STUDIES ON PORCINE MYOSTATIN IN 5’ PROMOTER REGION

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INTRODUCTION
Myostatin gene (also called GDF-8), which is a member of TGF-β superfamily, is a negative regulator of skeletal muscle mass. It was first identified by Mcpherron et al. (1997). Transgenic mice produced by gene knockout without the myostatin gene were 200-300 % bigger than common mice. Mutant homozygotes for this gene could reproduce normally and there was no abnormality in other tissues and organisms. As soon as the result was published, the researchers became interested in this gene and made a lot of studies. The following investigations showed that the double muscle characteristics of Belgian Blue and Piedmontese all resulted from mutations in this gene (Gonzalez et al., 1998; Grobet et al., 1998; Grobet et al., 1997). Strail et al. (1999) found that there was a T→A mutation at the nt position of 607 of genomic sequence (accession number AJ133580). Sequence analysis showed that this T→A mutation gave rise to a digestion site of Dra I and polymorphism of digestion fragments. Based on this alteration, we analyzed the polymorphism in Yorkshire, Landrace, Duroc, Junmu and Minzhu pig breeds to reveal the relationship between the gene and muscle development.

MATERIALS AND METHODS
Ear tissue samples. Pig’s ear tissues came from Lanxi pig-farm, Heilongjiang, Military Supplies University of People’s Liberation Army of China, and pig-farm of Northeast Agricultural University.

Major reagents. Taq DNA polymerase, dNTPs, DL2000 marker were bought from Takara CO. Ltd. Restriction endonuclease were bought from Promega CO. Ltd. DNA extracting. Ear tissue was broken, digested with proteinaseK overnight, extracted by phenol, chloroform and isopentanol respectively, precipitated with cool-ethanol, rinsed in 75 ethanol, dissolved in TE buffer and stored at –20℃.

Primer design and PCR reactions. Primer was designed according to the sequence of myostatin gene in Genbank (accession number AJ133580). F : 5’-TCT GAG GGC AAA CTG CAT TAT C-3’, R : 5’-AGC AAC AAT CAG CAT AAA CAG G-3’. PCR volume was 25 μl. PCR conditions : 94℃, 30s, 55℃, 45s, 72℃, 1 min, 30 times, 72℃, 7 min.

Digestion reaction. Mixed 10 μl PCR product with 5U Dra I to 20 μl of final volume, incubated 2 hours at 37℃, examined by 2 % agarose gel electrophoresis.

RESULTS AND ANALYSIS
DNA extracting and PCR amplification. Genomic DNA was tested by 0.7 % agarose gel electrophoresis and we could see a perfect band in UV. DNA fragment amplified with our primer should be 1024bp according to pig myostatin gene. PCR products were tested by 1 % agarose gel
electrophoresis. Results showed that there was a specific band in 1024bp areas. Moreover, all amplifications got specific PCR products.

**PCR product digestion and phenotype analysis.** A fragment of 419bp and a few smaller fragments were detected after PCR product was digested by Dra I in wild type, while a fragment of 353bp and a few smaller fragments were revealed in mutant type for the increasing of Dra I digestion site. Results of electrophoresis showed that digestion products were similar to that concluded from theory, and PCR products were digested completely (figure 1).

![Figure 1. 5' promoter region of porcine myostatin gene digested by Dra I](image)

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>TT</th>
<th>TA</th>
<th>AA</th>
<th>T</th>
<th>A</th>
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<tr>
<td>Landrace</td>
<td>26</td>
<td>0.9231(24)</td>
<td>0.0769 (2)</td>
<td>0</td>
<td>0.9616</td>
<td>0.0384</td>
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<tr>
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<td>34</td>
<td>0.9412 (32)</td>
<td>0.0588 (2)</td>
<td>0</td>
<td>0.9706</td>
<td>0.0294</td>
</tr>
<tr>
<td>Duroc</td>
<td>27</td>
<td>0.8889(24)</td>
<td>0.1111 (3)</td>
<td>0</td>
<td>0.9444</td>
<td>0.0555</td>
</tr>
<tr>
<td>Junnu</td>
<td>40</td>
<td>0 (40)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Minzhu</td>
<td>36</td>
<td>0.3056(11)</td>
<td>0.3889 (14)</td>
<td>0.3056(11)</td>
<td>0.5000</td>
<td>0.5000</td>
</tr>
</tbody>
</table>

**Polymorphism analysis on 5' promoter region of porcine myostatin gene.** Digestion analysis on 163 pigs of 5 breeds in this region showed that the T allele dominates in Yorkshire, Landrace, Duroc and there was no mutant homozygote in 87 pigs. There was no allele A detected in 40 individuals of Junnu breed, while the frequencies of these three genotypes was about equal in Minzhu (table 1). The result of X² analysis showed that the distribution of the three genotypes was very different between Minzhu and the other breeds.

**DISCUSSION**

We analyzed polymorphisms of 5’ promoter region of porcine myostatin gene of five different breeds by PCR-RFLP. The allele T dominates in the imported lean-type pig breeds such as Yorkshire, Landrace and Duroc. No allele A was detected in 40 individuals of Junnu breed and...
the frequencies of the three phenotypes was about equal in Minzhu breed. This indicated that this mutation may be related to the expression of the myostatin gene. Yorkshire, Duroc, Landrace are imported lean-type breeds. Junmu was also a breed with a high lean percentage. With the result from Jiang Yunliang (2001), we concluded that this mutation might be a molecular marker of a porcine lean percentage QTL.

REFERENCES