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QTL mapping for teat number in an Iberian by Meishan pig intercross

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Abstract

The aim of this study was to investigate chromosomal regions affecting the number of teats in pigs. An experimental F_2 cross between Iberian and Chinese Meishan lines was used for this purpose. These two breeds proceed from independent domestication processes and present great differences in teat number. A genomic scan was conducted with 117 markers covering the 18 porcine autosomes. Linkage analyses were performed by interval mapping, using the animal model to estimate QTL and additive polygenic effects. Complementary analyses with models fitting two QTL were also carried out. The results showed three genomewide significant QTL, mapping on chromosomes 5, 10 and 12, whose joint action control up to 30% of the phenotypic variance of the trait. Meishan alleles had a positive additive effect on teat number and a positive additive x additive epistatic interaction was detected between QTL on chromosomes 10 and 12. Two positional candidate genes have been identified for QTL on chromosomes 5 and 10, and their molecular analysis could improve the knowledge of the genetic architecture of teat number

Introduction

Actually breeding schemes use dam lines with high reproductive performance that have been developed by intense selection of Western-type breeds or by introgression of genes from hyperprolific Chinese breeds. In these lines litter size exceeds in many cases the number of functional nipples of the sow. A better understanding of the genetic control of this trait would offer the opportunity to improve selective breeding programs.

Several genome scans for quantitative trait loci (QTL) have identified a number of porcine chromosomal regions associated with the number of teats (Rohrer, 2000; Wada *et al*. 2000; Hirooka *et al*. 2001, Holl *et al*. 2004). The present study is a genome scan for QTL affecting teat number in pigs, based on an experimental F_2 intercross between Iberian and Meishan breeds. Great differences in teat number between the two breed of pigs have been reported, and there is previous evidence of epistatic effects for this trait. Both parental lines differ substantially for the trait, with means of 17.33 and 10.05 teats for the Meishan breed (Desprès *et al*. 1992) and the Guadyerbas strain of Iberian pigs (Toro *et al*. 1986), respectively.

Material and Methods

A three-generation intercross was generated by mating three Iberian boars, from the genetically isolated Guadyerbas strain (Toro *et al*. 2000), with 18 Meishan sows (Domaine du Magneraud, INRA, France). Animals with records on the number of morphological teats by sex and generation are summarized in Table1.

Purebred grandparents, F_1 reproducers and 272 F_2 females were genotyped for 115 microsatellites and two RFLP markers. Markers provided a coverage of the 18 autosomes being 17.4 cM the average marker interval (sex-averaged map distance).

Mixed model techniques, which allow to accommodate polygenic effects, have been used for performing this QTL study. If ignored, the polygenic variance might be considered as a part of the residual variance of the model, thus reducing the power of QTL detection (Nagamine & Haley, 2001). We used Qxpak software (Pérez-Enciso & Misztal, 2004), which implements these features and computes the required identity by descent probabilities using all animals in the pedigree simultaneously. Alternative QTL alleles fixed in each parental lines were assumed.

Two single-QTL Mendelian models (AD and A) were used. Model AD fitted the mean, sex, polygenic random effect, additive (*a*) and dominant (*d*) QTL fixed effects and residual random effect. The dominant QTL effect was excluded in the additive model A. In addition to single QTL analyses, more complex two-QTL analyses were performed. A model fitting additive effects of two QTL located at different positions of the same chromosome was compared with a single-QTL model in some relevant cases. A bi-dimensional scan was also carried out, fitting effects of two QTL at 1 cM intervals for two locations on different selected chromosomes. This model included the additive effects of both QTL but it did not allow for interaction between them.

Likelihood ratio (LR) tests were calculated, comparing the appropriate reduced and full models, to assess the significance of QTL effects. Nominal P values can be calculated assuming a chi-square distribution of the LR test. Significance thresholds were calculated using the procedure described by Nezer *et al*. (2002). This approach yields genomewise critical values of LR tests with 2 d.f. of 24.32, 19.69, 16.44 and 15.00, associated with type I errors α = 0.001, 0.01, 0.05 and 0.10, respectively. The corresponding critical values of LR tests with 1 d.f. are 20.80, 16.34, 13.27 and 11.93.

Additional LR tests were performed fitting models to test interaction between pairs of QTL mapping in different chromosomes (Varona *et al*. 2002). We compared the likelihood of the data under the hypothesis of epistasis between loci with that under H_0 (at the most likely QTL position on each chromosome detected by bi-dimensional scan).

Results and Discussion

The whole-genome scan using single-QTL models showed statistical evidence for QTL affecting teat number on pig chromosomes 5, 10 and 12. The likelihood obtained under the alternative hypothesis (models AD or A) was compared with the likelihood obtained under the null hypothesis (no-QTL model). The estimates of the QTL locations, their additive (*a*) and dominant (*d*) effects, LR test values and their levels of significance are presented in Table 2. The additive effect is expressed as half the difference between the homozygous Meishan and Iberian genotypes, and the dominant effect as the difference between the heterozygous and the average of both homozygous genotypes. Meishan alleles had a positive additive effect on teat number for QTL on SSC5, SSC10 and SSC12. Dominant effects were not significant, and the removal of the dominance term (model A) improved the statistical significance of the LR test.

Chromosome 5. A graphical representation of results for this chromosome is shown in Figure 1. The maximum LR test value on SSC5 (LR = 18.81) was located at position 29 cM, in the interval between microsatellites *SJ024* and *SWR453*. The additive fraction of the phenotypic variance explained by this QTL was 11.5 %. Previously, only Lee *et al*. (2003) reported the existence of a suggestive QTL for teat number in a Wild Boar x Pietrain intercross which mapped to a similar position on SSC5 (near marker *SWR453*).

Chromosome 10. The most probable position for a QTL on SSC10 was located around 72 cM, in the interval between markers *SW1991* and *SW1626*, being 14.6% the fraction of the phenotypic variance explained by this QTL. In the same chromosomal region significant QTL with effects on teat number had been consistently detected (Rohrer, 2000; Hirooka *et al*. 2001

and Dragos-Wendrich *et al*. 2003). The profile of LR test values obtained from single-QTL analysis along this chromosome reveals two local maxima (Figure 2). However an additional QTL was not significant in the test of the two-QTL model versus the single-QTL model.

Chromosome 12. The profile of LR test values along this chromosome presented a multimodal picture with two local maxima of similar magnitude at positions 67 and 96 cM (Figure 3). The most likely position was located at 67 cM, in the interval between markers *SW874 and SW1956*. The additive fraction of the phenotypic variance explained by this QTL was 5.9%. Significant QTL with effects on teat number have been detected in chromosomal regions close to the other local maximum found in our study (Hirooka *et al*. 2001; Yue *et al*. 2003). But the hypothesis of two-QTL for teat number on SSC12 was rejected in our study.

In order to account for possible variation caused by different chromosomes, bi-dimensional analyses were carried out for pairs of chromosomes in which QTL had been detected in this study. Results concerning to pairs SSC5/SSC10 and SSC5/SSC12 did not modify substantially those obtained from the single-QTL analyses (Table 3). However, when SSC10 and SSC12 were simultaneously analyzed the estimated position of QTL on SSC12 was 87 cM, delimited by microsatellites *SW1956* and *S0106* and it is partially coincident with the one reported by Hirooka *et al*. (2001).

Non-allelic interactions between loci are not usually fitted in models for QTL detection in livestock populations. In this experiment, possible interactions between pairs of QTL were tested. When the full model fitted the four possible interaction effects between QTL mapping on SSC10 and SSC12, the LR test value exceeded the 5% level of significance of a chi-square distribution with 4 degrees of freedom: LR= 10.00 ($P < 0.040$), and the hypothesis of no interactions can be rejected. The four estimated interaction effects were: $I_{axa} = 0.53 \pm 0.18$, $I_{axd} = 0.24 \pm 0.30$, $I_{dxa} = 0.10 \pm 0.28$, and $I_{dxd} = 0.14 \pm 0.14$. A more significant result was obtained when the alternative model only fitted the I_{axa} effect: LR= 8.14 ($P < 0.004$), being $I_{axa} = 0.51 \pm 0.18$. The interaction of Meishan alleles of these two QTL increases the number of teats. No evidence of epistatic interactions was obtained between the other two pairs of QTL identified in the bi-dimensional scan. The usefulness of the detected QTL in selective breeding must be evaluated by further studies to determine possible epistatic effects or undesirable correlated effects on other productive traits.

As QTL regions have become clearly identified, the analysis of positional candidate genes might elucidate mutations causing QTL effects (Ciobanu *et al*. 2001). From the homology

between porcine chromosome 5 and human chromosome 12, the *WNT inhibitory factor 1* gene (*WIF1*) could be proposed as positional candidate to explain the QTL on SSC5. The Wingless Type (WNT) proteins are a family of extracellular signalling molecules involved in the embryonic development of many organisms, with particular reference to mouse mammary gland (MacMahon, 1992). Recently, Nonneman and Rohrer (2004) have constructed a comparative map of chromosome human 10p and porcine 10q. They identified 17 loci from human chromosome that map to SSC10q, where several economically important QTL have been reported, including the above quoted QTL for teat number. The *Enhancer of Polycomb 1* gene (*EPC1*) maps close to marker *SW1991* and might be a strong positional candidate gene to explain the effect of SSC10 QTL on teat number.

The analysis of these two identified positional candidate genes could open new perspectives to the knowledge of the genetic architecture of teat number.

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Table 1 Number of pigs (N) with teat number records by sex and generation. Mean, standard deviation (SD), minimum and maximum number of teats.

Generation	Sex	N	Mean	SD	Minimum	Maximum
F1	Males	15	14.00	1.36	11	16
	Females	112	13.49	1.00	12	16
F2	Males	489	13.55	1.38	10	18
	Females	512	13.55	1.24	10	17

Table 2 Significant results of single QTL analyses for teat number using animal models fitting additive (*a*) and dominant (*d*) QTL effects (Model AD) or additive QTL effects (Model A)

Chromosome	Model	LR	Genomewise significant level	Position cM	*a* (s.e)	*d* (s.e.)
5	AD	19.13	P < 0.050	29	0.62 (0.14)	0.11 (0.19)
	A	18.81	P < 0.010	29	0.63 (0.14)	-
10	AD	34.61	P < 0.001	71	0.69 (0.12)	0.08 (0.16)
	A	34.39	P < 0.001	72	0.71 (0.12)	-
12	AD	15.53	P < 0.100	70	0.46 (0.12)	0.18 (0.16)
	A	14.44	P < 0.050	67	0.45 (0.12)	-

LR, likelihood ratio test values

Table 3 Results of bi-dimensional QTL scans using animal models fitting additive effects (*a*) of QTL located on different chromosomes

Pairs of analysed chromosomes	QTL on	LR	Genomewise significant level	Position cM	*a* (s.e)
5,10	SSC5	16.01	P < 0.050	33	0.55 (0.14)
	SSC10	29.10	P < 0.001	70	0.63 (0.12)
5,12	SSC5	18.54	P < 0.010	32	0.60 (0.14)
	SSC12	14.85	P <0.050	69	0.46 (0.12)
10,12	SSC10	34.18	P < 0.001	71	0.70 (0.12)
	SSC12	15.56	P < 0.050	87	0.45 (0.11)

LR, likelihood ratio test values for each one of the QTL additive effects

Figure 1. Likelihood-ratio test profiles across SSC5 for single QTL additive model.

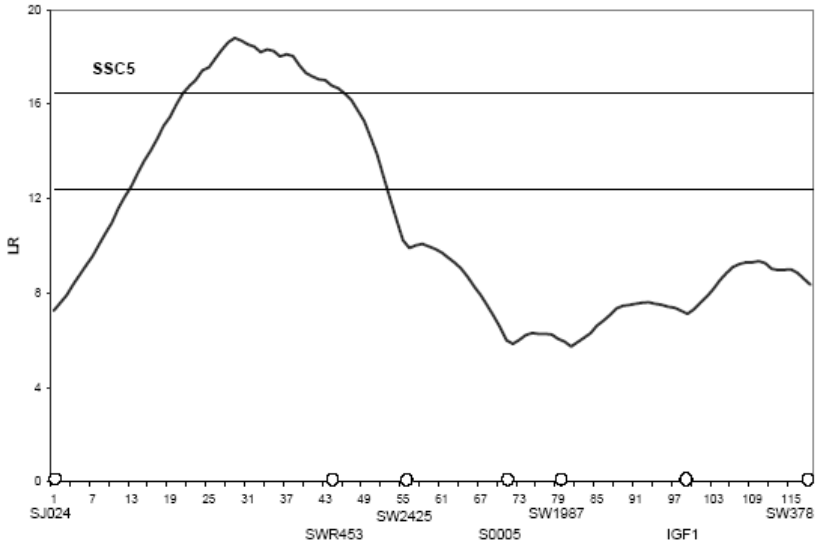


Figure 2. Likelihood-ratio test profiles across SSC10 for single QTL additive model.

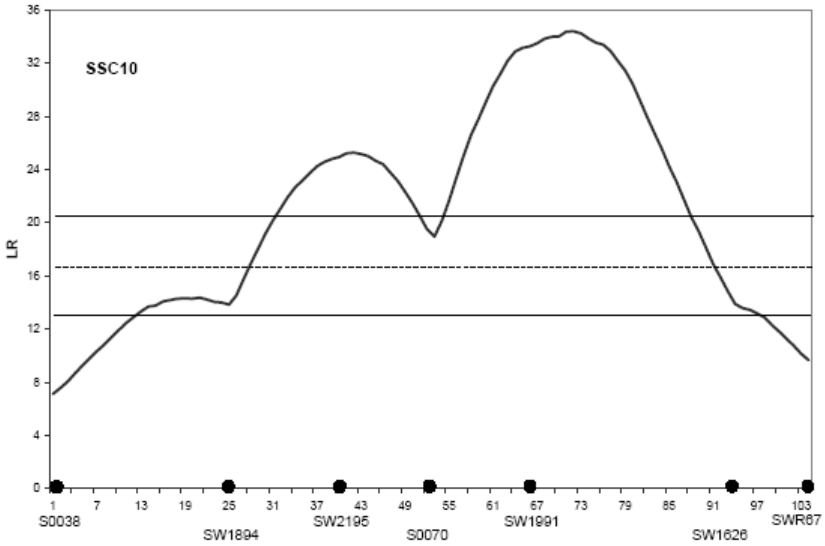
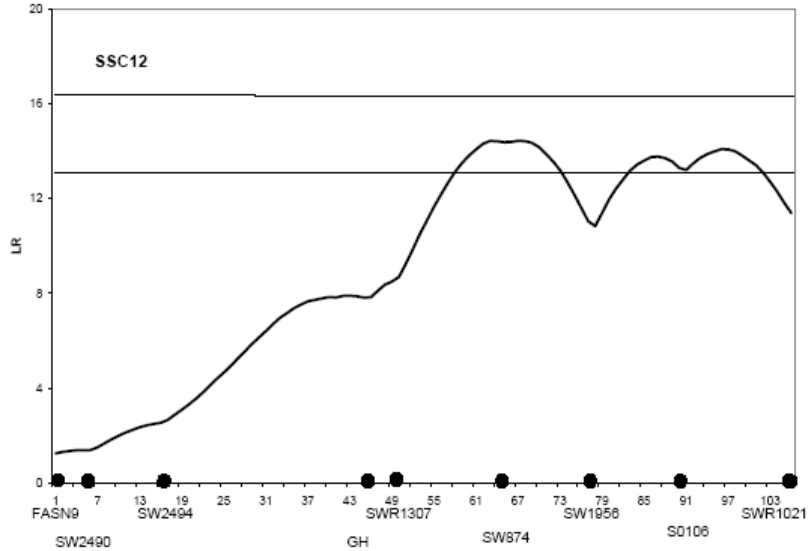


Figure 3. Likelihood-ratio test profiles across SSC12 for single QTL additive model.



Critical values of LR tests with 1 d.f. are 20.80, 16.34, 13.27 and 11.93