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Short Communication

Association analysis of alpha-amylase (AMY) and cathepsin L (CTSL) SNPs with growth traits in giant tiger shrimp *Penaeus monodon*

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Abstract

Alpha-amylase (AMY) and cathepsin-L (CTSL) were selected as candidate genes for SNP discovery for growth traits of *P. monodon*. Six SNPs were found in AMY and three in CTSL in *P. monodon*. Association analyses for the candidate SNPs with important economic traits were performed in populations. That allele A at CTLS-213 SNP, AA, and GA, tended to be associated with increased body weight. Shrimps with genotype GG had significantly smaller CL, CW, and CH values than those with GT and TT genotypes ($P < 0.05$). While CTLS-820 SNP was found to be significantly associated with CH and FSL ($P < 0.05$). These SNPs will be valid for marker-assisted selection breeding programs in *P. monodon*.

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Introduction

The giant tiger shrimp, *Penaeus monodon*, is one of the largest prawns among the shrimps. It has the advantages of fast growth, strong adaptability, and high nutritional value. It is one of the three largest shrimp breeds in the world at present and is also an important traditional high-quality shrimp resource in China and Southeast Asia. Many previous genetic studies have been performed on the penaeid shrimp (Ciobanu *et al.* 2010; Du *et al.* 2010; Yu *et al.* 2015). However, progress in genetic and biotechnology research has been slow because the currently available information on the correlation between genotypic and phenotypic variations is insufficient (Jung *et al.,* 2013; Prasertlux *et al.,* 2015).

Single nucleotide polymorphisms (SNPs) are the most abundant type of DNA sequence polymorphism which has been proven useful in genetic studies (Yu *et al.* 2014). The polymorphism of the amylase gene is significantly related to the growth of aquatic animals, and it is believed that molecular markers of the amylase gene have potential application value in breeding. The existing studies on prawns show that amylase is an important digestive enzyme of prawns. The amylase gene plays an important role in growth and development. The Cathepsin L gene is an important gene related to the growth of shrimp, which plays an important role in the molting process of shrimp growth and development. The Cathepsin L gene plays an important role in the growth, digestion, embryonic development, ovarian maturation, protein degradation, and energy supply of aquatic animals.

In conclusion, amylase and cathepsin L genes are considered important candidate genes related to the growth traits of shrimp. Analysis of gene-based SNP is one of the efficient approaches for discovering genes that significantly contribute to production traits (Liu & Cordes 2004). Alpha-amylase (*AMY*) and cathepsin L (*CTSL*) genes involved in a large number of important biological functions (proteolysis, digestion, immune response, intermolt cycle, etc.) were selected to identify useful SNPs for association studies and to evaluate their effects on production traits in penaeid shrimp (Glenn *et al.* 2005). These candidate genes are promising for the future development of molecular marker-assisted selection (MAS) in *P. monodon* breeding.

Materials and Methods

Healthy black tiger shrimp, *P. monodon,* were obtained from the experimental base of the South China Sea Fisheries Research Institute in Shenzhen (Guangdong, China). Thirty-two *P. monodon* individuals from four sample populations were used to identify SNPs. A different population of *P. monodon* was developed from larval shrimp and raised in tanks available to the association study. This population consisted of 300 shrimps harvested in the adult stage and had a mean body weight of (39.52 ± 2.12) g. The *P. monodon AMY* (Gene Bank accession no. KU308415) and *CTSL* (Gene Bank accession no. KP998481) cDNA sequences were used to design four sets of PCR primers **(Table1)**.

Firstly, amplification and sequencing of *AMY* and *CTSL have performed* on 32 *P. monodon* genomic DNA samples. The PCR reaction systems were as follows: 20 ng of genomic DNA, 3.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer, 0.5 U Ex Taq DNA polymerase (TaKaRa, Dalian, China), PCR buffer and added ddH_2O to a total volume of 20 ml. The thermocycling programs setting were 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 56~60 °C for 30 s, 72 °C for 1 min, and a 10 min extension at 72 °C. The PCR products were analyzed through electrophoresis in 1.5% agarose gel and sequenced (Invitrogen, Guangzhou, China).

Table 1 Primers sequences used in SNP screening experiment

Primers	Sequence (5′–3′)	Application	Product length
AMY-1F	ATGGTCAGGTTCAGCAACGCCGTG	Fragment amplified	563bp
AMY-1R	CTTGCCCGTCGTTAGTCTGTTTCTGG		
AMY-2F	GGGACAACCGCCTCGACTGGATGG	Fragment amplified	650bp
AMY-2R	AGTAGTCAGGGAAACTATTGACAGTGC		
CTSL-1F	GAGATTCGGGGTAAACATTAGAAGA	Fragment amplified	479bp
CTSL-1R	GAGAAAGCCCAGCAAGAGCC		
CTSL-2F	TTCCGCAAGACTGGTCAGCT	Fragment amplified	437bp
CTSL-2R	TTTACCAGCCAGTAGTCTGTGCC		
SNP-213	TTTTTTTTTTTTTTTTTTTTTTTTGAACAAGCACAAGATTGCCA	SnaPshot	45bp
SNP-784	TTTTTTTTTTTTTTTTAGCCACAGACACTGGGCCAAC	SnaPshot	38bp
SNP-820	TTTTTTTTTTTTTTTTTTTTTTTTTTTTGCTGTAGAACTGGAATGACTC	SnaPshot	50bp

Results

The *AMY* gene fragment amplified by primers AMY-1 and AMY-2 contained six polymorphisms at nucleotides 267, 1371, 1488, 2229, and 2381 of the *P. monodon* sequence. The *P. monodon CTSL* gene fragment contained three polymorphisms at nucleotides 213, 784, and 820 sites.

For genotyping, all 300 prawns were genotyped by SnapShot (ABI 3730XL, USA) of PCR products for the *CTSL* SNPs. The primer sequences used in the SnaPshot experiment are shown in table1. Three genotypes were founded, AA/GA/GG for CTSL-213 and CC/CT/TT for CTSL-820, respectively. CC and CT genotypes were identified on CTSL-784.

The association analyses between growth traits (Body weight, BW; Body length, BL; Carapace length, CL; Carapace width, CW; Carapace height, CH; First pleon segment length, FSL; Second pleon segment length, SSL and telson length, TL) **(Figure 1)** and CTLS genotypes (CTSL-213, CTSL-784, and CTSL-820) were performed by using the Proc GLM (general linear model, GLM) in the PASW Statistics20.0 software, with genotype treated as a fixed effect. The correlation of candidate SNPs *CTSL* genotypes with growth traits were calculated and prominently displayed in **Table 2**.

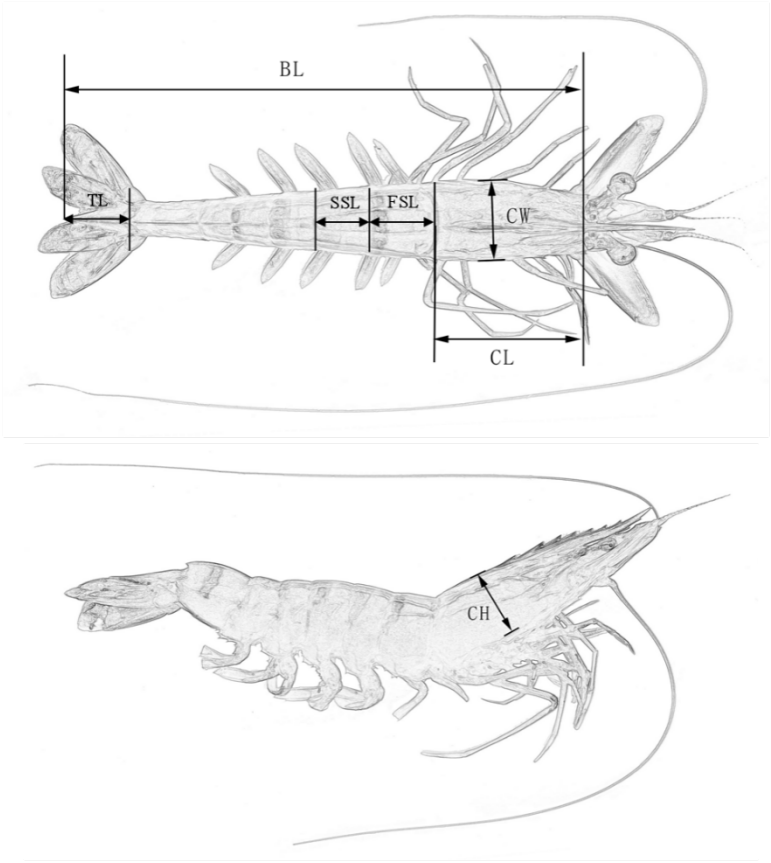

Figure 1 The phenotypic and growth Traits of shrimp *Penaeus monodon* (Body length, BL; Carapace length, CL; Carapace width, CW; Carapace height, CH; First pleon segment length, FSL; Second pleon segment length, SSL and telson length, TL)

Table 2 Correlation of candidate SNPs CTSL genotypes with growth traits

SNP	Genotype	Number	Frequency	BL (mm)	CL (mm)	CW (mm)	CH (mm)	FSL (mm)	SSL (mm)	TL (mm)	W (g)
CTSL-213	AA	129	43%	140.50±11.20^{a}	41.97±4.87^{a,b}	20.45±2.66^{b}	23.37±2.27^{b}	15.88±1.60^{a}	15.12±1.55^{a}	21.25±1.94^{a}	39.99±1.36^{b}
	GA	136	45.3%	140.87±11.38^{a}	42.99±4.01^{b}	20.90±6.05^{b}	23.30±2.51^{b}	16.08±1.55^{a}	15.55±1.69^{a}	21.53±2.35^{a}	39.83±1.11^{b}
	GG	35	11.7%	138.40±10.69^{a}	41.00±3.71^{a}	19.54±1.91^{a}	22.42±2.15^{a}	15.88±1.45^{a}	15.40±1.42^{a}	21.20±1.71^{a}	38.03±1.54^{a}
CTSL-784	CC	277	92.3%	141.43±11.05^{a}	42.31±4.37^{a}	20.58±2.34^{a}	23.26±2.36^{a}	15.98±1.56^{a}	15.36±1.62^{a}	21.38±2.11^{a}	39.53±1.19^{a}
	CT	23	7.7%	140.59±12.03^{a}	42.21±3.98^{a}	20.45±2.53^{a}	22.95±2.51^{a}	15.81±1.46a	15.22±1.47^{a}	21.18±2.21^{a}	39.56±1.91^{a}
CTSL-820	CC	251	83.6%	140.38±11.02^{a}	42.35±4.38^{a}	20.64±2.36^{a}	23.29±2.34^{b}	15.89±1.56^{a}	15.33±1.61^{a}	21.38±2.12^{a}	39.51±3.17^{a}
	CT	32	10.6%	141.09±13.15^{a}	42.40±5.08^{a}	20.18±2.41^{a}	23.00±2.46^{b}	15.84±1.56^{a}	15.68±1.53^{a}	21.25±2.34^{a}	40.33±2.87^{a}
	TT	17	5.7%	139.25±10.94^{a}	41.56±3.55^{a}	20.25±2.14^{a}	22.47±2.89^{a}	16.60±1.50^{b}	16.00±1.63^{a}	21.43±1.50^{a}	40.80±3.06^{a}

Discussion

At present, shrimp genetic breeding mainly depends on traditional breeding methods such as cross-breeding and family breeding, which is an inefficient, difficult, and long cycle. The combination of molecular marker-assisted breeding and traditional breeding is an important development direction of shrimp breeding in the future. However, due to the short time of decoding the shrimp genome information, the effective molecular markers are very limited. Developing more molecular markers associated with economic traits is the demand of shrimp genetics and breeding. Therefore, this study carried out the development of growth trait genes and markers, excavated molecular markers related to the growth traits of *P. monodon*, and provided important reference information for shrimp breeding.

The association analyses revealed no significant association of CTLS-784 with growth traits in the present population. However, a trend was observed that allele A at CTLS-213 SNP, AA, and GA, tended to be associated with increased body weight. The mean BWs of the three *CTSL* genotypes in the population were as follows: A/A, 39.99 ± 1.36 g; G/A, 39.83 ± 1.11 g; G/G, 38.03 ± 1.54 g. Furthermore, shrimps with genotype GG had significantly smaller CL, CW, and CH values than those with genotype GT and TT ($P < 0.05$). While CTLS-820 SNP was found to be significantly associated with CH and FSL ($P < 0.05$), shrimps with TT genotype had a smaller CH (22.47 ± 2.89mm) and longer FSL (16.60 ± 1.50mm) compared to those with CC or CT genotypes. In the end, the SNP markers identified and verified here will be a valuable resource for the *P. monodon* genetic study and selective breeding programs. Further studies need to be conducted to find more SNPs in both genes and to perform association analyses with growth-related traits in a large and diverse population.

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