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SINGLE NUCLEOTIDE POLYMORPHISMS IN SUPPRESSOR OF CYTOKINE SIGNALLING-2 GENE AND ASSOCIATION WITH FEED CONVERSION RATIO AND GROWTH IN PIGS

E. Piper[1], Y. Chen[1], Y. Zhang[2], B. Tier[2], H. Graser[2], B. Luxford[3], C. Moran[1]
[1]Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney, NSW 2006
[2] Animal Genetics and Breeding Unit[*], University of New England, Armidale, NSW 2351
[3]QAF Meat Industries, Corowa, NSW 2646

SUMMARY
The Suppressor of Cytokine Signalling-2 (SOCS2) is the main negative regulator of somatic growth through the mediation of growth hormone signalling (GH/IGF-1). Knock-out and naturally mutant mice have high growth phenotypes. We have mapped the porcine SOCS2 gene to chromosome 5q, located closely to a reported QTL for food conversion ratio (Lee *et al.*, 2003). Here we report five single nucleotide polymorphisms identified by sequencing of the promoter region and exon 1. One PCR-RFLP assay was designed for genotyping the polymorphism at position 1667(A/G). Association analyses were performed in an Australian mapping resource pedigree (PRDC-US43) for a number of traits (feed conversion ratio, backfat, IGF-1 level and growth traits) and showed significant effects on average daily gain on test (ADG2) ($p<0.01$) and marginal association with feed conversion ratio (FCR) ($p<0.08$).

INTRODUCTION
Cytokines regulate diverse biological processes which control growth, development, homeostasis and immune function, by activating cell surface receptor complexes through Janus kinase (JAK) and the signal transducers and activators of transcription (STATs). Suppressors of cytokine signalling (SOCS) are negative regulators that control excessive cytokine effects (Flores-Morales *et al.*, 2006). The Suppressor of Cytokine Signalling-2 (SOCS2) is one of eight SOCS proteins and the main negative regulator of somatic growth through the mediation of growth hormone signalling (GH/IGF-1). SOCS-2-deficient mice were indistinguishable from their wild-type littermates until weaning at three weeks of age but subsequently grew more rapidly and adult mice generated to be deficient in SOCS2 are on average 40% larger than their wild-type counterparts (Metcalf *et al.*, 2000). Similar phenotypes have also been observed from a naturally occurring mutant strain of *high-growth* (hg) mice. The hg phenotype is the result of a spontaneous deletion within chromosome 10, resulting in the inactivation of the SOCS2 locus. The deletion occurs in the second exons of the SOCS2 and plexin C1 Gene (Wong *et al.*, 2002). SOCS2 not only regulates GH receptor signalling, but also has important actions in the regulation of other processes unique to somatic growth such as metabolism, development of the central nervous system, cancer, the response to infection and the regulation of other cytokine-dependent pathways. The porcine SOCS2 gene has been mapped on chromosome 5q (Piper *et al.*, 2005) and located closely to a reported QTL for food conversion ratio (Lee *et al.*, 2003). The function of SOCS2 and the location near the chromosome 5 QTL region for food conversion ratio make the porcine SOCS2 a positional candidate gene for FCR and growth in pigs.

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MATERIALS AND METHODS

Six primers (Table 1) were designed to amplify the promoter region, exon 1 and exon 2 of the porcine SOCS2 gene (GenBank Accession No: AY312266). *Pf1059 – pr1606* flanked the promoter region of SOCS-2, *pf1446-pr1820* flanked exon 1, and *pf3547-pr4908* flanked exon 2 of SOCS-2. PCR amplification was performed in 25 µl containing 25ng of template DNA, 1XPCR buffer, 2.0mM MgCl2, 2.0mM dNTPs, 20pm of each primer (forward and reverse). Touchdown PCR was carried out by denaturing at 95°C for 10 min followed by 48 cycles of amplification with each cycle consisting of 40 s at 94°C for denature, 1 min for annealing at temperatures from 65-55°C and 1 min at 72°C for extension.

Table1. Primers for amplification of SOCS2 gene

Primers	Postion*	Sequence 5' → 3'
pf1059 forward	1060	cggtgcagagtgtgagatg
pr1606 reverse	1607	agtgagaaccccaaaacgaa
pf1446 forward	1447	acccaaccctccactttctc
pr1820 reverse	1821	gtgtgactaagctcccgca
pf3547 forward	3458	agaggcacccgaaggaac
pr4908 reverse	4909	tgaaaaacaggcaggtatcc

PCR products from 4 sires were purified from agarose gel with JETquick spin columns (Genomed, Germany) and cloned into the TOPO vector with a TOPO^{TM} TA cloning Kit (Invitrogen, USA). Plasmid DNA was purified with UltraClean^{TM} Mini Plasmid Prep Kit^{TM} (MO BIO Laboratories, Inc. USA). Sequencing was carried out on the ABI Sequencer (ABI3730). Single Nucleotide Polymorphisms (SNP) were identified using Sequencher (Gene Codes, Demo Version 4.5).

A PCR-RFLP assay was designed with forward primer 5'-GACTTCTAAGGACGCGTTGC-3' and reverse primer 5'-AGATGCCGAGAGACAACAGG-3' and restriction enzyme SauII digestion. The primers corresponded to bp 1608 to 1627 and 2401 to 2420 of published sequence (GenBank accession number AY312266) to amplify a region of 812 bp around SNP scocs2_1667 (A/G). Allele A of scocs2_1667 has two fragments of 502bp and 312bp while allele G has three fragments of 62bp, 312bp and 440bp.

Association analysis was carried out in an Australian resource pedigree consisting of 430 progeny of eight sires bred at QAF Meat Industries, Corowa, NSW, between 1999 and 2000. Growth and production traits were used for the analysis, including feed conversion ratio (FCR), average daily gain to test (ADG1), average daily gain on test (ADG2), lifetime average daily gain (ADG3), average daily gain to weaning (ADG21), backfat depth (mm) on test measured with real time ultrasound (BF) and IGF-1 levels (IGF-1) measured at weaning. Phenotypic data were corrected with the model with sex, sire, test group as fixed effects and weight into test as a co-variable. The variance analysis was carried out using R package (Copyright 2005, The R Foundation for Statistical Computing, Version 2.2.0 2005-10-06 r35749). The linear model included sire and SNP genotype as fixed effects (y =

sire-group + genotype + error). Further variance analysis was carried out using a model fitted with additive and dominant effects (y = sire-group + a + d + error).

RESULTS AND DISCUSSION

Five SNPs were identified in the proximal promoter region at positions 1110(T/C), 1163(A/G), 1483(C/G), 1667(A/G) and 1779(A/G). Analysis of potential transcription factor binding sites was performed by searching the Transfac6.2 database (http://www.gene-regulation.com/) using MatInspector V7.4.4 software (Cartharius *et al.*, 2005). A PCR-RFLP assay was designed with forward primer 5'-GACTTCTAAGGACGCGTTGC-3' and reverse primer 5'-AGATGCCGAGAGACAACAGG-3' and restriction enzyme SauII digestion. 8 sires, 63 dam and 400 progeny were genotyped for this SNP.

Variance analysis of SOCS2_1667 showed significant genotype effect on average daily gain on test (ADG2) and marginal association with feed conversion ratio (FCR) (Table 2). The dominance effects were significant for both ADG2 and FCR, while additive effects on ADG2 and FCR were not significant. Homozygous animals for the A allele had higher average daily and lower FCR than homozygous G/G and heterozygous G/A.

Table 2. SOCS2_1667 genotype effects expressed as deviations from GG genotype

	Trait[1]						
Genotype	FCR	IGF-1	ADG1	ADG2	ADG3	ADG21	BF
	2.53[8]	9.55	515.96	1115.52	743.34	310.45	6.77
	± 0.23[9]	±2.14	±58.31	±139.40	±78.10	±42.96	±1.15
	402[10]	410	388	411	420	394	426
GA	0.02	0.12	6.00	-9.19	-1.10	0.37	0.00
	(0.03)[6]	(0.27)	(7.89)	(18.50)	(10.03)	(5.85)	(0.15)
AA	-0.61	0.18	18.00	56.85	19.53	-6.78	-0.15
	(0.04)	(0.38)	(11.34)	(26.05)	(14.04)	(8.37)	(0.21)
N[2]	402	410	388	411	420	394	426
P[3]	0.08	0.87	0.28	0.01	0.20	0.59	0.68
A[4]	0.03	0.09	9.00	28.43	9.76	-3.39	-0.08
	(0.02)	(0.19)	(5.67)	(13.02)	(7.02)	(4.18)	(0.10)
D[5]	0.049	0.04	-3.00	-37.62	-10.87	3.76	0.08
	(0.03)*[7]	(0.22)	(6.65)	(15.36)**	(8.23)	(4.90)	(0.12)

[1] FCR = food conversion ratio (kg), IGF-1 = insulin like growth factor 1 (µg/ml), ADG1 average daily gain to test (g/day), ADG2 = average daily gain on test (g/day), ADG3 = lifetime average daily gain (gm/day), ADG21 = average daily gain to weaning (g/day), BF = backfat depth (mm).
[2] Total number of progeny in the analysis. [3] probability associated with an F test for the effect of genotype. [4] Additive effect. [5] Dominance effect. [6] Standard errors of estimates in parentheses.
[7] Test *p<0.05, ** p<0.01. [8] phenotypic mean of the trait. [9] standard deviation.
[10] number of observations.

Porcine SOCS2 gene has been mapped on chromosome 5q (Piper *et al.*, 2005) and a number of suggestive and significant QTLs have been mapped on porcine chromosome 5 in the proximal region of SOCS2, including meat colour and pH (Malek *et al.*, 2001b), backfat (Malek *et al.*, 2001a) and FCR (Lee *et al.*, 2003).

SOCS2 is able to regulate the cytokine-dependent JAK/STAT signalling pathway and affect somatic growth through the GH/IGF-1 axis. Detailed analysis of SOCS2 promoter *in vitro* and *in vivo* demonstrated its direct transcriptional regulation by STAT5b upon GH stimulation and the responsive element located in the first intron of human SOCS2 (Vidal *et al.*, 2007). In our analysis of porcine SOCS2 gene, only a single putative STAT5 binding site was identified within intron 1. SNP SOCS2_1667 in the pig SOCS2 promoter region is located on a putative binding site of nuclear hormone receptor TR2 (1664-1682, agagaagGGTCaccgcggc), which is one of the ligand-regulated transcription factors that modulate gene expression in response to small, hydrophobic hormones, such as retinoic acid and thyroid hormone.

The porcine SOCS2_1667 polymorphism showed no significant genotype effect on ADG21 (from birth to wean) and ADG1 (from weaning to test) and demonstrates similar phenotype effects observed in SOCS2-deficient mice.

Further industry wide validation is necessary to estimate SOCS2 gene effects and as a potential genetic marker for growth and food conversion efficiency. A novel set of SNPs in porcine SOCS2 need to be identified in order to find haplotype/casual mutation that affects daily gain and feed conversion efficiency. The genotype of porcine SOCS2 gene and its relation to its expression and expression of genes in JAK/STAT pathway needs to be further investigated to elucidate its function and physiological consequence.

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