

SABRE Integrating Award SABRE WP9



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SSC7-Androstenone QTL: identification of causal and target genes

SABRE partner:

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Castration of male pigs is a common practice in pig production. It reduces aggressive behaviour, facilitates animal management and, above all, reduces the occurrence of boar taint, a strong and unpleasant perspiration-like and urine-like odour and flavour detected in the cooked meat of a small proportion of mature boar carcasses. Boar taint primarily derives from the accumulation of androstenone and skatole in fat tissue [see (Robic *et al.* 2008; Zamaratskaia & Squires 2009) for a review]. A quantitative trait locus (QTL) for boar fat androstenone levels has been identified near the SSC7 centromere in a Large White x Meishan cross. No gene known to be involved in androstenone metabolism was found in this region, except possibly *TEAD3* which is able to stimulate the transcription of *HSD3B* in the human placenta (Peng *et al.* 2004).

1- *TEAD3*: candidate gene involved in this QTL

In human *TEAD3* is a transcription activator, known to be predominantly expressed in placenta and that could play a role in the regulation of the transcription of 3beta-hydroxysteroid dehydrogenase/isomerase (*HSD3B1*) gene. This enzyme is required for the biosynthesis of all active steroid hormones and is involved in the degradation of androstenone in the liver.

The 5'-flanking region of porcine *HSD3B* was examined by *genomatix* to evaluate the possibility of a transcription regulation by *TEAD3*. Following to this analysis it could be hypothesized that *HSD3B* would be regulated by *TEAD3* in pig species. Moreover we found a good correlation between expression of *HSD3B* and *TEAD3* in the liver of Duroc animals [see §2].

Variations were screened in sequences related to *TEAD3*: in exons, in flanking sequences of exons and in the promoter region. A SNP was characterized at 725pb at 5' of the first exon. This SNP was tested on several pig populations and we concluded that it was not the causal mutation.

Funds from the SABRE integrating award were used to finance my stay in Norway to genotype Norwegian Landrace and Duroc (one week in December 2008). The others populations were genotyped in October 2009 by funds obtained with the EU program, ALCASDE. Funds from the SABRE integrating award were also used to pay a training course on bioinformatics analysis of promoters' sequences.

2- Large expression studies

Using Fluidigm, a new high-throughput technology, the expression of 25 genes from the QTL region was measured in a single real-time PCR experiment. Backcrosses were produced to isolate the Chinese haplotype in a European genetic background. The expression of 25 genes from the QTL region was studied in the testes and livers of 5-month old backcross boars with the aim of identifying the causal gene.

This study found six significantly down-regulated genes (*C6ORF106*, *C6ORF81*, *CLPS*, *SLC26A8*, *SRPK1* and *MAPK14*) in the testes of MS-LW backcross boars. In the livers, none of the genes were significantly up- or down-regulated, including *TEAD3*, previously designated as a good candidate to explain this QTL.

It was very important to invalidate completely the hypothesis that an over-expression of *TEAD3* in liver was at the source of this QTL. In the BC pigs the range of variation of *TEAD3* expression was narrow (1 to 3.2X,) whereas the variation in the *HSD3B* level, as previously characterised at the protein level (Moe et al. 2007a), was broader (1 to 13X) in the Norwegian Duroc population. Thereby a new Fluidigm experiment was performed to evaluate the transcript quantity of *TEAD3* and *HSD3B* in the livers of BC and N. Duroc pigs: no significant variations in *HSD3B* or *TEAD3* expression were detected. However the correlation between *HSD3B* and *TEAD3* expression was examined. As expected, the gene expression of *TEAD3* and *HSD3B* in the livers of N. Duroc pigs seemed to vary more than in those of BC pigs the results showed strong correlation between the expression of *TEAD3* and *HSD3B* in the liver of Duroc animals. Nevertheless it was not possible to detect a significant correlation in the liver of BC animals and in testes of all animals. Inside these batches of samples, the changes measured were very low and the precision of the measurements may not be sufficient.

I had originally planned to evaluate the activation of *HSD3B* gene by studying proteins. The new equipment arrived at the genopole of Toulouse (<http://genopole-toulouse.prd.fr/index.php?id=57>) gave us new perspectives. Funds from the SABRE integrating award were not used for this large expression study performed in Toulouse in October 2009 to February 2010. Nevertheless samples from livers and testes from Norwegian Duroc boars were provided by our partners and the collaboration has been constant and intense. A short visit in Norway in June 2010 was also financed by funds to discuss of results and prospects.

3- Conclusion

At the beginning of this study *TEAD3* appeared as a possible candidate gene for the androstenone QTL.

A SNP was characterized in the promoter region of *TEAD3* and was tested on several pig populations. We concluded that it was not the causal mutation.

We expected to show an over-expression of *TEAD3* correlated to an over-expression of *HSD3B* in the liver of MS-LW BC pigs. It was only possible to show the correlation between *TEAD3* and *HSD3B* expression in the liver and this correlation was characterised in Norwegian Duroc pigs.

Our data suggest the existence of a new gene regulating the expression of *HSD3B* that is a key enzyme for androstenone degradation in liver.

My stay in Norway and the collaboration with Norsvin partners were very fruitful. The course about promoters' analysis was a great opportunity for me to learn new techniques. This new collaboration led to 1 poster at ISAG and two articles:

Robic Annie, Fève Katia, Billon Yvon, Larzul Catherine, **Grindflek Eli**, Riquet Juliette. Expression profile of 25 genes located in SSC7q1.2 in regards of androstenone accumulation in backcross animals. ISAG, Edinburgh, July 2010 (poster)

Robic Annie, Fève Katia, Larzul Catherine, Billon Yvon, **van Son Maren**, Liaubet Laurence, Sarry Julien, Milan Denis, **Grindflek Eli**, Bidanel Jean Pierre, Riquet Juliette. Expression levels of 25 genes in liver and testis located in a QTL region for androstenone on SSC7q1.2.. Animal Genetics, in press.

Robic Annie, Larzul Catherine, **Grindflek Eli**, Chevillon Patrick, Hofer Andreas, Fève Katia, Iannuccelli Nathalie, Prunier Armelle, Milan Denis, Riquet Juliette. Molecular characterization of the porcine *TEAD3* (TEF-5): Evaluation of a promoter mutation as the causal mutation of a QTL affecting androstenone level in boar fat. Submitted in December 2010.

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