

Haplotype Analysis of *Ferritin Heavy Chain* Affects Stillborn Piglets on Large White X Landrace Crossbred Sows

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Abstract

The objective of this experiment was to identify the novel SNP on porcine *FTH* genes. Moreover, their association with litter size traits in commercial pigs will be analyzed. 1,361 Large White x Landrace crossbred sows were bled and DNA was extracted. The records included total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets stillborn (SB) and number of piglets mummified (MM). The PCR primers were designed throughout the porcine *FTH*. PCR products were performed and screened for single nucleotide polymorphisms (SNPs) by using polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP). Consequently, differentially heteroduplex shift were cloned and sequenced. SNPs including c.596C>T, c.634A>G c.677T>C and c.699T>C were found. According to these SNPs, there were proved by PCR-RFLP technique, *BsuRI*-c.596C>T, *Hin6I*-c.643A>G, *MspI*-c.677T>C and *MspI*-c.699T>C. Six different haplotypes for the SNPs of *MspI* at nucleotide position *MspI*-c.677T>C and *MspI*-c.699T>C were presented. The TCTC haplotype was significantly associated with increasing SB. It was conclude that *MspI*-*FTH* haplotype significantly associated with SB in commercial pigs which may used for marker-assisted selection on pig breeding program.

Keywords: Litter size traits, Ferritin heavy chain, PCR-SSCP, Stillborn piglets

1. Introduction

Reproductive performance, especially litter size in term of total number of piglet born (TNB) and number of piglet born alive (NBA) are one of the most economically important traits in pig production. It is well known that litter size is affected by a set of factors such as genetics, farm, feeds, season and mating boars, which account for a large part of the phenotype variance. From a genetic point of view, litter size could be controlled by numerous genes in complicated physiological networks such as those affecting ovulation rate, fertilization rate, embryo survival and uterine capacity (Li *et al.*, 2009). Litter size is low heritability approximately 0.09 for NBA that affecting slowly changed in genetic drift (Rothschild, 1996) and difficult to measure phenotypically by traditional breeding method which have used animal selection on the basis of observable phenotype representing the collective effect of all genes and the environment (Merks *et al.*, 2000). Furthermore, litter size trait is sex-limited and is not measurable until sexual maturity (Spötter and Distl, 2006). This breeding method is

the-state-of-the-art and setback over 3-5 years or up to 10 generation that gain of about 0.6 piglets within-line selection and 0.8 piglets due to immigration (Bolet *et al.*, 2001).

Markers can be used to localize genes responsible for qualitative traits and they can also be used to detect genes with substantial effects on quantitative traits. In this case the approach is referred to as QTL mapping (de Vries *et al.*, 2000). Indeed, this approach has been used in relation to TNB and NBA. On the other hand, the candidate gene approach can be relatively straightforward compared to the QTL approach. Polymorphisms were used to look for associations across populations, when associations are identified the resulting marker can potentially be used directly in breeding programs (de Vries *et al.*, 2000). Significant QTL regions for litter size are located on SSC8 (King *et al.*, 2003) and on SSC11 (Cassady *et al.*, 2001). Putative QTL regions are located on SSC6 (Wilkie *et al.*, 1999), SSC7, SSC12, SSC14 and SSC17 (de Koning *et al.*, 2001).

Bertani *et al.* (2004) identified transcriptional candidate genes for fertility in anterior pituitary gland of pigs. Ferritin heavy chain (*FTH*) was found highly expressed between sows selected for ovulation rate and embryo survival compared with the control line, 42% and 10%, respectively. *FTH* is involved in the folate pathway by enhancing expression of serine hydroxymethyltransferase and de novo thymidine biosynthesis (Oppenheim *et al.*, 2001). Supplementation of folic acid during gestation leads to increases in litter size, primarily due to improved embryo and fetal survival (Lindemann, 1993). Ferritin is controlled by a common cytosolic protein, iron regulatory protein, which binds to the iron-responsive element in the 5-prime untranslated region of the H- and L-ferritin mRNAs (Leibold and Munro, 1988; Eisenstein, 2000). Moreover, Pham *et al.* (2004) identified *FTH*, the primary iron storage factor, as an essential mediator of the antioxidant and protective activities of nuclear factor kappa-B. Manipulation of *FTH* by knock-out method in the mouse affected on lethal during embryonic development (Ferreira *et al.*, 2001). *FTH* was characterized as a novel modulator in *TGF-β1-induced* epithelial-mesenchymal transition. In response to TGF-beta1, there was a dramatic decrease in the FHC levels, which caused iron release from FHC and, therefore, increased the intracellular labile iron pool (Zhang *et al.*, 2009). Nucleotide sequence of porcine *FTH* has been identified (GenBank accession No. NM 213975.1) and was mapped on Ssc2p14-17 between the loci SW2443 and SW240 with distances of 42.8 and 20.2 cM on the sex averaged map (Ponsuksili *et al.*, 2002) that encode the 181 amino acids. The *FTH* gene is a good candidate gene for fecundity in mammals. However the information on *FTH* marker for litter traits of pig is limited. Then, the aims of this study were to identify the novel SNP on porcine *FTH* genes. Moreover, their association with litter size traits in commercial pigs would be analyzed.

2. Materials and Methods

2.1 Animals and data

A totally 1,361 Large White X Landrace crossbred commercial sows including 5,102 parities were bled and recorded in terms of TNB which was calculated as NBA plus SB and plus MM on 1st parity to 5th parity for association study, the descriptive statistics was shown as table 1. Primary data were plotted and 20 sows were selected from extremely tails of population into 2 groups; 10 High- (>15 piglets/litter) and 10 low sows (<8 piglets/litter) for screening SNPs. Associated study was analyzed between candidate gene and phenotypic traits. Litter size trait was analyzed as TNB and NBA. Mortality traits were SB and MM.

Blood samples were collected from jugular vein with 0.5M EDTA anti-coagulant and extracted by using Chelex®. Briefly, 20 µl of blood was lysed twice, centrifuge at 2,000 rpm 1 min, added Chelex® solution over the pellet and incubated overnight. The solution samples were boiled at 95°C for 5 minutes and stored at 4°C until analyzed (Walsh *et al.*, 1991).

2.2 Polymerase Chain Reaction-Single Strand Conformation Polymorphism

The polymerase chain reaction-single strand conformation polymorphisms (PCR-SSCP) were used to screen SNPs (Orita *et al.*, 1997) on nucleotide sequence of porcine *FTH* genes. Briefly, amplification by PCR was performed with primers (Table 2). Standard PCR was performed in a final reaction volume of 20 µl by using 50.0 ng of genomic DNA sample, 1X NH₄SO₄ buffer, 0.4 µmol of primer, 0.5 µl of 0.2 mmol dNTPs, 1.5 mmol MgCl₂, 13.4 µl dH₂O and 0.25 unit *Taq* DNA polymerase (Fermentas). PCR conditions were optimized by trial and error technique. For verification prior to SSCP analysis, PCR products of the expected size were visualized on ethidium bromide-stained 6% polyacrylamide gels. The PCR products were denatured for 4 min at 95°C and immediately chilled on ice before loading to the SSCP gel. Five and 10% polyacrylamide (49:1 acrylamide to bis-acrylamide) with or without 5% glycerol TBE gels are run with 1X TBE on a vertical electrophoresis system with or without ice bath and constant power at 50 mA and 100 V for 3 hrs. Acrylamide concentration and temperature were optimized for each fragment to obtain maximum separation of allelic variations. Gels were stained by Silver Staining Standard Protocol and dried by Gel Slab® for 4 hr at 55°C.

2.3 Cloning and sequencing

PCR product would be cloned into pGEM-T Easy Vector system. The cloned PCR fragment compared with PCR product obtained from genomic DNA. Three clones with the same size of inserted fragment would be sequenced on the CEQ™ 8000 Genetic Analysis System, Beckman Coulter by using GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, USA).

2.4 Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

After the common sequence variations observed in a population have been determined by DNA sequencing, PCR strategies could be designed to elucidate the particular allele present in each individual sample. Similarly, amplification of a region surrounding a naturally occurring restriction enzyme site, followed by digestion with the appropriate enzyme, can allow a rapid genotyping determination to be performed. 6% polyacrylamide gel was stained by silver staining standard protocol and visualized genotyping.

2.5 Statistic analysis

Allele and genotype frequencies of *FTH* were determined by the total count of an allele and genotype divided by twice the number of observations. The genotype frequencies of polymorphisms were examined for deviations from Hardy-Weinberg equilibrium by χ^2 tests. Test of linkage disequilibrium between pairwise genotype combinations of the SNPs were also performed using χ^2 procedure. Association of SNP markers with litter size were analyzed with general linear model included fixed effect of genotype, breed and parity number, and residual as following: $Y_{ijk} = \mu + \text{genotype}_i + \text{parity}_k + e_{ijk}$, where μ denoted the average normalized of population; genotype_i denoted the fixed effect of genotype i ; breed_j denoted the fixed effects of breed j ; parity_k denoted the fixed effect of parity k ; and e_{ijk} denoted the residual effects of genotype i , breed j and parity k .

3. Results

3.1 Discovered SNPs novel of *FTH* gene

PCR-SSCP was used to screen SNPs on nucleotide sequence of porcine *FTH* gene. The PCR products were denatured at 95°C for 4 min and immediately chilled on dried ice before loading to the SSCP gel. Five and 10% polyacrylamide (49:1 acrylamide to bis-acrylamide) with or without 5% glycerol TBE gels were ran with 1X TBE on a vertical electrophoresis system with or without ice bath and constant power at 50 mA and 120 V for 3 hrs. Acrylamide concentration and temperature were optimized for each fragment to obtain maximum separation of allelic variations. Gels were stained by silver staining standard protocol and dried by Gel Slab® for 3 hr at 55°C. The polymorphism of *FTH* was shown as Figure 1. Lane 10 showed differentially heteroduplex shift between low- and high-TNB sows. Subsequently, Lane 10 polymorphic band was selected to clone into pGEM-T Easy Vector System and sequence analyzed on the CEQ™ 8000 Genetic Analysis System, Beckman Coulter by using GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, USA). The sequences were screened homology with BLAST software (Altschul *et al.*, 1997).

Four SNPs in *FTH* were found including *Bsu*RI-c.596C>T, *Hin*6I-c.643A>G, *Msp*I-c.677T>C and *Msp*I-c.699T>C. Consequently, these polymorphic sites were proved by using PCR-RFLP technique in extremely low- and high-TNB of sampling population. Similarly, 2.5 μ l of the PCR product of c.596C>T, c.634A>G and c.699T>C was digested with 0.25 units of *Bsu*RI, *Hin*6I and *Msp*I, respectively, by manufacturing instruction. The PCR-RFLP fragments of each candidate genes were ran on 6% polyacrylamide gel electrophoresis and stained with standardized silver staining protocol. *Bsu*RI-c.596C>T and *Hin*6I-c.634A>G polymorphism were not significantly between low- and high-TNB sows. On the other hand, the only 6 different haplotypes for the SNPs of *Msp*I-*FTH* at nucleotide position c.677T>C and c.699T>C were presented.

3.2 Genotype and allele frequencies for the investigated SNPs for *FTH* gene

Frequencies for the *FTH* gene in 1,361 parent stock of commercial sows with 5,102 parity records in Thailand were shown in Table 3. *TT*, *TC* and *CC* allele frequency was 0.22, 0.35 and 0.43, respectively. The haplotype frequencies of the two SNPs were not distributed according to Hardy-Weinberg equilibrium proportion ($P < 0.01$). Therefore, the population is in linkage disequilibrium at these haplotypes under consideration ($P < 0.01$).

3.3 Association study between *FTH* genotype and litter size related traits

The association between *Msp*I-*FTH* haplotype and litter size traits in terms of TNB, NBA, SB and MM piglets were analyzed as shown as Table 4. The unfavorable TC/TC haplotype was significantly higher SB than other haplotypes. No associated effects between *Msp*I-*FTH* haplotypes and litter size traits were found including TNB, NBA and MM.

4. Discussions and Conclusions

Compared with 12,599 Thai Landrace sows recorded between's 1993-2005, TNB and SB was 10.03 ± 2.64 and 0.21 ± 0.59 (Imboonta *et al.*, 2007). While, Moeller *et al.*, (2004) stated between's 1997-1999 that TNB was 11.05 ± 1.49 , with range 1-25 pigs, and NBA was 9.96 ± 1.38 , with range 0-21 pigs. In present study, 2006-2008, 1,361 Large White x Landrace sows records showed 11.30 ± 3.00 , 10.02 ± 3.00 and 0.88 ± 1.14 piglets/litter in TNB, NBA and SB, respectively. These parameters exhibited the genetic progress in Thai's swine commercial that tended to be favorable for white breeds and widely used by the commercial to produced F_1 crossbred gilts resulting from imported nucleus herd. However, SB may increase in order to improvement. Addition, the main direction of selection for this founder pigs was the meat deposition in carcass and growth rate, but was not considered in the piglet number and its related traits.

4.1 SNPs discovery techniques

PCR-SSCP technique could be detected DNA polymorphisms and mutations at multiple places in DNA fragments. The principle of this method was based on the fact that the electrophoretic mobility of nucleic acids in non-denaturing gels was sensitive to both size and shape (Orita *et al.*, 1997). Unlike double strand DNA, single strand DNA was flexible and would adopt a conformation determined by intramolecular interactions and base stacking that was uniquely dependent on sequence composition (Orita *et al.*, 1989). This conformation could be affected when even a single base is changed. Conformational changes could be detected as alterations in the electrophoretic mobility of the single-stranded DNA in non-denaturing polyacrylamide gels (Armstrong, 1998). As a mutation scanning technique, though, PCR-SSCP was more often used to analyze the polymorphisms at single locus (Sunnucks *et al.*, 2000). The SSCP technique has proven useful for detection of mutations and polymorphisms in a number of studies (Hayashi, 1992; Skinner *et al.*, 1996; Orita *et al.*, 1997). Although, this technique has limitations, detection of single base substitutions is less sensitive in larger DNA fragments (Fujita and Silver, 1994). In present study, the primers designed were not exceeded 300 bp and the PCR fragments were separated on a 6, 9 and 12% of acrylamide:bis-acrylamide (49:1) concentration and 5% additive glycerol for sensitivity to detect single-base changes (Orita *et al.*, 1989). The classical PCR-SSCP analysis for the detection of mutation was found in the *CYP11B1* gene (Skinner *et al.*, 1996). Moreover, two PCR-SSCP polymorphisms, a T-C transition in the third exon and an A-C transversion in the fifth exon were found that positive associated with milk production traits in dairy cattle (Yao *et al.*, 1996). In addition, PCR-SSCP polymorphism in exon 10 of growth hormone receptor associated with average daily gain from birth to weaning in sheep (Valeh *et al.*, 2009).

4.2 FTH polymorphisms discovery and association

In the present study, candidate genes were chosen because they have been shown to be involved in the regulation of metabolism. These functions seemed the most likely to have an effect on the litter size merit of the animals. SNPs within this gene were examined for effects on these traits in commercial sows. *FTH* was chosen based on their biological functions to be evaluated for association between polymorphisms and litter traits, because of differentially expressed gene in the anterior pituitary gland of sows selected of long-term for increased ovulation rate and embryo survival that was confirmed by using Northern hybridization (Bertani *et al.*, 2004), and the ferroxidase activity of the ferritin molecule which could transform the more toxic Fe(II) into the less toxic Fe(III) that was essential for detoxification of iron and/or for maintaining its bioavailability during embryonic development (Ferreira *et al.*, 2000). These functions seemed the most likely to have an effect on the fetuses development. Developing fetuses must depend on iron crossing the maternoplacental barrier that was accomplished via endometrial secretion of uteroferrin. Uteroferrin secreted by the endometrium was taken up by the areolae of the placenta and released to fetal circulation that is binding receptor at fetal liver. The Fe of the uteroferrin molecule was used for hemoglobin formation and liver was the primary site for hematopoiesis activity in developing fetuses (Mahan and Vallet, 1997). The highest level of *FTH* gene expression at 9.5 days of gestation was found in the heart suggests that the embryos could also die of heart failure because of massive iron deposition (Ferreira *et al.*, 2000). While, *FTH*^{-/-} mutant embryo survive at least to the 62-cell stage that should have enough iron to ensure DNA replication and heme synthesis (Ferreira *et al.*, 2000). Disruption of uroporphyrinogen III synthase was lethal between 2- and 4-cells stage, suggesting that heme synthesis takes place at very early stages (Bensidhoum *et al.*, 1998) and the faster blood cell development on early gestation period could be beneficial to fetal survival in swine (Vallet *et al.*, 2003). Moreover, *FTH* is involved in the folate pathway by enhancing expression of serine hydroxymethyltransferase and de novo thymidine biosynthesis (Oppenheim *et al.*, 2001). The role of *FTH* in modulating intracellular free iron concentrations, and its ability to enhance the expression of a cytoplasmic serine hydroxymethyltransferase (cSHMT), may enable coordinate regulation of folate and iron metabolism and potentially enable the regulation of one-carbon metabolism by iron (Woeller *et al.*, 2007). CUGBP1, a protein known to interact with the α and β subunits of eukaryotic initiation

factor2, binds to its consensus sequence on the 3'-UTR of cSHMT and ferritin, which has been shown to bind mRNA at 5'-UTR. The physical interaction between CUBGP1 and FTH recruits the translation initiation machinery to the cSHMT IREs (Woeller *et al.*, 2007). Supplementation of folic acid during gestation leads to increases in litter size traits, primarily due to improved embryo and fetal survival (Lindemann, 1993). A day 24-40 of gestation is a critical time progression for fetal erythropoiesis in pigs as well as survival in a crowded uterine environment. The uterine environment had little overall effect on erythropoiesis in white crossbreed pigs but did affect fetal survival, and crowding may compromise erythropoiesis in small fetuses (Pearson *et al.*, 1998). Selection for increased ovulation rate has been very successful. However, the increased in ovulation rate did not result in increases in litter size (Wilson *et al.*, 1999). Selection for uterine capacity improved fetal survival primarily during the time period between d 25 and d 45 that had a significant influence on subsequent fetal growth and survival in a crowded uterine environment (Freking *et al.*, 2007).

Johnson *et al.* (1999) showed strong evidence of a genetic component to embryonic survival ($h^2 = 0.14$) and a positive genetic correlation with litter size ($r_g = 0.35$) that increased SB and MM fetuses (Freking *et al.*, 2007), however, MM sows were not significantly different in this study. Moreover, several factors, such as parity, piglet birth weight, sex and birth assistance have been shown to be associated with variations in SB (Canario *et al.*, 2006). In fact, the excessively high ovulation rate of these animals may result in uterine crowding and excessive competition for nutrients and oxygen, which may negatively affect the growth rate and body composition of the survivor (Vonnahme *et al.*, 2002). SNPs within *FTH* gene was examined for effects on these traits in commercial sows.

However, the informations of *FTH* marker for piglet number and mortality traits in pig were limited. Present study, *FTH* primers were designed throughout mRNA sequence (accession No. NM213975.1). *FTH* was amplified from genomic DNA using PCR. The PCR products were screening for polymorphisms by using PCR-SSCP. Heteroduplex were found and cloned into pGEM-T Easy Vector System and subsequently automated sequencing on the CEQ™ 8000 Genetic Analysis System, Beckman Coulter by using GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, USA). The sequences are screened homology with BLAST software (Altschul *et al.*, 1997) and genotyping by using PCR-RFLP technique. Monomorphism was found in *BsuRI*-c.596C>T and *Hin6I*-c.634A>G. The novel SNPs were found in *MspI*-c.677T>C and *MspI*-c.699T>C haplotype polymorphism which occurred in 3'-UTR of *FTH*. Element in the 3'-UTR and the poly (A) tail of many genes have been shown to influence translation either positively or negatively (Woeller *et al.*, 2007). All of new polymorphisms detected in the current study were in the exonic region, indicating that the exonic region of the *FTH* gene are much more variable than the intronics, in the porcine *FTH* located at c.677T>C and c.699T>C of exon 3. Thus far, only one exonic polymorphism has been detected in the pig (Ponsuksili *et al.*, 2002). Detected polymorphisms occurred in 3'-UTR of *FTH* which perhaps the target binding site of micro RNA (miRNA) that mRNA translation was inhibited or mRNA was degraded (Williams, 2008).

The magnitude of the differences in genotype distribution of homozygous TTTT and CCCC haplotypes was low frequency as well as 42 and 166 animals, respectively. The effects of selection, different frequencies in the founder pigs, and random genetic drift could have caused allele frequencies to differ within this population. Random genetic drift is a non-directional force that acted to change allele frequencies and random genetic drift alone caused the order of favorable allele frequencies of this locus. In the absence of selection, the amount of drift depends on allele frequencies and effective population size (Linville *et al.*, 2001). After drift begins, it is most probable that an allele frequency would be continued to change in the same direction (Falconer and Mackay, 1996). The genotype frequencies of the two SNPs were not distributed according to Hardy-Weinberg equilibrium proportions ($P < 0.01$). Pairwise comparison of haplotype of *MspI*-c.677T>C and *MspI*-c.699T>C revealed the existence of significant linkage disequilibrium between the SNPs ($P < 0.01$).

Both SNPs haplotype showed associations with mortality traits that SB with TCTC haplotype was 19.27 and 25.69% higher than homozygous TTTT and CCCC haplotype ($P < 0.04$), respectively, and was 18.35 and 22.94% higher than heterozygous TTTC and TCCC haplotype ($P < 0.04$), respectively. The piglet number and its related traits were decline on subsequent parity (Imboonta *et al.*, 2007). Here, TCTC haplotype was significantly focused on SB that were due to effects of uterine environment in late gestation, length of the farrowing period, and management at parturition, that may result in SB pigs (Moeller *et al.*, 2004). Iron deficiency, however, is associated with an increased incidence of SB in pigs (Moore *et al.*, 1965). The availability of iron during erythropoiesis is a critical aspect of mammalian homeostasis (Tori and Tori, 2002). Although no evidence exists for a link between *FTH* haplotype and increased incidence of SB, it is possible that alterations in iron homeostasis are involved (Bertani *et al.*, 2004). Moreover, mitochondrial ferritin may be induced under conditions of pathologic iron accumulation in heme-synthesizing cells (Tori and Tori, 2002). Interestingly,

transient transfection assays demonstrated that ferritin H repressed synthesis of the beta-globin, suggesting that ferritin may play a role in hemoglobin switch (Broyles *et al.*, 2001).

This *FTH* candidate gene study concluded that *BsuRI*-c.596C>T and *Hin6I*-c.634A>G monomorphisms were found. Novel haplotype analysis of *MspI*-c.677T>C was found together with *MspI*-c.699T>C that associated with increasing SB. It remains unclear whether the *MspI*-c.677T>C and *MspI*-c.699T>C haplotype polymorphisms have direct impact on the function of the *FTH* gene and thus in the modulation of SB. Associations between genetic polymorphisms and litter traits in sows are an important step into understanding the genetics of complex traits that are commercially important. Although the results of this study suggest that *FTH* gene influence economically important traits, the mechanisms of these genetic changes remain unclear because most of them do not cause amino acid changes. It is possible that they affect the transcription of the genes, splicing, mRNA stability, or even translation. As more knowledge is available on how non-coding sequences affect gene function, it may become apparent how these SNP are contributing to variation in these traits. It is also possible that they are linked to other causative mutations that have not been found yet. In the present study not only revealed the primary function of the *FTH* gene in the pig, but also benefited the selection of sows for low SB by identifying the molecular genetic markers.

It was concluded that molecular approach, PCR-SSCP, has been potentially SNPs detection in commercial sows. This study was found that haplotype of *MspI*-*FTH* associated with SB on gilt and sows by using PCR-SSCP technique. However, the polymorphisms in the gene studied may not directly affect the trait. These polymorphisms could be markers linked with causative mutation within the gene or a closely linked gene. It would be of interest to examine the association between *MspI*-*FTH* and these traits in other populations and to extend research with a large number of animals to confirm the results of this study. Moreover, it should be focused at the analysis of high number of genes in order to identify gene interaction network and to describe metabolism of gene activities.

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Table 1. Phenotype data of piglet number related traits on parent stock of commercial pig

Trait	Abbr.	Units	N	Min	Max	Mean	SD
Total number of piglets born alive	TNB	Piglets	5,102	1.00	20.00	11.30	3.00
Number of piglets born alive	NBA	Piglets	5,102	0.00	19.00	10.02	3.00
Stillborn piglets	SB	Piglets	5,102	0.00	13.00	0.88	1.14
Mummified piglets	MM	Piglets	5,102	0.00	13.00	0.39	1.15

Table 2. Primers sequence and PCR condition of *FTH*

primer	Forward primer Reverse primer	Condition
<i>FTH1</i>	ggtttctgctcaacagtgc tcagcccattctcccagtc	(95C 30s/58C 45s/ 72C 60s)x 38 cycles
<i>FTH2</i>	tgagaaactgatgaagctgc ggtaacccgaacatgcatg	(95C 30s/58C 45s/ 72C 45s)x 30 cycles
<i>FTH3</i>	tcagagccagccctcacc gatattctgaaggaagattcg	(95C 30s/60C 45s/ 72C 45s)x 38 cycles
<i>FTH4</i>	aaaccggagcgcatgac gtgggggtcattttgtcag	(95C 30s/58C 30s/ 72C 45s)x 32 cycles
<i>FTH5</i>	tgtgtgacttcattgagacg ggaatggtacactaaggaaag	(95C 30s/58C 45s/ 72C 45s)x 38 cycles
<i>FTH6</i>	ggcggtttctgcttcaac ggtgagggtgctctga	(95C 30s/60C 45s/ 72C 60s)x 28 cycles
<i>FTH7</i>	cgaatcttctcagagatc tcacgtcatcgcgctcc	(95C 30s/60C 45s/ 72C 60s)x 38 cycles
<i>FTH8</i>	ctgacaaaaatgaccccccac gcgtctcaatgaagtcacac	(95C 30s/60C 30s/ 72C 30s)x 32 cycles
<i>FTH9</i>	tgggtgaccacattaccaac aggtgacggtaacccgaaac	(95C 30s/60C 45s/ 72C 60s)x 38 cycles

Table 3. Genotype and allele frequency of porcine *MspI-FTH*

Haplotype	Genotype frequency	Cumulative frequency	Allele frequency	χ^2
TT/TT	42	42	f(a) = 0.22 f(b) = 0.35 f(c) = 0.43	5,970.38 (df = 5) P<0.0001
TT/TC	260	302		
TC/CC	259	561		
TC/TC	41	602		
TC/CC	593	1,195		
CC/CC	166	1,361		

Table 4. Piglet number traits on parent stock of commercial pigs on *MspI-FTH*

Traits	Haplotype					
	TT/TT	TT/TC	TT/CC	TC/TC	TC/CC	CC/CC
TNB	11.32±0.21	11.46±0.09	11.31±0.09	11.61±0.26	11.36±0.06	11.18±0.12
NBA	10.23±0.21	10.22±0.09	10.16±0.09	10.13±0.24	10.18±0.06	10.05±0.13
SB	0.88±0.09 ^a	0.89±0.04 ^a	0.80±0.03 ^a	1.09±0.12 ^b	0.84±0.02 ^a	0.81±0.05 ^a
MM	0.21±0.05	0.35±0.03	0.35±0.03	0.40±0.08	0.35±0.02	0.31±0.04

^{a, b} LSMeans±SEM within row with different superscripts significantly differed (P<0.05).

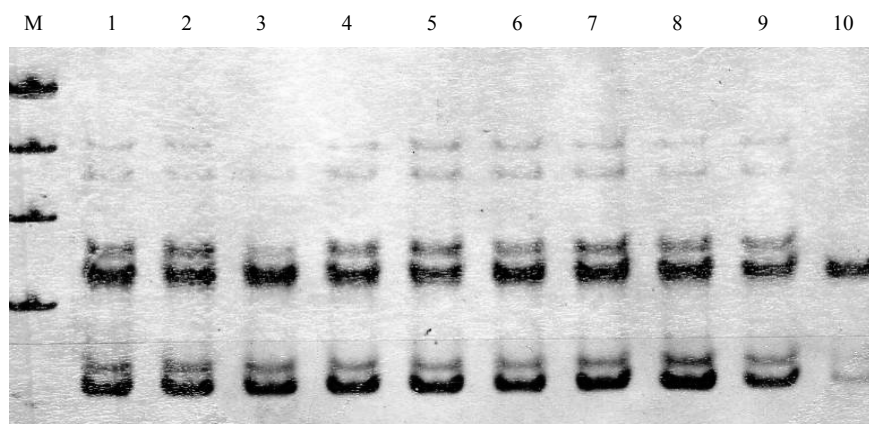


Figure 1. PCR-SSCP analysis of *FTH*. M = markers 100 bp, lane 1-5 = low TNB sows, lane 6-10 = high TNB sows