Short Communication

Investigation of effects of three candidate genes on leg action and fat deposition traits in pigs

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Abstract

Data from 188 sows were used in the current study to examine the effects of *high mobility group AT-hook1* (*HMGA1*), *transcription factor 7-like-2* (*TCF7L2*) and *insulin-like growth factor binding protein 3* (*IGFBP3*) genes on leg action and fat deposition traits, and further to explore the possible relationships between these genes on both traits. The candidate genes used in the study are known for their roles in fat deposition and growth. Overall leg action was scored on a scale of 1 (good movement) to 9 (leg weakness). Fatness traits included 10th rib backfat (BF10), adjusted 10th rib backfat to 125 kg (adjBF10) and last rib backfat (last BF), measured by ultrasonic imaging approach. The association analyses between single nucleotide polymorphisms (SNPs) and traits were performed using PROC MIXED procedures of SAS. The results showed that the associations between *HMGA1*, *TCF7L2* and *IGFBP3* genotypes with fat deposition traits were mostly suggestive in this limited data set. Leg action was also suggestively associated with *IGFBP3* gene effects but was not associated with *HMGA1* and *TCF7L2* genes. Thus, *IGFBP3* AA homozygote individuals tended to have had better movements (5.40), and were fatter when compared to GG homozygotes (5.84). The results from this study suggest a possible association between the *IGFBP3* gene effects on both leg action and fatness. Therefore, further studies must be carried out in several populations, and using larger data to demonstrate these results conclusively.

Keywords: Candidate genes, overall leg action, fatness, association, pig
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Leg and feet soundness play a major economic role in the commercial pig industry. Next to reproductive failure, feet and leg abnormalities are the most frequent reasons for culling of young sows (Boyle *et al*., 1998). Culling rates due to these problems accounted for 11% of the reasons for total disposal in Ireland, and range from 10 to 40% in Canada (Chagnon *et al*., 1991) and the USA (Irwin *et al*., 2000). In Denmark, up to 72% of culls were attributed to locomotive system problems (Kirk *et al*., 2005). Genetic improvement for leg action traits has in the past been challenging, since these traits have been shown to exhibit low to moderate heritability (Webb *et al*., 1983; Huang *et al*., 1995; Jørgensen & Andersen, 2000; Nikkilä *et al*., 2008). High fat deposition in pigs has been found to be associated with low lean meat production (Chen *et al*., 2002). Genetic progress for reduced fatness, using traditional breeding methods has been successful. Conversely, selection for reduced fatness has been shown to have antagonistic relationships with leg action traits (Huang *et al*., 1995; Stern *et al*., 1995; Jørgensen & Andersen, 2000; Nikkilä *et al*., 2008). Marker assisted selection (MAS) may be an effective strategy in improving these traits of economic importance and at the same time overcome pleoitropic effects in genes.

The current study was carried out to examine the effects of *high mobility group AT-hook1 (HMGA1)*, *transcription factor 7-like-2 (TCF7L2)* and *insulin-like growth factor binding protein 3* (*IGFBP3*) genes on leg action and fat deposition in pigs, and, furthermore, to explore the possible relationships between these genes on both traits. In previous studies, porcine *HMGA1* and *TCF7L2* genes had been shown to have associations with fat measurements, and were mapped on *Sus scrofa* chromosomes 7 and 14, respectively

(Kim *et al*., 2004; Du *et al*., 2008). Insulin-like growth factor binding protein 3 is a member of the IGFBP family. Binding proteins prolong the half-life of insulin-like growth factors, and inhibit or stimulate the growth promoting effects of the IGFs on the cell culture (Duan, 2002; Mohan & Baylink, 2002; Mote, 2008).

A total of 188 commercial Large White and Landrace intercross females from Newsham Choice Genetics were used in this study. When the animals weighed about 125 kg, overall leg action was measured on a scale of 1 (excellent movement) to 9 (severe leg weakness) by an experienced scorer. Fatness traits including 10th rib backfat (BF10), adjusted 10th rib backfat (AdjBF10) and last rib backfat (Last BF) were measured using an ultrasonic imaging approach with a Pie Medical 200 (Classic Medical Supply Inc., Tequesta, FL). Standard protocols were followed to extract DNA from the sows using the DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA, USA). The detailed information about single nucleotide polymorphisms (SNPs) and genotyping analyses is presented in Table 1. The association analyses between SNPs and traits were carried out using the PROC MIXED procedures of SAS (2006). Statistical models of analyses included genetic line, scoring date and genotype as fixed effects; sire within line as a random effect and body weight as a covariate.

Table 1 PCR primers, amplification conditions, polymorphism locations and restriction enzymes for porcine SNPs

Gene	SNP type	SNP location	Primer sequences (5' → 3') (Forward/Reverse)	T_m (°C)	Product size (bp)	Restriction enzyme	Digestion pattern
HMGA1	C>T	Intron 4	AGAAGGAGCCCAGCGAAGT/ ACAGTGCTCACCCAATGGC	60	700	*Nae*I	700/580+120
TCF7L2	A>G	Intron 10	AGAAAGGAAAGGGTGCAGGT/ GCGATAACTTGTCAGCACGA	60	314	*Bsr*I	314/192+122
IGFBP3	A>G	Intron 2	CAAGTCTCAAGCACGGACAC/ GCCAGGGGCTCTCTCTTTT	59	438	*BsaH*I	438/326+112

The association results between *HMGA1*, *TCF7L2* and *IGFBP3* genotypes with fat deposition traits and leg action score are presented in Table 2. Leg action was suggestively associated with the *IGFBP3* gene (P <0.2), but was not associated with *HMGA1* and *TCF7L2* genes (P >0.2). On the basis of these results the association between overall leg action and *IGFBP3* indicates that, in this population, AA homozygote individuals had better movements (5.40) compared to GG homozygotes (5.84). The association between the *HMGA1* gene with BF10 and adjBF10 were suggestive (P <0.1), but was not indicative with Last BF (P >0.2). Thus, CC homozygote individuals deposited more fat compared to lean TT homozygotes. Kim *et al*. (2004) reported significant associations between this gene and backfat measurements in Landrace, Large White and Duroc × Large White populations. However, they found no associations between *HMGA1* and backfat measurements in a Duroc population. These differences in gene markers and their effects on traits in different populations could be in part due to the genetic background and population sizes considered. The *TCF7L2* gene also had a suggestive effect on BF10 and adjBF10 (P <0.2), but had no association with last BF (P >0.2). On the contrary, Du *et al*. (2008) found significant associations between *TCF7L2* with last BF (P <0.05) and BF10 (P <0.01) in a Berkshire and Yorkshire intercross population.

The candidate genes *HMGA1*, *TCF7L2* and *IGFBP3* have been demonstrated in other pig populations for their transcriptional regulation roles in fat deposition and growth (Duan, 2002; Mohan & Baylink, 2002; Kim *et al*., 2004; Du *et al*., 2008; Mote. 2008). Observations in the field have long suggested that there may be unfavourable genetic correlations between fat deposition traits and overall leg action in pigs. In the present study the results from the association analyses between *HMGA1*, *TCF7L2* and *IGFBP3* genes were suggestive for most fat deposition traits. However, IGFBP3 was the only candidate gene found to have a suggestive effect on leg action (P <0.2), probably due to a limited dataset. On the basis of these findings, allele A in *IGFBP3* is desirable for leg action but unfavourable for fatness. Thus, *IGFBP3* transcription factors involved in the regulation of fat deposition may also function in the transcriptional activation of leg

soundness. Although this association was indicative in this study, it is in agreement with previous findings that the genetic correlations between leg action and fat deposition measurements are antagonistic in pigs (Huang *et al*., 1995; Jørgensen & Andersen, 2000; Nikkilä *et al*., 2008). On the other hand, the association between the favourable alleles for both leg action and fatness in *HMGA1* and *TCF7L2* (T and G, respectively) suggests that for these genes less fatness may be associated with better leg soundness. These associations would be ideal in pig breeding since the objective is to breed pigs that deposit less fat and have good overall leg action.

Previously, *HMGA1*, *TCF7L2* and *IGFBP3* gene effects on leg action have not been reported for pigs. Results from this study suggest a possible association between the effects of these candidate genes and both leg action and fatness. Because of the limited sample size, further investigations are required in several populations, and using larger population size, to demonstrate these associations conclusively.

Table 2 The association results between *HMGA1*, *TCF7L2* and *IGFBP3* genotypes with fat deposition traits and overall leg action

Gene	Trait	Genotypic least squares means (s.e.)			P-value
		TT	CT	CC	
HMGA1	Overall leg action	5.05 (0.27)	5.39 (0.37)	5.92 (0.84)	0.51
	BF10 (cm)	1.29 (0.02)	1.32 (0.03)	1.49 (0.08)	0.09
	AdjBF10 (cm)	1.19 (0.02)	1.22 (0.03)	1.37 (0.07)	0.08
	Last BF (cm)	1.15 (0.02)	1.18 (0.03)	1.22 (0.06)	0.53
		AA	AG	GG	
TCF7L2	Overall leg action	5.59 (0.65)	5.05 (0.29)	5.19 (0.33)	0.74
	BF10 (cm)	1.41 (0.06)	1.27 (0.03)	1.32 (0.03)	0.13
	AdjBF10 (cm)	1.29 (0.06)	1.17 (0.02)	1.22 (0.03)	0.18
	Last BF (cm)	1.20 (0.05)	1.15 (0.02)	1.17 (0.02)	0.71
		AA	AG	GG	
IGFBP3	Overall leg action	5.40 (0.38)	4.79 (0.31)	5.84 (0.50)	0.17
	BF10 (cm)	1.33 (0.04)	1.34 (0.03)	1.23 (0.05)	0.23
	AdjBF10 (cm)	1.22 (0.03)	1.23 (0.03)	1.14 (0.04)	0.22
	Last BF (cm)	1.19 (0.03)	1.18 (0.02)	1.10 (0.04)	0.13

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