Mapping and Investigation of Novel Candidate Genes for Fatness, Growth, and Feed Intake in the Pig

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ASL-1672

Summary and Implications

Five new candidate genes for fatness, growth, and feed intake traits were studied. The genes were chosen based on their presumed biological action for a given trait of interest. A molecular genetics polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) approach was used to identify genetic differences (polymorphisms) in the porcine *melanocortin-4 receptor* (*MC4R*), *melanocortin-5 receptor* (*MC5R*), *cocaine and amphetamine-regulated transcript* (*CART*), *peroxisome proliferator activated receptor _* (*PPAR_*), and *prepro-orexin* genes. These genes were genetically mapped using several markers on porcine chromosomes (SSC) 1, 6, 12, 13, and 16, respectively. All five genes also were physically mapped with a pig/rodent somatic cell hybrid panel. The physical locations of all five genes are as follows: *MC4R* (SSC1q22-27), *MC5R* (SSC6q24-(1/2)q31), *prepro-orexin* (SSC12p13-p11), *PPAR_* (SSC13q23-q41), and *CART* (SSC16q21). The localization of these genes is reasonably consistent with previous chromosome painting results, indicating conserved (similar) regions between human and pig chromosomes. We also looked at the effect of these genes on traits of interest. The effect of a *MC4R* polymorphism was investigated in a large population of pigs from several commercial lines. *MC4R* genotypes were significantly associated with fatness, growth rate, and feed intake traits. Further studies on the effect of these candidate genes are underway.

Introduction

An enormous amount of information on the structure and function of genes has provided valuable resources to study the genetic factors influencing economically or biologically important traits in livestock species. Studying candidate genes of known biological action is a useful method to identify genes controlling traits of interest. These important genes can be used to improve livestock through the immediate application of marker-assisted selection (4).

It is well established that the brain, specifically the hypothalamus, is a major site where various central nervous system signals are integrated to affect the expression of complex hormonal and neuroendocrine functions, such as food intake and energy homeostasis. The functions of the genes we are studying are as follows. MC4R is a receptor expressed in the brain and mediates the effects of leptin, one of the important signaling molecules in regulation of energy balance and energy homeostasis (7). MC5R mediates the effects of adrenocorticotropic hormone (ACTH)/ melanocortin stimulating hormones (MSH) on exocrine gland functions, including thermoregulation, immunomodulation, and sexual behavior (8). Prepro-orexin (5) and CART (3) are neuropeptides involved in the regulation of food intake in several mammalian species. PPAR_ is a member of the nuclear receptor superfamily and regulates the expression of several genes encoding proteins involved in adipocyte differentiation and fat deposition (6). The localization of these genes in the pig genome would be useful for quantitative trait loci (QTL) analyses for economically important traits, including fatness, growth rate, and feed intake. In addition, the identified polymorphisms with functional significance within these genes will give better insights into the molecular basis of food intake and energy balance.

Materials and Methods

The genes investigated are listed in Table 1. Physical gene mapping was carried out with amplification by the polymerase chain reaction (PCR) on a pig-rodent somatic cell hybrid panel (9). The polymorphisms within these genes were revealed with direct sequencing of PCR products from individuals representing different breeds. Single nucleotide polymorphisms were genotyped as PCR-restriction fragment length polymorphisms (RFLPs) on agarose gels. The PiGMaP reference families (1) were used for two and multipoint linkage analyses using the CRI-MAP program (2). An interesting *MC4R* polymorphism was genotyped in more than 1,700 animals from PIC USA to investigate the association of this gene with the phenotypic traits.

Results and Discussion

The *MC4R* gene was physically mapped to SSC1q22-q27and showed significant linkage to several markers on porcine SSC1. The most closely linked marker (recombination distance and

LOD score in parentheses) determined by two-point linkage analysis is SO313 (0.00, 17.76). A multipoint linkage analysis produced the best map order of the *MC4R* gene between linked markers (with distance in Kosambi cM): *FGF7-5.6-MEF2A*-5.8-*MC4R*-5.9-*SO313.*

A *MC4R* missense mutation was identified in a region highly conserved among melanocortin receptor (MCR) genes. To determine if there was an association of this *MC4R* polymorphism with phenotypic variation, we tested the mutation in a large number of individual animals from several different pig lines from PIC USA. Analyses of growth and performance test records showed significant associations of *MC4R* genotypes with backfat and growth rate in a number of lines, as well as feed intake overall (Table 2). It is probable that the variant amino acid residue of the *MC4R* mutation (or a closely linked mutation) causes a significant change of the MC4R function.

The porcine *MC5R* was physically assigned to SSC6q24-(1/2) q31. The *MC5R* gene was most closely linked to *S0059* on SSC6 (recombination distance = .05 and LOD = 12.43). The best map order of the *MC5R* gene produced by the multipoint linkage analysis with other linked markers is as follows:

GPI-7-*PGD*-19-*S0059*-5-*MC5R*-7-A*DCYAP1*. Preliminary investigations to date do not show an association between MC5R genotype and performance traits.

The prepro-orexin gene was physically mapped to SSC12p13-p11. The porcine prepro-orexin gene significantly linked (cM distances and LOD score in parenthesis) to *PRKAR1A* (12.5, 4.7), *GH1-1* (9.7, 8.1) and *BRCA1* (11.4, 5.85). The human prepro-orexin has not been mapped, but we can predict that the human prepro-orexin gene is located on human chromosome 17 (q21-q22) based on information from our mapping and previous chromosomal painting studies. This shows how pig gene mapping can help the human genome project. We plan to see how prepro-orexin is associated with feed intake.

The porcine *PPAR_* gene was physically assigned to porcine chromosome 13 (SSC13) to the region 13q23-q41 with 0.8 probability. The most closely linked markers (cM, LOD) were *S0222* (5.9 cM, 8.17), *S0021* (2.7 cM, 12.06), *S0223* (0 cM, 11.74), *Sw937* (2.2 cM, 12.52), *TF* (2.2 cM, 6.20), and *S0281* (4.0 cM, 11.76). Studies are underway to see how this gene is related to backfat and intramuscular fat.

The *CART* gene was physically localized to porcine chromosome 16 (SSC16) q21 and was most closely linked to *S0077* on SSC16 with recombination frequencies of .00 and a LOD score of 3.91, respectively. The best map order of *CART* gene produced by the multi-point linkage analysis with other linked markers is as follows:

S0077-6-*GHR*-.4-*CART*-2-*C9*-4-*S0298*. Preliminary analyses suggest it may be associated with feed intake differences.

Conclusions

- The localization of these genes is reasonably consistent with previous chromosome painting results, indicating conserved regions between human and pig chromosomes.
- The genes can serve as anchor markers for comparative mapping studies that could provide important insights for identification of the conserved synteny and comparative analyses of QTLs.
- The localization of these candidate genes in the pig genome improves the power of analyses for quantitative traits associated with fatness, growth rate, and feed intake traits.
- The *MC4R* polymorphism has immediate value as a genetic marker for both growth and backfat traits.
- The study of these genes has provided new insights for the molecular understanding of energy homeostasis and fat metabolism in the pig.
- Further studies on the roles of these genes in performance traits in the pig are underway.

Acknowledgements

This work was supported in part by the Iowa Agriculture and Home Economics Experiment Station, PIC USA and PIC International Group, State of Iowa and Hatch funds, the Norwegian Pig Breeders Association, and the Research Council of Norway. DNA from PiGMaP families and bioinformatics support was kindly provided by Dr. Alan Archibald (BBSRC, Roslin, UK) and distributed by the USDA Pig Genome Coordinator. DNA from the somatic cell hybrid panel was kindly provided by Dr. M. Yerle, INRA, France. The authors are grateful to Dr. T. Short, Dr. G. Plastow and Dr. E. Wilson from PIC for supplying data and DNA, Dr. Zhiliang Hu for linkage analyses, Ms. Jeannine Helm for technical assistance, and Ms. Shannon Kenealy and Ms. Jennifer Seifert for genotyping. Patent applications are pending for the use of these genes to improve genetically improve pigs.

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Table 1. Summary of mapping methods and chromosomal assignments of the genes mapped in this study. Genes are ordered by their chromosomal location in the pig genome.

Gene	Mapping methods[a]	Type of polymorphism	Physical location	Linked to loci[b]	
MC4R	Linkage/SCHP	*Taq*I	1q22-27	*SO313*	(0.00, 17.76)
MC5R	Linkage/SCHP	Allele-specific PCR	6q24-(1/2)q31	*S0059*	(0.05, 12.43)
Prepro-orexin	Linkage/SCHP	*Nla*III	12p13-p11	*GH1-1*	(9.7, 8.1)
*PPAR*γ	Linkage/SCHP	*Bsg*I	13q23-q41	*S0021*	(2.7, 12.06)
CART	Linkage/SCHP	*Hae*III	16q21	*S0077*	(0.00, 3.91)

[a]SCHP – somatic cell hybrid panel.
[b]Recombination fraction and LOD score in parentheses.

Table 2. Effect of *MC4R* genotype on several production traits in pigs from 4 commercial lines.

MC4R	Days[a]	BF[b]	ADG[c]	Feed Intake[d]
11	169.9 +/- .9	11.1 +/- .2	871.9 +/- 10.2	1.94 +/- .07
12	166.9 +/- .8	11.6 +/- .2	885.1 +/- 8.9	2.03 +/- .06
22	164.6 +/- .9	12.0 +/- .2	908.8 +/- 9.3	2.11 +/- .06
	P< .001[e](1,720)[f]	P< .001 (1,720)	P< .001 (1,194)	P< .01 (231)

[a]Days to 110 kg.
[b]Teneth rib backfat (mm).
[c]Daily gain (gm/day).
[d]Feed intake (kg/day).
[e]Level of significance.
[f]Number of animal tested in parentheses.