

## Mapping five new candidate genes in the pig

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#### Summary and Implications

Five new candidate genes for growth and carcass traits have recently been mapped in the pig by using either linkage analysis or analysis of a hybrid cell line panel. The genes mapped include the very long chain acyl-CoA dehydrogenase gene (*ACADVL*) mapped to pig chromosome 12, the adenylate cyclase activating peptide, pituitary 1 gene (*ADCYAP1*) on chromosome 6, the calpain large polypeptide L3 gene (*CAPN3*), the myocyte-specific enhancer factor 2A gene (*MEF2A*) on chromosome 1, and the thyroid stimulating hormone receptor gene (*TSHR*) on chromosome 7. All five genes have the potential to influence carcass traits in the pig. Future studies will be conducted to investigate if any of the genes actually do influence these traits.

#### Introduction

Genetic mapping in the pig has progressed rapidly in recent years and the number of genetic markers on recent maps sum to approximately 1,700. However, only 200–250 of these are genes, with the balance being anonymous pieces of DNA. This should be compared with 10,000–15,000 genes mapped in the human. The total number of pig genes is expected to be 90,000–100,000. Therefore, there is still a need for mapping more genes in the pig.

Genes and markers on the pig genome maps have been mapped using three different methods. Linkage maps are made by observing the inheritance of single gene alleles in families. Physical mapping of a gene can be performed by preparing chromosome spreads from cells and hybridizing with a DNA probe. This method is known as fluorescent *in situ* hybridization (FISH). Finally, the physical location can be determined using specially prepared and characterized hybrid cell lines containing parts of pig chromosomes and a complete genome of the mouse or hamster. Determining presence or absence of a pig gene in each cell line in a panel of hybrid cell lines allows mapping of the gene to a region within a pig chromosome.

This report includes mapping of the genes for very long chain acyl-CoA dehydrogenase (*ACADVL*); calpain large polypeptide L3 (*CAPN3*); and the thyroid stimulating

hormone receptor (*TSHR*) gene by using linkage analysis; and for adenylate cyclase activating peptide, pituitary 1 (*ADCYAP1*), and myocyte-specific enhancer factor 2A (*MEF2A*) by using a hybrid cell line panel.

The genes investigated in this study include one gene encoding an enzyme involved in fat metabolism (*ACADVL*), two genes with a possible role in muscle-specific gene expression (*CAPN3* and *MEF2A*), one gene encoding a hormone releasing peptide (*ADCYAP1*), and one gene encoding a hormone receptor (*TSHR*). Genetic variation in the expression of these genes could, therefore, influence carcass traits in pigs.

#### Materials and Methods

The strategy employed here includes several steps: (1) polymerase chain reaction (PCR) primers were designed from available DNA sequences (mostly from other species) and thereafter, PCR conditions were optimized for DNA amplification of the gene; (2) the amplified product was investigated with the purpose of finding polymorphisms; (3) genotypes were obtained from animals in the PiGMAP families. These animals have been genotyped for many markers by investigators in an international collaboration, and linkage analysis in these families, therefore, has high probability for identifying linked markers and indirectly the chromosomal location of the genes. If steps 2 and 3 failed, physical map position was obtained using a hybrid cell line panel for pig gene mapping developed at INRA-Toulouse in France. The PCR primers and PCR conditions will be published separately.

#### Results and Discussion

Polymorphisms were here identified within PCR fragments from the *ACADVL* (0.6 kb) and *CAPN3* (1.6 kb) genes and *TSHR* (3.5 kb). The PCR fragments from *ADCYAP1* (0.6 kb) and *MEF2A* (approx. 1.2 kb) used here were investigated using DNA sequencing from several animals and from animal pools, without detecting any polymorphisms. These two genes were, therefore, mapped using the hybrid cell line panel. Map details for these genes are given in Table 1.

Table 2 displays allele frequencies of the genes *ACADVL*, *CAPN3*, and *TSHR*. In all three, there is a large difference in allele frequency between the Meishan and commercial breeds. Most of the PiGMAP families were developed using crosses between Meishan and Large White animals. It was, therefore, easy to map these three genes. The *CAPN3* polymorphism appears to be fixed in the commercial breeds whereas the *ACADVL* polymorphism has both alleles present (Table 2). The

polymorphism in the *ACADVL* gene can, therefore, be used for association studies within commercial breeds.

When comparing gene maps from different species, it has been realized that some genes from a chromosome in species A also were found together on a chromosome in species B. It also was found that the order is not always conserved. From a sufficiently detailed comparative map of two species, it would be possible to guess which genes known in the well-investigated species (e.g., human) were present between two genes in the less-studied species (e.g., pig). This study adds genes to the swine gene map that have already been mapped in the human and thereby increases the information content of the comparative map. The *ACADVL* gene on pig chromosome 12 and human chromosome 17 supports the overall similarity of these two chromosomes and will help determine if the gene order has changed. In a similar way, *CAPN3* was expected to map to pig chromosome 1 and *TSHR* to pig chromosome 7. Different regions on human chromosome 18 are expected to correspond to pig chromosomes 1 and 6. *ADCYAP1* maps to a region of human chromosome 18 expected to correspond to pig chromosome 1, so the map position on pig chromosome 6 was somewhat unexpected. Preliminary linkage data supporting the chromosome 6 position of

*ADCYAP1* has been obtained. Regions of human chromosome 15 are expected to correspond to human chromosomes 1 and 7. *MEF2A* is from a region of human chromosome 15 expected to map to pig chromosome 7, but it mapped to pig chromosome 1 instead. Another gene (the insulin-like growth factor 1 receptor [*IGF1R*] gene) from the same region of human chromosome 15 also has been mapped to pig chromosome 1 (1,2). The *MEF2A* gene location is, therefore, helping define the extent of a new region on the comparative map. It will be interesting to see if association studies involving these markers will identify a significant influence on pig traits.

### References

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**Table 1. Mapping details for five genes.**

Gene	Mapping method	Porcine chromosome	Human chromosome	Size of PCR fragment (kb)	Restriction enzyme
<i>ACADVL</i>	linkage	12	17p11.2-p11.13	0.6	StuI
<i>ADCYAP1</i>	physical	6q24-q31	18pter	0.6	—
<i>CAPN3</i>	linkage	1	15q15.1-q21.1	1.6	HincII
<i>MEF2A</i>	physical	1q11-q17	15q26	1.2	—
<i>TSHR</i>	linkage	7	14q31	3.5	MboI

**Table 2. Frequency of allele 1 for each of three genes in 10 breeds of swine.**

Breed	LW <sup>1</sup>	Me <sup>1</sup>	Wb <sup>1</sup>	D <sup>2</sup>	Y <sup>2</sup>	H <sup>2</sup>	CW <sup>2</sup>	L <sup>2</sup>	Mi <sup>2</sup>	F <sup>2</sup>
No. animals	11	9	2	7	7	7	5	8	3	4
<i>ACADVL</i> allele 1 frq.	0.95	0.28	1.00	0.93	0.85	0.88	1.00	0.81	—	—
<i>CAPN3</i> allele 1 frq.	0.00	0.39	0.00	—	0.00	0.00	0.00	0.00	0.00	0.50
<i>TSHR</i> allele 1 frq.	0.09	1.00	0.00	—	—	—	—	—	—	—

<sup>1</sup> Grand parental animals from the European PiGMaP families (LW=Large White, Me=Meishan, Wb=wild boar)

<sup>2</sup> Unrelated animals from Iowa State University (Y=Yorkshire, H=Hampshire, CW=Chester White, L=Landrace, Mi=Minzu, F=Fengjing)