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SNP analysis of Molting related genes in *Penaeus monodon* and *Litopenaeus vannamei* shrimp (Brief report)

(SNP-Analyse von Genen zur Häutung bei Garnelen, *Penaeus monodon* und *Litopenaeus vannamei*)

Background: Molting is a very important growth period in shrimp. During the intermolt cycle, the accumulations of carbohydrate protein provide steady growth. The molt-inhibiting hormone (MIH) and the crustacean hyperglycemic hormone (CHH) belong to a large neuropeptide family, which are involved in the regulation of the length of the intermolt period, keeping shrimp in the intermolt stage (GU et al., 2000; YODMUANG et al., 2004). There are two kinds of MIH (MIH1 and MIH2) and one CHH. Therefore, the *MIH1*, *MIH2* and *CHH* genes are candidate genes for detecting polymorphisms associated with the molting pathway and growth

Procedures:

Primer sequences:

The *P. monodon MIH1* and *MIH2* (GenBank accession no. AY496454 and AY496455, respectively), *L. vannamei CHH* (GenBank accession no. AY434016) gene sequences were used to design 4 sets of PCR primer for SNP discovery and genotyping.

MIH1- F: 5'-GGT CTG TCC TCA CTT TCG TT-3'
MIH1- R: 5'-CAC ACG CAC TAC CTT CTT GT-3'
MIH2-F: 5'-CGC GTA TTC CCC TAT GTA CC-3'
MIH2-R: 5'-GCG TGA CCG CTA GAC CTT AT-3'
CHH-1-F: 5'-AAA CGC TCG CTC TTC GAC-3'
CHH-1-R: 5'-TCC ATA GAT GAC GGA CCT G-3'
CHH-2-F: 5'-CGA GTG TGT GAC GAC TGT TTC-3'
CHH-2-R: 5'-TAA AGA TGG GTG CCT TGG AC-3'

PCR conditions:

The PCR amplification used standard lab protocols. PCR program: initial denaturation for 5 min at 94 ºC, 35 cycles each 30 s at 94 ºC, 35 s at 58 ºC and 35 s at 72 ºC and 5 min final extension at 72 ºC, in a Peltier Thermal Cycler (MJ Research).

SNP identification, genotyping and association analyses:

The population we used for identification of SNPs (single nucleotide polymorphisms) in *MIH1* and *MIH2* was a *P. monodon* full-sib mapping population (PM) developed by the Australian Institute of Marine Science and the Commonwealth Scientific and Industrial Research Organization (http://www.aims.gov.au/shrimpmap). The SNPs found in the two MIH genes were genotyped in the PM population consisting of 41 progeny. Two *L. vannamei* populations, LV1 and LV2 (76 animals in LV1 and 30 in LV2, respectively), were used to identify SNPs in *CHH*. Both of the two populations were developed from post larval shrimp and raised in small tanks at Iowa State University. The trait of body weight (BW) was measured at the post larval stage in population LV1, whereas BW of animals in population LV2 was recorded at later ages (GLENN et al., 2005). Two SNPs were found in *L. vannamei CHH* and genotyped in

both of the LV1 and LV2. Association analyses between the two SNPs and BW in the LV1 and LV2 populations were performed using PROC GLM in SAS (SAS Institute, Cary, NC, USA) with genotype as the fixed effect.

Results: One SNP was found in each of the two MIH genes (Table). Due to the fact that all the 41 full-sib animals in PM were heterozygous for both the SNPs, neither association analysis nor linkage mapping could be performed in the population. However, the SNPs we found in *MIH1* and *MIH2* are worth being evaluated in other *P. monodon* populations. Two SNPs were found in *L. vannamei CHH*. One of the SNPs detected in the *CHH* gene fragment amplified by primers CHH-1-F and CHH-1-R causes an amino acid change (C to Y) and affects the recognition site of *Pml* I (Table). Association analyses revealed that neither of the 2 SNPs had a significant effect on BW in both of the LV1 and LV2 populations. In the future, larger populations will allow precise estimates of the effects of the 2 SNPs on production traits in *L. vannamei* shrimp.

Table
Polymorphism detected in MIH1, MIH2 and CHH genes

Gene (GenBank accession no)	Population[1] (No. of animals)	Primer	SNP Position[2]	Restriction enzyme	Genotype frequency
CHH (AY434016)	LV1 (76)	CHH-2-F/R	Intron 1 A153G	*EcoR* V	0.00G/G 0.40A/G/ 0.60A/A
		CHH-1-F/R	Exon 1 G59A	*Aci* I	0.00A/A 0.42A/G 0.58G/G
	LV2 (30)	CHH-2-F/R	Intron 1 A153G	*EcoR* V	0.23G/G 0.50A/G/ 0.27A/A
		CHH-1-F/R	Exon 1 G59A	*Aci* I	0.30A/A 0.43A/G 0.27G/G
MIH1 (AY496454)	PM (41)	MIH1-F/R	Exon 2 T304G	*Pml* I	1.00 T/G
MIH2 (AY496455)	PM (41)	MIH2-F/R	Intron 1 T106C	*Nla* III	1.00 C/T

[1] LV = *L. vanname*, PM= *P. monodon*
[2] Nucleotide positions are numbered according to the first base of each gene as in GenBank

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