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The X Chromosome harbors quantitative trait loci for backfat thickness and intramuscular fat content in pigs

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Genetic markers have been used in experimental crosses in pigs to dissect genetic variation in quantitative traits (e.g., Andersson et al. 1994; Knott et al. 1998; Rohrer and Keele 1998). We reported recently on the search for autosomal quantitative trait loci (QTL) for fatness traits in an experimental intercross between the obese Chinese Meishan breed and lean Dutch White production lines (De Koning et al. 1999; Rattink et al. 2000). In this paper, the analysis to look for QTL on the X Chromosome (Chr) in this cross is presented.

Briefly, 19 Meishan boars were mated to 120 sows of White lines from five Dutch breeding companies. From the F_1 animals, 39 F_1 boars and 124 F_1 sows were randomly selected to generate the F_2 generation. The F_2 animals (n = 1293), their F_1 parents, and the Meishan grandfathers were genotyped for five microsatellite markers located in the non-pseudoautosomal region of the X Chr. The percentage of fat within the muscle (intramuscular fat, IMF) and backfat thickness (BFT) of the *Musculus longissimus* was recorded on 785 F_2 animals after slaughter at a live weight of approximately 90 kg (for details see Janss et al. 1997). For QTL analysis, interval mapping by regression was carried out following a line cross analysis as described previously (De Koning et al. 1999) where the founder lines were assumed to be fixed for different QTL alleles. The model was extended to account for differences between sexes at the X Chr. The contrast between the Meishan and the White allele for the estimation of the QTL effect was calculated within male and female F_2 offspring separately because of the design of the cross. Male F_2 offspring carry only a maternal copy of the X Chr originating from the Meishan or from the White grandparents, whereas all female offspring inherited in addition the paternal copy of the X Chr from the White lines. Furthermore, the model was accommodated to take into account that the X Chr cannot recombine in male F_1 parents.

The female genetic map calculated from our data (Fig. 1) shows the same marker order but differences in distances between markers compared with the map of Rohrer et al. (1996). An increase in length of roughly 10 cM (Kosambi) is observed between markers *SW2534* and *SW2456* (37.8 cM versus 27.6 cM) as well as between *SW2476* and *SW1943* (21.5 cM versus 9.8 cM). However, a smaller distance is observed on our map in the interval between *SW2456* and *SW2476* (9.4 cM versus 19.8 cM). Genotypes were evaluated independently by two different persons, and sensitivity analysis did not point towards specific families causing these differences (data not shown). The number of informative meioses in this study ranges from 1551 (*SW2476*) to 1854 (*SW2534*). As the mapping population of Rohrer et al. (1996) consisted of 94 F_2 animals and only female parents contribute mapping information, their estimates of recombination frequencies are expected to be inaccurate. This is the likely explanation for the observed differences.

Figure 1 shows the test statistics of the QTL analysis for IMF and BFT and the threshold levels along the genetic map of the X Chr. For both fatness traits, a genomewide significant ($p < 5\%$) QTL is detected. The estimated position of the QTL is different between the two traits, but they are in adjacent marker intervals at 60 cM for BFT and 69 cM for IMF (Table 1). From these data we cannot conclude whether there are two separate loci or the same QTL affects both traits. As expected, the alleles from the obese Meishan breed increase the percentage of intramuscular fat as well as backfat thickness. The estimated size of the QTL effects is smaller in females (1.02 mm BFT, 0.13% IMF) than in males (1.47 mm BFT, 0.21% IMF) for both traits (Table 1). This might be caused by random inactivation of the X Chr in females. For every chromosomal interval, the effect in females is estimated as the contrast between animals that inherited two X Chr intervals derived from the White lines and those that are expected to be heterozygous for the interval, with one copy from Meishan and another copy from the White lines. Random X inactivation is known to occur quite early in development, and therefore larger parts of a tissue might originate from the same precursor cell and have the same X chromosomal inactivation imprint (Migeon 1994). Therefore, some females are expected to have inactivated the Meishan-derived X Chr in the relevant tissues and express the White-derived X Chr. On the other hand, these differences in effects between the sexes could reflect any other biological mechanism of sexually dimorphic gene expression, which can also be caused by interaction with autosomal genes (e.g., Davey et al. 1999). The observed effect on BFT is comparable in size to the effect of the halothane mutation at the porcine ryanodine receptor gene (Fujii et al. 1991) in a Wild boar x Large White cross (Knott et al. 1998). They estimated that the mutation, which is not present in our families, reduces back fat by 1.63 mm in heterozygous males.

A QTL affecting backfat thickness in pigs at a similar location on the X Chr has been reported by Rohrer and Keele (1998). In a Meishan x White composite backcross, a significant QTL is detected for different back fat measurements. The additive effects reported across sexes range from 0.292 to 0.419 cm increase of BFT for the Meishan allele, depending on the area of the carcass where back fat is measured. These estimates are much larger than in our study. This might be partly owing to a higher slaughter weight (100 kg) in the study of Rohrer and Keele (1998) because back fat accumulates exponentially with age. Furthermore, in the current study male animals were boars, whereas Rohrer and Keele (1998) investigated castrates. It is known that castrates accumulate more fat, and consequently QTL effects might be more pronounced. Also, the locations sampled along the spine differ be-

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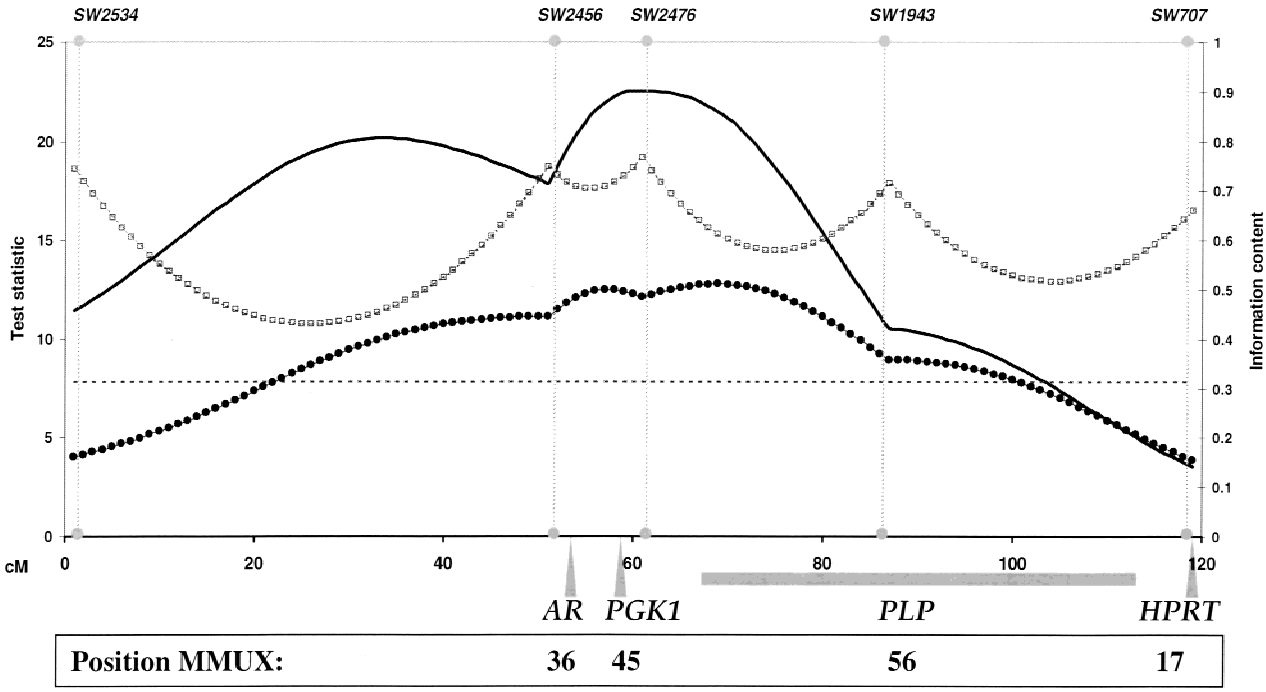

Fig. 1. Test statistic profile for Chr X. BFT is indicated as a solid line, IMF as a circled line. The 5% genomewise significant threshold determined by permutation is shown as a dotted line, information content as a squared line. Position of genes (androgen receptor, AR; phosphoglycerate kinase 1, PGK1; proteolipid protein, *PLP;* hypoxanthine phosphoribosyltransferase, *HPRT*) on the porcine and murine genetic map (http://www.informatics.jax.org) are indicated below. Porcine *PLP* gene according to the cytogenetic map position.

Table 1. QTL analysis for backfat thickness and intramuscular fat content on chromosome X. Test statistics, QTL position. and estimated QTL effects for sexes separately.

Marker bracket	Test statistic[a]	Position (cM)	QTL effect	
			Male	Female
Backfat thickness (mm)			(n = 483)	(n = 307)
SW2456/SW2476	22.56***	60	1.47 ± 0.249	1.02 ± 0.321
Intramuscular fat content (%)			(n = 479)	(n = 296)
SW2476/SW1943	12.79***	69	0.21 ± 0.046	0.13 ± 0.060

[a]*** $p < 0.0001$ (test statistic not exceeded in 10,000 permutations according to Churchill and Doerge 1994).

tween the studies. In summary, although the traits measured for backfat thickness are not identical between studies, it is very likely that the same QTL was identified in both experiments, whereas in a Wild boar x Large White cross, reported by Knott et al. (1998), the X chromosomal QTL effect on BFT only approached the suggestive threshold. This QTL maps around 25 cM distal to the marker *SW707* and is likely different from those detected in this study.

Total genome scans investigating QTLs for intramuscular fat on the X Chr in pigs have not been reported so far.

In mice, a QTL for adiposity and the weights of individual fat depots in males has been detected on the X Chr (York et al. 1997). The most likely QTL position is located close to the marker *DXMit174* (position 58) near the proteolipid protein (*PLP*) gene. Recently, another X-linked QTL has been described by Taylor et al. (1999) influencing obesity only in males (*Obq6*, most likely position 23.5). The comparative map of the X Chr indicates rearrangements in gene order (Fig. 1) that are also visible on the human-mouse comparative map (Chen et al. 1999). The androgen receptor (AR; Seifert et al. 1999), the phosphoglycerate kinase 1 (*PGK1;* Rohrer 1999), and the *PLP* gene (Baumgartner et al. 1999) map in the same order on the murine X Chr to positions 36 (*Ar*), 45 (*Pgk1*), and 56 (*Plp*). However, the hypoxanthine phosphoribosyltransferase (*HPRT*) gene maps at 0 cM from SW707 (Hu et al. 1997) but is located at position 17 on *MMUX*. Because of the low resolution of the comparative map and the lack of precision of QTL positions in all three studies, we cannot conclude whether the effects observed by York et al. (1997) and Taylor et al. (1999) may be located in an interval homologous to our study.

In human, the Wilson Turner Syndrome, a mental disease associated with obesity, has been mapped to HSA Xp21.1-q22 (Wilson et al. 1991), a region partially homologous to the QTL area in this study (Hu et al. 1997; Chen et al. 1999). However, the genes underlying these loci associated with obesity in mouse and human remain unknown.

In conclusion, a region on the porcine X Chr has been detected harboring loci that considerably affect backfat thickness and intramuscular fat content in both sexes. These findings provide evidence of major genes for obesity and carcass composition in pigs showing nonmendelian inheritance and expression.

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