# Association of a Single Nucleotide Polymorphism in the 5'-Flanking Region of Porcine *HSP70.2* with Backfat Thickness in Duroc Breed

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**ABSTRACT :** Higher environmental temperature affects the economic performance of pigs. Heat shock protein 70 has been shown to play an important role in thermoresistance. The purpose of this study was to assess the effect of a single nucleotide polymorphism in the 5'-flanking region of porcine *HSP70.2* on growth performance in Taiwanese Duroc. The genotype of this nt 393 polymorphic site could be verified by digestion with *Bsa* WI restriction enzyme of a PCR product. Pigs with TT and TC genotypes have thinner backfats than those with CC type ($p<0.05$). The result suggested that the polymorphic *Bsa* WI site in the 5'-flanking region of porcine *HSP70.2* may be used as a marker for the early selection of ultrasonic backfat thickness in Duroc pigs. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 1 : 100-103)***

**Key Words :** Pigs, Duroc, Heat Shock Protein 70, Growth Performance, Polymorphism

## INTRODUCTION

Higher ambient temperature is important for pigs because it affects their growth and carcasses (Holme et al., 1967). Generally, food intake and growth rate both decrease markedly at high temperature (Heitman et al., 1949; Heitman et al., 1958). Backfat depths measured above the eye muscle were significantly increased by hot treatment (Holmes, 1971). Living organisms are capable of producing heat shock proteins (HSPs) in response to environmental insults such as temperature elevation (Lindquist et al., 1988). The 70 kDa HSP (HSP70) has been demonstrated to be related to thermoresistance (Johnston et al., 1988; Riabowol et al., 1988).

A complete nucleotide sequence of porcine *HSP70.2* (heat inducible form) was reported by Peelman et al. (1992). The gene is located on chromosome 7 cen-p1.1 within the major histocompatibility complex (Nunes et al., 1993; Dezeure et al., 1993). Restriction fragment length polymorphism analysis with *Pst* I and *Pvu* II (Ruohonen-Lehto et al., 1993; Dezeure et al., 1993) have shown limited polymorphisms of *HSP70.2*. In addition, five polymorphisms (nt 44, 232, 250, 345, and 393) have been detected in the 5'-flanking region of porcine *HSP70.2* (Schwerin et al., 1999; Chen et al., 2000). Two of these sites appeared to be associated with meat quality (Schwerin et al., 1999) and birth weight (Maak et al., 1998). A recent report suggested that inactivation of the GC box site in the porcine *HSP70.2* promoter resulted in a significant decrease of heat-induced transcription compared to the corresponding wild-type promoter (Schwerin et al., 2001). The objective of the present study is to assess the effect of a single nucleotide polymorphism in the 5'-flanking region of porcine *HSP70.2* on growth performance in Taiwanese Duroc.

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## MATERIALS AND METHODS

### Animals

The animals used in this study included 88 unrelated purebred Duroc pigs which were from private seedstocks located in Taiwan. Genomic DNA was extracted from blood using a DNA Isolation Kit for mammalian blood (Boehringer Mannheim, IN, USA). The isolated genomic DNA was stored at -20°C until used for polymerase chain reaction (PCR) amplification.

### Analysis of restriction fragment length polymorphism

To investigate the polymorphism in the 5'-flanking region of *HSP70.2* one fragment was amplified and digested with *Bsa* WI restriction enzyme. The PCR amplification was performed in a final volume of 50 μl containing 100 ng of genomic DNA, 2 μM of each primer, 200 μM of each dNTP, 1×PCR reaction buffer, and 1unit of Taq polymerase (Viogene, CA, USA). The primers were as follows: 5'-GGG GCT TGA GGA AAA AAA-3'; 5'-GGC GAT GAT CTC CAC CTT GC-3'. The reaction was loaded onto a Perkin-Elmer 9600 thermal cycler (Norwalk, CT, USA) under the following conditions: 1 cycle at 94°C for 10 min; 40 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min; 1 cycle at 72°C for 10 min. The amplified fragment (227 bp) was purified and then digested with restriction enzyme *Bsa* WI in a total volume of 15 μl containing 10 μl purified PCR product, 1.5 μl BSA

(1 mg/ml), 1.5 μl buffer and 4 units of enzyme (New England Biolabs, MA, USA) at 65°C for 3 h and then examined by electrophoresis on 2% agarose gel. The gels were stained with ethidium bromide and photographed.

**Growth performance**

The boars were performance tested in cool season, hot season, and season between the two seasons (intermediate) season. The procedures of performance test were describe by Huang et al. (1995) and the economic traits measured were

ADG: average daily gain (kg/day) calculated as:
ADG=(end-test body weight - start-test body weight)/(end-test age - start-test age)

FE: feed efficiency calculated as:
FE=(total feed consumption during test)/(end-test body weight - start-test body weight)

BF: ultrasonic backfat thickness: Backfat thickness was measured on both sides of the following position: behind the scapula, last rib, and lumbar vertebra. BF was corrected for weight as follows (Chyr, 1980):
BF=average backfat thickness (mm)×[1+0.0088184×(110-end-test body weight (kg))]

AGE: adjusted age at 110 kg body weight.
AGE = end-test age - [(end-test body weight - 110)/ADG]

**Statistical analysis**

The effect of genotypes of the 5'-flanking region of porcine *HSP70.2* on growth performance traits was analyzed using the following model:

$$Y=\mu+G_i+S_{ij}+e_{ijk}$$

Where μ is the mean value common to all records; $G_i$ is the effect of the ith genotype; $S_{ij}$ is the effect of the jth season; and $e_{ijk}$ is the random error.

The analysis was performed by the GLM procedure of SAS (SAS institute, 1989). The significance of differences among genotypes was determined by the least-squares means method using season-corrected data.

## RESULTS

**Effect of polymorphism in the 5'-flanking region of porcine *HSP70.2* on performance measures**

The effect of genotypes of the 5'-flanking region of porcine *HSP70.2* on growth performance traits was evaluated. Since the season effect was significant on the performance trait, the effect of genotypes on growth performance was determined based-on season-corrected data. Table 1 summarized the association of porcine *HSP70.2* with ADG, BF, AGE and FE. Statistical analyses of animals from purebred farms in Taiwan revealed significant differences between genotypes with regards to the growth performance of backfat thickness. The A

**Table 1.** Effect of the nt 393C/T polymorphism in the 5'-flanking region of HSP70.2 on growth performance in Duroc pigs

| Category | | n | Growth performance | | | |
|---|---|---|---|---|---|---|
| | | | ADG (kg) | BF (cm) | AGE (day) | FE |
| 44 | | | | | | |
| | AA | 1 | 0.893±0.349 | 1.28±0.15 | 139.5±9.4 | 2.14±0.18$^{ab}$ |
| | AC | 11 | 0.952±0.109 | 1.49±0.05 | 155.8±2.9 | 1.95±0.056$^{a}$ |
| | CC | 76 | 1.038±0.050 | 1.41±0.02 | 150.8±1.3 | 2.08±0.026$^{b}$ |
| 232 | | | | | | |
| | AA | 1 | 0.893±0.349 | 1.28±0.15 | 139.5±9.4 | 2.14±0.18$^{ab}$ |
| | AC | 11 | 0.952±0.109 | 1.49±0.05 | 155.8±2.9 | 1.95±0.056$^{a}$ |
| | CC | 76 | 1.038±0.050 | 1.41±0.02 | 150.8±1.3 | 2.08±0.026$^{b}$ |
| 250 | | | | | | |
| | AA | 55 | 1.039±0.061 | 1.43±0.03$^{b}$ | 153.0±1.6$^{b}$ | 2.04±0.03$^{a}$ |
| | A- | 28 | 1.018±0.068 | 1.42±0.03$^{b}$ | 148.2±1.8$^{a}$ | 2.08±0.03$^{a}$ |
| | -- | 5 | 0.945±0.158 | 1.28±0.07$^{a}$ | 156.8±4.2$^{b}$ | 2.24±0.08$^{b}$ |
| 345 | | | | | | |
| | CC | 2 | 0.940±0.245 | 1.42±0.11 | 142.4±6.6$^{a}$ | 2.01±0.13$^{a}$ |
| | TC | 11 | 0.951±0.109 | 1.49±0.05 | 155.8±2.9$^{b}$ | 1.95±0.06$^{ab}$ |
| | TT | 75 | 1.038±0.050 | 1.41±0.02 | 150.8±1.3$^{ab}$ | 2.09±0.03$^{b}$ |
| 393 | | | | | | |
| | CC | 41 | 0.992±0.057 | 1.45±0.02$^{b}$ | 151.3±1.6 | 2.06±0.03 |
| | TC | 38 | 1.092±0.070 | 1.39±0.03$^{a}$ | 150.0±1.9 | 2.11±0.04 |
| | TT | 9 | 1.006±0.120 | 1.31±0.05$^{a}$ | 154.0±3.3 | 2.00±0.06 |

$^{a,b}$ Least-squares means differ significantly ($p<0.05$) for genotypes at the same site with different superscripts.

deletion homozygotes at nt 250 and TC heterozygotes and TT homozygotes at nt 393 displayed the thinner backfat thickness ($p<0.05$). The polymorphism did not have significant effect on the other performance traits.

**A PCR-RFLP in the 5'-flanking region of porcine *HSP70.2***

Although all five polymorphic sites were associated with growth traits, only the nt 393 could be detected quickly by RFLP. Figure 1 shows the PCR-RFLP at nt 393. After *Bsa* WI digestion, the 227 bp long PCR product was cleaved into two fragments of 157 and 70 bp (variant T) each or was still the original fragment (variant C).

## DISCUSSION

The development of genetic maps of swine has allowed identification of quantitative trait loci (QTL) affecting a variety of traits of economic importance. While these results are promising with respect to potential improvement of pork production through marker-assisted selection procedures, it would be of significant interest to identify the genes and specific allelic polymorphisms underlying the observed variation. This achievement would allow development of specific DNA-based genetic tests to evaluate individual animals for merit and genetic potential, as well as to increase understanding of the genetic basis underlying non-disease producing variability among populations. Several QTL were identified on the distal portion of the long arm of SSC1, representing correlated traits of early growth rate (from 8 to 18 weeks), weight at 26 weeks, backfat thickness at 14 and 26 weeks of age, and age at puberty. However, backfat thickness QTL on SSC2 and SSC7 in Meishan × Large White and Landrace (F2) cross which was reported by Rattink et al. (2000) is in the same locus as this study indicated.

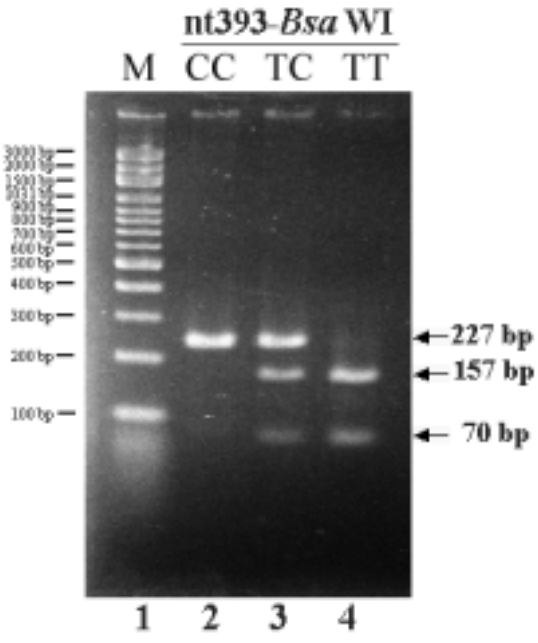


**Figure 1.** Characterization of *Bsa* WI polymorphism in the 5'-flanking region of porcine *HSP70.2*. Amplification products of genomic DNA from three different individuals with the three possible genotypes are shown. The PCR products were digested with *Bsa* WI at nt 393 as indicated. Lane 1: DNA molecular marker, 2: homozygous CC, 3: heterozygous TC, and 4: homozygous TT.

The results of the present study confirmed the presence of nt 393 polymorphism in the 5'-flanking region of *HSP70.2*. Our previous study (Chen et al., 2000) indicated the presence of this polymorphism in three pig breeds. We found that the "T" allele was mostly in the heterozygous form. Homozygotes TT appeared only in Duroc pigs, not in Landrace and Yorkshire. Therefore, in the present study the relationship of this polymorphic site with growth performance was examined only in Duroc pigs. Promoter variants differing in potential cis-acting elements and showing impaired binding of corresponding transcription factors were described in the inducible porcine *HSP70.2* (Schwerin et al., 2001). Mutation of the GC box in the *HSP70.2* promoter resulted in a significant decrease of heat-induced transcription compared to the corresponding wild-type promoter. Therefore, other variants in the 5'-flanking region of porcine *HSP70.2* could be associated with mRNA abundance and protein translation level.

The results of this study suggest that the nt 393 single nucleotide polymorphism in the 5'-flanking region of porcine *HSP70.2* may be used as a marker for early selection of ultrasonic backfat thickness in Duroc pigs.

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