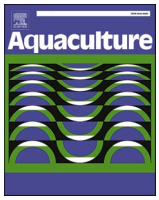
Aquaculture 586 (2024) 740778

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Identification of SNPs and candidate genes associate with growth performance in all-female mandarin fish (*Siniperca chuatsi*) by a genome-wide association study

Dingrui Liu [a,b,1], Jin Zhang [a,1], Zhenjiang Zou [a], Chen Long [a], Jiaqi Lin [a], Junyan Zeng [a], Jingpeng Hou [a], Linqiang Han [c], Yanlin Jiang [d], Shuisheng Li [a,*], Yong Zhang [a,b,*]

[a] *State Key Laboratory of Biocontrol and School of Life Sciences, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Guangdong Provincial Key Laboratory of Aquatic Economic Animals and Guangdong Provincial Engineering Technology Research Center for Healthy Breeding of Important Economic Fish, Sun Yat-Sen University, Guangzhou 510275, China*

[b] *Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266373, China*

[c] *Guangdong Liangshi Aquatic Seed Industry Co., Ltd, Foshan 528100, China*

[d] *Guangzhou Nansha Fishery Industrial Park Co., Ltd., Guangzhou 510000, China*

ARTICLE INFO

Keywords:
Mandarin fish
GWAS
Growth
SNP
Selective phenotyping

ABSTRACT

The all-female mandarin fish (Dinggui I, *Siniperca Chuatsi*) was recently approved by Ministry of Agriculture and Rural Affairs of the People's Republic of China as a new strain of mandarin fish (GS-04-001-2021). Select breeding of fast-growing lines is an important goal of the mandarin fish industry. However, single nucleotide polymorphisms (SNPs) associated with growth of Dinggui I mandarin fish remains unclear. Therefore, a genome-wide association study (GWAS) was performed on 5 growth traits (body weight, body length, whole length, body height and body thickness) of 192 mandarin fish. A total of 49,940 SNPs were identified by ddRAD-seq. Among them, a total of 6 significant SNPs were detected on multiple chromosomes. The proportion of phenotypic variance explained for these SNPs ranged from 9.66% to 19.47% for growth traits. Specifically, 4 SNPs were associated with all traits. Several candidate genes (*AKT2*, *CCNT2*, *EPB41L2* and *CAMKMT*) were identified based on these SNPs. The loci were verified in 100 individuals, indicating the reliability of candidate SNPs. Our results contribute to understanding of growth traits and the development of molecular marker-assisted selection in all-female mandarin fish.

1. Introduction

Mandarin fish (*Siniperca chuatsi*) belongs to Serranidae family, Perciformes order (Han et al., 2021). It mainly distributes in east Asian countries including China, Vietnam and South Korea (Zhu et al., 2022). According to China fishery yearbook, the aquaculture production and output value of mandarin fish in China have exceeded 0.4 million tons and 4 billion US dollars in 2022, making it one of the most economically valuable and promising freshwater fish in China. Due to this great market demand, select breeding of fast-growing lines has always been the main goal of the mandarin fish industry.

Growth performance is an extremely important trait for aquatic animal breeding (Guan et al., 2020) as it directly determines the production and output (Ashton et al., 2019; Shiratsuchi et al., 2020). Mandarin fish has a special feature whereby females grow particularly faster than males before sexual maturity because of sexual dimorphism (Wang et al., 2006). Our lab managed to determine the critical period of sex development of mandarin fish (Zhu et al., 2022; Liu et al., 2023; Han et al., 2020) and consequently produce the all-female mandarin fish (Dinggui I, *Siniperca chuatsi*) (Fig. 1), making it feasible to conduct the first GWAS of growth traits on mandarin fish.

Genome-wide association analysis (GWAS) can statistically analyze the relationship between quantitative trait loci (QTLs) and complex economic traits (Zhou et al., 2018), and has become the most effective

* Corresponding authors at: State Key Laboratory of Biocontrol and School of Life Sciences, Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Guangdong Provincial Key Laboratory of Aquatic Economic Animals and Guangdong Provincial Engineering Technology Research Center for Healthy Breeding of Important Economic Fish, Sun Yat-Sen University, Guangzhou 510275, China.

E-mail addresses: lshuish@mail.sysu.edu.cn (S. Li), lsszy@mail.sysu.edu.cn (Y. Zhang).

[1] These two authors contributed equally to this work

https://doi.org/10.1016/j.aquaculture.2024.740778
Received 21 November 2023; Received in revised form 23 January 2024; Accepted 5 March 2024
Available online 7 March 2024

Fig. 1. The all-female mandarin fish (Dinggui I, *Siniperca chuatsi*).

method to identify trait linked genetic markers in aquaculture species. The genetic markers obtained by GWAS can be directly used for genome-wide marker related selection (MAS) and genome selection (GS) (Matoba et al., 2020). The completion of mandarin fish genome (He et al., 2020) lays the foundation for GWAS, QTLs mapping and trait association candidate gene mining in genome-wide. Previous studies investigated the growth traits of mandarin fish based on transcriptome and non-reference genomic genetic map in recent years (Sun et al., 2017; Liu et al., 2020a, 2020b). However, the identification of candidate genes related to growth traits of mandarin fish is less advanced, given that there is only one published study on screening growth-related SNPs and candidate genes of mandarin fish by GWAS (Zhou et al., 2022). Therefore, it is of great importance to study growth-related SNPs for mandarin fish.

In this study, a genome-wide association study (GWAS) was performed on 5 growth traits (body weight, body length, whole length, body height and body thickness) of 192 mandarin fish for identification of SNPs related to growth of all-female mandarin fish (Dinggui I, *Siniperca chuatsi*). These results contribute to the genetic basis of growth-related traits and the development of molecular marker-assisted selection in all-female mandarin fish.

2. Materials and methods

2.1. Fish and rearing conditions

In total, 2000 fertilized Dinggui I mandarin fish fertilized eggs were obtained from Guangdong Liangshi Aquatic Seed Industry Co., Ltd., Foshan, China. These fertilized eggs were hatched and cultured in a concrete-walled ponds (15 × 15 × 2 m^3) for 5 months. During the experiment, the water temperature, dissolved oxygen, pH value and total ammonia nitrogen were maintained at 28–32 °C, 6.0–7.5 mg/L, 7.5–8.5, < 0.1 mg/L and < 0.05 mg/L, respectively. Commercial compound feeds (Guangdong JieDa feed Co., Ltd., Guangzhou, China) were used to feed mandarin fish twice a day (6:00 am and 19:00 pm).

2.2. Sample collection and trait measurement

After the 5-month culture, 192 individuals were randomly selected and anesthetized with MS-222. Growth-related traits including body weight (BW), total length (TL), body length (BL), body height (BH) and body thickness (BT) were measured. The dorsal fins were then collected and kept in liquid nitrogen, then saved in the −80 °C ultra-frozen refrigerator until DNA extraction.

2.3. DNA extraction, library constructing and sequencing

HiPure Tissue DNA Mini Kit (Magen, Guangdong, China) was used for extracting genomic DNA from dorsal fin tissues. Qubit 3.0 was used to quantify the concentration of genomic DNA. After detecting the integrity by running 1% agarose gel electrophoresis, the ddRAD-seq libraries were constructed according to the published protocol (Aguirre et al., 2023; Peterson et al., 2012). Illumina NovaSeq PE150 sequencing were then performed.

2.4. SNP discovery and genotyping

High quality clean data were obtained from raw reads after filtering and trimming. Three steps were conducted, (1) reads linked with barcode sequence were removed; (2) reads with unidentified nucleotides that is >10% were removed; (3) low quality reads with >50% bases harboring Phred quality scores of ≤20 were removed. Then, clean reads were mapped to the reference genome of mandarin fish (GCA_011952085.1) using Bowtie version 2.0 (Langmead and Salzberg, 2012). BCFtools v1.8 (Li et al., 2009) was used to call SNPs with minimum quality >30. VCFtools (Danecek et al., 2011) was used to get SNPs with the depth < 3, minor allele frequency (MAF) < 0.05, missing position <10%, and number of alleles >2. Finally, plink v1.9 (Purcell et al., 2007) was used to conduct the linkage disequilibrium (LD).

2.5. Genome-wide association study on growth-related traits

TASSEL5.0 (http://www.maizegenetics.net/tassel) was used to analyze the genotypic and phenotypic data of the 192 individuals by using the general linear model (GLM) and calculate the phenotypic variances explained (PVE) and F values (degree of freedom). Kinship analysis and principal component analysis (PCA) were conducted to analyze the population structure by TASSEL5.0. Genome-wide significant threshold of -log10(p value) for significant association was set as 6.0 by calculating the formula p value = 0.05/N, where N represents the number of total markers used for association analysis (Wang et al., 2023).

2.6. Candidate gene annotation

Candidate genes were obtained by searching 500 kb up- and downstream of the SNPs. The coding sequences of candidate genes were annotated by BLAST 2.2.24+ (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/) against the Swiss-Prot database and Non-Redundant Protein Sequence Database (NR).

2.7. Verification of SNP markers by sanger sequencing

A total of 100 individuals with extreme phenotypes (the biggest 50 individuals and the smallest 50 individuals) were used to verify the effectiveness of SNP 20:31934096. The primer pair was designed by Primer5 (Premier Biosoft International, Palo Alto, CA, USA). PCR was performed following the mix of a total reaction volume of 20 μL, including 3 μL 10 ng/μL genomic DNA, 5 μL pure water, 1 μL 10 μM forward and reverse primers and 10 uL 2 × PCR Master Mix (ThermoFisher, USA). The PCR condition was set as follows: 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s and 72 °C for 10 min. The PCR products were then sequenced using an ABI 3730XL sequencer (Applied Biosystems, USA). Snapgene (http://snapgene.com) was used to analyze genotypes of the SNPs.

2.8. Ethics statement

All animal experiments were approved by the Committee of Animal Research and Ethics of Sun Yat-sen University.

3. Results

3.1. Statistics of growth-related traits

Five growth traits of 192 individuals were analyzed. As shown in Table 1, the experiment group (192 individuals) has an average body weight of 192.99 ± 75.35 g, body length of 18.35 ± 2.94 cm, body height of 6.38 ± 0.86 cm, body thickness of 3.01 ± 0.60 cm and whole

Table 1
Growth traits of experiment group.

Traits	WL/cm	BL/cm	BW/g	BH/cm	BT/cm
Mean	21.77	18.35	192.99	6.38	3.01
Standard deviation	3.06	2.94	75.35	0.86	0.60

Note: WL stands for whole length; BL stands for body length; BW stands for body weight; BH stands for body height; BT stands for body thickness.

length of 21.77 ± 3.06 cm (mean ± SD). As shown in Fig. 2, all five growth traits of the experiment group were in accordance with normal distribution. The complete trait data for all 192 individuals were shown in Supplementary Table S1.

3.2. Sequencing data and characterization of SNPs

As shown in Fig. 3, A total of 120 Gb of high-quality sequencing data with 43.27% GC contents were obtained. The average Q20 and Q30 were 96.6% and 92.2%, respectively. The overall read alignment rate for each individual was 91.17 ± 0.89% (mean ± SD, n = 192). A total of 49,940 SNPs were called after filtering. SNPs were unevenly distributed in all the 24 chromosomes. The average number of SNPs per chromosome was 2080, with the most SNP number located on the Chr5 and the least number located on the Chr22.

3.3. Analysis of population structure

Principal component analysis (PCA) and kinship analysis were conducted to examine genetic relatedness among individuals. As shown in heatmap (Fig. 4A), individuals from the experiment group demonstrated a very weak relatedness with each other. All the values distributed within the scope of −0.2– 0.01. The result of principal component analysis demonstrated that all the individuals distributed randomly (Fig. 4B). The result indicated that the experiment was ideal and suitable for the following GWAS analysis.

3.4. Genome-wide association analysis of growth traits

To reveal the relationship between candidate SNPs and growth traits, the GLM model was used for analysis. The genomic inflation factors (λ) of body weight, whole length, body length, body thickness and body height were 1.041, 1.072, 1.066, 1.071 and 1.024, respectively (Fig. 5). By applying Bonferroni correction method, the p values of 1.00 × 10e −6 and 1.00 × 10e −4 were regarded as the genome-wide significant and suggestive p-value threshold, respectively.

Based on the siginificant threshold, a total of 6 SNPs were found to be significantly associated with growth traits (Fig. 6 and Table 2). For body weight, two significant associated SNPs were observed on chromosome 14 and 20 (Fig. 6 and Table 2). One of them located on 13,719,188 bp on chromosome 14 (p value =2.45 × 10e −08, F = 35.06, PVE = 19.47%). The other one located on 31,934,096 bp on chromosome 20 (p value = 8.11× 10e −07, F = 15.58, PVE = 17.90%). Interestingly, except for body weight, the SNP 14:13719188 was both significantly associated with body length (p value =6.53× 10e −08, F = 32.67, PVE = 17.86%), body height (p value =8.47× 10e −08, F = 32.05, PVE = 18.00%), body thickness (p value =5.29× 10e −07, F = 27.72, PVE = 16.16%) and whole length (p value =7.93× 10e −08, F = 32.21, PVE = 17.64%). The SNP 20:31934096 was both suggestively associated with body length (p value = 8.03× 10e −06, F = 12.81, PVE = 14.60%), body height (p value = 1.55× 10e −05, F = 12.03, PVE = 14.49%), body thickness (p value = 1.48× 10e −05, F = 12.08, PVE = 14.59%) and whole length (p

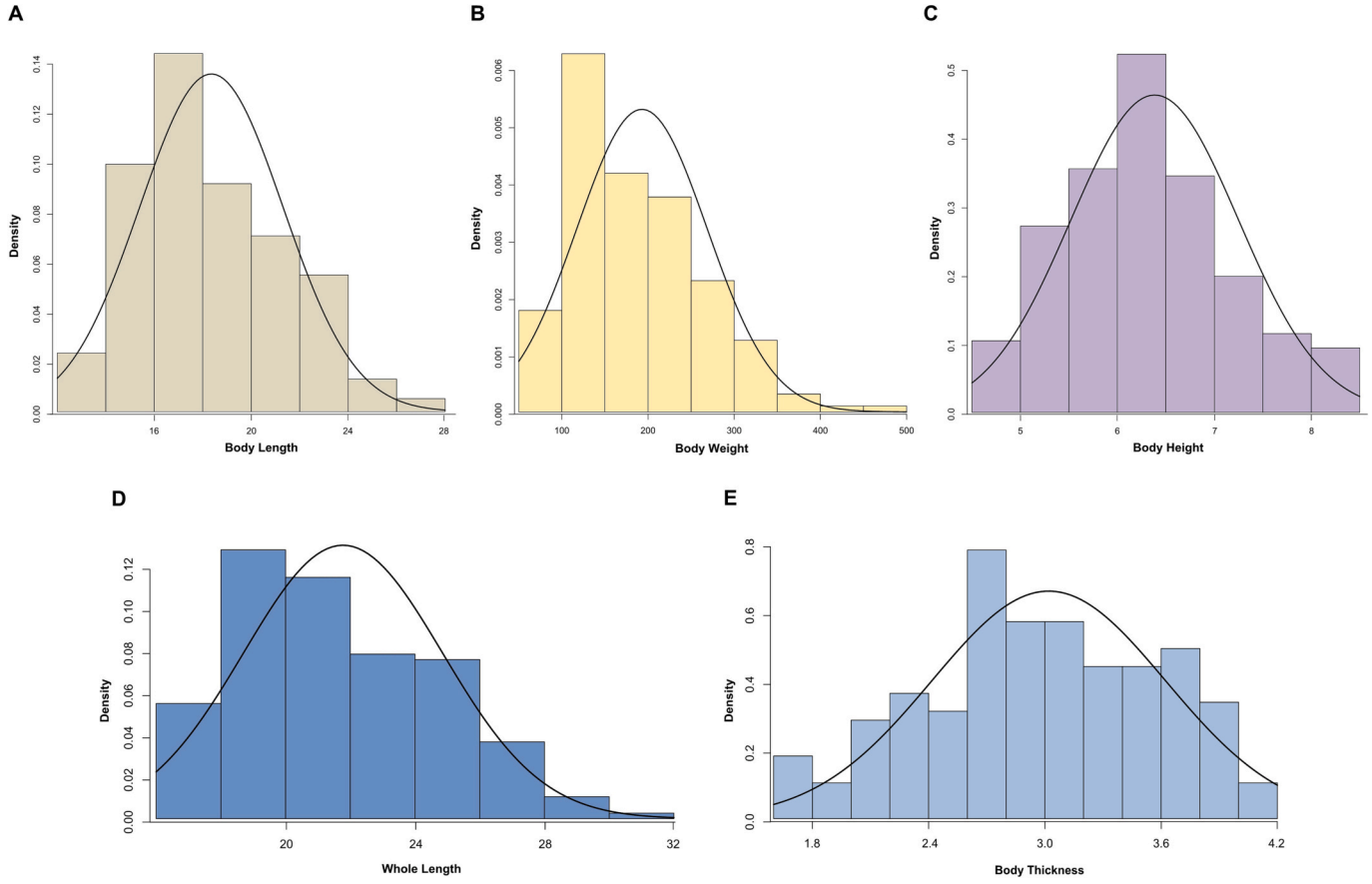


Fig. 2. Histograms and fitting curves of five growth traits of all-female mandarin fish (Dinggui I, *Siniperca chuatsi*). Histogram and fitting curve of body length (A), body weight (B), body height (C), whole length (D) and body thickness (E).

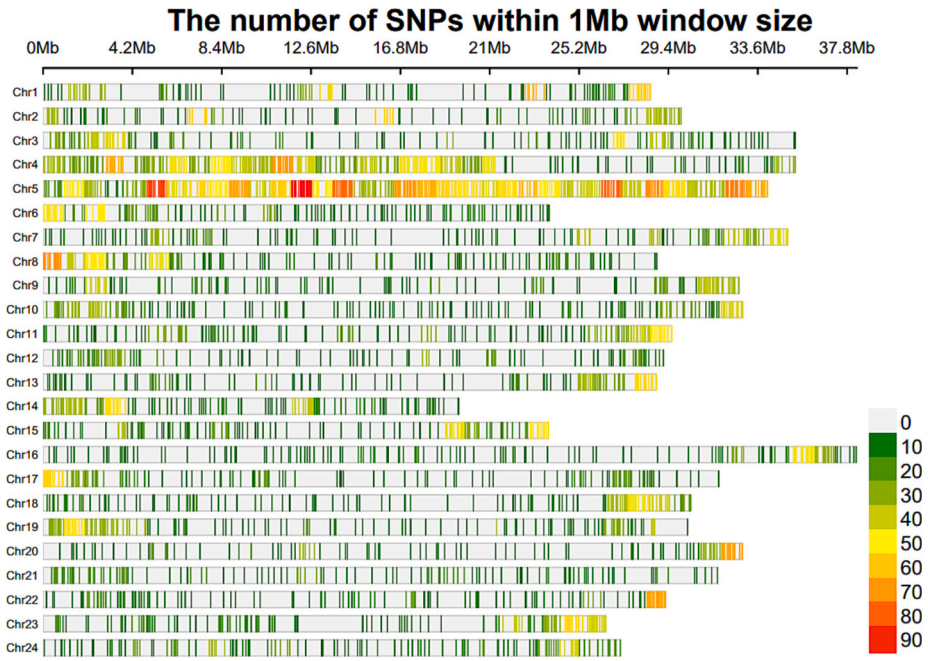


Fig. 3. Distribution of SNPs across chromosomes of all-female mandarin fish (Dinggui I, *Siniperca chuatsi*). Notes: Red bars and green bars indicate high density and low density in chromosomes, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

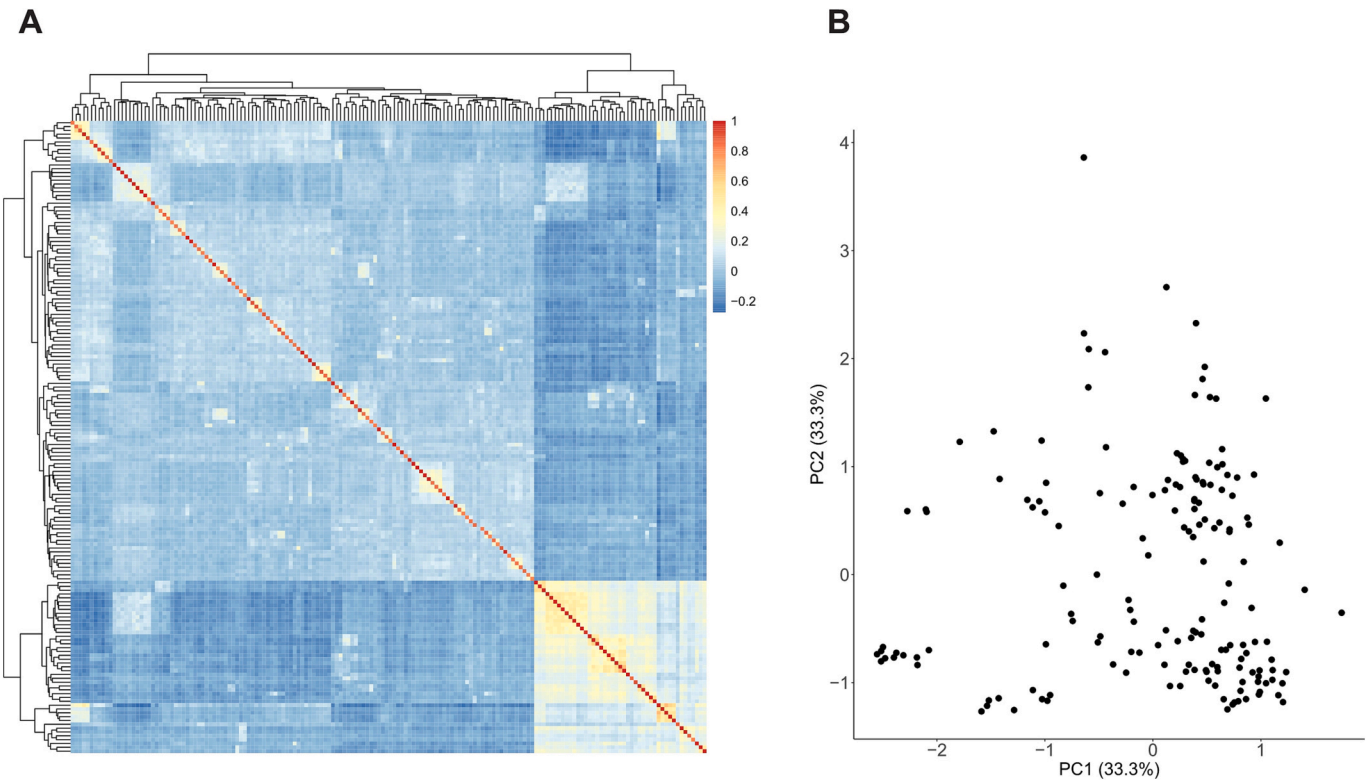


Fig. 4. Analysis of population genetic relatedness. A. The heatmap of genetic relatedness. The blue box and red box indicate low kinship and high kinship, respectively. B. Principal component analysis of genetic structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

value = 7.44× 10e −06, F = 12.90, PVE = 14.67%). Four of these SNPs were associated with all five growth traits (Fig. 7 and Supplementary Fig. S1). Overall, the PVEs for these SNPs ranged from 9.66% to 19.47%.

3.5. Verification of significant SNP markers

SNP 20:31934096 was selected to verify the reliability of SNPs. Sanger sequencing was used to genotype the extreme group (the 50 biggest individuals and the 50 smallest individuals). Two alleles (A/G)

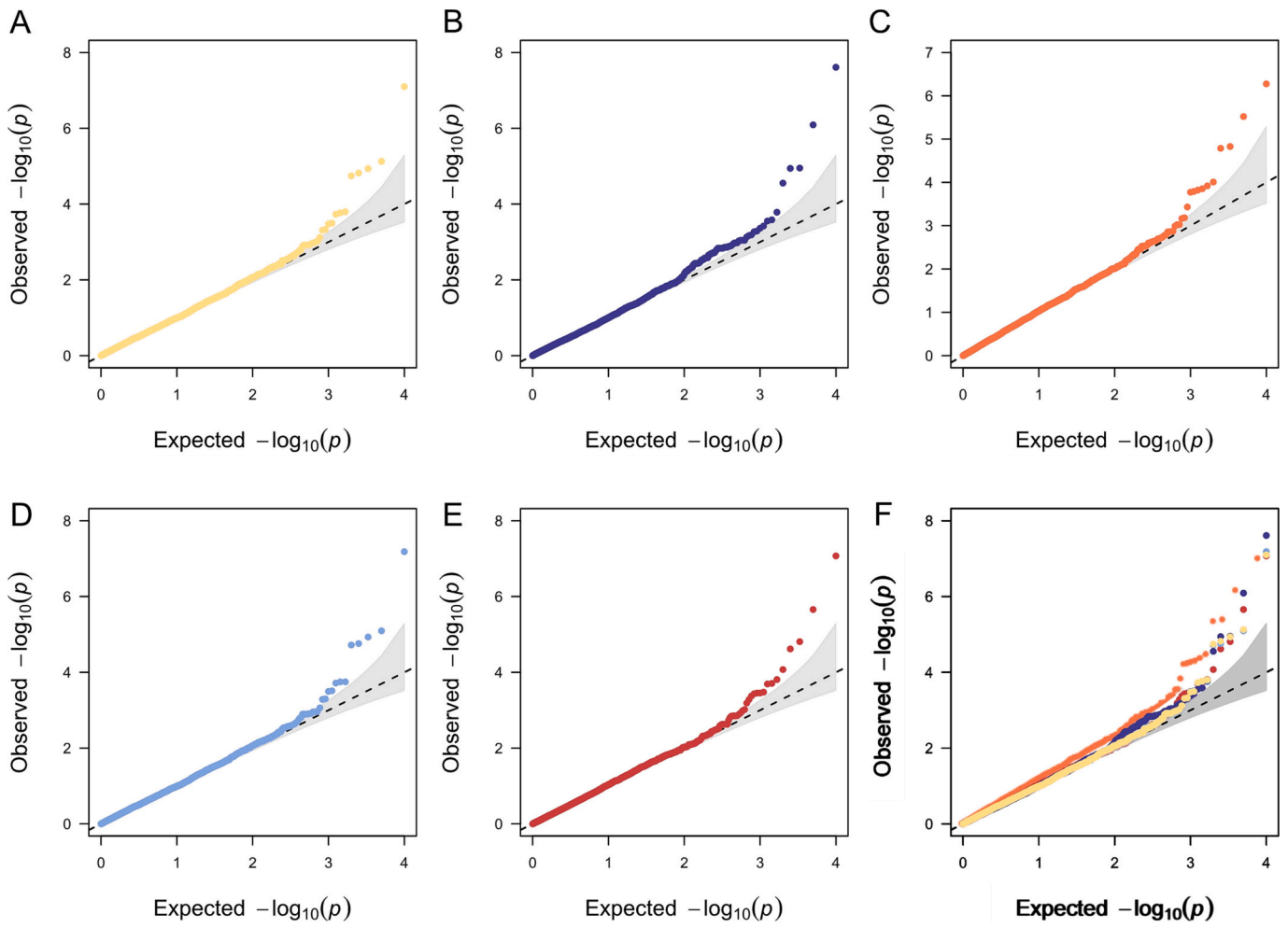


Fig. 5. QQ plots of SNPs associated with different growth traits. A. The QQ plot of SNPs associated with whole length. B. The QQ plot of SNPs associated with body weight. C. The QQ plot of SNPs associated with body thickness. D. The QQ plot of SNPs associated with body length. E. The QQ plot of SNPs associated with body height. F. The QQ plot of SNPs associated with all traits.

and three genotypes (AA/AG/GG) in the locus were observed, in which 'A' allele counted for 25% and the 'G' allele counted for 75% of the genotypic data. The 'A' allele was superiorto the 'G' allele. Analysis of the SNP genotyping data from the sanger sequencing and high-throughput sequencing showed that the SNP marker was significantly associated with the growth-related traits ($p = 0.0151$; $p = 0.0134$; Chi-square test) (Fig. 8 and Supplementary Table S3). The result of sanger sequencing was in accordance with high-throughput sequencing, demonstrating the reliability of the SNPs.

3.6. Candidate genes related to growth-related traits

Candidate genes within ±500 Kb windows of significant SNPs were obtained. In total, 39 genes were functionally annotated (Supplementary Table S2). Among which, multiple genes were annotated, including *MGAT5*, *AKT2*, *CCNT2*, *INADL*, *WDR49*, *NWD1* and *CAMKMT*. Specifically, several of the genes are known to be related to growth and muscle fiber development (*AKT2*, *CCNT2*, *EPB41L2* and *CAMKMT*).

GO and KEGG analysis on annotated genes showed that these genes were enriched to 37 different GO terms and 111 KEGG terms (Fig. 9). It is found that growth-related terms like cellular process, metabolic process and development process were enriched by GO analysis. Similar results were observed in KEGG analysis as carbohydrate metabolism and amino acid metabolism were enriched by KEGG analysis.

4. Discussion

Mandarin fish (*Siniperca Chuatsi*) is an economically important freshwater fish (Shen et al., 2021) with the annual yield of mandarin fish staying above 300,000 tons since 2016. In 2021, the all-female mandarin fish (Dinggui I, *Siniperca chuatsi*) was approved as a new strain of mandarin fish in China (GS-04-001-2021). However, thus far, there has only been one study on growth-related SNPs and candidate genes of mandarin fish (Zhou et al., 2022). In their study, obtained SNPs showed a relatively less significance and the phenotypic variation explained by the obtained SNP was not shown. In this study, the first GWAS analysis of growth traits on all-female mandarin fish (Dinggui I, *Siniperca chuatsi*) was conducted. The results contribute to the understanding of the complex genetic architecture of growth and breeding of all-female mandarin fish.

4.1. Analysis of population structure

The genetic relatedness among individuals was examined through the principal component analysis (PCA) and kinship analysis. The kinship values of all the individuals from the experiment group were <0.01, demonstrating a very weak relatedness among the group. In principal component analysis, the result showed that individuals distributed randomly, indicating a weak kinship as well. Thus, the experiment group was suitable for the following GWAS analysis. Similar results were shown in Brown-marbled grouper (*Epinephelus*

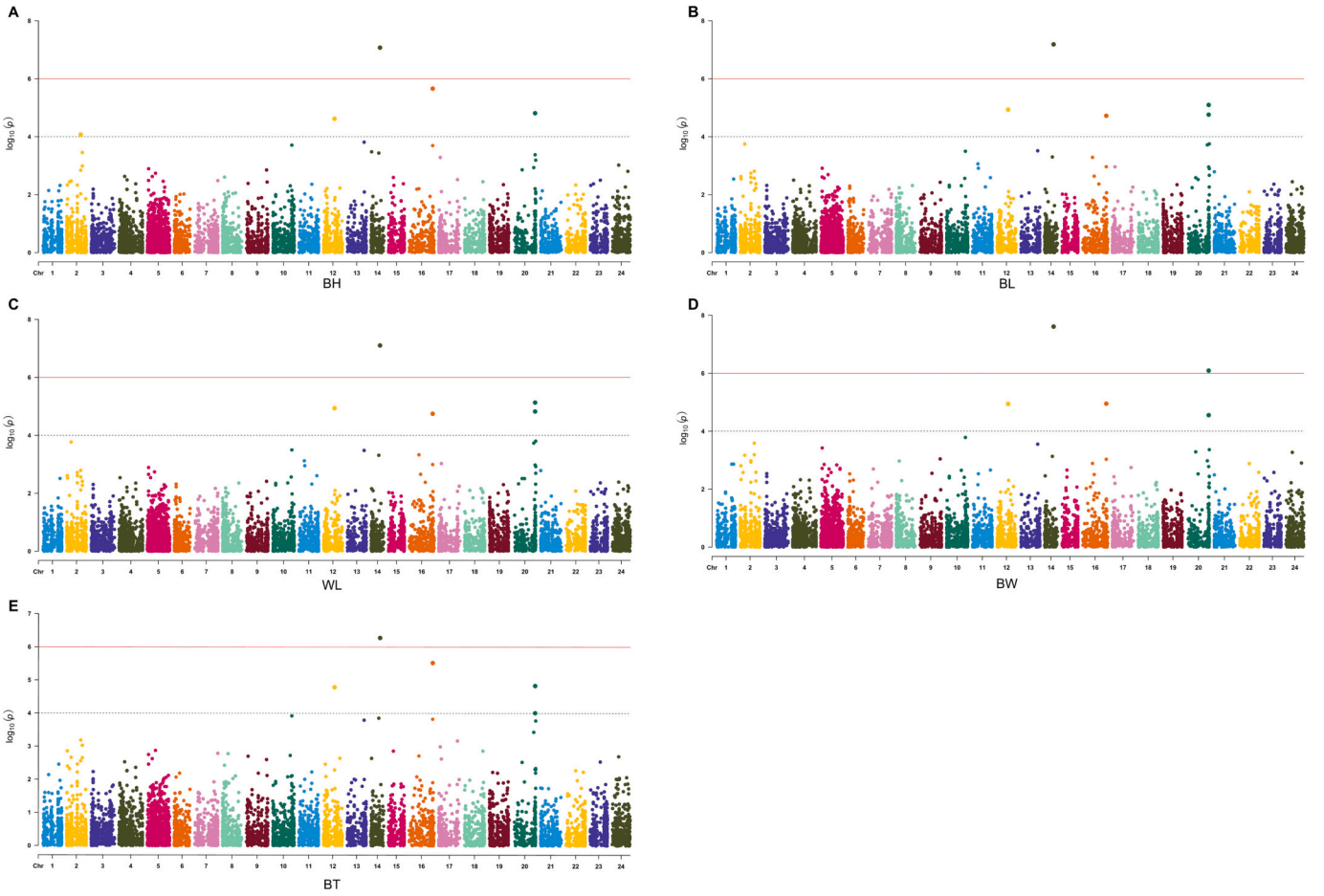


Fig. 6. Manhattan plot for (A) body height, (B) body length, (C) whole length, (D) body weight and (E) body thickness.
Note: The red line denotes the genome-wide significance threshold and the black dashed line denotes the suggestive threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fuscoguttatus) (Yang et al., 2020), leopard coral grouper (*Plectropomus leopardus*) (Wang et al., 2023), olive flounder (*Paralichthys olivaceus*) (Omeka et al., 2022), European seabass (*Dicentrarchus labrax*) (Oikonomou et al., 2022), golden pompano (*Trachinotus ovatus*) (Zhu et al., 2023) and Chinese longsnout catfish (*Leiocassis longirostris*) (Mou et al., 2022).

4.2. Detection of SNPs related to growth

Growth is a complex trait regulated by multiple genes. The growth of mandarin fish can be altered by various SNPs. A previous GWAS analysis on growth-related SNPs in mandarin fish (Zhou et al., 2022) reported that the obtained SNPs showed a relatively less significance and the phenotypic variation was not explained. Therefore, it is of great importance to detect growth-related SNPs for mandarin fish.

In current study, a total of 6 SNPs were found to be significantly associated with growth traits. Four of these SNPs were associated with all five growth traits. Overall, the PVEs for these SNPs ranged from 9.96% to 19.47%. Thus, the obtained SNPs were able to explain the phenotypic variation well and are suitable for application in further investigation.

In aquaculture industry, body weight has always been the most important growth trait because of its connection to economic benefits. Two significant SNPs related to body weight were obtained in this study, locating on chromosome 14 and chromosome 20 respectively. The PVE of these two markers was higher than grass carp (*Ctenopharyngodon Idella*; 10.4–12.3%) (Hao et al., 2023), bighead catfish (*Clarias macrocephalus*; 1.02–6.59%) (Chaivichoo et al., 2023) and Atlantic salmon (*Salmo salar*; 0.3– 12%) (Tsai et al., 2015). Interestingly, except for body weight, the SNP 14:13719188 was significantly associated with all growth traits, showing a great relationship with growth performance. By contrast, SNP 20:31934096 was suggestive of all growth traits.

SNPs obtained in this study distributed on multiple different chromosomes, suggesting that the growth of mandarin fish is controlled by multiple genes. To verify the credibility of SNP 20:31934096, the fast-growing group (n = 50) and the slow-growing group (n = 50) were selected for verification. The result showed that two alleles (A/G) and three genotypes (AA/AG/GG) were observed in the locus. The 'A' allele was superior while the 'G' allele was inferior allele for growth. Thus, individuals with AA genotype showed better growth performance. Previous studies have suggested potential regulatory effects of intergenic SNPs on gene expression (Lyu et al., 2021). Although SNP 20:31934096 is located in the intergenic region of the *INADL* gene, further evidence is required to establish its specific influence on the expression of *INADL*. This gene is known to play a role in the proliferation, differentiation, and maturation of muscle cells, as well as in the regulation of their metabolic activity (Barbáchano et al., 2016). Given the importance of *INADL* in muscle growth and development in fish, it is crucial to investigate the potential regulatory impact of SNP 20:31934096 on the expression of this gene.

Interestingly, similar results were observed in human, with a significant relationship between SNP (rs1056513) in *INADL* and body weight (Comuzzie et al., 2012), which is consistent with our results. Our result showed that SNP 20:31934096 is a reliable growth-related genetic

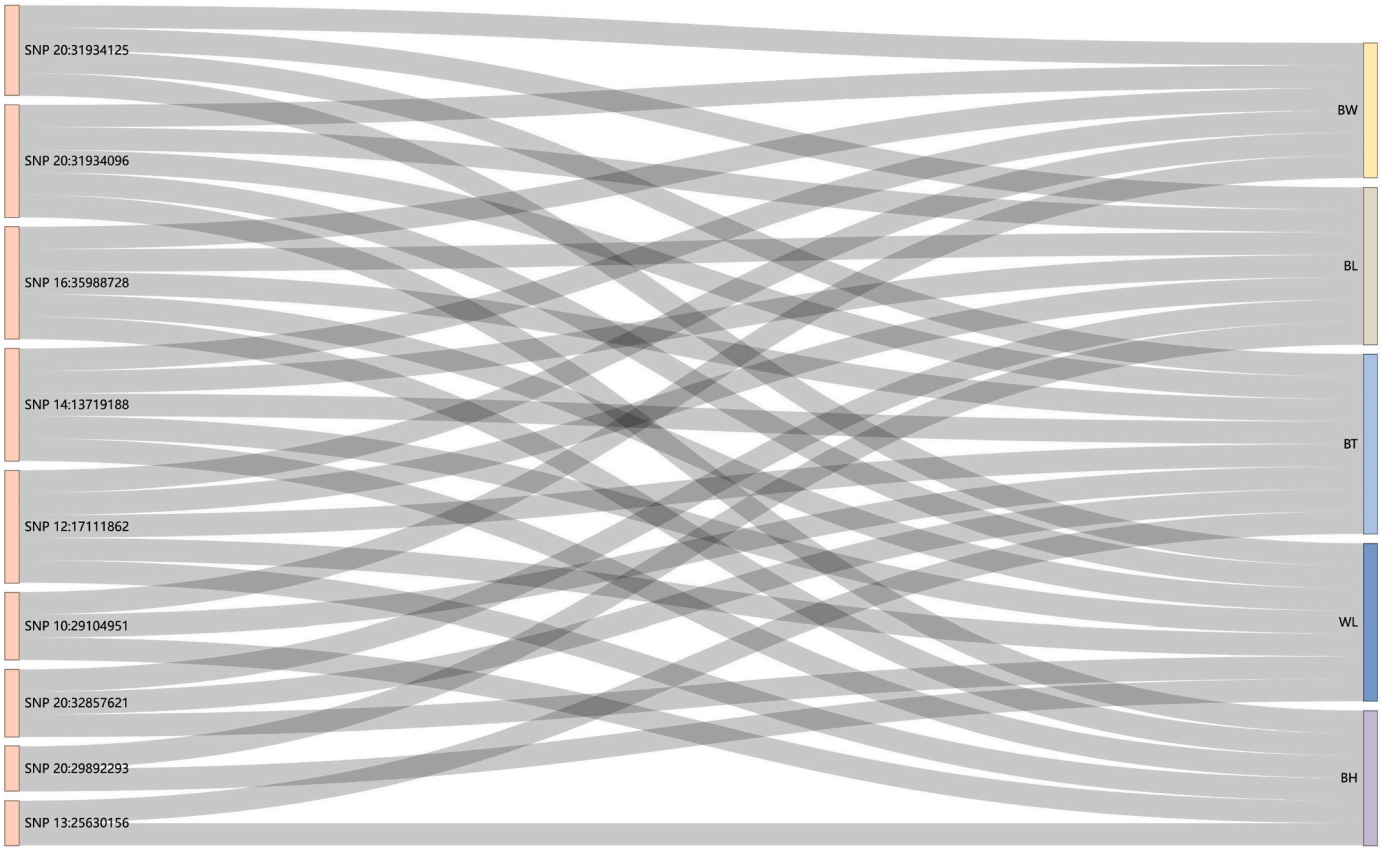


Fig. 7. The relationship between SNPs and growth traits.
Note: The left side represents for SNP markers (Marker names were shown as chromosome/position) and the right side represents for different growth traits.

Table 2
The identified SNPs associated with growth traits.

Trait name	SNP ID	Chr	*p* value	F	PVE%	Location
Whole Length	SNP 14:13719188*	14	7.93e −08	32.21	17.64	Intergenic
	SNP 20:31934096	20	7.44e −06	12.90	14.67	Intergenic
	SNP 12:17111862	12	1.16e −05	20.69	11.92	Intergenic
	SNP 20:31934125	20	1.51e −05	12.04	13.66	Intergenic
	SNP 16:35988728	16	1.80e −05	11.75	12.43	Intron
Body Weight	SNP 14:13719188*	14	2.45e −08	35.06	19.47	Intergenic
	SNP 20:31934096*	20	8.11e −07	15.58	17.90	Intergenic
	SNP 16:35988728	16	1.12e −05	12.30	13.50	Intron
	SNP 12:17111862	12	1.14e −05	20.73	12.36	Intergenic
	SNP 20:31934125	20	2.79e −05	11.32	13.53	Intergenic
Body Thickness	SNP 14:13719188*	14	5.29e −07	27.72	16.16	Intergenic
	SNP 16:35988728	16	3.01e −06	13.84	14.99	Intron
	SNP 20:31934096	20	1.48e −05	12.08	14.59	Intergenic
	SNP 12:17111862	12	1.63e −05	19.95	12.00	Intergenic
	SNP 20:31934125	20	9.78e −05	9.88	12.10	Intergenic
Body Length	SNP 14:13719188*	14	6.53e −08	32.67	17.86	Intergenic
	SNP 20:31934096	20	8.03e −06	12.81	14.60	Intergenic
	SNP 12:17111862	12	1.17e −05	20.69	11.93	Intergenic
	SNP 20:31934125	20	1.74e −05	11.88	13.51	Intergenic
	SNP 16:35988728	16	1.90e −05	11.69	12.39	Intron
Body Height	SNP 14:13719188*	14	8.47e −08	32.05	18.00	Intergenic
	SNP 16:35988728	16	2.20e −06	14.21	15.27	Intron
	SNP 20:31934096	20	1.55e −05	12.03	14.49	Intergenic
	SNP 12:17111862	12	2.41e −05	19.10	11.52	Intergenic
	SNP 2:21350095	2	8.45e −05	16.37	9.66	Intron

Note: Chr: Chromosome; F: degree of freedom; PVE %: phenotypic variance explained; SNP ID was shown as chromosome/position; The '*' denotes genome-wide significant level.

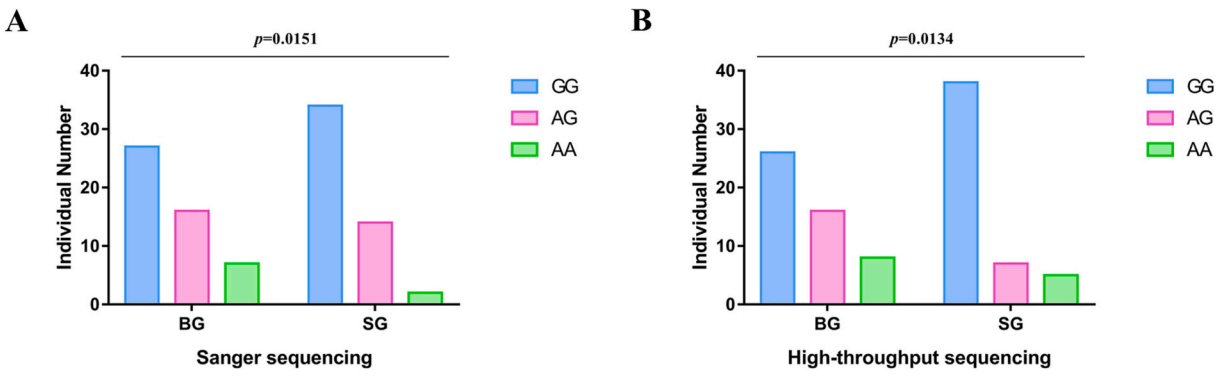


Fig. 8. Verification of the SNP 20:31934096. A: Sanger sequencing; B: High-throughput sequencing. Note: BG represents biggest group and SG represents smallest group.

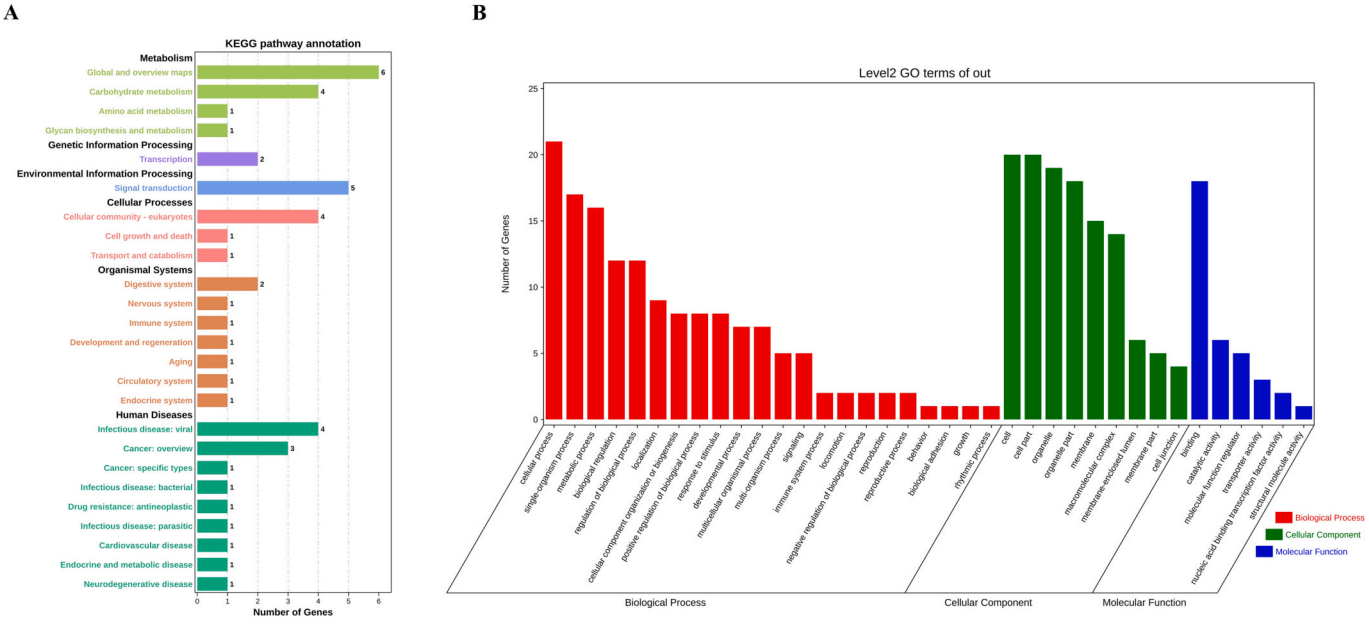


Fig. 9. Enriched GO and KEGG terms for the candidate genes for growth related SNPs. A: KEGG enriched analysis; B: GO enriched analysis.

marker for mandarin fish and it is vital for the development and improvement of the select breeding in mandarin fish.

4.3. Candidate genes related to growth-related traits

In this study, a total of 39 candidate genes within ±500 kb windows of siginificant SNPs were annotated, several of which were related to growth and muscle fiber development (*AKT2*, *CCNT2*, *EPB41L2* and *CAMKMT*).

AKT2 encodes a protein kinase involved in insulin signaling and glucose metabolism (Vergadi et al., 2017). In fish growth, *AKT2* promotes body weight gain and organ development by regulating cell metabolism, protein synthesis and growth factor signaling (Zhang et al., 2017). In muscle development, it regulates the proliferation and differentiation of muscle cells and promoting muscle formation and development (Chen et al., 2020).

CCNT2 encodes protein kinase subunit, is one of the components of P-TEFb, a transcriptional regulatory protein complex that participates in the regulation of gene transcription together with other proteins (Tian et al., 2021; Zhao and Hou, 2022), thereby increasing the transcription rate and expression level of genes, which in turn regulate cell proliferation and growth. *CCNT2* also plays an important role in muscle development (Cottone et al., 2006).

EPB41L2 encodes a muscle-specific structural protein involved in the morphological stability and function of muscle cells (Parra et al., 1998). It participates in the remodeling of cytoskeleton and regulation of cell morphology, and maintains the structural integrity and stability of muscle cells (Taylor-Harris et al., 2005; Yuan et al., 2021), which regulate cell proliferation and expression of growth-related genes, thereby promoting fish growth and development (Kelly and Reversade, 1997).

CAMKMT encodes a Ca2+/calmodulin-dependent protein kinase modifying enzyme involved in the regulation of calcium signaling pathways (Ma et al., 2016; Berridge, 2016). It mediates calcium signal through the phosphorylation and regulation of target protein activity, thus affecting muscle cell function and development (Zhang et al., 2013).

Thus, the result of this study indicated that obtained SNPs probably alter the growth of mandarin fish through growth-related genes *AKT2*, *CCNT2*, *EPB41L2* and *CAMKMT*. Therefore, future work should investigate these genes and pathways in more detail.

5. Conclusion

In conclusion, this study identified 6 significant SNPs related to growth of all-female mandarin fish (Dinggui I, *Siniperca Chuatsi*). The

proportion of phenotypic variance explained for these SNPs ranged from 9.65% to 19.46% for growth traits. Specifically, 4 SNPs were associated with all traits. Several candidate growth-related genes (*AKT2*, *CCNT2*, *EPB41L2* and *CAMKMT*) were identified. These results provide a theoretical basis for improving mandarin fish select breeding and can help improve the development of the mandarin fish industry.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740778.

CRediT authorship contribution statement

Dingrui Liu: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Jin Zhang:** Methodology, Investigation, Data curation. **Zhenjiang Zou:** Methodology, Data curation. **Chen Long:** Methodology, Data curation. **Jiaqi Lin:** Methodology, Data curation. **Junyan Zeng:** Methodology, Data curation. **Jingpeng Hou:** Methodology, Data curation. **Linqiang Han:** Methodology, Data curation. **Yanlin Jiang:** Methodology, Data curation. **Shuisheng Li:** Writing – review & editing, Supervision, Conceptualization. **Yong Zhang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request. Supplementary data has been attached.

Acknowledgements

This work was supported by the Key-Area Research and Development Program of Guangdong Province (2021B0202020001), Seed Industry Development Project of Agricultural and Rural Department of Guangdong Province (2022SPY-00-004).

References

Aguirre, N.C., Filippi, C.V., Vera, P.A., Puebla, A.F., Zaina, G., Lia, V.V., Marcucci Poltri, S.N., Paniego, N.B., 2023. Double digest restriction-site associated DNA sequencing (ddRADseq). Technol. Methods Mol. Biol. 2638, 37–57. https://doi.org/10.1007/978-1-0716-3024-2_4.

Ashton, D.T., Ritchie, P.A., Wellenreuther, M., 2019. High-density linkage map and QTLs for growth in snapper (*Chrysophrys auratus*). G3 (Bethesda, Md.) 9 (4), 1027–1035. https://doi.org/10.1534/g3.118.200905.

Barbáchano, A., Fernández-Barral, A., Pereira, F., Segura, M.F., Ordóñez-Morán, P., Carrillo-de Santa Pau, E., González-Sancho, J.M., Hanniford, D., Martínez, N., Costales-Carrera, A., Real, F.X., Pálmer, H.G., Rojas, J.M., Hernando, E., Muñoz, A., 2016. SPROUTY-2 represses the epithelial phenotype of colon carcinoma cells via upregulation of ZEB1 mediated by ETS1 and miR-200/miR-150. Oncogene 35 (23), 2991–3003. https://doi.org/10.1038/onc.2015.366.

Berridge, M.J., 2016. The inositol trisphosphate/calcium signaling pathway in health and disease. Physiol. Rev. 96 (4), 1261–1296. https://doi.org/10.1152/physrev.00006.2016.

Chaivichoo, P., Sukhavachana, S., Khumthong, R., Srisapoome, P., Chatchaiphan, S., Na-Nakorn, U., 2023. Genome–wide association study and genomic prediction of growth traits in bighead catfish (*Clarias macrocephalus* Günther, 1864). Aquaculture 562, 738748. https://doi.org/10.1016/j.aquaculture.2022.738748.

Chen, M., Ji, C., Yang, Q., Gao, S., Peng, Y., Li, Z., Gao, X., Li, Y., Jiang, N., Zhang, Y., Bian, X., Chen, C., Zhang, K., Sanchis, D., Yan, F., Ye, J., 2020. AKT2 regulates development and metabolic homeostasis via AMPK-depedent pathway in skeletal muscle. Clin. Sci. 134 (17), 2381–2398. https://doi.org/10.1042/CS20191320.

Comuzzie, A.G., Cole, S.A., Laston, S.L., Voruganti, V.S., Haack, K., Gibbs, R.A., Butte, N. F., 2012. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One 7 (12), e51954. https://doi.org/10.1371/journal.pone.0051954.

Cottone, G., Baldi, A., Palescandolo, E., Manente, L., Penta, R., Paggi, M.G., De Luca, A., 2006. Pkn is a novel partner of cyclin T2a in muscle differentiation. J. Cell. Physiol. 207 (1), 232–237. https://doi.org/10.1002/jcp.20566.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27 (15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Guan, W.Z., Qiu, G.F., Liu, F., 2020. Transcriptome analysis of the growth performance of hybrid mandarin fish after food conversion. PLoS One 15 (10), e0240308. https://doi.org/10.1371/journal.pone.0240308.

Han, C., Zhu, Q.Y., Lu, H.M., Wang, C.W., Zhou, X.N., Peng, C., Zhang, Y., 2020. Screening and characterization of sex-specific markers developed by a simple NGS method in mandarin fish (*Siniperca chuatsi*). Aquaculture 527, 735495. https://doi.org/10.1016/j.aquaculture.2020.735495.

Han, C., Wang, C.W., Ouyang, H.F., Zhu, Q.Y., Huang, J.J., Han, L.Q., Zhang, Y., 2021. Characterization of dmrts and their potential role in gonadal development of mandarin fish (*Siniperca chuatsi*). Aquacult. Rep. 21, 100802 https://doi.org/10.1016/j.aqrep.2021.100802.

Hao, Y.H., Jia, X.W., Yuan, L., Liu, Y.T., Gui, L., Shen, Y.B., Li, J.L., Xu, X.Y., 2023. Genome-wide association study reveals growth-related SNPs and candidate genes in grass carp (*Ctenopharyngodon idella*). Aquaculture 577, 739979. https://doi.org/10.1016/j.aquaculture.2023.739979.

He, S., Li, L., Lv, L.Y., Cai, W.J., Dou, Y.Q., Li, J., Tang, S.L., Chen, X., Zhang, Z., Xu, J., Zhang, Y.P., Yin, Z., Wuertz, S., Tao, Y.X., Kuhl, H., Liang, X.F., 2020. Mandarin fish (Sinipercidae) genomes provide insights into innate predatory feeding. Commun. Biol. 3 (1), 361. https://doi.org/10.1038/s42003-020-1094-y.

Kelly, G.M., Reversade, B., 1997. Characterization of a cDNA encoding a novel band 4.1-like protein in zebrafish. Biochem. Cell Biol. 75 (5), 623–632.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with bowtie 2. Nat. Methods 9 (4), 357–359. https://doi.org/10.1038/nmeth.1923.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 Genome Project Data Processing Subgroup, 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25 (16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

Liu, X., Zeng, S., Liu, S., Wang, G., Lai, H., Zhao, X., Bi, S., Guo, D., Chen, X., Yi, H., Su, Y., Zhang, Y., Li, G., 2020a. Identifying the related genes of muscle growth and exploring the functions by compensatory growth in mandarin fish (*Siniperca chuatsi*). Front. Physiol. 11, 553563 https://doi.org/10.3389/fphys.2020.553563.

Liu, F., Zhan, W., Xie, Q., Chen, H., Lou, B., Xu, W., 2020b. A first genetic linage map construction and QTL mapping for growth traits in *Larimichthys polyactis*. Sci. Rep. 10 (1), 11621. https://doi.org/10.1038/s41598-020-68592-0.

Liu, S.Y., Han, C., Huang, J.J., Zhu, Q.Y., Liu, D.R., Han, L.Q., Li, S.S., Li, G.F., Lin, H.R., Zhang, Y., 2023. Screening and characterization of X chromosome-specific markers in mandarin fish (*Siniperca chuatsi*). Aquaculture 562, 738833. https://doi.org/10.1016/j.aquaculture.2022.738833.

Lyu, S., Yang, P., Liu, Y., Song, T., Zhang, Z., Shi, Q., Chen, F., Liu, X., Li, Z., Ru, B., Cai, C., Xie, J., Lei, C., Chen, H., Xu, Z., Wang, E., Huang, Y., 2021. Genetic effects of *MOGAT1* gene SNP in growth traits of Chinese cattle. Gene 769, 145201. https://doi.org/10.1016/j.gene.2020.145201.

Ma, X., Guan, L., Xuan, J., Wang, H., Yuan, Z., Wu, M., Liu, R., Zhu, C., Wei, C., Zhao, F., Du, L., Zhang, L., 2016. Effect of polymorphisms in the CAMKMT gene on growth traits in Ujumqin sheep. Anim. Genet. 47 (5), 618–622. https://doi.org/10.1111/age.12455.

Matoba, N., Akiyama, M., Ishigaki, K., Kanai, M., Takahashi, A., Momozawa, Y., Ikegawa, S., Ikeda, M., Iwata, N., Hirata, M., Matsuda, K., Murakami, Y., Kubo, M., Kamatani, Y., Okada, Y., 2020. GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. Nat. Hum. Behav. 4 (3), 308–316. https://doi.org/10.1038/s41562-019-0805-1.

Mou, C.Y., Li, Y., Zhou, J., Li, Q., Zhou, B., Wei, Z., Luo, H., Ke, H.Y., Duan, Y.L., Zhai, W. T., Huang, Z.P., Zhao, H., Zhao, Z.M., Du, J., Ye, H., Zhang, L., 2022. Genome-wide association study reveals growth-related markers and candidate genes for selection in Chinese longsnout catfish (*Leiocassis longirostris*). Aquaculture 560, 738513. https://doi.org/10.1016/j.aquaculture.2022.738513.

Oikonomou, S., Kazlari, Z., Papapetrou, M., Papanna, K., Papaharisis, L., Manousaki, T., Loukovitis, D., Dimitroglou, A., Kottaras, L., Gourzioti, E., Pagonis, C., Kostandis, A., Tsigenopoulos, C.S., Chatziplis, D., 2022. Genome Wide Association (GWAS) analysis and genomic heritability for parasite resistance and growth in European seabass. Aquacult. Rep. 24, 101178 https://doi.org/10.1016/j.aqrep.2022.101178.

Omeka, W.K.M., Liyanage, D.S., Lee, S., Lim, C., Yang, H., Sandamalika, W.M.G., Udayantha, H.M.V., Kim, G., Ganeshalingam, S., Jeong, T., Oh, S.R., Won, S.H., Koh, H.B., Kim, M.K., Jones, D.B., Massault, C., Jerry, D.R., Lee, J., 2022. Genome-wide association study (GWAS) of growth traits in olive flounder (*Paralichthys olivaceus*). Aquaculture 555, 738257. https://doi.org/10.1016/j.aquaculture.2022.738257.

Parra, M., Gascard, P., Walensky, L.D., Snyder, S.H., Mohandas, N., Conboy, J.G., 1998. Cloning and characterization of 4.1G (EPB41L2), a new member of the skeletal protein 4.1 (EPB41) gene family. Genomics 49 (2), 298–306. https://doi.org/10.1006/geno.1998.5265.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7 (5), e37135. https://doi.org/10.1371/journal.pone.0037135.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81 (3), 559–575. https://doi.org/10.1086/519795.

Shen, Y.W., Li, H.Y., Zhao, J.L., Tang, S.J., Zhao, Y., Bi, Y.H., Chen, X.W., 2021. The digestive system of Mandarin fish (*Siniperca chuatsi*) can adapt to domestication by feeding with artificial diet. Aquaculture 538, 736546. https://doi.org/10.1016/j.aquaculture.2021.736546.

Shiratsuchi, S., MacDonald, C., Srinivasan, M., Jones, G.P., 2020. Sexual dimorphism in the horn size of a pair-forming coral reef butterflyfish. PLoS One 15 (10), e0240294. https://doi.org/10.1371/journal.pone.0240294.

Sun, C., Niu, Y., Ye, X., Dong, J., Hu, W., Zeng, Q., Chen, Z., Tian, Y., Zhang, J., Lu, M., 2017. Construction of a high-density linkage map and mapping of sex determination and growth-related loci in the mandarin fish (*Siniperca chuatsi*). BMC Genomics 18 (1), 446. https://doi.org/10.1186/s12864-017-3830-3.

Taylor-Harris, P.M., Keating, L.A., Maggs, A.M., Phillips, G.W., Birks, E.J., Franklin, R.C., Yacoub, M.H., Baines, A.J., Pinder, J.C., 2005. Cardiac muscle cell cytoskeletal protein 4.1: analysis of transcripts and subcellular location - relevance to membrane integrity, microstructure, and possible role in heart failure. Mamm. Genome 16 (3), 137–151. https://doi.org/10.1007/s00335-004-2436-7.

Tian, R., Guan, X., Qian, H., Wang, L., Shen, Z., Fang, L., Liu, Z., 2021. Restoration of NRF2 attenuates myocardial ischemia reperfusion injury through mediating microRNA-29a-3p/CCNT2 axis. BioFactors 47 (3), 414–426. https://doi.org/10.1002/biof.1712.

Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Gharbi, K., Stear, M.J., Matika, O., Bishop, S.C., Houston, R.D., 2015. Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. BMC Genomics 16, 969. https://doi.org/10.1186/s12864-015-2117-9.

Vergadi, E., Ieronymaki, E., Lyroni, K., Vaporidi, K., Tsatsanis, C., 2017. Akt signaling pathway in macrophage activation and M1/M2 polarization. J. Immunol. 198 (3), 1006–1014. https://doi.org/10.4049/jimmunol.1601515.

Wang, X.Q., Li, C.W., Xie, Z.G., Fan, W.J., Zhang, J.S., 2006. Studies on the growth difference of the male and female *Siniperca chuatsi*. Freshw. Fish. 3, 34–37.

Wang, T., Wu, X., Song, L.L., Yang, Y., Gong, S.R., Zeng, L.L., Tao, Y.H., Zhong, C.Y., Meng, Z.N., Liu, X.C., 2023. Identification of candidate growth-related SNPs and genes using GWAS and transcriptome analyses in leopard coral grouper (*Plectropomus leopardus*). Aquaculture 574, 739677. https://doi.org/10.1016/j.aquaculture.2023.739677.

Yang, Y., Wu, L., Wu, X., Li, B., Huang, W., Weng, Z., Lin, Z., Song, L., Guo, Y., Meng, Z., Liu, X., Xia, J., 2020. Identification of candidate growth-related snps and genes using GWAS in Brown-marbled grouper (*Epinephelus fuscoguttatus*). Mar. Biotechnol. 22, 153–166. https://doi.org/10.1007/s10126-019-09940-8.

Yuan, J., Xing, H., Li, Y., Song, Y., Zhang, N., Xie, M., Liu, J., Xu, Y., Shen, Y., Wang, B., Zhang, L., Yang, M., 2021. EPB41 suppresses the Wnt/β-catenin signaling in non-small cell lung cancer by sponging ALDOC. Cancer Lett. 499, 255–264. https://doi.org/10.1016/j.canlet.2020.11.024.

Zhang, L., Liu, J., Zhao, F., Ren, H., Xu, L., Lu, J., Zhang, S., Zhang, X., Wei, C., Lu, G., Zheng, Y., Du, L., 2013. Genome-wide association studies for growth and meat production traits in sheep. PLoS One 8 (6), e66569. https://doi.org/10.1371/journal.pone.0066569.

Zhang, D., Wang, J., Zhou, C., Xiao, W., 2017. Zebrafish akt2 is essential for survival, growth, bone development, and glucose homeostasis. Mech. Dev. 143, 42–52. https://doi.org/10.1016/j.mod.2017.01.004.

Zhao, D., Hou, Y., 2022. Long non-coding RNA nuclear-enriched abundant transcript 1 (LncRNA NEAT1) upregulates cyclin T2 (CCNT2) in laryngeal papilloma through sponging miR-577/miR-1224-5p and blocking cell apoptosis. Bioengineered 13 (1), 1828–1837. https://doi.org/10.1080/21655979.2021.2017653.

Zhou, Z., Chen, L., Dong, C., Peng, W., Kong, S., Sun, J., Pu, F., Chen, B., Feng, J., Xu, P., 2018. Genome-scale association study of abnormal scale pattern in Yellow River carp identified previously known causative gene in European mirror carp. Mar. Biotechnol. 20 (5), 573–583. https://doi.org/10.1007/s10126-018-9827-3.

Zhou, Y., Fu, H.C., Wang, Y.Y., Huang, H.Z., 2022. Genome-wide association study reveals growth-related snps and candidate genes in mandarin fish (*Siniperca chuatsi*). Aquaculture 550, 737879. https://doi.org/10.1016/j.aquaculture.2021.737879.

Zhu, Q., Han, C., Liu, S., Ouyang, H., Liu, D., Zhang, Z., Huang, J., Han, L., Li, S., Li, G., Lin, H., Zhang, Y., 2022. Development and gene expression analysis of gonad during 17α-methyltestosterone-induced sex reversal in mandarin fish (*Siniperca chuatsi*). Aquacult. Rep. 23, 101049 https://doi.org/10.1016/j.aqrep.2022.101049.

Zhu, F.Q., Sun, H.B., Jiang, L.M., Zhang, Q.Q., Liu, J.X., 2023. Genome-wide association study for growth-related traits in golden pompano (*Trachinotus ovatus*). Aquaculture 572, 739549. https://doi.org/10.1016/j.aquaculture.2023.739549.