Molecular Cloning and Polymorphism Analysis of PmFGF18 from *Pinctada fucata martensii*

Ruijuan Hao¹,², Chuchu Mo¹, Linda Adzigbli¹,³*, Chuangye Yang¹,4,5,*, Yuewen Deng¹,2,4,5 and Qingheng Wang¹,4,5  

¹ Fisheries College, Guangdong Ocean University, Zhanjiang 524088, China; haorj0729@outlook.com (R.H.); mochuchu2020@163.com (C.M.); Clalinda72@gmail.com (L.A.); dengyw@gdou.edu.cn (Y.D.); wangqingheng_haida@163.com (Q.W.)  
² Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Zhanjiang 524013, China  
³ Department of Marine Biology, University of Rostock, 18059 Rostock, Germany  
⁴ Guangdong Science and Innovation Center for Pearl Culture, Zhanjiang 524088, China  
⁵ Guangdong Provincial Engineering Laboratory for Mariculture Organism Breeding, Zhanjiang 524088, China  
* Correspondence: yangcy@gdou.edu.cn  
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Abstract: Fibroblast growth factor 18 (FGF18) plays an important functional role in skeletal growth and development. The FGF18 gene was characterized in pearl oyster *Pinctada fucata martensii* (PmFGF18) with the full-length sequence containing an open reading frame of 714 bp encoding 237 amino acids. The domain analysis of PmFGF18 showed a distinctive FGF domain, with a high similarity to FGF18 protein sequences from *Crassostrea gigas* (43.35%) and *C. virginica* (37.43%). PmFGF18 expression was revealed in all analyzed tissues with a significantly higher expression level in the fast-growing group than the slow-growing group. The analysis of PmFGF18 polymorphism demonstrated 33 SNPs (single nucleotide polymorphisms) in the CDS and promoter region of PmFGF18 sequence. Association analysis revealed 19 SNPs (2 SNPs from CDS and 17 SNPs from the promoter region) associating significantly with growth traits. Among the associated SNPs, one SNP g.50918198 A>C was verified in the other breeding line. Therefore, PmFGF18 can be utilized as a candidate gene for growth, and its related SNPs could be used in selective breeding of *P. f. martensii* for the improvement of growth traits.  

Keywords: FGF18; SNP; growth traits; *Pinctada fucata martensii*

1. Introduction  

The pearl oyster *Pinctada fucata martensii*, cultured for nucleated marine pearls, is naturally found in Japan, South China, and Australia [1]. The size of marine pearls is affected by the growth of pearl oysters [2,3]. This condition has prompted considerable interest in culturing fast-growing populations appropriate for producing pearls [4,5]. Growth traits, as quantitative traits regulated by multiple genes, have been reported in the construction of genetic linkage maps and SNP (single nucleotide polymorphism) identification of associated candidate genes [6–10]. Multiple genetic linkage maps have been researched for the genetic mechanism of the growth traits of bivalves, including *C. gigas*, *Hyriopsis cumingii*, *Chlamys farreri*, and *P. f. martensii* and many genes participating in the construction of growth traits have been obtained [11–13]. In order to explore the genetic mechanism of growth traits, the polymorphism of growth-associated genes, including the amylase gene, the insulin-related peptide gene from *C. gigas* [14–16], and myostatin genes from *C. farreri* [17], *Argopecten irradians* [18] and *C. nobilis* [19] have been widely researched.  

The fibroblast growth factor (FGF) family regulates the development of brain patterning, branching morphogenesis, and limbs [20]. In mammals, 18 FGFs (FGF1–10 and FGF16–23) are
known and have been categorized into 6 subfamilies in accordance with the variations in sequence
caracters [21] and FGF18 exhibits an essential function in skeletal growth and development [22,23].
FGF18 has also been observed to stimulate the growth of costal chondrocytes of neonatal rats and the
proteoglycan synthesis of mature primary porcine and human articular chondrocytes [24,25]. In a rat
model of osteoarthritis, intra-articular injection of FGF18 increased cartilage formation which showed
its potential in biomineralization [26]. In the invertebrate, several FGF18 genes were identified in
Mizuhopecten yessoensis [27] and Harpegnathos saltator [28], but the special functions of FGF18 have not
been widely researched. In the present study, one FGF18 gene of P. f. martensii was identified and we
further analyzed its expression pattern and polymorphism to show its potential role in the growth
traits’ construction and pearl oyster breeding.

2. Materials and Methods

2.1. Samples Used in this Study

In the present study, P. f. martensii was sampled and acquired from Xuwen, Zhanjiang, Guangdong
Province, China (20°25′ N, 109°57′ E). Tissue expression analysis required the adductor muscle (A),
gonad (GO), gill (GI), marginal zone of mantle (ME), pallial zone of mantle (MP), and central zone of
mantle (MC), having been sacrificed from pearl oysters from base stock. Thirty oysters from slow- (shell
length: 56.81 ± 3.87 mm) (fifteen) and fast-growing groups (shell length: 72.19 ± 3.42 mm) (fifteen)
(based on the shell length of pearl oysters) from base stock were also obtained for the expression level
analysis of PmFGF18 with adductor muscle as samples to explore PmFGF18 expression levels in the
two groups.

2.2. Method for the Gene Cloning and Sequence Analysis of PmFGF18

The FGF18 gene sequence was obtained from the genome data of P. f. martensii [29]. The RACE
method was utilized to acquire 5′ and 3′ untranslated regions (UTRs) of the specified sequence with a
SMART RACE cDNA amplification kit (TaKaRa, Dalian, China). The primers utilized in the present
study are shown in Table 1. The analytical tools used for PmFGF18 full-length sequence included
open reading frame (ORF) Finder, SMART for conserved domain analysis, ClustalX for aligning
the sequence of FGF18 genes from species including C. virginica (XP_022288219.1), M. yessoensis
(OWF47747.1), C. gigas (XP_011453678.1), Homo sapiens (AAC62240.1), Danio rerio (AAQ22394.1),
Mus musculus (AAC62239.1), and Bos taurus (AAI20281.1). MEGA 6 was utilized for the phylogenetic
analysis of FGF18 from different species with neighbor joining (NJ) method and tested for reliability
over 1000 bootstrap replicates.

| Primers     | Sequence (5′–3′)                     | Application |
|-------------|-------------------------------------|-------------|
| 5′-outer    | TTGTTGAAAGCGAGGTACAGGTTAGATT        | Outer PCR   |
| 5′-inner    | GTCCTTGATGCCTCAGTTCAGATT            | Inner PCR   |
| 3′-outer    | CTACCCCGTCATCTGCTCGTTAAAGGAA        | Outer PCR   |
| 3′-inner    | GATGTCACCTTTCCAAAATCTGACTGC         | Inner PCR   |
| PmFGF18-A   | GAATTACCACCGCTCTGCTGCTGCGCC        | qRT-PCR     |
| PmFGF18-S   | CGTGGGAAATTGATACGTTGATC             | qRT-PCR     |
| GAPDH-A     | CGTGGTTATATATGCGGAGGTGTC           | qRT-PCR     |
| GAPDH-S     | CGAGATGGCTGCCGACTGTTGAGTT          | qRT-PCR     |

2.3. PmFGF18 Expression Level

The isolation of total RNA from fast- and slow-growing groups and different tissues was
performed with TRIzol reagent from Invitrogen (Carlsbad, CA, USA) [30]. The M-MLV First-strand
cDNA Synthesis Kit (Invitrogen) was used to synthesize cDNA which was then used as a template
for qRT-PCR analysis by using the primers described in Table 1. The 2^–ΔCt method was utilized for the expression level analysis of PmFGF18 in detected tissues and different groups with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as a reference gene [3]. The expression level of PmFGF18 in the development was analyzed with the data from the transcriptome of P. f. martensii development.

2.4. SNP Polymorphism of PmFGF18

In order to explore the polymorphism of PmFGF18, the fifth-generation selected line of P. f. martensii for rapid growth was used. The selected line was designated as “Haixuan NO1”, and the development of the line was described in detail by Du et al. (2015) [31]. The fifth-generation selected line of P. f. martensii for yellow shell color line was used for the verification of SNPs. The adductor muscle of samples was obtained for DNA extraction with the DNA Kit of Marine Animals from TIANGEN. The shell length, shell width, and shell height of each sample were measured using a digital caliper (0.02 mm accuracy) [32]. Total weight and shell weight of each sample were measured using an electronic balance (0.01 g accuracy). The exon and promoter sequences of PmFGF18 whose mutation would influence the protein structure and gene expression [33,34] from sampled pearl oysters were genotyped by target resequencing with a filter of SNP with a minor allele frequency lower than 5% [10], and the genome of P. f. martensii was utilized as a reference [29]. The expected heterozygosity (He), observed heterozygosity (Ho), and allele frequency of the identified SNPs were calculated using PopGene 32 (version 3.2). PIC (Polymorphic information content) of SNPs was evaluated using PIC_CALC software according to its instruction. PIC can be divided into three types, namely, low (PIC < 0.25), medium (0.25 < PIC < 0.5), and high polymorphisms (PIC > 0.5). Comparison of the means of growth traits among different genotypes was conducted one-way ANOVA (analysis of variance) with Duncan test or T-test in both lines using SPSS version 17.0. Significance level for the analysis was specified at P < 0.05.

2.5. Data Analysis

ANOVA was used for tissue expression data, and t-test was used for the relative expression analysis between the fast- and slow-growing groups. Significance level for all analyses were specified at P < 0.05. Data analyses were accomplished on SPSS 17.0 (IBM, Chicago, IL, USA).

3. Results

3.1. Cloning and Bioinformatic Analyses of PmFGF18

PmFGF18 had a full-length sequence of 2534 bp with 248 bp 5′ UTR and 1572 bp 3′ UTR (Figure S1) and an ORF of 714bp encoding 237 amino acids. The PmFGF18 protein sequence held an estimated molecular mass of 28.09 kDa and contained a classic FGF domain located at 45–178 amino acids.

3.2. PmFGF18 Structure and Protein Homology Analysis

The homologous analysis of PmFGF18 with sequences of FGF18 from C. virginica (XP_022288219.1), M. yessoensis (OWF47747.1), C. gigas (XP_011453678.1), H. sapiens (AAC62240.1), D. rerio (AAQ22394.1), M. musculus (AAC62239.1), and B. taurus (AAI20281.1) by using Clustal X2 exhibited the conserved functional domain of PmFGF18 (Figure 1A). PmFGF18 shared 43.35% identity with C. gigas, 37.43% identity with C. virginica, and 33.14% identity with M. yessoensis. The predicted organizational structure of PmFGF18 and that of different species (C. gigas, C. virginica, M. yessoensis, H. sapiens, M. musculus, D. rerio, and B. Taurus) also showed the conservation of PmFGF18 (Figure 1B). The phylogenetic tree analysis showed that these FGF18 proteins were divided into invertebrate and vertebrate branches which conformed to the traditional taxonomy (Figure S2).
Figure 1. Multiple sequence alignment and structural organization of PmFGF18 amino acid sequence and other species. (A) shows the multiple sequence alignment of PmFGF18. Dark blue background indicates conserved aa; pink background indicates aa with strong similarity; light blue indicates aa with weak similarity. The numbers on the right present the total amino acid of each protein. (B) represents the structural organization of FGF18 among different species. The red region represents the signal peptide. Accession number for the sequences used in this alignment and domain analysis are as follows: C. gigas (XP_011453678.1), C. virginica (XP_022288219.1), M. yessoensis (OWF47747.1), H. sapiens (AAC62240.1), M. musculus (AAC62239.1), D. rerio (AAQ22394.1), and B. taurus (AAI20281.1).

3.3. Expression Analysis of PmFGF18

PmFGF18 mRNA expressed in the adductor muscle, gill, marginal zone of mantle, pallial zone of mantle, gonad, and central zone of mantle of P. f. martensii, which presented its wide existence in P. f. martensii (Figure 2A). A significantly higher expression level of PmFGF18 was recorded in the marginal zone of mantle than in other tissues. The expression pattern of PmFGF18 during development presented a wide expression in development and a higher expression level in the late development (Figure S3). The expression level of PmFGF18 in the fast- and slow-growing groups indicated that PmFGF18 expressed higher in the fast-growing group than in the slow-growing group (P < 0.05, Figure 2B).
In total, 33 SNPs were detected, and the polymorphism of SNPs showed that He ranged from 0.0578 to 0.5004, and Ho ranged from 0.0595 to 0.5756. PIC analysis found 21 loci with a low polymorphism level and 12 loci with a medium polymorphism level. HWE analysis indicated that 8 loci significantly deviated from HWE (P < 0.05) determined through one-way ANOVA, and the bar represents the standard deviation. (B) Expression pattern of PmFGF18 in the fast- and slow-growing groups. Bars with different letters indicate significant differences (P < 0.05).

3.4. PmFGF18 Polymorphism Analysis and SNP Identification

In total, 33 SNPs were detected, and the polymorphism of SNPs showed that He ranged from 0.0578 to 0.5004, and Ho ranged from 0.0595 to 0.5756. PIC analysis found 21 loci with a low polymorphism level and 12 loci with a medium polymorphism level. HWE analysis indicated that 8 loci significantly deviated from HWE (P < 0.05) (Table 2).

### Table 2. Genetic diversity based on SNP polymorphisms in PmFGF18.

| Location | Number | Locus     | Ho     | He     | HWE     | PIC     |
|----------|--------|-----------|--------|--------|---------|---------|
| Promoter | SNP1   | 50916942 T > C | 0.1023 | 0.0999 | 0.532498 | 0.094861 |
|          | SNP2   | 50916948 T > G | 0.0992 | 0.0971 | 0.576594 | 0.092275 |
|          | SNP3   | 50916962 G > A | 0.0885 | 0.0875 | 0.75112  | 0.083585 |
|          | SNP4   | 50916963 T > C | 0.0901 | 0.0889 | 0.724239 | 0.084909 |
|          | SNP5   | 50916970 T > C | 0.0947 | 0.093  | 0.647506 | 0.088691 |
|          | SNP6   | 50916971 T > A | 0.0947 | 0.093  | 0.647506 | 0.088691 |
|          | SNP7   | 50916977 A > G | 0.0962 | 0.0944 | 0.623226 | 0.089835 |
|          | SNP8   | 50916978 A > T | 0.0977 | 0.0958 | 0.59959  | 0.091139 |
|          | SNP9   | 50916986 A > G | 0.0977 | 0.0958 | 0.59959  | 0.091139 |
|          | SNP10  | 50916996 T > A | 0.0977 | 0.0958 | 0.59959  | 0.091139 |
|          | SNP11  | 50917667 A > C | 0.1924 | 0.1862 | 0.396673 | 0.168744 |
|          | SNP12  | 50917717 A > G | 0.5756 | 0.4769 | 0    | 0.362973 |
|          | SNP13  | 50917735 A > T | 0.0611 | 0.0592 | 0.426239 | 0.057391 |
|          | SNP14  | 50917780 A > T | 0.5603 | 0.4734 | 0.000003 | 0.361182 |
|          | SNP15  | 50917839 A > T | 0.0779 | 0.0805 | 0.396651 | 0.077234 |
|          | SNP16  | 50917897 T > C | 0.1267 | 0.1214 | 0.26371  | 0.11401  |
|          | SNP17  | 50918021 T > C | 0.5496 | 0.5    | 0      | 0.37479  |
|          | SNP18  | 50918198 A > C | 0.4763 | 0.4221 | 0.001006 | 0.332859 |
|          | SNP19  | 50918226 G > A | 0.1771 | 0.174  | 0.646955 | 0.158772 |
|          | SNP20  | 50918333 C > T | 0.4366 | 0.3989 | 0.01529  | 0.319141 |
|          | SNP21  | 50918342 C > A | 0.5099 | 0.4647 | 0.012993 | 0.356514 |
|          | SNP22  | 50918354 T > G | 0.5099 | 0.4663 | 0.016496 | 0.35739 |
|          | SNP23  | 50918376 C > A | 0.0473 | 0.0462 | 0.541862 | 0.045206 |

Figure 2. Expression pattern of PmFGF18 from P. f. martensii. (A) Expression pattern of PmFGF18 in different tissues. ME: Marginal zone of mantle; MP: Pallial zone of mantle; MC: Central zone of mantle; A: Adductor muscle; GI: Gill; GO: Gonad; Different letters indicate significant differences (P < 0.05) determined through one-way ANOVA, and the bar represents the standard deviation. (B) Expression pattern of PmFGF18 in the fast- and slow-growing groups. Bars with different letters indicate significant differences (P < 0.05).
The association analysis between identified SNPs and the growth traits of individuals from “Haixuan NO1” indicated that 19 SNPs (2 SNPs from exon region and 17 SNPs from the promoter region) of PmFGF18 were significantly associated with growth traits (P < 0.05) (Table 3). As shown in Table 3, the shell width of the genotype TT of g. 50916942 T > C was significantly larger than that of the genotype TC (P < 0.05). The shell width and weight of the genotype GG of g. 50916962 A > G were significantly larger than those of the genotype AG (P < 0.05). The shell length of the genotype AA and AC of g. 50918198 A > C were significantly larger than those of the genotype CC (P < 0.05) (Table 3).

| SNP   | SNP Name       | Genotype | Sample Number | Shell Length (mm) | Shell Width (mm) | Shell Height (mm) | Total Weight (g) | Shell Weight (g) |
|-------|----------------|----------|---------------|-------------------|------------------|-------------------|------------------|------------------|
| SNP1  | 50916942 T > C| TT       | 587           | 67.29 ± 6.73      | 24.58 ± 2.12a    | 68.42 ± 7.24      | 45.87 ± 9.18     | 20.75 ± 3.99     |
| SNP2  | 50916948 T > G| TT       | 589           | 67.29 ± 6.72      | 24.58 ± 3.13a    | 68.42 ± 7.22      | 45.85 ± 9.19     | 20.74 ± 3.99     |
| SNP3  | 50916962 A > G| TT       | 596           | 67.29 ± 6.72      | 24.58 ± 2.12a    | 68.45 ± 7.21      | 45.90 ± 9.23     | 20.76 ± 4.00     |
| SNP4  | 50916963 T > C| TT       | 595           | 67.30 ± 6.72      | 24.58 ± 2.12a    | 68.45 ± 7.22      | 45.92 ± 9.22     | 20.77 ± 4.00     |
| SNP5  | 50916970 T > C| TT       | 592           | 67.32 ± 6.73      | 24.58 ± 3.12a    | 68.46 ± 7.23      | 45.92 ± 9.25     | 20.77 ± 4.01     |
| SNP6  | 50916971 T > A| TT       | 592           | 67.32 ± 6.73      | 24.58 ± 2.12a    | 68.46 ± 7.23      | 45.92 ± 9.25     | 20.77 ± 4.01     |
| SNP7  | 50916977 A > G| TT       | 591           | 67.30 ± 6.73      | 24.58 ± 2.13a    | 68.45 ± 7.22      | 45.98 ± 9.21     | 20.73 ± 3.99     |
| SNP8  | 50916978 A > T| TT       | 589           | 67.32 ± 6.62      | 24.59 ± 2.12a    | 68.46 ± 7.22      | 45.90 ± 9.20     | 20.76 ± 3.98     |
| SNP9  | 50916986 A > T| TT       | 590           | 67.30 ± 6.73      | 24.59 ± 2.12a    | 68.44 ± 7.23      | 45.90 ± 9.20     | 20.76 ± 4.00     |
| SNP10 | 50916996 A > T| TT       | 590           | 67.29 ± 6.74      | 24.58 ± 2.12a    | 68.43 ± 7.24      | 45.88 ± 9.21     | 20.75 ± 4.00     |
| SNP12 | 50917177 A > G| TT       | 586           | 67.30 ± 6.62      | 24.63 ± 2.21     | 68.97 ± 6.85a     | 46.39 ± 9.34     | 20.83 ± 4.05     |
| SNP13 | 50917235 A > T| TT       | 615           | 67.21 ± 6.68      | 24.57 ± 2.10a    | 68.39 ± 7.20      | 45.77 ± 9.13     | 20.67 ± 3.95     |
| SNP14 | 50917280 A > T| TT       | 68             | 67.65 ± 6.98      | 23.34 ± 3.82b    | 69.69 ± 6.67      | 45.13 ± 9.20     | 20.32 ± 3.92     |
| SNP17 | 50918021 T > C| TT       | 138           | 67.81 ± 6.83      | 24.44 ± 2.66b    | 68.23 ± 8.16      | 45.83 ± 9.65     | 21.05 ± 4.22a    |
| SNP18 | 50918198 A > C| AA       | 301           | 68.03 ± 6.85a     | 24.57 ± 2.67     | 68.56 ± 7.79      | 46.33 ± 9.69     | 21.02 ± 4.11     |
| SNP19 | 50919201 C > G| CC       | 42             | 65.03 ± 5.43b     | 24.70 ± 2.11     | 67.86 ± 4.33      | 45.89 ± 9.12     | 20.97 ± 3.67     |
| SNP20 | 50926498 G > T| EXON     | 50926498      | 67.56 ± 6.76      | 24.66 ± 1.89a    | 68.74 ± 6.63      | 46.03 ± 8.94     | 20.77 ± 3.91ab   |

Note: Ho: the observed heterozygosity; He: the expected heterozygosity; PIC: the polymorphism information content; HWE: Hardy-Weinberg equilibrium.
Table 3. Cont.

| SNP    | SNP Name | Genotype | Sample Number | Shell Length (mm) | Shell Width (mm) | Shell Height (mm) | Total Weight (g) | Shell Weight (g) |
|--------|----------|----------|---------------|-------------------|------------------|------------------|-----------------|-----------------|
| SNP26  | 50918498 A > G | AA       | 145           | 67.96 ± 6.79a     | 24.62 ± 1.88     | 68.26 ± 8.00     | 45.80 ± 9.51    | 20.82 ± 4.11a   |
|        |          | GG       | 141           | 66.37 ± 6.12b     | 24.47 ± 1.87     | 67.86 ± 7.32     | 44.83 ± 8.39    | 19.96 ± 3.39b   |
|        |          | AG       | 369           | 67.40 ± 6.86ab    | 24.46 ± 2.52     | 68.78 ± 6.76     | 46.04 ± 9.25    | 20.85 ± 4.05a   |
| SNP28  | 50918729 T > C | TT       | 142           | 68.22 ± 6.78a     | 24.49 ± 2.64     | 68.49 ± 8.04     | 46.15 ± 9.63    | 21.10 ± 4.18    |
|        |          | CC       | 149           | 66.48 ± 6.16b     | 24.48 ± 1.93     | 67.93 ± 7.16     | 45.23 ± 8.83    | 20.29 ± 3.65    |
|        |          | TC       | 364           | 67.28 ± 6.85ab    | 24.51 ± 2.22     | 68.68 ± 6.82     | 45.77 ± 9.07    | 20.62 ± 3.96    |
| SNP32  | 50926642 G > A | GG       | 616           | 67.23 ± 6.86     | 24.58 ± 2.10a    | 68.40 ± 7.19     | 45.81 ± 9.15    | 20.69 ± 3.96    |
|        |          | AG       | 39            | 68.44 ± 7.07     | 23.24 ± 3.84b    | 69.52 ± 6.81     | 44.43 ± 8.86    | 20.00 ± 3.65    |
| SNP33  | 50926661 G > A | GC       | 616           | 67.23 ± 6.68     | 24.58 ± 2.10a    | 68.40 ± 7.19     | 45.81 ± 9.15    | 20.69 ± 3.96    |
|        |          | AG       | 39            | 68.44 ± 7.07     | 23.24 ± 3.84b    | 69.52 ± 6.81     | 44.43 ± 8.86    | 20.00 ± 3.65    |

Note: Mean values with different letter within a column are significant different (P < 0.05).

3.6. SNP Verification in the Yellow Shell Color Line

One SNP g. 50918198 A > C associated with the growth of *P. f. martensii* in the “Haixuan NO1” was chosen for the polymorphism analysis of *PmFGF18* in the yellow shell color line for SNP verification. The association analysis of a yellow shell color line showed that shell lengths of the genotype AA of g. 50918198 A > C were significantly larger than those of the genotype CC and AC (P < 0.05) (Figure 3C,D). The shell heights of the genotype AA of g. 50918198 A > C were significantly larger than those of the genotype AC (P < 0.05) (Figure 3C,D). Therefore, the genotype AA of g. 50918198 A > C showed significantly larger shell length than genotype CC in both lines (P < 0.05) (Figure 3A,B).

![Figure 3](image-url)

**Figure 3.** SNP g.50918198A > C verification in two lines. (A,B) and (C,D) showed the result of the “Haixuan NO1” and yellow shell color line, respectively. Bars with different letters indicate significant differences among different genotypes (P < 0.05).

4. Discussion

Mollusca is the second largest invertebrate phylum, and its external exoskeleton or shell protects it and represents the size of individuals [35]. The success of the mollusk species can be ascribed...
partially to their exoskeleton [36], which provides defense and support functions. FGF18 regulates cell proliferation and differentiation positively in osteogenesis and negatively in chondrogenesis [22] which indicates its potential in biomineralization and shell size construction. In the present study, the PmFGF18 primary sequence presented a signal peptide and an FGF domain corresponding to the result of other species. The multiple sequence alignment of FGF18 from diverse bivalves also demonstrated a conservation of their amino acid sequence and primary structure, and further indicated that the gene cloned in the present study was PmFGF18. These results showed the functional potential of PmFGF18 to the general FGF18 function.

FGF18 plays a central role in skeletal growth and development [22,23]. In invertebrates, the sequence information of some FGF18 genes is limited in their characterization and functions. This study observed the expression of PmFGF18 at various developmental stages and diverse pearl oyster tissues, showing its wide existence in the pearl oyster. PmFGF18 showed a high expression level in shell formation-associated tissues especially in the marginal zone of mantle, which indicated its potential role in the prismatic layer formation and was consequently involved in shell size construction [3,37,38].

In the research of our group, multiple genes and SNPs from growth-related genes were analyzed to obtain genes that participate in the growth of pearl oysters; we reported that some growth-related genes presented a relatively higher expression level or RPKM in the fast-growing group than in the slow-growing group [29,31,39,40]. Adductor muscles of shellfish which are the main muscular system in bivalve molluscs and connected to their shells have been widely used to research the growth genes expression pattern [39,41,42], and researchers who focused on the growth of shellfish also utilized the adductor muscle as the detected tissue for the omics analysis [29,31,43]. In the present study, adductor muscle was utilized and it was shown that PmFGF18 showed a significantly high expression level in the fast-growing group compared with the slow-growing group. Therefore, PmFGF18 hold the potential in regulating the growth traits of *P. f. martensii*.

SNP, as a molecular marker, has been widely utilized in the genetic architecture analysis of complex economic traits and promotes the selection accuracy and efficiency in economic animals and plants [44,45]. In order to analyze the potential of PmFGF18 in the genetic breeding of pearl oysters, 33 SNPs from PmFGF18 were identified. Correlation or association analysis between SNP markers and economic traits reaching a significant level may imply the existence of relation between the markers and related traits [46], which was also used to analyze the association among SNPs from PmFGF18 and growth traits. In Zhikong scallops, the SNP (c. 1815C.T) in the 3′ UTRs of the TGF-b type I receptor gene was associated with the growth of scallops, and the genotype TT possessed higher growth traits than the genotype CC or CT in a full-sib family [42]. In *C. nobilis*, SNP g.-579A/C in the promoter region of an MSTN gene was significantly associated with body size, adductor muscle mass, and soft tissue [19]. Three SNPs from PmOSR1 gene showed significant association with the shell size of *P. f. martensii* [47]. 19 SNPs from PmFGF18 were significantly associated with growth traits in the present study. Among them, growth traits of genotype AA from g. 50918198 A > C showed the better traits compared with the other two genotypes in both lines. Thus, the alteration of the genetic sequence of specific genes may lead to the variation in correlated traits [48] and the selection of SNPs from these genes may promote the efficiency of breeding progress.

In the promoter region, the SNP observed had the possibility of influencing FGF18 expression. In chickens, the SNPs in E-box3 and E-box4 changed the myostatin expression [49]. In pigs, SNP interrupted a putative MEF3 binding site related to MSTN expression [50]. In *PmFGF18*, the capability of the identified SNPs to affect the efficiency of the gene transcription is unknown and requires further research. The current research provides key and vital molecular information to progress our knowledge on the biological roles and evolution of the *PmFGF18* gene and will be valuable for future research required for the genetic enhancement of *P. f. martensii*. 
5. Conclusions

PmFGF18 may exhibit a functional role in the growth of pearl oysters. The characterization of the full length of PmFGF18 showed its conserved structures at a primary level. Expression analysis demonstrated the wide distribution of PmFGF18 in all sampled tissues. PmFGF18 was expressed significantly higher in the fast-growing group compared with the slow-growing group. In total, 33 SNPs were identified in the promoter and exon sequences of PmFGF18, and 19 SNPs were significantly associated with the growth traits of pearl oysters from “Haixuan NO1”. The growth traits of genotype AA from g. 509198 A > C showed the better traits compared with the other two genotypes in both lines. This study demonstrated the function potential of PmFGF18 in the body size construction of pearl oysters and provided a potential molecular marker for the selective breeding and genetic improvement of the pearl oyster P. f. martensii.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-1312/8/11/896/s1, Figure S1: Nucleotide sequence analysis of PmFGF18, Figure S2: Phylogenetic tree of FGF18 among different species. Figure S3: Expression pattern of PmFGF18 during development in the development transcriptome.

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