lost the typical vertebrate green-sensitive opsin gene (*rh2*) and replaced it with a copy of the green-sensitive *LWS* opsin(Liu et al. 2019). The loss of LWS by Noctua reduces the effect of green light aiming(Liu et al. 2018; Zaccardi et al. 2006).

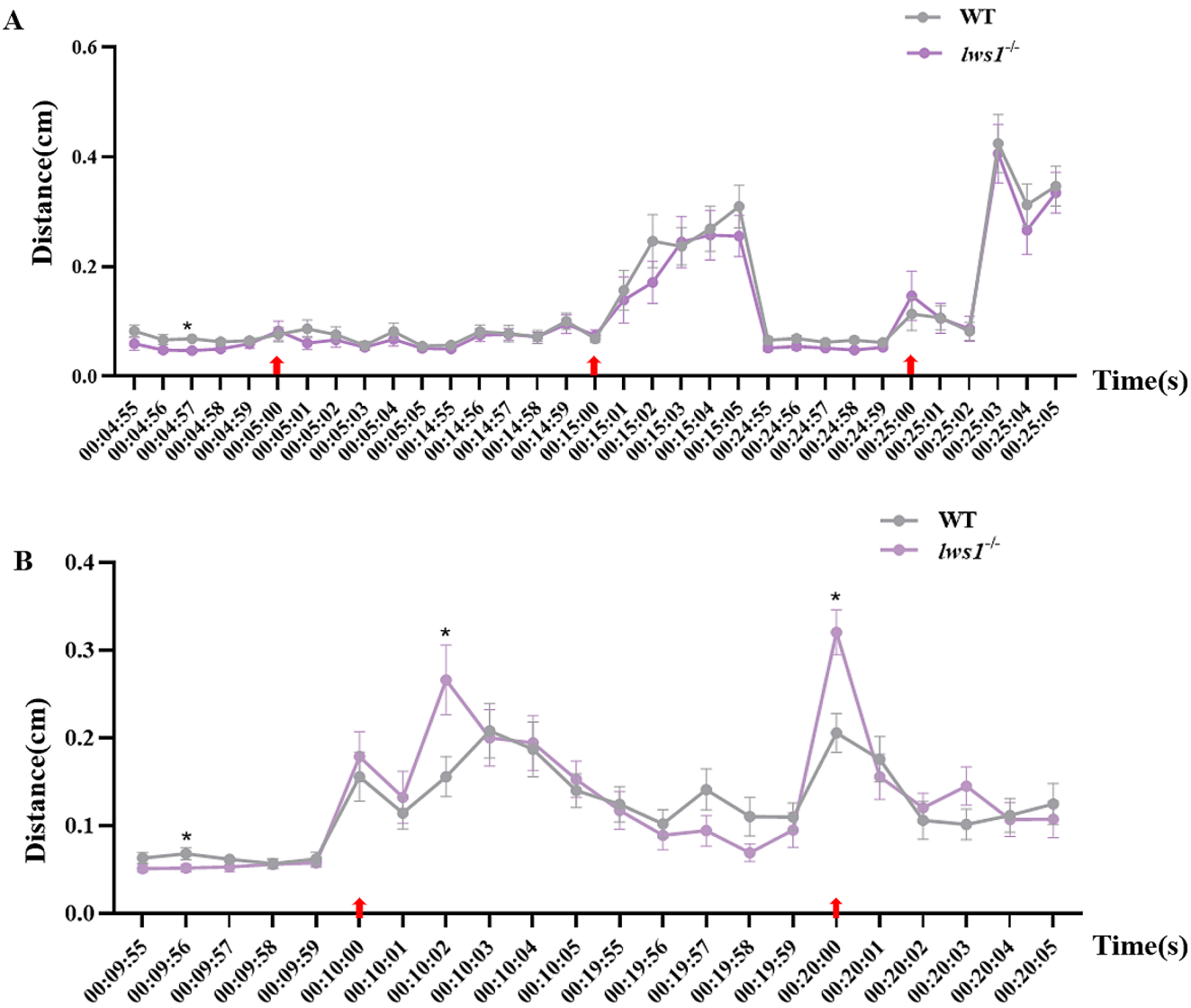

Fig. 8 Behavioral response of zebrafish larvae during light-dark transitions. *Lws1*$^{-/-}$ and WT larvae's distance travelled curves for 5s before and after switching the light on (A) or off (B). The red arrow represents the moment when the light is switched on or off. The data is expressed as: mean ± standard error (mean ± SEM, n=24), with a marker (*) reflecting statistically significant differences ($p < 0.05$)

For zebrafish, the ability to catch prey is highly dependent on the rapid development of the visual system (Bianco et al. 2011; Muto and Kawakami 2013). In feeding behavior studies, a significant increase in feeding was found in *lws1*$^{-/-}$ larvae. The increase in feeding may be related to a significant increase in the expression of green light gene (*rh2-1*) and phototransduction genes in *lws1*$^{-/-}$, suggesting that *lws1*$^{-/-}$ has high photosensitivity, which improves larval feeding success. Previous studies have found that green light promotes growth in red-finned Eastern leatherback turtles (*Takifugu rubripes*) and feeding and survival rates in haddock larvae (*Mellanogrammus aeglefinus*), while red light inhibits growth (Kim et al. 2016; Villamizar et al. 2011). In addition, the up-regulation of the appetite gene (*agrp*) and the down-regulation of the anorexia gene compared to WT further suggests that LWS1 plays an important role in the regulation of feeding in zebrafish larvae (Karakatsouli et al. 2007). Light exposure is inextricably linked to changes in hormone regulation and body metabolism in fish (Head and Malison 2000). The significantly lower *npy* gene in *lws1*$^{-/-}$ larvae than in WT may be the result of adaptive adjustments in the digestive and metabolic systems of the fish (increased secretion of digestive enzymes and hormones in response to higher intake) resulting from chronic overfeeding in *lws1*$^{-/-}$ larvae.

Light and dark motion tests and responses to light stimuli are effective methods for reflecting the integrated function of the visual pathway (Zhang 2021, Cai 2018, Guo 2021). We assessed the photosensitive behavior of the larvae after knocking down the *lws1* gene using an alternating light/dark

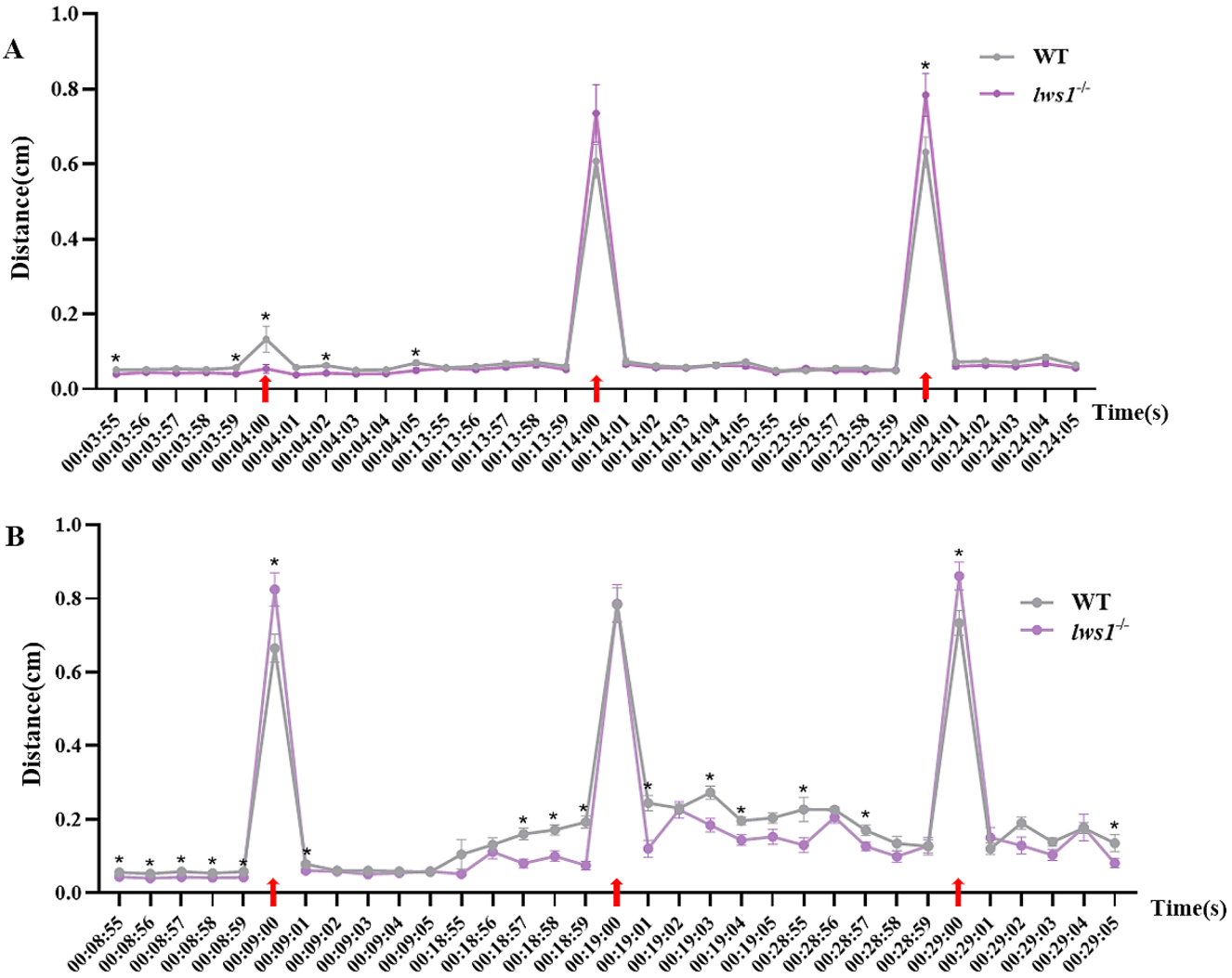

Fig. 9 Behavioral response of zebrafish larvae to vibratory stimuli. Curves of the distance travelled by zebrafish litters during the dark phase (A) and the light phase (B) as a function of time, *i.e.*, the distance travelled from 5s before to 5 s after the onset of each tapping stimulus. Red arrows represent the duration of vibratory stimulation in light or dark conditions. The data is expressed as: mean ± standard error (mean ± SEM, n=24), with a marker (*) reflecting statistically significant differences ($p < 0.05$)

behavioral test method (Hu et al. 2019). During alternating light and darkness, zebrafish larvae exhibit a range of different behavioral swimming patterns in response to environmental factors (Kalueff et al. 2013).

The cumulative distance travelled by zebrafish during the 30 min of behavioral testing was significantly lower in the *lws1*$^{-/-}$ larvae than in the WT. The cumulative distance travelled by zebrafish larvae during the dark phase was significantly lower than during the light phase, and previous studies have also found a preference for dark environments in adults (Serra et al. 1999; Tuz-Sasik et al. 2022), which larvae will strongly avoid (Steenbergen et al. 2011). It has also been observed that when zebrafish larvae are placed to light, their swimming steering angle rises as the light fades over time (Burgess and Granato 2007). As the darkness persist, this overactive steering angle levels off and the larvae begin to swim normally (Burgess and Granato 2007; MacPhail et al. 2009). However, previous experiments have reported that the cumulative distance travelled by zebrafish larvae in behavioral tests was higher in the dark and relatively lower in the light (Jarema et al. 2015; Steele et al. 2018; Leuthold et al. 2019). Cumulative differences in swimming distances between dark and light conditions in zebrafish may be related to the experimental procedure or the developmental stage of the fish, but specific factors need to be further investigated.

The cumulative distance travelled by WT and *lws1*$^{-/-}$ larvae did not change significantly between 5 s before and after switching on the light, and the distance travelled by zebrafish larvae did not change much at the moment of switching on the light because, once acclimatised to darkness, larvae show a "freezing" response to the sudden

introduction of light, which reduces their swimming distance and speed (MacPhail et al. 2009). The cumulative distance of *lws1*$^{-/-}$ larvae was significantly higher than that of WT when the light was turned off, which may be due to the greater sensitivity of *lws1*$^{-/-}$larvae to the light perception of the environment.

In addition, motor responses to stimuli in juvenile zebrafish are co-mediated by lateral hair cells and highly myelinated Mautener neurons at the back of the brain (Kohashi 2012, Mekdara 2018, Wang 2017). Under light or dark conditions, the cumulative distance of *lws1*$^{-/-}$ larvae after vibration stimulation (Banerjee et al. 2022) was significantly higher than that of WT, suggesting that *lws1* knockout may promote the function of lateral hair cells and highly myelined Mautener neurons in the rear of the brain of larva Showed increased sensitivity to the environment, but further study is needed.

In conclusion, we revealed the evolution of the zebrafish *lws* gene and the first construction of a zebrafish *lws1* mutation model, and further investigated the effect of the *lws1* gene on the visual behavior of larvae. These studies help to reveal the role of lws1 genes in the regulation of behavior and may also expand the understanding of zebrafish as a model organism.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10142-024-01333-y.

Acknowledgements No.

Author contributions Di-Mei Xu supplement and analyzed experimental data, wrote the original draft. Fa-Rui Chai designed and performed the experiment, analyzed experimental data. Ke Lu participated in some of the experiments. Xu-Fang Liang designed and supervised the experiment and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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