# Genome Scan For Androstenone And Estron Sulphate Using The 60K Porcine SNPchip

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## Introduction

Male pigs used for pork production are normally castrated early in life to prevent boar taint in the meat. High levels of 16-androstene steroids (Patterson, 1968) are one of the main compounds causing boar taint. Androstenone is synthesised along with e.g. estrogens, which are known to be important female sex hormones as well as important in regulating the normal function of the adult male reproductive tract and male fertility (reviewed by Hess (2003)). The sex hormone estradiol is one of the main estrogens, and estron sulphate, which is the reservoir for the active compound of this hormone, was included in the project to study common genomic regions affecting levels of androstenone and estrogens. A genome scan for androstenone and estron sulphate, using a porcine 60K SNP panel (Ramos et al. (2009)), were performed in the two populations in Norway; Landrace and Duroc.

## Material and methods

**Animals, data collection and map constructions.** In total, 1154 purebred Landrace and 841 purebred Duroc male pigs tested in NORSVIN's three boar testing stations were included in this study. The boars were slaughtered at 100 kg live weight, and blood samples were taken before slaughter for plasma suspension and DNA extraction. Samples of subcutaneous adipose tissue were collected from the neck for androstenone measurement. Furthermore, pedigree information was available for all individuals, spanning six generations of parents. The Landrace material descended from 86 sires whereas the Duroc material descended from 68 sires. The levels of androstenone were analysed at the Norwegian School of Veterinary Sciences (NVH) by a modified time-resolved fluoroimmunoassay (Tuomola et al. (1997)), using antibody produced by Andresen (1974). Level of estrone sulphate in plasma were analysed at the Aker University Hospital, using a radioimmunoassay (Diagnostic System Laboratories, Inc., Webster, TX, USA). Genotyping was performed at CIGENE using the iScan platform with the PorcineSNP60 array according to manufacturer's instructions. Image intensity data processing, clustering and genotype calling were performed using the genotyping module in the Genome Studio software (Illumina, San Diego, CA, USA). The

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average call rate across samples was 0.994, and 18 samples were excluded from analysis due to unacceptable call rates (<0.75). The order of markers were determined by the porcine sequence Build 9 (Sanger Institute) followed by multipoint linkage analyses using a modified version of the CriMap software (Green et al. (1990)). Recombination units were then transformed to map distances using the Haldane mapping function. All the non-informative markers were removed from the map and one map per breed were used in the analyses.

**Statistical analyses:** A combined Linkage Disequilibrium and Linkage Analysis (LDLA) was conducted using a statistical model that included the fixed effects of test-station, age at entering the test, number of days in test, number of litter mates born alive, as well as a random polygenic effect and a random haplotype effect. Polygenic effects were included to account for the family structure present in the data and were assumed to have a covariance matrix proportional to the pedigree based relationship matrix. LDLA followed the approach of Meuwissen and Goddard (2007), except that haplotypes were either assumed completely correlated or uncorrelated (rather than fitting a more differentiating IBD matrix, G). Based on experiences with IBD matrix calculations it was decided that, if the number of identical SNPs equal to the left and to the right of the putative QTL position was $\geq$ 10, a haplotype pair was considered completely correlated. Otherwise it was considered uncorrelated. A log-likelihood ratio test–statistic was calculated as LnLikratio = $LnLik_{model\ incl.\ haplo} - LnLik_{model\ excl.\ haplo}$. Under the null-hypothesis of no QTL effect, 2*LnLikratio was assumed to follow a chi-squared distribution with one degree of freedom. Chromosome-wide P-values were obtained by the approach of Piepho (2001). Genome-wide P-values were obtained by multiplying the chromosome-wide P-values by 18, i.e. by the number of porcine chromosomes.

**Table 1: QTL for androstenone in fat detected in both Norsvin Landrace and Duroc.**

| SSC | QTL | Breed | LnLikratio | Chr_P | Genome_P | Bp_Conf.Int. |
|---|---|---|---|---|---|---|
| 2 | 2a | L | 12.73 | 0.002 | 0.03 | 29857261-36933179 |
| 2 | 2a* | D | 9.06 | 0.002 | 0.05 | 36635374-53585648 |
| 2 | 2b* | L | 9.11 | 0.01 | NS | 101434879-101965552 |
| 2 | 2b | D | 21.12 | <0.00001 | 0.00001 | 101553334-101726085 |
| 3 | 3* | L | 14.19 | 0.0003 | 0.005 | 43465517-51614240 |
| 3 | 3* | D | 11.07 | 0.001 | 0.02 | 38071895-39437782 |
| 7 | 7a* | L | 7.37 | 0.001 | 0.02 | 34490889-41880530 |
| 7 | 7a* | D | 9.10 | 0.003 | 0.05 | 33573817-40151324 |
| 7 | 7b* | L | 4.59 | 0.05 | NS | 52834343-75085186 |
| 7 | 7b* | D | 12.36 | 0.0006 | 0.01 | 62824472-64957850 |
| 7 | 7c* | L | 4.74 | 0.04 | NS | 82256385-88308701 |
| 7 | 7c* | D | 9.95 | 0.002 | 0.04 | 80809151-81568888 |
| 15 | 15* | L | 14.2 | 0.0004 | 0.008 | 42524157-55922873 |
| 15 | 15 | D | 4.85 | 0.03 | NS | 46670062-50070033 |

[α] Significance at chromosome wide level (p<0.05) for estronsulphate in the androstenone QTL regions is marked with *, NS=Non-Significant.

## Results and discussion

Average androstenone levels in fat were 1.17 µg/g (SD=1.10) and 3.22 µg/g (SD=2.69) for Landrace and Duroc populations, respectively. The LDLA was conducted, in addition to genome wide associations studies (GWAS) (results not shown), to utilize the linkage information, filter spurious associations from GWAS and refine the QTL intervals. With the LDLA method 7 QTLs for androstenon were detected with genome-wide significance level in at least one of the breeds and at least chromosome-wide significance level in the other breed. Additionally, 15 QTLs were found to be genome-wide significant in one of the breeds only. This is in agreement with the large genetic differences found previously in these populations (Moe et al. (2007); Moe et al. (2009)). QTLs for androstenone in fat showing up in both breeds are presented in Table 1 and Figure 1. The QTLs reaching chromosome-wide significance levels for estron sulphate in one or both breeds are presented in Table 1. This kind of results is important for future genetic selection against androstenone without simultaneously declining genetically correlated fertility traits.

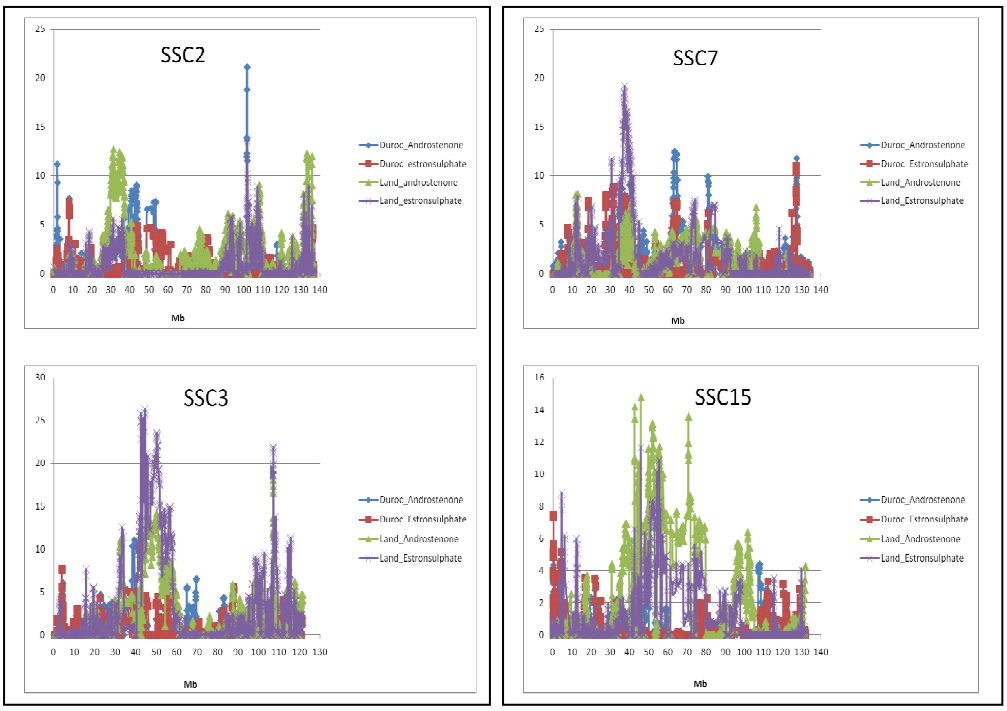


**Figure 1: QTL profile for androstenone and estron sulphate from LDLA analyses in Norsvin Duroc and Landrace. LnLikratio values are shown on the verticals.**

The QTL regions detected in SSC2, SSC3 and SSC7 are supported by studies in other breeds (Quintanilla et al. (2003); Lee et al. (2004)). Our results, however, seem to detect three different QTLs on SSC7 (Figure 1). Among these the candidate genes *P450 21-hudroxylase*

*(CYP21)* and *Cytochrome P450 subfamily XIA polypeptide 1 (CYP11A1)* are located in the two regions 7a and 7b, respectively. Interestingly, both *CYP21* and *CYP11A1* are previously found to be differentially expressed in testes (Grindflek et al. (2010)), although no significant associations for SNPs within the genes have been detected (Quintanilla et al. (2003); Grindflek et al. (2010)). No QTLs have previously been found on SSC15. The QTLs presented in Table 1 are all highly supporting the GWAS result obtained in the study.

## Conclusion

These results revealed that several chromosomal regions are involved in genetic control of androstenone level. Differences between breeds were clearly shown, although 7 QTL regions for androstenone in fat were detected in both Norwegian Landrace and Duroc. More precise mapping of QTL positions were obtained due to the highly improved density of the marker map. In all regions, suggestive or significant QTL for estron sulphate were also detected, something that might be problematic in genetic selection against boar taint.

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