RESEARCH REPORT
ANIMAL SCIENCE

**Title:** Large-Scale Association Analyses of Candidate Genes for Feed Efficiency Traits in Pig – **NPB #08-011**

**Investigator:** Max F. Rothschild

**Institution:** Iowa State University

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**Industry Summary**

This study was conducted with the objectives of identifying genes which impact feed efficiency in pigs, quantifying those effects, and informing producers of how to utilize this information in selection programs. The purpose of this research was to identify markers that can be used in marker-assisted selection (MAS) to improve feed efficiency. Feed intake data are expensive to collect on individual pigs, so such trait data frequently are not included in a selection index despite the potential economic gains. By identifying genetic markers which can be used for selection, producers can have that benefit added to their bottom lines without the expense and hassle of collecting feed intake data.

About ten years ago, Iowa State University (ISU) started developing a randomly selected line and a line selected for decreased residual feed intake (RFI), a measure of the difference between what an animal actually consumes and the average amount of feed required for that animal's levels of maintenance and growth. To establish the two lines, a single foundation population of Yorkshire pigs was divided by randomly splitting litters. For this gene marker association study, a total of 730 animals from the first seven generations of these lines were genotyped.

To improve the speed and reduce the cost of genotyping, a tool known as the PorcineSNP60 BeadChip (SNP chip) was developed with the assistance of the grant PI, Max Rothschild, and other researchers. The single

nucleotide polymorphism (SNP) chip simultaneously genotypes 64,232 genetic markers in a single pig at a cost of approximately 0.3 cents per genotype. In conjunction with NPB grant #08-190, this SNP chip was used in this project to facilitate a more thorough analysis of the porcine genome and produce more significant results than would have been otherwise achieved by looking at individual candidate genes.

A computer program written by Drs. Dorian Garrick and Rohan Fernando at ISU was used to determine which of the 64,232 markers were associated with RFI. In addition, data on average daily feed intake (ADFI), average daily gain (ADG), and 10th-rib backfat (BF) were analyzed in this population to determine effects of the same 64,232 markers.

Results of this research look very promising for producers. The SNP chip works extremely well in many populations and produced an average call rate (useable genotypes) of 99.6% in this study. Statistical analyses showed that a genetic marker on chromosome 2 had the largest marker effect for RFI; markers on chromosomes 3, 10, and 15 also had significant effects on RFI, and many smaller effects were also identified throughout the genome. Markers with large effects on ADFI were found on chromosomes 1, 3, 4, 7, 9, and 16. Markers on chromosomes 1, 6, 7, 12, 14, and 15 had the largest effects on ADG. The gene *MC4R*, located near the significant markers on chromosome 1 for ADFI and ADG, was previously known to have large effects on both traits. A currently unmapped marker had the largest effect for BF, which accounted for over 3% of the genetic variance in the trait. The genetic markers with the largest effects on BF and known positions in the genome were on chromosomes 9, 12, 13, and 15. Additional research will be carried out on the genes near these markers to develop final markers for selection that maximize the effects for producers.

By selecting animals using genetic markers associated with residual feed intake, producers could reduce feed costs without the expense and difficulty of measuring feed intake and without reducing growth rates. Taken together, selection for the various markers identified in this study could save producers several dollars per pig in the near future.

For additional information, contact Dr. Rothschild at mfrothsc@iastate.edu or call (515) 294-6202.

**Scientific Abstract**

Residual feed intake (RFI) measures how much feed an animal consumes compared to how much would be expected based on maintenance and growth requirements. Iowa State University developed two lines of Yorkshire pigs. The select line was selected for reduced RFI over 6 generations. A control line that originated

from the same population was randomly selected for 5 generations prior to 1 additional generation of selection for increased RFI. A total of 730 animals (395 select, 335 control) were genotyped with the PorcineSNP60 BeadChip. After removing fixed markers and SNPs with call rates below 80%, a total of 55,533 SNPs remained for analyses with genomic positions based on the Sscrofa9 genome build. Genetic effects were fitted using a Bayesian model averaging approach (Bayes-C) that simultaneously fitted various combinations of 250-300 SNPs. Many SNPs were fitted more often than expected by chance, reflecting associations with the traits analyzed. For RFI, SNPs near *SLC5A12* on SSC2 and *SUCLG1* on SSC3 showed association, for example. Several SNPs near the *MC4R* gene on SSC1 showed some of the largest effects on both average daily feed intake (ADFI) and average daily gain (ADG), though other significant regions were also identified for both traits. An unmapped SNP had the largest SNP effect on $10^{th}$-rib backfat (BF), accounting for over 3% of the estimated genetic variance. Markers on chromosomes 13 and 15 had the largest effects on BF. More research needs to be done to validate the new significant regions, but results look promising based on the combination of previously identified genes and new candidate regions.

**Introduction**

The high genetic correlation between growth and feed intake has increased feed requirements as pigs have been selected for increased growth rate. The largest variable cost in swine production today is feed. Boggess *et al.* (2009) claimed that around $500 million dollars annually could be saved by swine producers in the US by reducing the average feed:gain ratio from 2.75 to 2.45. Significant variability exists between pigs in the amount of feed intake required to achieve the same rate of growth. Residual feed intake (RFI) is a measure of the difference between what an animal actually consumes and the average amount of feed required for that animal's levels of maintenance and growth. It is believed that animals can be successfully selected for both increased growth and reduced RFI, to reduce the feed costs per-unit gain.

Iowa State University has selected pigs for decreased RFI over six generations. A single foundation population of Yorkshire pigs was divided into two lines by randomly splitting litters to form a select line for low RFI and a randomly selected control line (Cai *et al.* 2008). Following five generations of random selection, the control line was subsequently selected for increased RFI. Phenotypic RFI was calculated as average daily feed intake (ADFI) – ($b_{1i}$*onTestWeight + $b_{2i}$*offTestWeight + $b_{3i}$*midTestWeight + $b_{4i}$*(average daily gain (ADG)) + $b_{5i}$*offTestBackFat (BF)), where the regression coefficients (*b*'s) were generation and line dependent. An animal model was used to estimate EBV from the phenotypic RFI observations (Cai *et al.* 2008). Estimated heritability for RFI calculated in this manner was 0.29 and RFI explained 34% of phenotypic variation in ADFI (Cai *et al.* 2008). These results indicate that significant financial progress could be made by selecting for growth and RFI if this could be done using SNPs without the expense of collecting feed intake data.

**Stated Objectives from original proposal**

The objectives of this research are to:

1) Develop a large panel of over 100 genetic markers from individual genes and/or genetic pathways important to feed efficiency in pig;
2) Test associations of these markers and determine their genetic effects for improved feed efficiency; and
3) Recommend ways to utilize important genetic markers in breeding programs for all producers.

**Materials & Methods**

*Genotyping*

Tail samples were collected from each animal at birth and used for DNA isolation. The Qiagen (Valencia, CA, USA) DNeasy blood & tissue kit was used for DNA isolation. A total of 730 animals were selected for genotyping from generations 0, 4, 5, and 6 of the Iowa State University RFI selection lines. Genotyped animals included a total of 716 animals with RFI data, with 329 control and 387 select animals. GeneSeek Inc. (Lincoln, NE, USA) completed the genotyping with the Illumina (San Diego, CA, USA) PorcineSNP60 BeadChip.

*Phenotyping*

Feed intake were measured on all animals using electronic FIRE Feeders (Osborne, KS, USA) donated by PIC (Hendersonville, TN, USA), as described in Cai et al. (2008). Animals were weighed at least every two weeks to compute ADG. At approximately 115 kg, 10th-rib BF was evaluated with an Aloka ultrasound machine (Corometrics Medical Systems Inc., Wallingford, CT, USA).

*Statistical analyses*

Quality control included the removal of all SNPs which were fixed in the entire population or had a quality control (QC) score less than 0.4 in greater than 20% of the population. A total of 55,533 SNPs remained for analysis (objective 1). Bayes C model averaging, as implemented in GenSel (http://bigs.ansci.iastate.edu) was used for data analyses (objective 2). The regression model used was: $Y = \mathbf{X}\beta + \mathbf{Z}u + e$, where **X** is an incidence matrix for fixed effects and **Z** is a matrix of SNP genotypes fitted as random effects. Fixed effects included line, sex, on-test group, pen fitted within group, and on-test age as a covariate, except for BF where on-test age was replaced with off-test weight. The prior probability that a SNP in **Z** has zero effect was set to 0.995, corresponding to about 300 non-zero SNP effects fitted in any particular realization of the Monte-Carlo Markov Chain (MCMC) used for the Bayesian analysis. Following a 10,000 iteration burn-in period, 40,000 MCMC iterations were run. Results were obtained in the form of a post burn-in posterior distribution for the effect of

every SNP fitted simultaneously with other informative SNPs. The posterior mean effect of each SNP across the chains was used to predict the genomic breeding value of every chromosomal fragment consisting of 5 contiguous loci. Each such chromosomal fragment's contribution to the additive genetic variance in the population was then derived, a statistic that has a multi-locus analogy to $2pq\hat{a}^2$, the gene frequency specific contribution to genetic variance of the substitution effect of a single locus. That variance was divided by an estimate of the total genetic variance to compute the proportion of genetic variance explained by the loci.

The most significant regions of the genome for RFI, ADFI, ADG, and BF were examined for genes based on build 9 of the porcine genome. Gene positions were taken from Ensembl (www.ensembl.org) identified by proximity to the most informative individual SNPs or 5-SNP windows and relation of the gene's function to the trait being analyzed.

**Results**

*Objective 1*

The development of the 64,232 SNP marker chip was a considerable feat of collaborative science. Efforts by the PI, Rothschild, working with other researchers helped to speed its development, and it was completed in late December 2008. This modification of the objective was approved when ***Large scale SNP association analyses of feed efficiency and longevity,*** **NPB #08-190** was funded.

*Objective 2*

In Figure 1 SNP association results are shown for RFI, ADFI, ADG, and BF. Sets of 250-300 markers explained 33% of phenotypic variation for RFI. For ADFI, ADG, and BF, 48%, 43%, and 69%, respectively, of the phenotypic variation was explained by sets of 250-300 markers.

The largest SNP effect and largest SNP window effects for RFI were located on *S. scrofa* chromosome (SSC) 2 near 32 megabases (Mb) [coordinate position]. None of the other traits analyzed had large SNP effects in this region of SSC2. Some of the largest genetic effects for ADFI were near *MC4R* on SSC1 and around 49 Mb [coordinate position] on SSC16 near *FGF18*. Some of the largest effects for ADG were near 17 Mb [coordinate position] on SSC6 and near *MC4R* on SSC1. Finally, for BF, the largest effect was from an unmapped SNP that lacked high LD with any currently mapped SNPs. The largest effect on BF from mapped SNPs was around 143 Mb [coordinate position] on SSC13. Many markers with smaller effects on each trait were also identified.

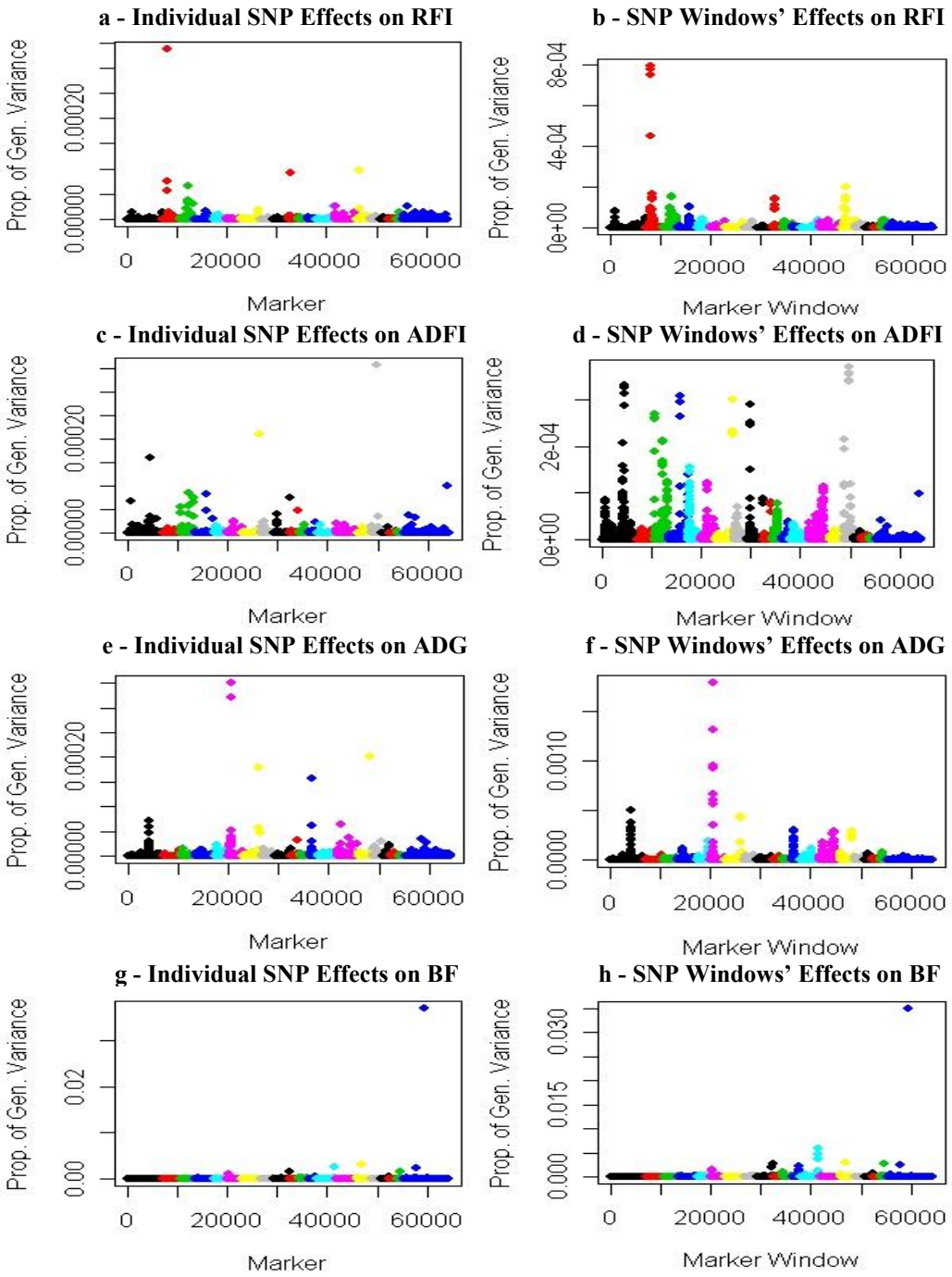


**Figure 1:** Proportion of genetic variance explained by each single marker (left side) or window of 5 consecutive markers in the genome (right side) for each trait. Each chromosome is a different color with SSC1 on the left, SSC18 in red on the right, followed by SSCX in green and unmapped markers in blue. Note: y-axes differ in scale.

*Objective 3*

So far citations including results from this grant have included:

Gorbach, D.M., W. Cai, J.C.M. Dekkers, J.M. Young, D.J. Garrick, R.L. Fernando, and M.F. Rothschild. 2009. Whole-genome analyses for genes associated with residual feed intake and related traits utilizing the PorcineSNP60 BeadChip. Pig Genome III Conference. November 2-4, Hinxton, UK. Abstract No. 11.

Gorbach, D.M., W. Cai, J.C.M. Dekkers, J.M. Young, D.J. Garrick, R.L. Fernando, and M.F. Rothschild. 2010. Genetic analysis of residual feed intake and its components based on the PorcineSNP60 BeadChip. The International Plant & Animal Genome XVIII Conference. January 9-13, San Diego, California. Abstract No. P614.

Information was also included in presentations at:

- The National Swine Improvement Federation (NSIF) conference in Nashville, TN in December 2009
- The Iowa Feed and Nutrition Seminar in Des Moines, Iowa in January 2010.

An article was also published in the February 15$^{th}$, 2010 issue of the National Hog Farmer. Further publication plans include presentation of results at the International Society of Animal Genetics (ISAG) conference in July 2010, the World Congress on Genetics Applied to Livestock Production (WCGALP) in August 2010, and submission of a larger paper to one of the major journals. Given the initial results, pork producers should continue to use *MC4R* to select for improvement in many of these traits.

**Discussion**

Use of this new SNP chip far exceeded the initial objective of 100 genetic markers. The genotyping call rate and genome-wide distribution of markers make it an exciting new tool for improving pig production.

The proportion of phenotypic variation explained by markers for RFI was close to the estimated heritability (0.29 - Cai *et al.* 2008) plus litter variance of 4% (Bunter *et al*. 2010). In the current study, litter was not fitted because few families had more than one genotyped offspring. Heritabilities for ADFI, ADG, and BF were estimated to be 0.51, 0.42, and 0.68, respectively, in this population (Cai *et al.* 2008) and corresponded closely with the proportions of phenotypic variance explained by markers, as obtained from these marker analyses. For each analysis, the similarity of results to the estimated heritability values indicates that most of the genetic variance was captured by 250-300 markers.

The single marker genetic variance values used to evaluate individual SNP marker significance would be estimates of the true genetic variance accounted for by the SNP, if the SNP was in linkage equilibrium with the other SNPs. The existence of linkage disequilibrium (LD) reduces the genetic variance explained by each SNP as multiple SNPs share the effect of each QTL due to LD. Thus, SNP windows more accurately capture the contributions of QTL to genetic variance and provide regions for further analyses.

This study confirmed previously known results, such as the effects of *MC4R* on ADFI and ADG, but also identified many new genetic effects which need further analyses to confirm. The SNPs with large effects on RFI have the potential to be used in marker-assisted selection (MAS) to reduce the feed intake requirements of pigs without negatively impacting other production traits and without the hassle and expense of collecting feed intake data on individual pigs. The economic impact of such a reduction would greatly benefit hog producers.

**References**

Boggess, M. (2009). *Pig Genome III Conference*.

Bunter, K.L., Cai, W., Johnston, D.J., *et al.* (2010). *J. Anim. Sci.* (in press).

Cai, W., Casey, D.S., and Dekkers, J.C.M. (2008). *J.Anim. Sci.*, 86:287–298.