Genotypes of 5′-flanking region in porcine heat-shock protein 70.2 gene affect backfat thickness and growth performance in Duroc boars

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Abstract

The 5′-flanking region in heat-shock protein 70.2 gene (HSP70.2) has been found to be associated with meat quality, birthweight, and semen quality in pigs. This study examines the relation between the genotype of five polymorphic sites in the 5′-flanking region of porcine HSP70.2 with backfat thickness and growth performance under different environmental conditions, especially the cool (low temperature 12–19 °C, high temperature 19–25 °C) and hot (low temperature 21–25 °C, high temperature 28–33 °C) seasons. A total of 216 Duroc boars were performance tested in both seasons using segregated early weaning entrance. Haplotypes of the 5′-flanking region of the animals were determined using the single nucleotide polymorphisms of their siblings and/or parents. Additionally, six haplotypes were found in the tested samples and used to construct 15 genotypes. The animals with the most prevalent nine genotypes were further analyzed to determine the interaction between genotype and season. Genotype significantly influenced backfat thickness ($P<0.001$). Furthermore, the interaction of genotype and season was found to influence both backfat thickness ($P<0.01$) and feed efficiency ($P<0.05$). Most of the variation in backfat thickness and feed efficiency was caused by pigs of certain genotypes raised during the cool season. Taken together, we suggest that variation of backfat deposition under different weather conditions may occur in Duroc boars with different genotypes of the 5′-flanking region of HSP70.2.

Keywords: Heat-shock protein 70; 5′-Flanking region; Single nucleotide polymorphisms; Backfat thickness; Growth performance; Duroc boars

1. Introduction

A complete nucleotide sequence of porcine heat-shock protein 70 gene (HSP70) was first reported by Peelman et al. (1992). The gene is located on chromosome 7 in the region of major histocompatibility complex and has 95% homology with human HSP70.
The expressed protein (HSP70) from the porcine heat-shock protein 70.2 gene (HSP70.2) is classified as an inducible form (Schwerin et al., 1999) and is related to heat stress or other stress induced response (Lindquist and Craig, 1988). The transcriptional region may control the expression of this inducible heat-shock protein gene (Tsukiyama et al., 1994). Schwerin et al. (1999) reported that the 5'-flanking region of HSP70.2 contains 13 polymorphic sites in four different breeds (German Landrace, Pietrain, German Large White, and German Saddleback). The polymorphisms in the 5'-flanking region of HSP70.2 were found to be associated with meat quality (color-brightness and conductivity), birthweight (Maak et al., 1998; Schwerin et al., 1999) and semen quality (Huang et al., 2002) in pigs. Meanwhile, the C/A transition in the inverted GC box (nt232) was found to be associated with color-brightness and birthweight (Maak et al., 1998). The polymorphic nt232 and an adenosine deletion right before the TATA box, nt250 site, jointly influenced conductivity of pork (Schwerin et al., 1999). Our previous study indicated that five nucleotide sites (nt44, nt232, nt250, nt345 and nt393) in this region were polymorphic in the three major breeds (Duroc, Landrace and Large White) in Taiwan (Chen et al., 2000; GenBank accession number AF139178).

In the hot and humid weather that lasted from April to October, pigs suffering heat stress might be more likely to express HSP70. The distribution of adipose tissues in pig changes under heat stress (Le Dividich et al., 1998). Furthermore, heat stress is associated with slower growth because of lower feed intake (Lopez et al., 1991; Rinaldo and Le Dividich, 1991; Becker et al., 1993). The Duroc breed is the major terminal sire used in commercial pig production in Taiwan (used in over 99% of breeding) because of its leanness and meat quality (Lo et al., 1992; Candek-Potokar et al., 2002). Understanding of the association between polymorphism in the 5'-flanking region of HSP70.2 and the leanness and growth performance of Duroc boars will benefit the pork industry and also help explore the function(s) of HSP70.2. This study investigates the association between polymorphism in the 5'-flanking region of porcine HSP70.2 and backfat thickness and growth performance in Duroc boars under different weather conditions.

2. Materials and methods

2.1. Animals

This study used a total of 216 purebred Duroc boars from 11 purebred farms around Taiwan. Genetic relationship among those tested boars was avoided as much as possible to ensure a random sample representing the Duroc population in Taiwan. The boars were sent to the Northern Central Testing Station (NCTS) at the Animal Technology Institute Taiwan (ATIT) in eight batches using the segregated early weaning (SEW) entrance method (July, September, October, November, December of 1999, and March, April, May of 2000). The body weight and age at entry were restricted to 4–7 kg (5.94 ± 1.35 kg in average) and 14–20 days (17.2 ± 2.1 days on average), respectively. The tested piglets were raised in the modular SEW nursery (Double L Group Inc., Dyersville, IA, USA) for approximately 42 days, then moved to the testing house.

2.2. Performance test

The performance test was described in detail by Huang et al. (1995). Briefly, the performance test started at a body weight of 30.0 ± 2.0 kg, and ended when the boars reached a body weight of 110.0 ± 2.0 kg. Each tested boar was kept in a single 4 m² pen, with animals in the same batch being kept in the same testing house. All the tested boars were fed the same diet of CP 18.5%, ME 3,140 kcal/kg, Ca 1.20%, P 0.80%, Lysine 1.06%, Methionine 0.41%, Cystine 0.30%, and Tryptophan 0.20% ad libitum. The daily temperature at the testing house and the daily feed intake were recorded. At the end of the performance test, five traits were recorded and calculated for each tested boar, namely: age in days at the start of the test (SAGE), average daily gain adjusted to 110 kg of body weight (ADG), feed efficiency (ratio of total feed intake to total body weight gain; FE), backfat thickness adjusted to 110 kg of body weight (BF), age in days adjusted to 110 kg body weight (A110).

2.3. DNA isolation and sequencing

Genomic DNA was isolated from blood using a DNA Isolation Kit for mammalian blood (Roche...
Diagnostics GmbH, Mannheim, Germany) as per the instructions of the manufacturer. The primers used in this study were designed according to the HSP70 sequence reported by Peelman et al. (1992), and the condition of the polymerase chain reaction was as described by Chen et al. (2000). The PCR products were purified from gels using a Gel Extraction Mini-prep Kit (Viogene, Sunnyvale, CA, USA), and sequenced in both the forward and reverse directions. Meanwhile, the nucleotide sequences were recorded using an automated DNA sequencer (ABI 377, Perkin-Elmer, Forster, CA, USA).

2.4. Determination of genotype

As noted in our earlier investigation, five nucleotide polymorphisms existed in the 5′-flanking region of HSP70.2 of three major pig breeds (Duroc, Landrace, and Yorkshire) in Taiwan, namely: nt44 (A/C), nt232 (A/C), nt250 (A/−, ‘−’ denotes deletion), nt345 (T/C), and nt393 (T/C) (Chen et al., 2000). To maximize the quantity of information in this association study, haplotypes of the region in homologous chromosomes for each individual were determined according to the single nucleotide polymorphism (SNP) of their siblings and/or parents. Consequently, the genotype of that region for each individual could be determined by the two haplotypes. For example, the SNP of the 5′-flanking region for a particular individual has C/C, C/C, A/−, T/T, and T/C on the nucleotide sites of nt44, nt232, nt250, nt345, and nt393, respectively. Two possibilities for haplotype combination are either CCATT/CC−TC or CCATC/CC−TT. After the SNPs of siblings and/or parents are verified, the correct haplotype combination can be determined and used as the genotype for the region.

2.5. Statistical analysis

The statistical model includes a season effect in the performance test, namely: cool (the whole test period between November and April with a low temperature range of 12–19 °C and a high temperature of 19–25 °C) and hot (between May and October with a low temperature range of 21–25 °C and a high temperature range of 28–33 °C). To achieve a clear contrast between the cool and hot seasons, the period of intermediate weather in which animals were tested across cool and hot conditions was excluded from the analysis. One hundred and eighty-three animals with the nine major genotypes were tested in either the cool or the hot seasons to provide information on the effect of interaction between season and genotype. Meanwhile, the performance traits of those 183 animals were compared in the statistical analysis.

The four performance traits (ADG, FE, BF, and A110) were analyzed by using a linear model with SAGE as a covariate, along with the test season, the genotype of the 5′-flanking region of HSP70.2, and interaction between the genotype and test season. Statistical analysis was conducted using the SAS GLM procedure (SAS Institute, 1989). The effects of regional genotype and its interaction with season were also compared using the least-squares means method.

3. Results

3.1. Frequency of haplotype and genotype of the 5′-flanking region of porcine HSP70.2

A total of 216 purebred Duroc boars were used to estimate the frequencies of haplotype and genotype of the 5′-flanking region of porcine HSP70.2. The 216 tested boars had 432 chromosomes with only six different haplotypes, which were termed hp1 to hp6 for simplicity. Table 1 lists the distribution of the six haplotypes. The frequencies of hp1, hp2, and hp3 added up to 88%. These three haplotypes should be the major combinations of the SNP in the 5′-flanking region of HSP70.2. The hp3 haplotype had the highest

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>CCATT</th>
<th>CC-TC</th>
<th>CCATC</th>
<th>CCACC</th>
<th>AAACC</th>
<th>CC-TT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple notion</td>
<td>hp1</td>
<td>hp2</td>
<td>hp3</td>
<td>hp4</td>
<td>hp5</td>
<td>hp6</td>
<td>hp6</td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>24.3</td>
<td>20.8</td>
<td>43.1</td>
<td>1.2</td>
<td>9.5</td>
<td>1.2</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* ‘−’ indicates the deletion of nucleotide at the nt250 site.*
frequency among all animals (43.1%). Conversely, the hp4 and hp6 haplotypes had the least frequency (1.2%). Combining two of these six haplotypes to construct different sets of individual genotypes revealed only 15 genotypes in the sample population (Table 2). Among the 15 genotypes, the nine major genotypes accounted for 202 (93.5%) of these 216 animals, and are listed as follows: hp1/hp1, hp1/hp2, hp1/hp3, hp1/hp5, hp2/hp2, hp2/hp3, hp2/hp5, hp3/hp3, and hp3/hp5.

3.2. Effects of genotype of the 5′-flanking region of porcine HSP70.2 on backfat thickness and growth performance

To examine the effect of SNPs in the 5′-flanking region of porcine HSP70.2 on backfat thickness and growth performance, 183 animals with the nine major genotypes were tested in either the cool or the hot seasons to provide information on the effect of interaction between season and genotype. Table 3 presents the effect of the genotypes of the 5′-flanking region of HSP70.2 on growth performance and BF. Genotypes had no significant effect on ADG, FE, and A110, and BF was the only trait influenced by the genotypes of the 5′-flanking region of HSP70.2 (P < 0.001). Notably, the boars with genotypes hp2/hp5, hp3/hp3, and hp3/hp5 exhibited significantly thicker BF than those with other genotypes, while animals with genotype hp2/hp3 showed moderate BF thickness. The hp2/hp2 genotypes and genotypes with at least one hp1 haplotype significantly reduced BF.

Since significant interaction occurred between genotype and season which affected BF (P < 0.01) and FE (P < 0.05), the differences among genotypes of the 5′-flanking region of HSP70.2 listed in Table 3 should be considered along with season effect. Based on the results of this interaction, the effects of the genotypes of this region on BF and FE can be determined within each season (Table 4). For BF, differences among genotypes actually occurred during the cool season rather than the hot season (P < 0.01). The animals

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ADG</th>
<th>FE</th>
<th>BF</th>
<th>A110</th>
</tr>
</thead>
<tbody>
<tr>
<td>hp1/hp1</td>
<td>0.987 ± 0.077</td>
<td>2.07 ± 0.05</td>
<td>1.35 ± 0.04</td>
<td>155.9 ± 2.1</td>
</tr>
<tr>
<td>hp1/hp2</td>
<td>0.994 ± 0.068</td>
<td>2.20 ± 0.05</td>
<td>1.35 ± 0.03</td>
<td>151.9 ± 1.9</td>
</tr>
<tr>
<td>hp1/hp3</td>
<td>1.068 ± 0.034</td>
<td>2.20 ± 0.02</td>
<td>1.39 ± 0.02</td>
<td>153.3 ± 0.9</td>
</tr>
<tr>
<td>hp1/hp5</td>
<td>0.987 ± 0.086</td>
<td>2.23 ± 0.06</td>
<td>1.37 ± 0.04</td>
<td>154.3 ± 2.3</td>
</tr>
<tr>
<td>hp2/hp2</td>
<td>0.971 ± 0.086</td>
<td>2.25 ± 0.06</td>
<td>1.31 ± 0.04</td>
<td>157.0 ± 2.3</td>
</tr>
<tr>
<td>hp2/hp3</td>
<td>1.011 ± 0.041</td>
<td>2.18 ± 0.03</td>
<td>1.41 ± 0.02</td>
<td>153.2 ± 1.1</td>
</tr>
<tr>
<td>hp2/hp5</td>
<td>0.976 ± 0.100</td>
<td>2.17 ± 0.07</td>
<td>1.53 ± 0.05</td>
<td>154.2 ± 2.7</td>
</tr>
<tr>
<td>hp3/hp3</td>
<td>0.977 ± 0.053</td>
<td>2.50 ± 0.04</td>
<td>1.48 ± 0.03</td>
<td>154.9 ± 1.4</td>
</tr>
<tr>
<td>hp3/hp5</td>
<td>0.960 ± 0.063</td>
<td>2.16 ± 0.04</td>
<td>1.48 ± 0.03</td>
<td>156.4 ± 1.7</td>
</tr>
</tbody>
</table>

Least-squares means differ significantly (P < 0.05) for genotypes with the same trait but different superscripts A–D.

a ADG, average daily gain adjusted to 110 kg of body weight; FE, feed efficiency as represented by the ratio of total feed intake to total body weight gain; BF, backfat thickness adjusted to 110 kg of body weight; A110, days of age adjusted to 110 kg body weight.
b Values in parentheses indicate the number of observations for those genotypes.
with the hp1/hp1 genotype had the thinnest BF in the cool weather, while those with hp3/hp3 and hp3/hp5 had a largest backfat thickness than the others. Meanwhile, the animals with hp1/hp3 and hp2/hp3 had intermediate BF. Finally, the animals with genotypes hp2/hp3, hp3/hp3 and hp3/hp5 had thicker BF in cool weather than in hot weather. Though no significant difference among genotypes existed in hot season, there was a significant genotypic effect on FE during the cool season. Boars with hp1/hp1 exhibited the best FE during the cool season, while those with hp2/hp2 had the worst FE. Regarding the effect of season within genotype, the hp1/hp1 and hp2/hp2 genotypes differed significantly between cool and hot weather.

4. Discussion

HSP70 is the most abundant and highly conserved heat shock protein in all organisms studied to date (Lindquist and Craig, 1988). The expression of HSP70 in eukaryotic cells is encoded by a multigene family and can be classified into constitutively expressed and stress-inducible forms (Lindquist and Craig, 1988; Welch, 1992). HSP70 has been shown to be important in thermotolerance (Lindquist and Craig, 1988). Meanwhile, an association has been demonstrated between polymorphism in the 5′-flanking region of porcine HSP70.2 and meat quality (color-brightness and conductivity), birthweight (Maak et al., 1998; Schwerin et al., 1999) and semen quality (Huang et al., 2002). This study further clarifies the effect of haplotype in the 5′-flanking region of porcine HSP70.2 on BF and growth performance in centrally tested Duroc boars in Taiwan. The results suggested that genotypes of the 5′-flanking region in porcine HSP70.2 affect BF and growth performance in Duroc boars.

Peelman et al. (1992) were the first to report the complete nucleotide sequence of porcine HSP70 gene. At least four genes regulate the expression of HSP70 (Nunes et al., 1993). Schwerin et al. (1999) found 13 polymorphic sites in the 5′-flanking region of porcine HSP70.2, and five polymorphic sites have been found in the same region in the Taiwanese pig population (Chen et al., 2000). However, the relationship between the HSP70 level or polymorphisms in this gene family and economic traits in pigs has received little attention. van Laack et al. (1993) failed to find any relationship between meat quality and the expression of either form of HSP70 in liver and muscle tissues. However, the 5′-flanking region of HSP70.2 appears to significantly influence meat quality (Schwerin et al., 1999), birthweight (Maak et al., 1998), and semen quality (Huang et al., 2002).

Brookes (1999) defined a SNP as “Single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), whereas the least frequency

Table 4
Least-squares means and comparison of different genotypes of the 5′-flanking region of porcine heat-shock protein 70.2 gene, focused on differences in feed efficiency and backfat thickness between the cool and hot seasons

<table>
<thead>
<tr>
<th>Genotypeb</th>
<th>FEa</th>
<th>BFb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool</td>
<td>Hot</td>
</tr>
<tr>
<td>hp1/hp1 (5, 5)</td>
<td>1.96 ± 0.08A,x</td>
<td>2.18 ± 0.08y</td>
</tr>
<tr>
<td>hp1/hp2 (8, 6)</td>
<td>2.27 ± 0.06BCD</td>
<td>2.13 ± 0.07</td>
</tr>
<tr>
<td>hp1/hp3 (22, 31)</td>
<td>2.22 ± 0.04BCD</td>
<td>2.17 ± 0.03</td>
</tr>
<tr>
<td>hp1/hp5 (3, 6)</td>
<td>2.29 ± 0.10BCD</td>
<td>2.17 ± 0.07</td>
</tr>
<tr>
<td>hp2/hp2 (4, 4)</td>
<td>2.39 ± 0.09 DX</td>
<td>2.11 ± 0.09y</td>
</tr>
<tr>
<td>hp2/hp3 (14, 25)</td>
<td>2.19 ± 0.05BC</td>
<td>2.16 ± 0.03</td>
</tr>
<tr>
<td>hp2/hp5 (3, 3)</td>
<td>2.25 ± 0.10BCD</td>
<td>2.13 ± 0.10</td>
</tr>
<tr>
<td>hp3/hp3 (7, 21)</td>
<td>2.30 ± 0.06CD</td>
<td>2.20 ± 0.04</td>
</tr>
<tr>
<td>hp3/hp5 (10, 6)</td>
<td>2.11 ± 0.05AB</td>
<td>2.22 ± 0.07</td>
</tr>
</tbody>
</table>

Least-squares means in the same column (within season) with different superscripts differ significantlyA–E (P < 0.05). Least-squares means for two seasons within a particular genotype for a trait with different superscripts differ significantlyx–y (P < 0.05).

a FE, feed efficiency; BF, backfat thickness.
b Values in parentheses represent the number of observations for those genotypes in cool and hot seasons, respectively.
allele has an abundance of 1% or greater”. SNPs are frequently adopted as candidates while seeking causative variation (Cargill and Daley, 2000). The 216 boars tested in this study were taken from 11 purebred farms around Taiwan. Consequently, the frequencies of the haplotypes in these tested boars might represent the possible distribution of the purebred Duroc population in Taiwan. These six haplotypes (Table 1) indicate that if an ‘A’ is found at the nt44 site, an ‘A’ and a ‘C’ will be found at the nt232 and nt345 sites, respectively. Meanwhile, if a ‘C’ is found at the nt44 site, a ‘C’ and a ‘T’ will be found at the nt232 and nt345 sites. Thus, the two main sites of variation across CC?T? (89.3%) and AA?C? (9.5%) were at sites nt250 (A/C) and nt393 (T/C).

According to several pig QTL mapping studies, BF was significantly influenced at the region of major histocompatibility complex (MHC) on chromosome 7 where the HSP70.2 was located (Milan et al., 1998; Moser et al., 1998; Rohrer and Keele, 1998; Wang et al., 1998; Rohrer, 2000). All the above QTL studies used Meishan based F2 cross as the test populations. Current QTL mapping projects have revealed limited information on association studies involving Durocs. Several candidates, namely, tumor necrosis factor α (TNFα) and Colipase, were suggested potential QTLs for BF in this region (Wang et al., 1998). Notably, TNFα and HSP70.2 were mapped into the same region of the MHC on chromosome 7. The results of the present investigation suggested that genotype of the 5’-flanking region of porcine HSP70.2 may affect BF (Table 3).

The seasonal differences observed among the different genotypes of the 5’-flanking region of HSP70.2 (Table 4), particularly during the cool season, should be attributed to differences in feed consumption. The daytime temperature during the hot season (May to October) in Taiwan is around 32–35 °C, with relative humidity of 85–95%, while during the cool season the daytime temperature and relative humidity are 22–25 °C and 75–80%, respectively. Meanwhile, feed consumption of the tested boars during the hot season decreased by approximately 0.4 kg/day per pig compared to the cool season. This result matched those of Verstegen and Close (1994). Thus, the BF increased more easily during the cool season than the hot season. However, BF was largely unchanged during the cool season in boars with the genotypes hp1/hp1, hp1/hp3, and hp1/hp5 (Table 4). Boars with the other three genotypes, namely hp2/hp3, hp3/hp3, and hp3/hp5, exhibited significantly increased BF during the cool season. Since the main difference between the two groups of genotypes is at the site of nt393, namely TT and TC in the first group and CC in the other group, nt393 might be the major SNP influencing fat deposition in Duroc pigs. However, the polymorphism at site nt250 may interfere with the effect of nt393. Recent studies of human obesity found a significant association between HSP70.2 and obesity by comparing 343 unrelated obese patients and 174 healthy controls (Chouchane et al., 2001). Patients with certain genotype of HSP70.2 (P2/P2) also displays significant differences in body mass and weight compared to patients without the genotype. Given different diets, humans with certain HSP70.2 genotypes were able to easily increase their body mass, implying that a specially designed feeding regime may be required for pigs with certain genotypes. However, the molecular mechanism(s) of how the genotypes of the 5’-flanking region of HSP70.2 influence fat deposition in both humans and swine needs further investigation.

In summary, differences in the genotype of the 5’-flanking region of HSP70.2 gene in Duroc boars might cause different backfat deposition under different weather conditions. Purebred Duroc pigs with genotype of hp1/hp1 for the HSP70.2 promoter might show excellent BF and FE performance during cool weather. In boars with the homozgyote (except for hp3/hp3), BF and FE do not significantly differ regardless of whether they were raised in the cool or hot seasons. Animals with a haplotype of hp3 might display thicker BF in cool weather, but still achieve acceptable growth and backfat in hot weather. Furthermore, the hp5 and hp2 haplotypes may balance the effects of hp1 and hp3 on those performance traits in different seasons.

Acknowledgements

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References