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Research Article

Insulin-Like Growth Factor 1 (*IGF1*) in the Chinese Soft-Shelled Turtle, *Pelodiscus sinensis*: Molecular Characterization, Expression Pattern, and Polymorphism Analysis

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Insulin-like growth Factor 1 (*IGF1*) plays a vital role in vertebrate growth, and its sexually biased expression may influence the development and evolution of sexual size dimorphism (SSD). To investigate the role of *IGF1* in regulating the growth of the Chinese soft-shelled turtle (*Pelodiscus sinensis*), this study quantified *IGF1* mRNA abundance across various tissues, phenotypes, and steroid hormone treatments using quantitative real-time polymerase chain reaction (qRT-PCR). Additionally, the polymorphism of the *IGF1* gene was analyzed and genotyped using the tetra-primer amplification refractory mutation system PCR (ARMS-PCR). The findings revealed that the full-length cDNA sequence of the *IGF1* gene (*PsIGF1*) in *P. sinensis* was 1790 bp, with the liver identified as the primary site of *PsIGF1* expression. The expression of *PsIGF1* was positively correlated with rapid growth and followed a pattern indicative of its involvement in sexually dimorphic growth. Treatment with 17α-methyltestosterone (MT) significantly increased hepatic *IGF1* gene expression, while 17β-estradiol (E_2) showed the opposite effect, suggesting that *IGF1* may be a critical factor underlying SSD in *P. sinensis*. Furthermore, a single-nucleotide polymorphism (SNP) identified in the 5′ untranslated region (UTR) of *PsIGF1* was significantly associated with growth traits in *P. sinensis* (p value < 0.05). These results provide a foundation for further research into the regulatory mechanisms of *IGF1* in the development and SSD of *P. sinensis*.

Keywords: insulin-like growth factor 1; *Pelodiscus sinensis*; sexual size dimorphism; single-nucleotide polymorphism; trait association analysis

1. Introduction

Growth is a key trait in aquaculture breeding and ranks among the most economically valuable traits for genetic improvement [1, 2]. In addition to interindividual growth differences, significant sex-specific disparities in growth rate were observed in aquatic organisms exhibiting sexual size dimorphism (SSD) [3]. This sexual growth difference provides a promising approach for optimizing aquaculture production. On one hand, the development of sex control technologies can significantly improve the yield of monosex cultures. On the other hand, understanding the mechanisms and molecular basis underlying allometric and sexual growth differences can facilitate the advancement of targeted growth control strategies, offering valuable insights for scientifically informed breeding practice [4].

The growth hormone/insulin-like growth factor 1 (GH/IGF1) axis, a primary regulatory component of the

hypothalamic–pituitary–liver axis, serves as the principal endocrine system governing individual growth. Insulin-like growth factor 1 (IGF1), a secreted growth factor with a structure resembling insulin, plays a key role in the somatotropic axis by functioning downstream of growth hormone (GH) [5]. The involvement of *IGF1* in vertebrate growth is well established, with studies consistently reporting a significant positive correlation between its expression levels and rapid growth in animals [6]. For example, elevated *IGF1* expression has been identified as a key factor driving the accelerated growth rates observed in triploid crucian carp (*Carassius auratus*) [7]. Species-specific differences in sex-biased *IGF1* expression, particularly in the liver, are believed to contribute to the evolution of SSD across species [8]. In *Cynoglossus semilaevis*, where females grow faster than males, significantly higher levels of *IGF1* mRNA were observed in females after 190 days of age, with serum *IGF1* levels in females nearly double than those in males. This finding highlights IGF1's potential role in endocrine regulation of sexually dimorphic growth [9]. The temporal and sex-specific expression of *IGF1* may be crucial in the development of SSD in reptiles. In the male-larger Madagascar ground gecko (*Paroedura picta*), high *IGF1* levels positively influence bone growth, with this effect being sex-specific and seemingly independent of sexual maturation [10]. Similar patterns have been observed in lizards: male-biased hepatic *IGF1* expression was found in male-larger brown anoles, whereas female-biased hepatic *IGF1* expression occurred in female-larger eastern fence lizards [11, 12]. These studies collectively demonstrate that the sex-biased expression of *IGF1* plays a significant role in regulating the development of SSD.

The Chinese soft-shelled turtle (*Pelodiscus sinensis*, Trionychidae, Testudines), a reptile of considerable aquacultural importance in China, is valued for its nutritional and medicinal properties [13]. Similar to most vertebrates, *P. sinensis* exhibits sexual dimorphism, characterized by morphological and physiological differences between sexes. These traits make it a promising model for studying the molecular mechanisms underlying gender-specific growth differences [14]. It is well-established that *IGF1* plays a key role in regulating vertebrate growth and may significantly impact the development and evolution of SSD [15]; however, its specific effects in turtles remain less understood. Therefore, a detailed investigation of the role and molecular regulatory mechanisms of the *IGF1* gene in both overall growth and sex-specific growth differences in *P. sinensis* could provide essential insights and a theoretical basis for understanding the mechanisms governing allometric growth and sexual dimorphism in this species.

2. Materials and Methods

2.1. Experimental Animals. An artificially bred population of Chinese soft-shelled turtles from the Hezhou Breeding Farm (Hunan, China) was selected for the study. The turtles were fed formula feed twice daily at 9:00 a.m. and 4:00 p.m. Feeding was discontinued from November to the following April during hibernation. Laboratory experiments were subsequently conducted on carefully selected cohorts of healthy adult and juvenile turtles.

2.2. Sample Collection, RNA Isolation, and First-Strand cDNA Synthesis. Tissues including liver, hypophysis, lung, muscle, spleen, kidney, heart, gonad, and intestine were collected from adult male and female *P. sinensis*. Individuals with a significant difference in body weight (BW) were selected from two populations (1-year-old and 3-year-old), which had been hatched at the same time and cultured under the same conditions, and liver tissue samples were collected. The average BW and number of samples are presented in Table S1.

Three experimental groups were established: 17β-estradiol (E_2), 17α-methyltestosterone (MT), and control (C). Each group comprised 45 individuals with an average weight of 35.42 g. The dosage of hormone administration (10 mg/kg) was selected based on a previous study [16], which reported that MT at this concentration significantly affected survival rate and represented a threshold dosage for juvenile turtles. Accordingly, both E_2 and MT were administered at 10 mg/kg in this study. To prepare the hormone solutions, 15 mg of E_2 or MT was first dissolved in 0.5 mL of absolute ethanol, followed by dilution with 4.5 mL of olive oil, yielding a final concentration of 3 μg/μL. The control group received a vehicle solution consisting of 0.5 mL of absolute ethanol diluted with 4.5 mL of olive oil.

Intramuscular injections of E_2 and MT were administered at a dosage of 10 mg/kg BW, while the control group received an equivalent volume of the vehicle solution. Liver tissues were collected at 0-, 6-, 12-, 24-, and 48 h post-injection and preserved in RNA keeper tissue stabilizer (Vazyme, R501) at −20°C. All sampling procedures were performed after anesthetizing the turtles with 150 mg/L of MS-222. The carotid artery was cut for bloodletting following anesthesia induction, ensuring that brain death is prioritized over tissue sampling. Total RNA was extracted using the total RNA Kit II (Omega, R6934), and reverse transcription was performed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fermentas, K1622, Waltham, MA, USA) following the manufacturer's protocols. The resulting cDNA products were stored at −20°C for subsequent analyses.

2.3. cDNA Amplification and Sequence Analysis of PsIGF1. Predicted mRNA sequences of the *IGF1* gene, derived from the genomic data of *P. sinensis*, were used as templates. Primers for amplification were designed using Primer Premier 6.0 software (Table 1). The 5′ and 3′ RACE fragments were amplified using the SMARTer RACE 5′/3′ Kit (TaKaRa, 634,858). PCR products were purified, cloned into a pEASY-T5 Zero Cloning Vector (TransGen Biotech, CT501), and confirmed via sequencing.

PsIGF1, the open reading frame (ORF) finder (https://www.ncbi.nlm.nih.gov/orffinder/), identified the ORF, while SMART (https://smart.embl-heidelberg.de/) was employed to analyze conserved domains. Multiple sequence alignments of the *PsIGF1* amino acid sequences from different

Table 1: Sequence information of primer.

Primer name	Primer sequence (5′-3′)	Annealing temperature (°C)	Purpose
IGF1-3′F	ATGTTTGCTTACCTTAACCAATTCTGCCACGG	68	Gene cloning
IGF1-5′R	TCAGTGTGGCGTTGAGCACGTACAGAGCGT		
qGAPDH-F	AGAACATCATTCCAGCATCCA	58	qPCR
qGAPDH-R	CTTCATCACCTTCTTAATGTCGTC		
qIGF1-F	GTCCTACATTCATCTCTTCTACCTT	58	
qIGF1-R	GCCTCTGTCTCCACATACGA		
IGF1-F	AATCTCACTGTCACTGCTAA	50	SNP detection
IGF1-R	AAGTGCAAAGTCTGGAAATG		
IGF1-NF	TTCTCATTATTTCTGCTAACC	64	SNP genotyping
IGF1-NR	GCAAAGTCTGGAAATGAATTA		
IGF1-WF	ATATGCTTCTGTGCTCTAGTT		
IGF1-WR	GTTGTTATCGGTATGTAATTGC		

species were performed using the ClustalW program (Version 2.0.10). A phylogenetic tree was constructed using MEGA 6 software and the neighbor-joining (NJ) method with 1000 bootstrap replications.

2.4. PsIGF1 Expression Across Various Tissues, Phenotypes, and Steroid Hormone Treatments. The CFX96 real-time PCR detection system was utilized to perform quantitative polymerase chain reaction (qPCR) assays, employing the SYBR Premix Ex Taq II (TaKaRa: RR820A). The reaction volume was set to 10 μL, consisting of 5 μL of SYBR qPCR mix, 0.5 μL of cDNA from each sample, 0.25 μL of primer pairs (10 μM), and 4 μL of double-distilled water. Thermal cycling conditions began with an initial incubation at 95°C for 5 min, followed by 40 cycles of 95°C for 5 s, and an annealing step at 58°C for 40 s. The expression levels of *PsIGF1* genes were normalized to the reference gene *GAPDH*. Primers were designed using Primer Premier 6.0 software (Table 1). The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression levels of the target genes. Statistical analyses were conducted using a *t*-test or one-way ANOVA in IBM SPSS Statistics 22 software, with a p value below 0.05 considered statistically significant.

2.5. Polymorphism Screening, Genotyping, and Growth Association Analysis. For SNP analyses, 210 juvenile and 269 adult individuals (105 males and 159 females) were randomly chosen from two groups of samples, which were hatched simultaneously and raised under identical rearing and management conditions for either 1 year (juvenile turtles) or 3 years (adult turtles). The genetic sex of all individuals was confirmed using previously reported sex detection techniques [17]. Growth traits, including BW, carapace length (CL), carapace width (CW), body height (BH), and apron width (AW), were measured. Genomic DNA was extracted using the EasyPure Genomic DNA Kit (Transgen Biotech, EE101, China), diluted to ~50 ng/μL with Elution Buffer, and stored at −20°C.

Comparing sequencing results from the *P. sinensis* transcriptome (accession number: SRP119729) with the reference genome (https://ftp.ensembl.org/pub/release-76/fasta/pelodiscus_sinensis/dna/) revealed a C/T mutation in the 5′ region of *PsIGF1*. This SNP was identified through PCR amplification and validated by Sanger sequencing. The tetra-primer amplification refractory mutation system PCR (ARMS-PCR) was used for genotyping. A set of reverse extension-specific primers targeting the SNP loci (IGF1-NF and IGF1-NR) and a set of reference primers flanking the SNP loci (IGF1-WF and IGF1-WR) were designed (Table 1). PCR amplification was conducted in a 25-μL reaction mixture containing 1.0 μL of genomic DNA (~50 ng/μL), 0.6 μL each of 10 μM primers IGF1-NF and IGF1-NR, 0.4 μL each of 10 μM primers IGF1-WF and IGF1-WR, 12.5 μL of instant PCR Kit 3.0 MIX, and 9.5 μL of ddH_2O. Thermal cycling conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing with a drop of 2°C per 5 cycles and 58°C for the remaining 15 cycles for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min, before storage at 4°C. The ARMS-PCR products were resolved on a 1.5% agarose gel. Homozygous genotypes were indicated by two bands, while heterozygous genotypes exhibited three bands.

A general linear model was applied to identify significant associations between genotypes and growth traits using SPSS 22.0 software. Tukey's honestly significant difference (HSD) test was conducted for further pairwise comparisons. A p value of < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of PsIGF1. The cDNA sequence of *IGF1*, including the ORF, was obtained from liver tissue of *P. sinensis* using rapid amplification of cDNA ends (RACE) PCR. The full-length cDNA of the *PsIGF1* gene spans 1790 bp, with an ORF of 462 bp encoding a protein of 153 amino acids (accession number: UVN25845.1). The 5′ untranslated region (UTR) is 232 bp long, while the 3′ UTR measures 1096 bp, including a 25 bp poly (A) tail (Figure S1).

Multiple sequence alignments of *PsIGF1* amino acid sequences with those of other vertebrates revealed a high degree of conservation across species, with minor variations mainly located in the signal peptide region. Notably, fish species showed an insertion of 12 amino acid residues in the C-terminal structure compared to other species. Similar to vertebrates, the mature IGF1 peptide consists of four regions (B, C, A, and D), featuring six conserved cysteine residues within the

core region and conserved recognition sequences for both the IGF1 receptor and binding proteins. These conserved elements underline the structurally and functionally preserved nature of IGF1 among diverse species (Figure 1(a)).

A phylogenetic tree was constructed using the NJ method to explore the evolutionary relationships of *PsIGF1* across taxa (Figure 1(b)). The IGF1 proteins grouped into six distinct clades: Chelonia, Crocodylia, Aves, Mammals, Amphibia, and Fish. These clusters, supported by high bootstrap values, highlight the close evolutionary relationship between the Chinese soft-shelled turtle and other members of the Chelonia group, consistent with their taxonomic classification.

3.2. Expression Pattern of PsIGF1 Across Various Tissues, Phenotypes, and Steroid Hormone Treatments. As shown in Figure 2(a), the highest *PsIGF1* mRNA expression was observed in the liver, followed by the male gonad. In peripheral tissues, including the spleen, lung, pituitary, ovary, intestine, muscle, kidney, and heart, *PsIGF1* mRNA expression was relatively low.

The expression of *PsIGF1* was further analyzed in different phenotypes of *P. sinensis* at juvenile and adult growth stages to investigate its role in growth regulation. During the juvenile stage, fast-growing individuals exhibited significantly higher *PsIGF1* expression levels compared to slow-growing individuals (p value < 0.01) (Figure 2(b)). At the adult stage, fast-growing individuals of both sexes showed significantly increased *PsIGF1* expression compared to their slow-growing counterparts (p value < 0.05) (Figure 2(c)). These findings indicate that *PsIGF1* may contribute to interindividual growth disparities in *P. sinensis*.

In Chelonia, there were opposite patterns of SSD and sexual differences in growth rate between *P. sinensis* (male-larger) and *Apalone spinifera* (female-larger). To further explore the relationship between *IGF1* and sexual growth differences of Chelonia, expression patterns in adult male and female liver tissues were analyzed using transcriptome data from *P. sinensis* (accession number: SRP119729) and *A. spinifera* [18]. In *P. sinensis*, male liver tissues exhibited significantly higher *IGF1* expression than female tissues (p value < 0.01). Conversely, in *A. spinifera*, female liver tissues showed significantly higher *IGF1* expression level than male tissues (p value < 0.05) (Table 2).

In response to MT, *PsIGF1* expression increased significantly at 12 h (p value < 0.05) before gradually decreasing. In contrast, E_2 treatment led to a significant downregulation of *PsIGF1* expression at 6 and 12 h (p value < 0.05) compared to the control group (Figure 3).

3.3. Polymorphism Identification, Detection, and Genotyping. A C/T mutation, designated as g.5710379C > T, was identified in the 5′ region of *PsIGF1* through transcriptome and genomic data analysis and subsequently confirmed by Sanger sequencing (Figure 4(a)). Genotyping was performed using ARMS-PCR. To address challenges associated with nonspecific amplification caused by incorporating four primers into a single reaction system, the concentration ratio of internal to external primers and the annealing temperature were adjusted during experimentation. Optimal PCR amplification for the three genotypes was achieved with an internal-to-external primer concentration ratio of 3:2, resulting in distinct bands and the highest accuracy for gene identification. The g.5710379C > T locus was genotyped in 479 individuals. Partial genotyping results are shown in Figure 4(b). Nonspecific PCR products and primer dimers were minimized, with a 634-bp external primer product serving as a positive control for amplification in all individuals. CC and TT genotypes produced an additional band at 318 bp and 357 bp, respectively, while CT genotypes exhibited bands at both 318 bp and 357 bp.

3.4. Identification of Molecular Markers Related to Growth Traits. A GLM was used to analyze associations between the SNP and growth traits using SPSS 22.0 software. Among 214 juvenile turtles (1-year-old), individuals with the TT genotype had higher average values for five growth traits compared to those with CC and CT genotypes. Significant differences in BW and BH were observed between TT and CC genotypes (p value < 0.05). In 3-year-old adult *P. sinensis*, TT genotypes similarly exhibited higher average values for all five growth traits compared to CC and CT genotypes. Male individuals showed significant differences in all five growth traits between TT and CT genotypes (p value < 0.05). However, no significant differences in average values of these traits were found among the three genotypes in female turtles (p value > 0.05) (Table 3).

4. Discussion

IGFs are a group of regulatory proteins characterized by conserved structural features. Primarily secreted by the liver, IGFs play a central role in regulating growth and development across organisms [19]. In this study, the full-length cDNA sequence of the *IGF1* gene was successfully isolated from *P. sinensis*. The precursor protein encoded a peptide chain comprising 153 amino acid residues, with the mature peptide consisting of four regions: B, C, A, and D. Within these regions, six cysteine residues formed three disulfide bonds, which are crucial for maintaining the secondary spatial structure of IGF, ensuring proper protein folding and normal physiological function. These six cysteine residues were highly conserved across species, highlighting the structural conservation of IGFs throughout evolution. Three-dimensional structural analyses using NMR and X-ray crystallography, combined with site-directed mutagenesis, highlighted the importance of Tyr24 and Tyr60 in IGF1R recognition, with Tyr31 also playing a significant role. Moreover, specific amino acids in the B domain (Positions 3, 4, 15, and 16) were essential for IGF binding to IGFBPs. Residues 49, 50, and 51 in the A domain were critical for recognition by all four IGFBPs [20, 21]. This study identified conserved receptor and binding protein recognition sites within the B and A regions of *PsIGF1*, suggesting functional similarities between *PsIGF1* in *P. sinensis* and IGFs in other vertebrates.

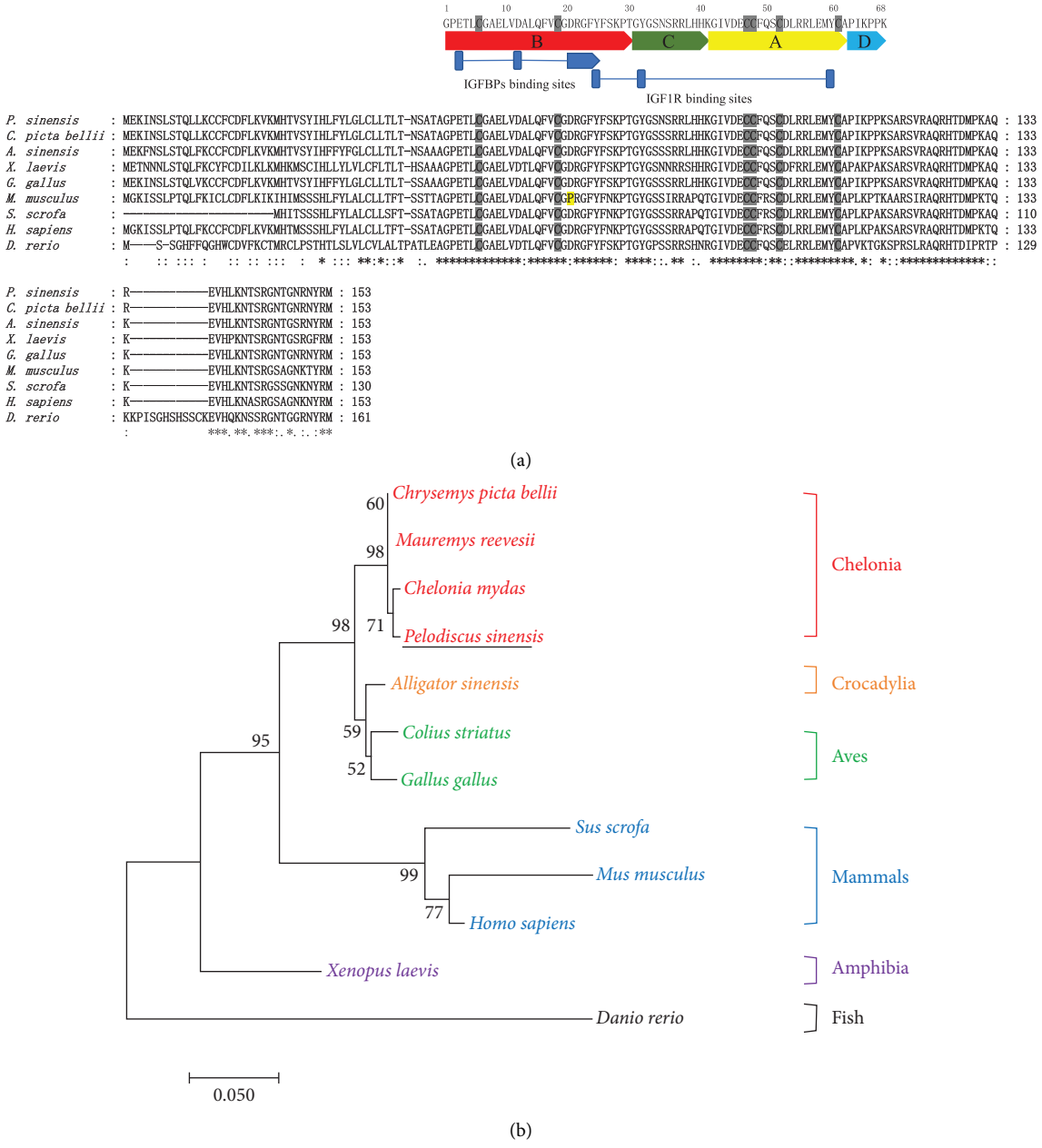

Figure 1: Multiple sequence alignment and phylogenetic tree of PsIGF1 amino acid sequence and other species. (a) Multiple alignment of amino acid sequences of IGF1. The identical, highly conserved and less conserved amino acid residues were indicated by (*), (:), and (.), respectively. The 6 conserved cysteine residues were shaded gray. The numbers on the right present the total amino acid of each protein. (b) Phylogenetic tree based on the putative amino acid of PsIGF1 and other species. The phylogenetic tree was constructed using the neighbor joining (NJ) method, and *P. sinensis IGF1* were underlined. GenBank accession number: *P. sinensis* (UVN25845.1), *Chrysemys picta bellii* (XP_005303753.1), *Mauremys reevesii* (XP_039345696.1), *Chelonia mydas* (XP_007072330.1), *Alligator sinensis* (NP_001273775.1), *Colius striatus* (KFP29405.1), *Gallus gallus* (AGG38005.1), *Sus scrofa* (XP_020946799.1), *Mus musculus* (NP_001300939.1), *Homo sapiens* (CAG46659.1), *Xenopus laevis* (NP_001156865.1), and *Danio rerio* (NP_571900.1).

The evolutionary history of turtles spans a vast timescale, reflecting their unique specialization in reptilian morphology [22]. Evidence suggests that turtles share a common ancestor with birds and crocodiles but diverged from this lineage approximately 248–267 million years ago [23]. Phylogenetic analysis of *IGF1* amino acid sequences revealed that *P. sinensis* clusters closely with other turtles, identified as its nearest evolutionary relatives. This cluster subsequently grouped with alligators and birds, followed by mammals (e.g., humans, rats, and pigs). Fish and amphibians remained the most distant relatives. These findings support the hypothesis that turtles may represent the sister group to the common ancestor of crocodiles and birds.

IGF1, a key regulator of growth and metabolism, is widely expressed across tissues, with the liver being the primary source of circulating *IGF1*, produced by hepatocytes

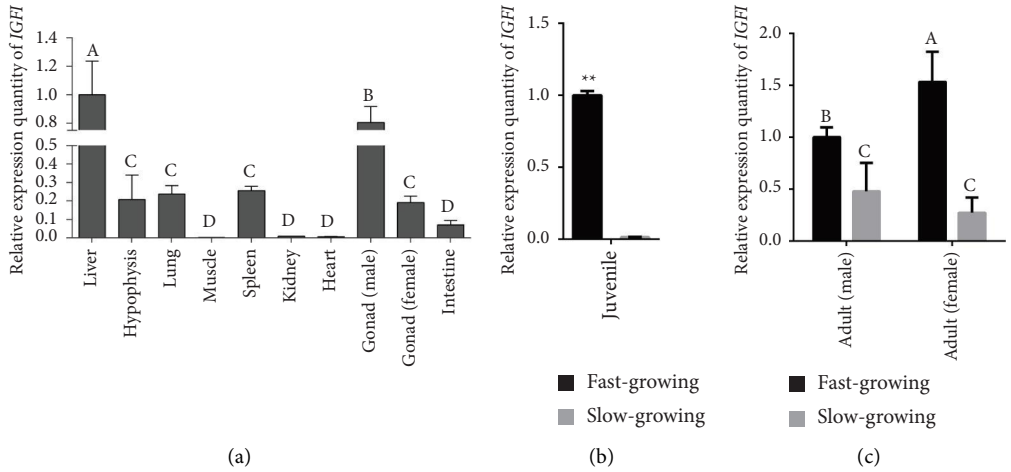

Figure 2: Expression pattern of *PsIGF1*. (a) Relative expression of *IGF1* gene in adult turtle tissues of *P. sinensis*. The relative expression level was represented by ratio, and all data were represented by mean ± SEM. Different letters indicated significant differences (p value < 0.05). (b) Relative expression of *PsIGF1* in different phenotypes at the juvenile stage. Data are presented as mean ± SEM. Significant differences between phenotypes are indicated by ** (p value < 0.01). (c) Relative expression of *PsIGF1* in different phenotypes at the adult stage. Data are presented as mean ± SEM. Different uppercase letters indicate significant differences among phenotypes (p value < 0.05).

Table 2: Expression of *IGF1* mRNA in male and female liver tissue of *P. sinensis* and *A. spinifera*.

Species	Log_2 (foldchange) (male vs. female)	p value
Pelodiscus sinensis	6.80	0.0023**
Apalone spinifera	−1.46	0.0386*

*p value < 0.05.
**p value < 0.01.

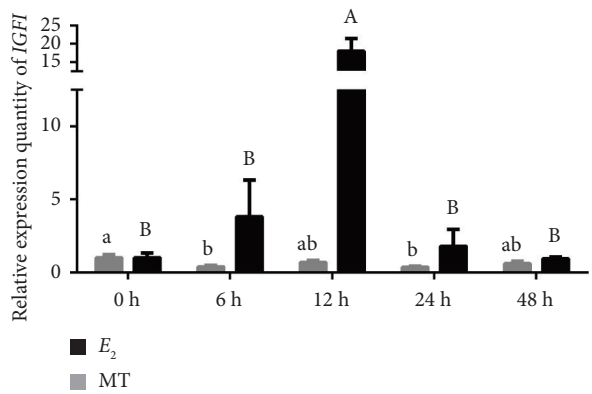

Figure 3: Relative expression level of the *PsIGF1* gene in juveniles *P. sinensis* treated with 10 mg/kg E_2/MT. The relative expression level was represented by ratio, and all data were represented by mean ± SEM. Different lowercase letters (a, b) above bars indicate significant differences (p value < 0.05) among time points within the E_2 treatment group. Different uppercase letters (A, B) indicate significant differences (p value < 0.05) among time points within the MT treatment group.

[24]. In the Chinese soft-shelled turtle *P. sinensis*, the liver was identified as the main site for *IGF1* expression, consistent with observations in other species [25]. Muscle tissue is another organ capable of producing *IGF1*, and studies have shown that *IGF1* effectively stimulates and supports muscle cell differentiation, thereby contributing to skeletal muscle development. Additionally, a positive correlation has been reported between *IGF1* levels and increased BW [26]. However, minimal *PsIGF1* expression was detected in the muscle tissue of *P. sinensis*, suggesting a limited role for *IGF1* in muscle tissue development and myocyte proliferation in adult turtles. This finding may reflect the species' characteristic slow growth rate.

The *IGF1* gene occupies a central position within the GH-IGF1 axis, functioning as a mediator of GH to promote animal growth. A strong positive correlation has been reported between *IGF1* expression levels and rapid growth in animals. For example, in *C. semilaevis*, female fish exhibited significantly higher serum IGF1 concentrations than males, and both female fish and supergiant male fish displayed elevated hepatic *IGF1* expression compared to typical males [9]. This study similarly found that fast-growing turtles at various growth stages showed significantly higher *PsIGF1* expression levels than slow-growing individuals. These findings suggest that the expression of *PsIGF1* is positively correlated with BW gain, highlighting its role as a growth-promoting factor in *P. sinensis*.

SSD is prevalent among reptiles and is often accompanied by sex-specific differences in growth patterns [27]. Interestingly, the direction of SSD can vary, even among closely related species. Research indicates that in nonavian reptiles, amphibians, and fish, sex-specific growth differences are primarily driven by differential gene expression during development, rather than directly by sex-linked genes [28]. Previous studies on lizards have highlighted male-biased liver *IGF1* expression in the male-larger *Anolis sagrei* [11], whereas female-biased liver *IGF1* expression was observed in the female-larger *Sceloporus undulatus* [12]. These findings suggest that *IGF1* expression may play a significant role in the development and evolution of sexual dimorphism in size among squamate reptiles. Furthermore,

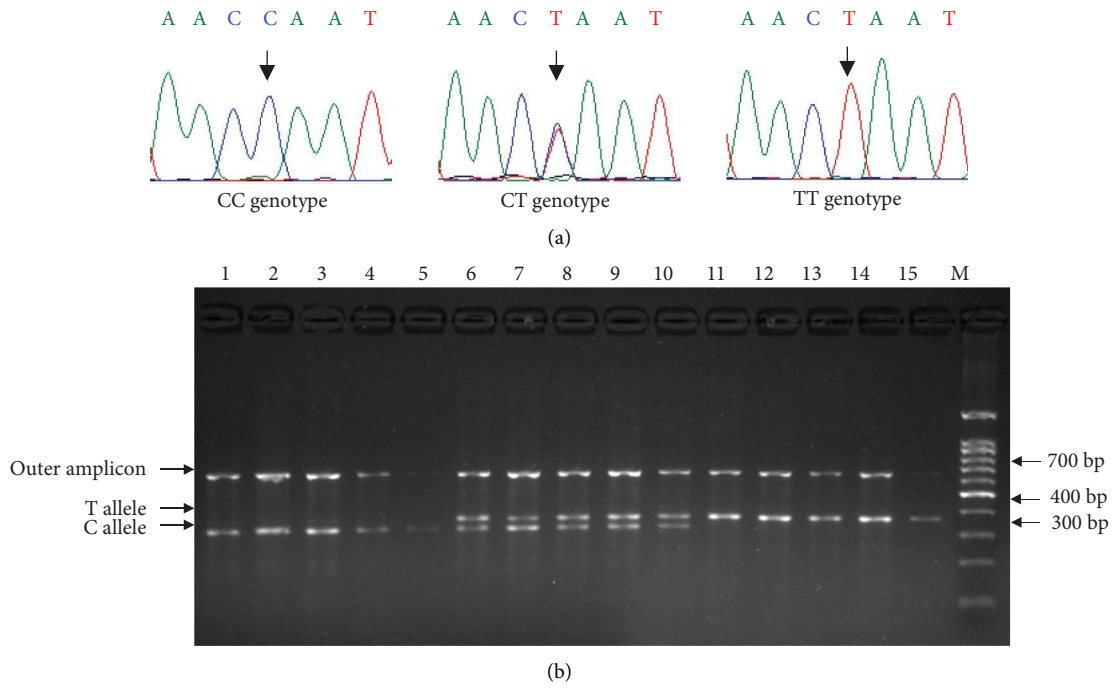

FIGURE 4: The genotypes and typing results of g.5710379C > T. (a) DNA sequencing map of SNP. (b) The result of ARMS-PCR. 1–5: CC genotype, 6–10: CT genotype, 11–15: TT genotype, and M: 100 bp marker. The image has been cropped to remove the white space surrounding the gel, while retaining the integrity of the original gel content.

TABLE 3: Association analysis results between genotype and growth-related traits.

SNP site	Age	Sex	Genotype	Number	BW (g)	CL (mm)	CW (mm)	BH (mm)	AW (mm)
g.5710379C > T	1-year-old	Mix	CC	76	32.85 ± 2.72^{b}	54.21 ± 1.61	49.45 ± 1.26	19.64 ± 0.64^{b}	7.82 ± 0.28
			CT	105	36.11 ± 2.27^{ab}	55.68 ± 1.29	50.84 ± 1.06	20.64 ± 0.58^{ab}	7.98 ± 0.24
			TT	29	43.69 ± 4.81^{a}	58.99 ± 2.54	53.83 ± 2.07	22.13 ± 1.13^{a}	8.77 ± 0.49
	3-year-old	Male	CC	46	443.53 ± 27.4^{ab}	145.52 ± 3.98^{ab}	123.76 ± 3.08^{ab}	45.01 ± 1.15^{ab}	24.34 ± 0.79^{ab}
			CT	47	404.58 ± 32.06^{a}	138.76 ± 4.51^{a}	117.69 ± 3.29^{a}	43.36 ± 1.39^{a}	23.42 ± 0.81^{a}
			TT	12	533.88 ± 39.39^{b}	160.2 ± 4.54^{b}	133.28 ± 3.64^{b}	48.95 ± 2.23^{b}	27.89 ± 1.2^{b}
		Female	CC	65	354.06 ± 23.89	131.2 ± 3.39	113.87 ± 2.66	42.06 ± 1.19	22.15 ± 0.71
			CT	77	324.98 ± 21.72	126.05 ± 3.11	109.59 ± 2.42	40.59 ± 0.99	21.15 ± 0.57
			TT	17	282.42 ± 39.33	120.51 ± 6.37	105.87 ± 5.04	38.07 ± 1.95	20.57 ± 1.17

Note: Data in the table are the mean ± SEM. The phenotypic traits were body weight (BW), carapace length (CL), carapace width (CW), body height (BH), and apron width (AW). Different lowercase letters within a column indicate significant differences (p value < 0.05).

studies have identified a significant increase in *IGF1* expression in males during the period when dimorphism develops, with high *IGF1* levels positively influencing bone growth, contributing to male-biased SSD in lizards [10]. In this study, species with opposing SSD patterns, male-larger *P. sinensis* and female-larger *A. spinifera*, exhibited correlations between *IGF1* expression levels and pronounced growth. These findings suggest that *IGF1* expression is positively correlated with rapid growth associated with SSD in Chelonia. This supports the hypothesis that differential expression of *IGF1* may be a critical factor driving gender-specific developmental processes, contributing to the occurrence of SSD in these species.

Sexual dimorphism becomes increasingly pronounced during the gonadal development stage following sex differentiation, likely influenced by variations in the secretion levels of sex steroid hormones. In lizards, the development of SSD has been attributed to circulating levels of male gonadal androgens, which promote growth in male-larger species and suppress it in female-larger species [12]. The regulation of growth by sex hormones may occur through modulation of *IGF1* gene expression, as suggested by related studies [29]. In male-larger species, testosterone (T) enhances the activity of the somatotrophic axis, increasing the expression of hepatic *IGF1* and peripheral *IGF1* receptors, whereas E_2 exerts the opposite effect. In the present study, a significant upregulation of *PsIGF1* expression was observed 12 h after treatment with MT in juvenile *P. sinensis*, while significant downregulation occurred at 6 and 24 h following treatment with E_2 (p value < 0.05). Given the positive correlation between *PsIGF1* expression levels and growth rates in the SSD of *P. sinensis*, it was hypothesized that sex hormones

may regulate growth by influencing *PsIGF1* expression. These findings suggest that the complex interaction between *PsIGF1* and sex steroid hormones could play a significant role in driving sexual growth differences. However, the precise regulatory mechanisms underlying this interaction require further investigation.

The role of *IGF1* in regulating growth and development has been linked to polymorphisms associated with growth performance across various species, including goose [26], buffalo [30], pig [31], Atlantic salmon (*Salmo salar* L.) [32], striped catfish (*Pangasianodon hypophthalmus*) [33], and European sea bass (*Dicentrarchus labrax, L.*) [34]. In the current study, a SNP, g.5710379C > T, was identified in the 5′ UTR region of the *PsIGF1* gene. Polymorphisms in this region can alter transcription factor binding sites, potentially affecting gene expression and mRNA abundance [35, 36]. Correlation analysis revealed that the g.5710379C > T genotype was significantly associated with two growth traits, BW and BH, in juvenile *P. sinensis* (p value < 0.05). At the adult stage, this SNP was significantly correlated with all five growth traits in male turtles (p value < 0.05), while no significant correlations were observed in females. A similar pattern was reported in broiler chickens, where an SNP within the 5′ UTR of *IGF1* was significantly associated with average weight, with its effect being more pronounced in males than in females [37]. The regulation of sex differences in gene expression has been shown to be conserved across vertebrates. For instance, a study on zebrafish using CRISPR/Cas9 gene editing revealed that loss-of-function mutations in the *IGF1* gene resulted in slowed growth rates in both male and female zebrafish. However, the regulatory mechanisms exhibited sex-specific differences [38]. The g.5710379C > T SNP was significantly associated with growth traits in male *P. sinensis* and could serve as a valuable molecular marker for assisted genetic breeding programs focused on males. Overall, *IGF1* is a promising candidate gene for improving the breeding of *P. sinensis*, with evidence suggesting potential sex-specific regulatory mechanisms influencing its expression and effects.

5. Conclusion

This study successfully isolated the full-length cDNA sequence of the *IGF1* gene from *P. sinensis*, spanning 1790 bp and encoding a peptide chain of 153 amino acid residues. Comparative amino acid analysis revealed a highly conserved structural framework and physiological functionality of IGF1 across various species. The expression level of *PsIGF1* showed a positive correlation with rapid growth, highlighting its role as a growth-promoting factor in *P. sinensis*. The *PsIGF1* expression pattern also aligned with its involvement in sexually dimorphic growth. Hepatic *IGF1* gene expression was upregulated by MT and downregulated by E_2, suggesting that differential *IGF1* expression may play a key role in gender-specific developmental processes in *P. sinensis*. Additionally, an SNP located in the 5′ UTR region of *PsIGF1* was found to be significantly correlated with growth traits (p value < 0.05). This SNP could serve as a valuable genetic marker for improving growth performance in MAS programs for *P. sinensis*. These findings provide important insights into the regulatory mechanisms underlying SSD and offer a basis for advancing genetic improvement of growth traits in *P. sinensis*.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Statement

All turtles involved in this study were sourced from a licensed aquaculture facility. All samplings and procedures were carried out in accordance with relevant guidelines of the Animal Protection Laboratory Animal Regulations and approved by the Animal Welfare Committee of Hunan Agricultural University, China (No. 21-0032). All experimental procedures adhered to the relevant guidelines and regulations. The informed consent was obtained from all owners involved in the study.

Disclosure

All listed authors have approved the manuscript for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Dan Zeng: experimental design and conduction, data analysis, and writing manuscript. Yangyang Tu, Gongwei Sun, and Mengying Chen: sample collection and data validation. Yunsheng Zhang: revising manuscript. Xiaoqing Wang and Liangguo Liu: experimental design and revising the final manuscript.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Table S1: The weight distribution for different age stage turtles. Figure S1: The full-length cDNA and deduced amino acid sequences of *PsIGF1*. The CDS was shown in capital letters, whereas the 5′and 3′ UTR were shown in lowercase letters. The deduced amino acid sequence was shown by single-letter code of amino acid below the CDS. Stop codon "TGA" at the end of ORF was marked with an asterisk. The

putative polyadenylation signal was boxed. The domain was shaded and shown by bent arrows below. The mutation was marked with "▲."

References

[1] C. Zhang, H. Wen, Y. Zhang, K. Zhang, X. Qi, and Y. Li, "First Genome-Wide Association Study and Genomic Prediction for Growth Traits in Spotted Sea Bass (*Lateolabrax maculatus*) Using whole-genome Resequencing," *Aquaculture* 566 (2023): 739194, https://doi.org/10.1016/j.aquaculture.2022.739194.

[2] C. Zhang, Y. Zhang, C. Liu, et al., "Genome-Wide Association Study and Genomic Prediction for Growth Traits in Spotted Sea Bass *(Lateolabrax maculatus*) Using Insertion and Deletion Markers," *Animal Research and One Health* 2, no. 4 (2024): 400–416, https://doi.org/10.1002/aro2.87.

[3] Y. Zhang, W. Zhang, Y. Jian, et al., "Identification of Candidate Genes and Pathways Involved in the Establishment of Sexual Size Dimorphism in the Olive Flounder (*Paralichthys olivaceus*) Using RNA-seq," *Aquaculture* 595 (2025): 741604, https://doi.org/10.1016/j.aquaculture.2024.741604.

[4] K. Rjiba, S. Mougou-Zerelli, I. H. Hamida, et al., "Additional Evidence for the Role of Chromosomal Imbalances and *SOX8, ZNRF3* and *HHAT* Gene Variants in Early Human Testis Development," *Reproductive Biology and Endocrinology* 21, no. 1 (2023): 2, https://doi.org/10.1186/s12958-022-01045-7.

[5] T. S. Schwartz and A. M. Bronikowski, "Evolution and Function of the Insulin and Insulin-like Signaling Network in Ectothermic Reptiles: Some Answers and More Questions," *Integrative and Comparative Biology* 56, no. 2 (2016): 171–184, https://doi.org/10.1093/icb/icw046.

[6] J. Lodjak and S. Verhulst, "Insulin-Like Growth Factor 1 of Wild Vertebrates in a life-history Context," *Molecular and Cellular Endocrinology* 518 (2020): 110978, https://doi.org/10.1016/j.mce.2020.110978.

[7] H. Zhong, Y. Zhou, S. Liu, et al., "Elevated Expressions of GH/IGF Axis Genes in Triploid Crucian Carp," *General and Comparative Endocrinology* 178, no. 2 (2012): 291–300, https://doi.org/10.1016/j.ygcen.2012.06.006.

[8] N. M. Tosto, E. R. Beasley, B. Wong, J. E. Mank, and S. P. Flanagan, "The Roles of Sexual Selection and Sexual Conflict in Shaping Patterns of Genome and Transcriptome Variation," *Nature Ecology & Evolution* 7 (2023): 981–993, https://doi.org/10.1038/s41559-023-02019-7.

[9] Q. Ma, S. Liu, Z. Zhuang, et al., "Molecular Cloning, Expression Analysis of Insulin-like Growth Factor I (IGF-I) Gene and IGF-I Serum Concentration in Female and Male Tongue Sole (*Cynoglossus semilaevis*)," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 160, no. 4 (2011): 208–214, https://doi.org/10.1016/j.cbpb.2011.08.008.

[10] B. Meter, L. Kratochvíl, L. Kubička, and Z. Starostová, "Development of male-larger Sexual Size Dimorphism in a Lizard: IGF1 Peak Long After Sexual Maturity Overlaps with Pronounced Growth in Males," *Frontiers in Physiology* 13 (2022): 917460, https://doi.org/10.3389/fphys.2022.917460.

[11] R. M. Cox, C. L. Cox, J. W. McGlothlin, et al., "Hormonally Mediated Increases in sex-biased Gene Expression Accompany the Breakdown of between-sex Genetic Correlations in a Sexually Dimorphic Lizard," *The American Naturalist* 189, no. 3 (2017): 315–332, https://doi.org/10.1086/690105.

[12] C. A. Duncan, W. S. Cohick, and H. B. John-Alder, "Testosterone Reduces Growth and Hepatic *IGF-1* Mrna in a female-larger Lizard, *Sceloporus Undulatus*: Evidence of an Evolutionary Reversal in Growth Regulation," *Integrative Organismal Biology* 2, no. 1 (2020): obaa036, https://doi.org/10.1093/iob/obaa036.

[13] D. Zeng, G. Xiong, X. Wang, et al., "Transcriptome Analysis and Identification of Genes and Single-Nucleotide Polymorphisms Associated with Growth Traits in the Chinese Soft-Shelled Turtle, *Pelodiscus sinensis*," *Journal of the World Aquaculture Society* 52, no. 4 (2021): 913–931, https://doi.org/10.1111/jwas.12780.

[14] D. Zeng, M. Chen, J. Zeng, et al., "Whole-Genome Resequencing Reveals Novel Sex-Related Markers and Candidate Gene in the Chinese Soft-Shelled Turtle (*Pelodiscus sinensis*)," *Journal of the World Aquaculture Society* 55, no. 4 (2024): 13069, https://doi.org/10.1111/jwas.13069.

[15] C. L. Cox, M. L. Logan, D. J. Nicholson, et al., "Species-Specific Expression of growth-regulatory Genes in 2 Anoles with Divergent Patterns of Sexual Size Dimorphism," *Integrative Organismal Biology* 4, no. 1 (2022): obac025, https://doi.org/10.1093/iob/obac025.

[16] H. Shen, *Effects of Feeding Steroid Hormones on the Growth, Serum Sex Hormones and Gonad Development of Pelodiscus sinensis Juvenile* (Xinjiang University, 2019).

[17] X. Zhou, H. Luo, D. Zeng, et al., "Sex-Relevant Genes in the Embryo Stage of Chinese soft-shelled Turtles as Revealed by RNA-Seq Analysis," *Animal Biology* 72, no. 2 (2022): 133–151, https://doi.org/10.1163/15707563-bja10073.

[18] Z. Luo, X. Li, D. Zeng, P. Wang, N. Peng, and X. Wang, "RNA-seq Transcriptome Analysis and growth-related Gene Screening of *Apalone spinifera*," *Genomics and Applied Biology* 38 (2019): 1480–1487.

[19] D. LeRoith and S. Yakar, "Mechanisms of Disease: Metabolic Effects of Growth Hormone and Insulin-like Growth Factor 1," *Nature Clinical Practice Endocrinology & Metabolism* 3 (2007): 302–310, https://doi.org/10.1038/ncpendmet0427.

[20] H. Duval, K. Rousseau, G. Eliès, et al., "Cloning, Characterization, and Comparative Activity of Turbot IGF-I and IGF-II," *General and Comparative Endocrinology* 126, no. 3 (2002): 269–278, https://doi.org/10.1016/s0016-6480(02)00002-3.

[21] A. Denley, L. Cosgrove, G. Booker, J. Wallace, and B. Forbes, "Molecular Interactions of the IGF System," *Cytokine & Growth Factor Reviews* 16, no. 4-5 (2005): 421–439, https://doi.org/10.1016/j.cytogfr.2005.04.004.

[22] R. R. Schoch, H. Sues, and R. Benson, "The Origin of the Turtle Body Plan: Evidence from Fossils and Embryos," *Palaeontology* 63, no. 3 (2020): 375–393, https://doi.org/10.1111/pala.12460.

[23] Z. Wang, J. Pascual-Anaya, A. Zadissa, et al., "The Draft Genomes of soft-shell Turtle and Green Sea Turtle Yield Insights into the Development and Evolution of the Turtle-specific Body Plan," *Nature Genetics* 45, no. 6 (2013): 701–706, https://doi.org/10.1038/ng.2615.

[24] R. D. Kineman, M. del Rio-Moreno, and A. Sarmento-Cabral, "40 YEARS of IGF1: Understanding the Tissue-specific Roles of IGF1/IGF1R in Regulating Metabolism Using the Cre/loxP System," *Journal of Molecular Endocrinology* 61, no. 1 (2018): T187–T198, https://doi.org/10.1530/jme-18-0076.

[25] M. Gao, Y. Chen, H. Fan, et al., "Transgenerational Effects on the Gene Transcriptome of Chicken Liver," *Animal Advances* 1 (2024): 0, https://doi.org/10.48130/animadv-0024-0003.

[26] J. Tang, M. Guo, J. Fu, et al., "Polymorphism Analysis and Expression Patterns of the *IGF1* Gene in the Shitou Goose," *Archives of Animal Breeding* 64, no. 2 (2021): 315–323, https://doi.org/10.5194/aab-64-315-2021.

[27] C. Manicom, R. Alford, T. W. Schoener, and L. Schwarzkopf, "Mechanisms Causing Variation in Sexual Size Dimorphism in Three Sympatric, Congeneric Lizards," *Ecology* 95, no. 6 (2014): 1531–1544, https://doi.org/10.1890/13-0693.1.

[28] A. Kostmann, L. Kratochvil, and M. Rovatsos, "Poorly Differentiated XX/XY Sex Chromosomes Are Widely Shared Across Skink Radiation," *Proceedings of the Royal Society B: Biological Sciences* 288, no. 1943 (2021): 20202139, https://doi.org/10.1098/rspb.2020.2139.

[29] K. M. Reindl and M. A. Sheridan, "Peripheral Regulation of the Growth hormone-insulin-like Growth Factor System in Fish and Other Vertebrates," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 163, no. 3-4 (2012): 231–245, https://doi.org/10.1016/j.cbpa.2012.08.003.

[30] M. A. El-Magd, A. A. Saleh, A. A. Nafeaa, S. M. El-Komy, and M. A. Afifi, "Polymorphisms of the *IGF1* Gene and Their Association with Growth Traits, Serum Concentration and Expression Rate of *IGF1* and *IGF1R* in Buffalo," *Journal of Zhejiang University-Science B* 18, no. 12 (2017): 1064–1074, https://doi.org/10.1631/jzus.b1600573.

[31] P. Niu, S. Kim, B. Choi, T. Kim, J. Kim, and K. Kim, "Porcine Insulin-like Growth Factor 1 (IGF1) Gene Polymorphisms Are Associated with Body Size Variation," *Genes & Genomics* 35, no. 4 (2013): 523–528, https://doi.org/10.1007/s13258-013-0098-0.

[32] H. Y. Tsai, A. Hamilton, D. R. Guy, and R. D. Houston, "Single Nucleotide Polymorphisms in the Insulin-like Growth Factor 1 (*IGF1*) Gene Are Associated with growth-related Traits in Farmed Atlantic Salmon," *Animal Genetics* 45, no. 5 (2014): 709–715, https://doi.org/10.1111/age.12202.

[33] T. T. H. Tran, H. T. Nguyen, B. T. N. Le, P. H. Tran, S. Van Nguyen, and O. T. P. Kim, "Characterization of Single Nucleotide Polymorphism in *IGF1* and *IGF1R* Genes Associated with Growth Traits in Striped Catfish (*Pangasianodon Hypophthalmus* sauvage, 1878)," *Aquaculture* 538 (2021): 736542, https://doi.org/10.1016/j.aquaculture.2021.736542.

[34] E. Özcan Gökçek and R. Işık, "Associations Between Genetic Variants of the Insulin-like Growth Factor I (IGF-I) Gene and Growth Traits in European Sea Bass (*Dicentrarchus Labrax, L.*)," *Fish Physiology and Biochemistry* 46, no. 3 (2020): 1131–1138, https://doi.org/10.1007/s10695-020-00779-8.

[35] D. Robledo, C. Fernandez, M. Hermida, et al., "Integrative Transcriptome, Genome and Quantitative Trait Loci Resources Identify Single Nucleotide Polymorphisms in Candidate Genes for Growth Traits in Turbot," *International Journal of Molecular Sciences* 17, no. 2 (2016): 243, https://doi.org/10.3390/ijms17020243.

[36] Z. Zeinaddini Meymand, S. Yeganeh, G. Rahimi-Mianji, and A. Farhadi, "Insulin-Like Growth Factor I Gene Polymorphism Associated with Growth Traits in Beluga (*Huso Huso*) Fish," *Iranian Journal of Fisheries Sciences* 16 (2017): 1257–1266.

[37] A. Al-Hassani, D. Al-Hassani, and I. A. Abdul-Hass, "Association of Insulin-like Growth factor-1 Gene Polymorphism at 279 Position of the 5′ UTR Region with Body Weight Traits in Broiler Chicken," *Asian Journal of Poultry Science* 9, no. 4 (2015): 213–222, https://doi.org/10.3923/ajpsaj.2015.213.222.

[38] N. Zeng, J. Bao, T. Shu, et al., "Sexual Dimorphic Effects of *igf1* Deficiency on Metabolism in Zebrafish," *Frontiers in Endocrinology* 13 (2022): 879962, https://doi.org/10.3389/fendo.2022.879962.