Fine Mapping Of Quantitative Trait Loci For Androstenone And Skatole Levels In Pig

G. Le Mignon[†], N. Iannuccelli[‡], A. Robic[‡], Y. Billon[§], J.P. Bidanel[†] and C.Larzul[†]

Introduction

Male pigs are currently castrated to prevent boar taint, an off-flavor mainly due to accumulation in fat tissues of skatole (produced in the colon from tryptophan) and/or androstenone (steroid pheromone synthesized in the testis). In Europe, some countries have already or would like to ban surgical castration for male pig without anesthesia. One of the alternatives is to raise entire males, considering that boar taint occurrence has to be reduced. Previous studies have demonstrated that selection against androstenone content in backfat was efficient (Robic *et al.* (2008)). Androstenone and skatole contents showed both moderate to high values for heritability, with a positive genetic correlation between the two traits (Robic *et al.* (2008) for review). However, both for practical reasons and a suspected unfavorable genetic relationship with reproduction traits, especially sexual maturity (Fouilloux *et al.* (1997)), such a selection has not been implemented in pigs (Zamaratskaia and Squires (2009)). Therefore, many studies have attempted to detect quantitative trait loci (QTL) affecting these traits, so that the QTL information could be utilized through marker-assisted selection (MAS). In the last ten years, several studies detected QTL by linkage analysis (LA) for androstenone or skatole content. Only few candidate genes have been fully studied. The most promising was a gene located on chromosome 14 (*CYP2E1*) for which polymorphism was associated with skatole content (Zamaratskaia and Squires (2009)). SNP micro-arrays now give the opportunity to genotype animals for thousand SNPs. The aim of this study was to precisely locate QTL for androstenone and skatole in a French Large White pig population.

Material and methods

Animal and measures. On the INRA experimental farm, 98 Large White sows were inseminated with 56 Large White boars, chosen as unrelated as possible. Each boar inseminated one or two sows. Male piglets were kept entire. A total of 580 male piglets were raised in pens till they reached 110 kg of live body weight and slaughtered in a commercial slaughterhouse. A backfat sample was taken the day of slaughter, frozen and kept at -20°C for androstenone and skatole measurements. Skatole was measured by HPLC, and

[†] : INRA, UMR1313, GABI, F- 78352 Jouy-en-Josas , France
[‡] : INRA, UR444, LGC, F-31326 Castanet-Tolosan, France
[§] : INRA, UE967, GEPA, F-17700 Surgères, France

androstenone, by GC-MS following Sole and Garcia Regueiro (2001). A total of 480 and 373 animals were measured for androstenone and skatole, respectively.

DNA extraction and marker genotyping. For AI boars, DNA was extracted from blood sample. For piglets, DNA was extracted from the tail, cut shortly after birth. Both sires and piglets were genotyped using the Illumina 64K SNP array.

Statistical analyses. Androstenone and skatole contents were corrected using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) taking into account slaughtering day as fixed effect and animal slaughter weight as covariate. SNPs were excluded for the following reasons: genotype call rate below 90%, 12 or more Mendelian errors, minor allele frequency (MAF) below 0.05. A total of 36188 SNPs passed quality criteria on the 19 chromosomes.

LDLA analysis. The QTL fine-mapping strategy was mostly based on the use of statistical methods combining linkage (LA) and linkage disequilibrium analysis (LDLA) describes by Druet *et al.* (2008). The model applied in this study is fully described by Tarres *et al.* (2009) with IBD10 acronym.
For linkage analysis, we have constructed the linkage map by considering the same markers order than physical map. Genetic distance between SNP have been extrapolated on physical distance (1Mb= 1cM).
In this study, windows of 6 markers (3 markers at the left and 3 markers at the right of the putative position) were considered to compute the IBD probabilities. IBD probability matrices were calculated at the midpoint of each bracket of flanking markers. Founder haplotypes were grouped into distinct clusters. Base haplotypes, i.e two haplotypes with IBD probabilities greater than 0.5, were considered to carry the same QTL allele and were grouped into the same cluster (Ytournel, personal communication).
Hypothesis tests for the presence of QTL were based on the asymptotic distribution of the likelihood ratio test (LRT) statistic, LRT=-2ln(Lnull-Lqtl), where Lnull and Lqtl were the maximized likelihoods under the null-model and the qtl-model respectively.

Results and discussion

Seven and eight significant QTL were found for androstenone and skatole, respectively, at the p<0.01 genome-wide level (table 1). The most significant QTL with a large effect were located on SSC1, 13 and SSC1, 8, 9 and 14 for androstenone and skatole, respectively.
These results were the first evidence of QTL for fat androstenone and skatole contents segregating in the French Large White population. A major gene affected androstenone level has been previously described by Fouilloux *et al.* (1997) in the French Large White population, and they ruled out the hypothesis that it was linked to SLA, on chromosome 7. The "low allele" had a dominant effect. In the present study, no QTL could be qualified as major gene, the largest effect being around 30% of the genetic variance. Previous QTL located by genome scan studies were first found in Meishan x Large White crossbreeding designs. QTL for androstenone level measured at slaughter were located on chromosome 2, 4, 7, 9, 13, 14. Additional QTL for androstenone measured from 100 day to 160 day of age were located on chromosome 3 and 9. In an outbred Landrace population, Varona *et al.* (2005) found no evidence on QTL for androstenone or skatole level, but they limited their

study to locus (covering from 10 to 40 cM) previously detected for growth or fatness traits in Meishan and Large White breeds (Quintanilla *et al.* (2003), Lee *et al.* (2004)).

In a F2 Hampshire x Landrace design, Markljung *et al.* (2008) reported only one QTL for androstenone level on chromosome 11. The discrepancy between studies was expected considering the different genotypes and design. In purebred populations, the only result from genome scan currently available was presented by Karacaören *et al.* (2009), reporting a unique QTL for skatole level in a Danish Landrace population on chromosome 14, at the exact location of gene CYP2E1. These results suggest together that QTL regions detected for boat taint could be very specific of the population studied.

Table 1: Androstenone and skatole QTL detected on the different chromosomes with LDLA approach.

Trait	Chromosome	Selected position (Mb)[a]	Block size (kb)	LRT[b]	Markers	% genetic variance[c]
Androstenone	1	20.83 (20.76-20.95)	19	11.33*	H3GA0001085/H3GA0001084/MARC0007613/ ALGA0001565/MARC0018943/ALGA0122320	12.51
		176.81 (176.75-176.94)	19	12.11*	INRA0005213/H3GA0003324/ALGA0006940/ ALGA0006943/ALGA0006948/ALGA0006952	33.08
		242.64 (242.60-242.81)	21	11.48*	ALGA0008410/ALGA0008413/H3GA0003852/ ALGA0008419/H3GA0003854/H3GA0003856	17.02
	9	66.87 (66.82-67.05)	23	10.84*	ALGA0053793/ALGA0105293/ALGA0117453/ MARC0085147/MARC0019765/ASGA0043753	5.79
	11	37.15 (37.12-37.77)	65	11.19*	ALGA0061932/DRGA0011136/MARC0058247/ DRGA0011138/DRGA0011139/ALGA0113843	14.9
	13	103.24 (103.15-103.35)	20	10.79*	MARC0053131/MARC0082257/MARC0045417/ H3GA0037397/ASGA0058988/H3GA0037401	17.46
		129.5 (129.45-129.71)	26	11.29*	DRGA0013255/ALGA0073099/ALGA0073101/ MARC0014344/ASGA0059475/INRA0041395	19.73
Skatole	1	109.5 (109.39-109.69)	30	10.05*	ASGA0089931/H3GA0002001/ASGA0004129/ DRGA0001438/MARC0008029/ALGA0005375	25.1
		288.75 (288.66-288.87)	21	13.8**	ALGA0010989/ASGA0008234/M1GA0002042/ ASGA0008247/ASGA0008250/H3GA0005387	29.78
	3	31.07 (31.05-31.26)	21	10.08*	ALGA0018524/ALGA0018526/MARC0009965/ MARC0064428/H3GA0009291/MARC0053230	23.14
	7	12.85 (12.75-13.04)	29	10.99*	ALGA0038731/ASGA0031322/ALGA0038747/ MARC0049053/ASGA0031337/ALGA0038752	14.9
	8	41.32 (41.20-41.60)	40	16.48**	ASGA0038797/ALGA0047848/ASGA0098917/ MARC0093074/H3GA0024861/H3GA0024862	28.21
	9	81.29 (81.25-81.43)	18	10.53*	ASGA0043958/INRA0032151/ASGA0043959/ MARC0073290/ALGA0054144/MARC0046912	40.69
	13	28.86 (28.79-29.01)	22	10.31*	ALGA0069514/ASGA0057195/ASGA0057192/ MARC0114673/ASGA0057206/MARC0012193	22.32
	14	139.45 (139.35-139.53)	18	13.95**	MARC0094389/M1GA0019394/M1GA0019399/ ASGA0067392/MARC0060367/ALGA0082666	49.87

[a]Position in Mb of the most significant QTL. In brackets are indicated the position of the haplotype of 6 markers.
[b]LRT statistics of trait with a significant QTL-effect: ns means not significant, *<0.01 and **<0.001 at chromosome-wide level.
[c]Percentage of genetic variance explained by the QTL at the selected position.

Conclusion

The aim of this analysis is now to select some SNP in LD with QTL for androstenone and/or skatole level so they can be used for routine marker assisted selection (MAS).

References

Druet T., Fritz S., Boussaha M. *et al.* (2008). *Genetics*, 178:2227-2235.

Fouilloux M.N., Le roy P., Gruand J. *et al.* (1997). *Genet. Sel. Evol.*, 29:357-366.

Karacaören B., de Koning D.J., Velander I. *et al.* (2009). In *proc. 60th EEAP*, Barcelone, Spain.

Lee, G.J., Archibald A., Law A.S. *et al.* (2004). *Anim. Genet*., 36:14-22.

Markljung E., Braunschweig M.H., Karlskov-Mortensen P. *et al.* (2008). *BMC Genetics*, 9:22.

Quintanilla , R., Demeure, O., Bidanel, J.P. *et al.* (2003). *J. Anim. Sci.,* 81:385-394.

Robic A., Larzul C. and Bonneau M. (2009). *Genet. Sel. Evol.,* 40:129-143.

Sole M. A. and Garcia Regueiro, J.A. (2001). *J. Agric. Food Chem*., 11:5303-5309.

Tarres J., Guillaume F. and Fritz S (2009). *BMC Proceedings*, 3:S3.

Varona L., Vidal O., Quintanilla R. *et al.* (2005). *J. Anim. Sci*., 83:301-307.

Zamaratskaia G. and Squires E.J. (2009). *Animal*, 3:1508-1521.