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Short communication. SNP analyses of the 5HT1R and STAT genes in Pacific white shrimp, *Litopenaeus vannamei*

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Abstract

Shrimp aquaculture is one of the growing animal industries. Breeding stocks with high disease-resistance and growth rate are of particular interest to shrimp breeders. Association studies are useful to find genetic markers for marker assisted selection of animals. Using six *Litopenaeus vannamei* individuals from a sample population, four single nucleotide polymorphisms (SNPs) were found in the 5HT1R (5-hydroxytryptamine receptor1) gene and one SNP in the STAT (signal transducer and activator of transcription) gene. However, the SNP in the STAT gene was homozygous in a different population used for association analyses. The association analyses revealed that allele C of two SNPs, C109T and C395G in 5HT1R, tended to be associated with increased body weight. Further studies need to be conducted using a large and diverse population sample.

Additional key words: growth, 5-hydroxytryptamine receptor1, signal transducer and activator of transcription.

Resumen

Análisis tipo SNP de los genes 5HT1R y STAT en el camarón del Pacífico, *Litopenaeus vannamei*

La industria de la producción de camarón es una de las industrias acuícolas que se encuentra en más crecimiento en la actualidad. Los estudios para encontrar marcadores genéticos son muy efectivos para la mejora de sus propiedades y de gran interés para los productores de camarón. En este trabajo se utilizaron seis individuos de una población de *Litopenaeus vannamei*, donde se encontraron cuatro polimorfismos de nucleótido único (SNPs) en el gen 5HT1R (5-hidroxitriptamina receptor1) y un SNP en el gen STAT (transductor de señal y activador de la transcripción). Sin embargo, el polimorfismo en el gen STAT resultó ser homocigoto en una población diferente utilizada para análisis de asociación. Los presentes análisis revelaron que el alelo C, en dos polimorfismos SNP (C109T y C395G) del gen 5HT1R, tiende a estar asociado con el aumento del peso corporal. Consideramos que hay necesidad de hacer nuevos estudios utilizando una muestra más amplia y diversa de la población en cuestión.

Palabras clave adicionales: crecimiento, receptor 1 de la 5-hidroxitriptamina, transductor de señal y activador de la transcripción.

Shrimp aquaculture is a rapidly growing industry generating billions of dollars per year (http://www.asicoaquaticmarkers.com/SustainabilityinShrimp.htm). Breeding stocks with high disease-resistance and growth rate are of particular interest to shrimp breeders (García *et al.,* 2009). The improvement of breeding stocks requires the advancements in shrimp genomics. However, the studies using single nucleotide polymorphisms (SNPs) on shrimp production traits were very few (Glenn *et al.,* 2005; Yu *et al.,* 2006). Recently, a computational program (SNPIDENTIFIER) was developed to predict SNPs using available expressed sequence tags (ESTs) in *Litopenaeus vannamei* (Gorbach *et al.,* 2009).

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Abbreviations used: 5HT1 (5-hydroxytryptamine), cDNA (complementary DNA), EST (expressed sequence tags), JAK (janus kinase), PCR (polymerase chain reaction), RFLP (restriction fragment length polymorphism), SNP (single nucleotide polymorphism), STAT (signal transducer and activator of transcription), WSSV (white spot syndrome virus).

The 5HT1 receptor (*5HT1R*) and signal transducer and activator of transcription (*STAT*) genes are potentially involved in the growth and immune response pathways. Hence they were chosen in this study to analyze the association of single nucleotide polymorphism (SNP) with growth in a shrimp population. The 5HT1R protein is a G protein coupled receptor which mediates the function of serotonin (5-hydroxytryptamine, 5HT). Serotonin is a biogenic neurotransmitter involved in a wide variety of physiological and behavioral functions, including reproduction, sleep, appetite, learning, pain perception, and circadian rhythm. In addition, it stimulates a crustacean hyperglycemic hormone (Ongvarrasopone *et al.,* 2006). The STAT protein is in the JAK/STAT pathway, which is involved in both growth and immunity. Earlier studies in many gene knockout models reported that the JAK-STAT signaling pathway is essential for postnatal growth (Schaefer *et al.,* 2001). Similarly it was found that WSSV (white spot syndrome virus) activated STAT proteins by phosphorylation but inhibited its transcription in shrimp (Chen *et al.,* 2008).

Six *L. vannamei* individuals belonging to a sample population were used for identification of SNPs. A different population of *L. vannamei* was developed from post-larval shrimp purchased from Oceanic Institute, Kona, HI, USA and grown at Iowa State University in small tanks, which limited the number of shrimp available to the association study. This population consisted of 76 animals which were harvested in the post-larval stage and had a mean body weight of 0.35 (±0.06) g. The *Peneaus monodon 5HT1R* (Gene Bank accession no. AY661549) and *STAT* (Gene Bank accession no. AY327491) cDNA sequences were used to design two sets of PCR primers. Amplification of *L. vannamei* genomic DNA with 5HT1Ra primers produced a 528-bp fragment; and the STAT primers produced an 800-bp fragment.

The primer sequences were: 5HT1Ra F: 5'-AAC CTG CTG GAG TAC CTG GA-3'; 5HT1Ra R: 5'-GAA GAA GTG GCT CTG GTT GG-3'; STATa F: 5'-ACA AAC ACA CGC TTC ACA GC-3'; and STATa R: 5'-CGG CCT TGT TCT GCA AAT-3'.

Initially, amplification and sequencing of *5HT1R* and *STAT* gene fragments were performed on six test *L. vannamei* genomic DNA samples from different populations. The 10 µL PCR mixture contained the following: 1 µL of genomic DNA (12.5 ng μL^{-1}), 2 µL of 5X PCR buffer (Promega), 0.5 µL of 10 mM dNTPs, 0.1 µL of each primer (25 pmol μL^{-1}), 0.05 µL of Taq DNA polymerase (5 U μL^{-1}) (Promega, Madison, WI, USA) and 6.25 µL of water. The PCR was done with the following conditions (PCR conditions/program): denaturation at 95°C for 2 min, then 38 cycles of denaturation at 95°C for 1 min, annealing at 61°C (5HT1Ra) or 52°C (STATa) for 30s and extension at 72°C for 30s followed by a final extension at 72°C for 4 min. Each PCR sample (2 µL) was subjected to 1.5% agarose gel electrophoresis. The PCR products of 5HT1R and STAT were sequenced by the sequencing facility at Iowa State University. Four exonic SNPs and one intronic SNP were identified in the 5HT1R and STAT gene fragments, respectively.

Later all 76 animals were genotyped by direct sequencing of PCR products for the 5HT1 receptor SNPs. However, the genotyping for the *STAT* gene was done by restriction fragment length polymorphism (RFLP), and some genotypes were confirmed by sequencing. To amplify a short sequence (297 bp) containing a SNP in the STAT gene fragment, specific primers were designed within the obtained sequence (800 bp) from earlier PCR amplified fragment.

Table 1. Polymorphisms of 5HT1R and STAT in *Litopenaeus vannamei*

Gene	Polymorphism	Restriction enzyme	Fragment length (bp)	No. shrimp genotyped	Genotype frequencies		
5HT1R	Exon 2 A88G	DS[1]	DS	76	0.00 A/A	0.01 A/G	0.99 G/G
	Exon 2 C109T	DS	DS	76	0.54 C/C	0.48 C/T	0.00 T/T
	Exon 2 C395G	DS	DS	76	0.28 C/C	0.48 C/G	0.24 G/G
	Exon 2 C398G	DS	DS	76	0.24 C/C	0.46 C/G	0.29 G/G
STAT	Intron 12 A165G	EcoRV	297 (A/A) 297, 167, 130 (A/G) 167, 130 (G/G)	76	1.00 A/A	0.00 A/G	0.00 G/G

[1] DS: direct sequencing.

Table 2. Association of 5HT1R polymorphisms with body weight in *Litopenaeus vannamei*

Polymorphism	Genotype	No. shrimp	Least Squares Mean (SD)	*P* value
Exon 2 C109T	C/C	41	0.41 (0.61)	0. 26
	C/T	34	0.27 (0.47)	
Exon 2 C395G	C/C	21	0.33 (0.61)	0.62
	C/G	36	0.41 (0.55)	
	G/G	18	0.25 (0.47)	

The specific primer sequences were STATsnp F: 5'-AGG AGG CGG AGA ACT TGT TT-3'; and STATsnp R: 5'-TGA CTA CAA CTG GCA AAG ACA-3'.

The PCR was done at an annealing temperature 62°C using same PCR protocol as mentioned above. The restriction digestion was done by using the EcoRV restriction enzyme according to the manufacturer's instructions (New England Biolabs, Beverly, MA, USA). The resulted digestion fragments were separated by electrophoresis on a 3% agarose gel and visualized using ethidium bromide and UV light. The restriction fragments were 297 bp, 167 bp and 130 bp (Table 1). The association analyses between body weight and 5HT1 receptor genotypes (C109T, C395G) were performed by using the Proc GLM in the SAS software, with genotype treated as a fixed effect.

The polymorphisms identified in the 5HT1 receptor gene were in exon 2. These exonic SNPs did not alter the amino acid sequence. The SNPs were found at nucleotides 88, 109, 395 and 398 of amplified 5HT1 receptor gene fragment (Table 1). The allele frequency of all 4 SNPs are presented in Table 1. The association analyses revealed that no significant association of 5HT1 receptor with body weight existed in the present population. However, a trend was observed, that allele C at SNPs, C109T and C395G, tended to be associated with increased body weight (Table 2). The RFLP genotyping results of STAT SNP showed that all 76 animals in this population were homozygous (AA), and were confirmed by direct sequencing. Hence further studies are recommended to find more SNPs in both genes and to perform association analyses with growth related traits in larger populations.

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