THE POLYMORPHISM OF GHR GENE ASSOCIATED WITH THE GROWTH AND CARCASS TRAITS IN THREE RABBIT BREDS

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ABSTRACT

Although the growth hormone receptor (GHR) has been widely proposed to affect the carcass traits in livestock, its potential role in domestic rabbits has not been intensively investigated. In the present study, a non-synonymous SNP was identified in exon 3 (c.106 G>C) of GHR gene, which resulted in the amino acid substitution (V36L). This SNP was subsequently genotyped by using PCR-SSCP method among the two hundred and sixty-six individuals from three rabbit breeds (51 Tianfu black rabbits, 104 Ira rabbits, and 111 Champagne rabbits). The allele frequencies across the three breeds were 0.71 for allele G and 0.29 for allele C, which suggested the moderate polymorphism. The association analyses revealed that the pH value of longissimus muscle after slaughter 24 h (LpH24) of CC genotype was significantly higher than GC and GG genotypes (P<0.01); the 84-day-weight, eviscerated weight, semi-eviscerated weight, eviscerated slaughter rate, and semi-eviscerated slaughter rate of CC genotype was significantly higher than GC genotype (P<0.05); the eviscerated slaughter rate and pH of hind leg muscle after slaughter 24 h (HpH24) of CC genotype was significantly higher than GG genotype (P<0.05). In contrast, GHR polymorphism had no significant influence on 28-day-weight, 35-day-weight, and 70-day-weight. This SNP of GHR gene could be applied with marker-assistant selection to improve carcass traits.

Key words: Rabbit, GHR gene, polymorphism, PCR-SSCP, growth traits, carcass traits.

INTRODUCTION

As an important endocrine factor, growth hormone (GH) regulates the metabolic procedures of growth and development. During the biological process of GH action, the first step is the binding to the growth hormone receptor (GHR), followed by the activation of the JAK-STAT pathway and expression of insulin-like growth factor 1 (IGF1) and the other target genes (Herrington and Carter-Su, 2001). Among them, GHR is a member of the cytokine / hematopoietin receptor superfamily (VanderKuur et al., 1994) and consists of three functional domains of the extracellular (ligand-binding) domain, the transmembrane domain and the cytoplasmic domain (signal-transducing). The binding of GH to GHR causes receptor dimerization and initiates signaling cascades via the cytoplasmic domain (Frank, 2001). The polymorphisms of GHR gene may affect the binding capacity to GH (Bai et al., 2011), which was considered as the candidate gene to influence the growth, development, and carcass traits in farm animals. The GG genotype at P4 locus (c.114G>T) of GHR gene was proposed to has superior growth traits in Boer goats (An et al., 2011). A SNP located in the intron 4 of the GHR gene had the largest effects on body weight and feed efficiency in beef cattle (Sherman et al., 2008). The F297Y polymorphism located in exon 8 was associated with milk yield (Waters et al., 2011). In rabbit, GHR gene consists of ten exons encoding 638 amino acids (Leung et al., 1987). One study reported the association between polymorphism of GHR gene in exon 10 and carcass traits in rabbit (Deng et al., 2008). In human, the deletion of exon 3 in GHR gene was associated with increased responsiveness to high-dose recombinant human GH (Binder et al., 2006), which suggested that exon 3 may be very
important for binding to GH. Here, we studied the genetic polymorphism of exon 1, exon 3, and exon 6 of *GHR* gene in three meat rabbits breeds; the association with growth and carcass traits was subsequently investigated.

**MATERIALS AND METHODS**

**Growth traits and carcass traits collection, and genomic DNA isolation**

A total of 266 commercial meat rabbits from three breeds including 51 Tianfu Black rabbits, 104 Ira rabbits, and 111 Champagne rabbits were collected. The nutritional levels and feeding management were previously described (Zhang et al., 2011). Data of growth and carcass traits were collected. Growth traits include 28-day-weight, 35-day-weight, 70-day-weight, 84-day-weight, while carcass traits include eviscerated weight, semi-eviscerated weight, eviscerated slaughter rate, semi-eviscerated slaughter rate, pH of longissimus muscle after slaughter 24 h (*LpH24*), pH of hind leg muscle after slaughter 24 h (*HpH24*), intramuscular fat content of longissimus muscle (*LF*), and intramuscular fat content of hind leg muscle (*HF*). Ear tissues were collected for genomic DNA extraction using AxyPrep Genomic DNA Miniprep Kit (Axygen, USA).

**Mutation screening and genotyping using PCR-SSCP**

24 samples were randomly selected from 266 individuals to scan the genetic polymorphism in the exon 1, exon 3, and exon 6 using direct sequencing method. Three PCR primer pairs (Table 1) were designed according to the *GHR* gene sequence (GenBank accession no. AF015252) using Primer 5. The PCR was performed with the following condition: one denaturation cycle at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 54-56 °C for 30 s and 72 °C for 40-60 s, and ended with an extension cycle at 72 °C for 10 min. The 30 µL reaction volume includes 15 µL 2× Taq PCR MasterMix (TIANGEN, Beijing, China), 60 ng DNA template, 9.6 µL ddH2O, 12 pmol of each primer. The purified PCR products were directly sequenced in both directions using the BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing was performed on a 3700 DNA sequencer (Applied Biosystems) according to the manufacturer’s instructions. The primer 2 was used to amplify the small fragment for SSCP analysis.

**Table 1.** Primer sequences, PCR product sizes, Tm value and location

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequences (5′→3′)</th>
<th>Product (bp)</th>
<th>Annealing (°C)</th>
<th>Location</th>
<th>Purpose</th>
</tr>
</thead>
</table>
| 1      | F: AATCC ACC TTCAACC CTATC  
R: CCGGAGACTTCTTACAATGGCC | 263          | 56             | Exon 1    | sequencing |
| 2      | F: TTGCC TGGGGCAGGGTCACT  
R: TGGAGACGCGGGGCTGGCCTACT | 186          | 54             | Exon 3    | Sequencing, PCR-SSCP |
| 3      | F: GCCGGGTACCGGGGCTTAGGT  
R: AGAGGGTGGTCTGGGGTAGGGG | 336          | 56             | Exon 6    | Sequencing |

**Data analysis**

The effects of genotypes on the traits were analyzed by the least-squares method as applied in the General Linear Model (GLM) procedure of SAS 9.2 program according to the following statistical model:

\[ Y_{ijk} = \mu + G_i + M_j + B_k + e_{ijkl} \]

Where \( Y_{ik} \) is a record of the trait, \( \mu \) is the overall mean of observations, \( G_i \) is the gender effect, \( M_j \) is the fixed genotype effect, \( B_k \) is the fixed breed effect, and \( e_{ijkl} \) is the residual error.
RESULTS AND DISCUSSION

Among the ten exons of rabbit GHR gene, exons 1, 3 and 6 were resequenced for three rabbit breeds in this study. No variation was identified in exon 1 and exon 6. In exon 3, we detected one non-synonymous mutation of c.106G>C, which leaded the change of valine to leucine (V36L). This V36L is located within the extracellular GH-binding domain of the GHR and highly conserved among all analyzed mammals (human, chimpanzee, rabbit, mouse, rat, dog, pig, sheep, goat, and cattle; data are not shown). Further more, the region of exon 3 was also shown to be conserved in mammalian species (Kelly et al. 1993). In human, the GHR isoform with the absence of exon 3 (d3-GHR) was associated with 1.7 to 2 times more growth acceleration induced by growth hormone than the full-length isoform (Dos Santos et al., 2004). So, we considered the V36L would play important biological role.

The frequencies of alleles G and C for the three breeds were 0.71 and 0.29, respectively (Table 2), which showed high heterozygosity (He) and moderate polymorphism information content (PIC). Effects of genotypes on the traits analyzed by the least-squares method are shown in Table 3. The pH of longissimus muscle after slaughter 24h (LpH24) of CC genotype was significantly higher than GC and GG genotypes ($P < 0.01$). The 84-day-weight, eviscerated weight, semi-eviscerated weight, eviscerated slaughter rate, and semi-eviscerated slaughter rate of CC genotype were significantly higher than GC genotype ($P < 0.05$). Meanwhile, the eviscerated slaughter rate and pH of hind leg muscle after slaughter 24h (HpH24) of CC genotype was significantly higher than GG genotype ($P < 0.05$). Nevertheless, CC genotype was slightly higher than GC and CC for other growth and carcass traits. The polymorphism of GHR was significantly associated with carcass traits in the rabbit which is consistently with former reports in other livestock (Sherman et al., 2008; An et al., 2011).

Table 2. The frequencies of allele and genotype of the c. 106G>C variation site

<table>
<thead>
<tr>
<th>Breed</th>
<th>n</th>
<th>GG frequency</th>
<th>GC frequency</th>
<th>CC frequency</th>
<th>Allele frequency</th>
<th>Genetic characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tianfu rabbit</td>
<td>51</td>
<td>21(0.4118)</td>
<td>27(0.5294)</td>
<td>3(0.0588)</td>
<td>0.6765 0.3235</td>
<td>0.5294 0.3419 0.7285</td>
</tr>
<tr>
<td>Iraq rabbit</td>
<td>104</td>
<td>50(0.4808)</td>
<td>27(0.2596)</td>
<td>27(0.2596)</td>
<td>0.6106 0.3894</td>
<td>0.2596 0.3625 1.9067</td>
</tr>
<tr>
<td>Champagne rabbit</td>
<td>111</td>
<td>73(0.6577)</td>
<td>36(0.3243)</td>
<td>2(0.018)</td>
<td>0.8198 0.1802</td>
<td>0.3243 0.2518 1.4193</td>
</tr>
<tr>
<td>Total</td>
<td>266</td>
<td>144(0.5414)</td>
<td>90(0.3383)</td>
<td>32(0.1203)</td>
<td>0.7105 0.2895</td>
<td>0.3383 0.3267 1.6988</td>
</tr>
</tbody>
</table>

He, Heterozygosity. PIC, Polymorphism information content, Ne, Effective number of alleles.

Table 3. Least square means of growth and carcass traits of different genotypes

<table>
<thead>
<tr>
<th>Traits$^1$</th>
<th>GG (144)</th>
<th>GC (90)</th>
<th>CC (32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpH24</td>
<td>5.72±0.01$^b$</td>
<td>5.73±0.01$^b$</td>
<td>5.74±0.02$^a$</td>
</tr>
<tr>
<td>84-day-weight (g)</td>
<td>2613±20$^{ab}$</td>
<td>2525±24$^b$</td>
<td>2632±43$^a$</td>
</tr>
<tr>
<td>eviscerated weight (g)</td>
<td>1336±12$^{ab}$</td>
<td>1305±14$^b$</td>
<td>1365±25$^a$</td>
</tr>
<tr>
<td>semi-eviscerated weight (g)</td>
<td>1447±13$^{ab}$</td>
<td>1414±15$^b$</td>
<td>1477±27$^a$</td>
</tr>
<tr>
<td>semi-eviscerated slaughter percentage (%)</td>
<td>0.570±0.002$^{ab}$</td>
<td>0.567±0.002$^b$</td>
<td>0.575±0.004$^a$</td>
</tr>
<tr>
<td>eviscerated slaughter percentage (%)</td>
<td>0.527±0.002$^b$</td>
<td>0.523±0.002$^b$</td>
<td>0.531±0.004$^a$</td>
</tr>
<tr>
<td>HPH24</td>
<td>5.78±0.01$^b$</td>
<td>5.79±0.01$^{ab}$</td>
<td>5.83±0.02$^a$</td>
</tr>
<tr>
<td>28-day-weight (g)</td>
<td>526±8$^a$</td>
<td>526±10$^a$</td>
<td>552±17$^a$</td>
</tr>
<tr>
<td>35-day-weight (g)</td>
<td>833±20$^a$</td>
<td>783±23$^a$</td>
<td>849±42$^a$</td>
</tr>
<tr>
<td>70-day-weight (g)</td>
<td>2174±15$^a$</td>
<td>2119±17$^a$</td>
<td>2179±31$^a$</td>
</tr>
<tr>
<td>LF (%)</td>
<td>0.0164±0.0007$^b$</td>
<td>0.0179±0.0008$^a$</td>
<td>0.0186±0.0015$^a$</td>
</tr>
<tr>
<td>HF (%)</td>
<td>0.0286±0.0014$^a$</td>
<td>0.0300±0.0015$^a$</td>
<td>0.0324±0.0028$^a$</td>
</tr>
</tbody>
</table>

$^1$HpH24 represents the trait of pH of hind leg muscle after slaughter 24 h, LpH24 for pH of longissimus muscle after slaughter 24 h, LF for intramuscular fat content of longissimus muscle, and HF for intramuscular fat content
of hind leg muscle. In the same row, different lowercase letters and capital letters mean significant difference at 0.05 and 0.01 levels, respectively.

**CONCLUSION**

We identified the c.106G>C polymorphism of rabbit GHR gene in exon 3, which resulted in the amino acid substitution (V36L). This SNP was suggested to associate significantly with carcass traits and could be applied with marker-assistant selection to improve carcass traits in rabbits.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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