Comparison Of Two Pig Pedigrees Regarding The Effect Of The IGF2 Mutation On Backfat.

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Introduction

Backfat thickness (BFT) is an economically important trait for which various QTL have been detected. Two analyses of similar Meishan x European F2 pedigrees carried out in France and in The Netherlands gave different results concerning the position and the imprinting status of a QTL detected in the telomeric part of SSC2. In 2000, de Koning et al. described a paternally expressed QTL at 36 cM on SSC2 whereas in 2002, Milan et al. described a QTL at 0 cM on SSC2, for which no imprinting effect was detected (Quintanilla et al., 2002). When the *IGF2*-intron3-G3072A mutation was described (Van Laere et al., 2003), Jungerius et al. (2004) showed that the paternally expressed QTL described by de Koning et al. was essentially explained by this mutation. On the contrary, Sanchez et al. (2006) showed that the *IGF2*-intron3-G3072A was not solely responsible for the growth and fatness QTL on SSC2. The aim of the present study was to combine these 2 pedigrees and understand the differences in QTL positions and imprinting status.

Material and methods

Animals and phenotypic data. For the French pedigree, 6 F1 were sires mated to 23 F1 dams. Six large half-sib families (61 to 133 F2 pigs per sire) were obtained. BFT was measured on the carcasses of 521 male F2 pigs. For the Dutch pedigree, only the 24 largest half-sib families were considered. The 24 sires were mated to 181 F1 dams. On average, each F1 sire produced 36 F2 pigs. BFT was measured on the carcasses of 565 F2 pigs. BFT was corrected for relevant fixed effects and covariates in each pedigreees prior to QTL detection.

Genotyping and QTL detection. Animals from both pedigrees were genotyped for a set of 10 microsatellites markers. The IGF2 3072 G>A mutation was genotyped on the F1 animals; the genotypes of F2 at the IGF2 mutation were inferred, when possible, based on their genotypes at the surrounding markers. The two pedigrees were jointly analysed using interval mapping with the QTLMAP software (Le Roy et al., 1998). A substitution effect was estimated for each F1 sire at the maximum of the test statistics. The confidence interval QTL

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position was empirically determined by the "Lod drop-off" method (Lander and Kruglyak, 1995). Additional QTL mapping analyses were performed after dividing the families into 2 sub-groups based on the genotype of the F1 males at the IGF2 mutation (A/G or G/G).

Analyses of variance. Analyses of variance were also carried out on F2 pigs to evaluate the effects of paternal and maternal IGF2 alleles on BFT. Different sub-groups of F2 pigs were also considered to check potential effects of maternal IGF2 alleles when heterozygous females were crossed with either homozygous and/or heterozygous males.

Simulation studies.
Simulation studies were done to check whether the observed differences concerning the imprinting status of the IGF2 gene could be due to the existence of a second QTL. First, the effect of the IGF2 mutation was estimated on the real dataset by setting the paternally inherited allele as a fixed effect in the QTL analysis of the combined pedigree. Then, assuming paternal expression of the IGF2 gene only, a QTL segregating at 44 cM was simulated in each pedigree. The q allele of the simulated QTL was fixed in the Meishan lines. Four frequencies of the Q allele of the simulated QTL were tested (100%, 75%, 50% and 25%, in the second grand-parental line) as well as 3 different effects (0.22, 0.32 or 0.42 s.d. unit). Two thousand simulations were run for each pedigree x frequency x effect situation. Additionally, 2000 simulations were performed for the combined pedigree assuming a frequency of 50% of the Q allele in both grand-parental lines and an effect of 0.32 s.d. unit. For each simulation, the effect of the maternal allele inherited at IGF2 on BFT was estimated with an analysis of variance.

Results and discussion

The QTL analysis of the combined pedigree gave a significant result for a QTL underlying BFT on SSC2, with a more likely position at 1 cM. The lod drop-off confidence interval spans from 0 to 47 cM, where previously published QTL had their most likely positions.

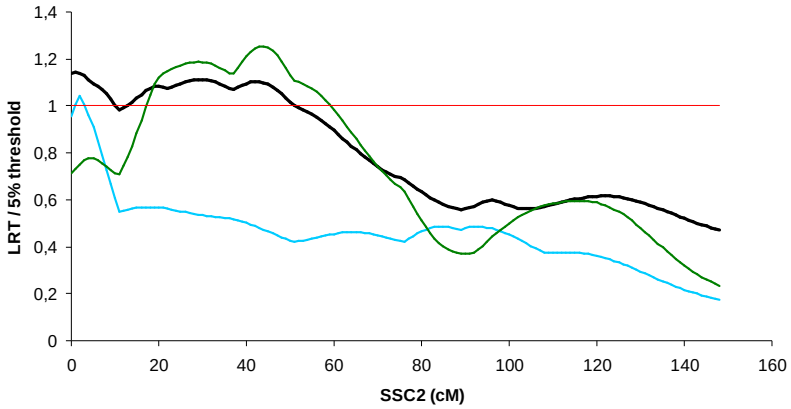

Figure 1: QTL mapping with the combined pedigree (black line), or with only G/G F1 males (green line) or with only A/G males (blue line) for BFT on SSC2 (cM). The LRT is presented in proportion to the 5% threshold on the chromosome.

The combined pedigree was then divided in 2 groups depending of the genotypes of the F1 males at the IGF2 mutation. Fourteen F1 males were A/G (with 434 F2 progeny) and the 16 remaining sires were G/G (with 652 F2 progeny). By analyzing these 2 groups, we demonstrated that the large significant region was in fact due to 2 different QTL.
We confirmed that IGF2 is the first QTL segregating around 1 cM. The effect of the IGF2 mutation was estimated at 0.48 s.d. unit in the combined pedigree. The second QTL was detected at 44 cM while analyzing the G/G F1 males families and is therefore not due to the effect of the IGF2 mutation (Figure 1). This second QTL had an effect of of 0.32 s.d. unit on BFT. Eleven of the 16 G/G F1 males were found to be heterozygous (Q/q).

Analyses of variance were performed to evaluate the effect of the maternal allele inherited at the IGF2 mutation. Within the G/G F1 males group, a significant effect of the allele inherited from the dam at the IGF2 mutation was observed (p-value = 0.0134) in the sub-group of F2 pigs having A/G F1 mothers. This significant effect is not in accordance with a paternal expression of the IGF2 gene.

Simulation studies were done to check whether the significant maternal effect at the IGF2 locus could be due to the existence of the second QTL. A QTL was simulated at 44 cM and assuming an effect of the paternal allele inherited at the IGF2 gene of 0.48 s.d. unit. We observed highly different results between the French and Dutch pedigrees whatever the considered frequency and effect of the QTL allele (Table 1). A significant effect of the IGF2 allele inherited from the mother was found in at least 32 % of the simulations run with the French pedigree whereas it was encountered in less than 10% of the simulations run with the Dutch pedigree. For the estimated frequency (75%) and effect (0.32 s.d. unit) of the second locus, a significant effect of the maternal IGF2 allele was observed in more than 50% of the simulations performed with the French pedigree, whereas it was observed in less than 4% of the simulations carried out with the Dutch pedigree.

Frequency of the simulated Q allele

Effect of the simulated Q allele	**0.25**	**0.50**	**0.75**	**1.00**
0.22	36.95% 6.45%	35.20% 4.30%	33.35% 3.75%	32.25% 4.60%
0.32	57.95% 8.05%	56.15% 4.55%	53.35% 3.45%	51.70% 5.4%
0.42	75.20% 9.75%	72.75% 4.90%	70.45% 3.25%	68.75% 5.70%

Table 1: Percentages of simulations leading to the detection of a significant effect of the allele inherited from the mother at the IGF2 mutation on BFT in the French and Dutch pedigrees. The effect was considered significant when p-value < 0.05.

When analyzing the French pedigree it is therefore highly likely to detect a false effect of the IGF2 maternal allele.
For the combined pedigree, the QTL was simulated with a Q allele frequency of 50% in each grand-parental line and an effect of 0.32 s.d. unit. A significant effect of the IGF2 allele transmitted by the F1 mothers appeared in 6.6% of the simulations.

Conclusion

These new analyses give some explanations for the conflicting results obtained from separate analyses of French and Dutch Meishan x European F2 pedigrees. The different QTL positions can be explained by the segregation of two genetically linked QTL, one of them being IGF2 gene. Concerning the imprinting status of the first QTL, a maternal effect of the IGF2 gene was hypothesized in the French pedigree where no imprinting effect was detected. Our simulation studies showed that the risk to detect a false significant effect of the maternal IGF2 allele in this pedigree is too high to support this hypothesis. Therefore, the statistical significant effect we observed is probably due to genetic linkage existing between the IGF2 gene and the second detected QTL. Finally, it is interesting to notice that the risk to detect a false significant effect of the maternal IGF2 allele is higher in the French pedigree than in the Dutch pedigree. This difference can be explained by the 2 pedigrees' structures: many little half-sib families versus a few large half-sib families.

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