

***Melanocortin-4 receptor (MC4R)* genotypes have no major effect on fatness in a Large White × Wild Boar intercross**

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Summary

The *melanocortin-4 receptor (MC4R)*, a G-protein coupled receptor, is implicated in mediating the effect of leptin on food intake and energy balance. A previous candidate gene study reported an association between an *MC4R* missense mutation (Asp298Asn) and fatness, growth and feed intake in pigs. To assess this association further, we analysed the segregation of this missense mutation in relation to variation in fatness traits using a Wild Boar × Large White intercross. The Wild Boar and Large White founders were homozygous for different *MC4R* alleles. The *MC4R* was assigned to the expected region on pig chromosome 1. The statistical evaluation did not reveal any indication of a significant effect on fatness related traits in this pedigree.

Keywords fatness, *MC4R*, pigs, quantitative trait loci.

After the positional cloning of the obesity (*ob*) gene encoding leptin (Zhang *et al.* 1994), considerable efforts have been made to decode the complex neural pathway that co-ordinate metabolic effects downstream of leptin. The *melanocortin-4 receptor (MC4R)*, a G protein-coupled receptor, is one important component of this pathway. The *MC4R* signalling is important for mediating the effect of leptin on food intake and energy homeostasis (Seeley *et al.* 1997). A targeted disruption of *MC4R* was found to cause large phenotypic effects on obesity related traits in mice (Huszar *et al.* 1997). Similarly, loss-of-function mutations cause a dominant form of obesity in humans (Vaisse *et al.* 1998; Yeo *et al.* 1998). Kim *et al.* (2000) used a candidate gene approach and reported that a missense mutation (Asp298Asn) in *MC4R* is associated with fatness, growth and feed intake traits in pigs. The present study was performed to further evaluate this possible association using our intercross between the European Wild Boar and Large White domestic pigs that previously have been used to identify major quantitative trait loci (QTLs) affecting fatness and growth (Andersson *et al.* 1994; Jeon *et al.* 1999).

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Melanocortin-4 receptor was genotyped across the pedigree using a *TaqI* polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) (Kim *et al.* 2000). The two European Wild Boar males were homozygous for allele 1 (Asp298), whereas the eight Large White founder sows were homozygous for allele 2 (Asn298). The latter allele was reported to be associated with increased backfat thickness, feed intake and growth (Kim *et al.* 2000). Allele frequencies in the population of 190 F_2 pigs were 0.48 (allele 1) and 0.52 (allele 2) and the genotype distribution did not deviate significantly from the expected 1 : 2 : 1 ratio ($\chi^2 = 2.15$, d.f. = 2, $P > 0.05$). The data were used to assign *MC4R* to pig chromosome 1 on the Nordic porcine genetic map (Marklund *et al.* 1996) using CRI-MAP (Green *et al.* 1990). Two point analysis revealed highly significant lod scores between *MC4R* and several markers on this chromosome (e.g. *CGA*, $\theta = 0.17$, LOD = 14.5; *GPII*, $\theta = 0.14$, LOD = 12.0; *S0082*, $\theta = 0.05$, LOD = 52.3; *S0155*, $\theta = 0.12$, LOD = 13.4). A multi-point linkage analysis assigned *MC4R* to the interval between *GPII* and *S0082* with great confidence (lod score support > 3; Fig. 1).

A single marker linear model was used for analysing the relationship between *MC4R* and fatness traits in our resource pedigree. In addition to the effect of the *MC4R* genotype, the model included the effects of sex, full-sib family and feed treatment within batch. Weight at slaughter was used as a covariate. Furthermore, a previously described QTL on pig chromosome 4 with a major effect on

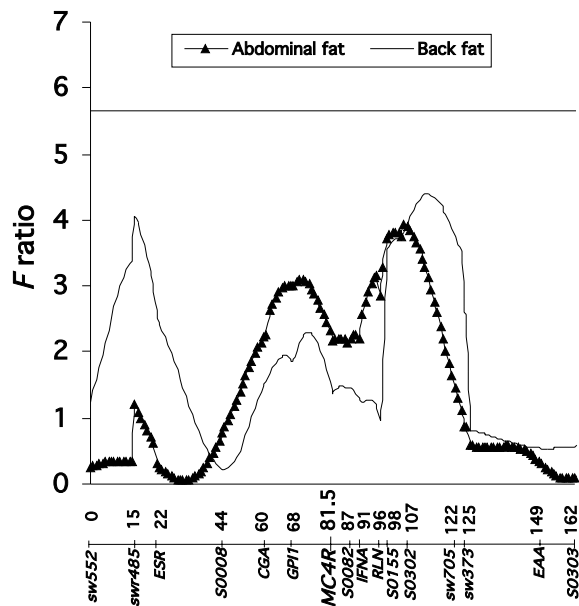


Figure 1 Test statistic curves for backfat thickness and abdominal fat content on pig chromosome 1. The marker map with the distances between markers in Kosambi cM is given on the x-axis. The y-axis represents the *F*-ratio testing the hypothesis of a single quantitative trait locus (QTL) at a given position on the chromosome. The horizontal line represents the 5% chromosome-wide significance threshold established by a permutation test.

fatness (Andersson *et al.* 1994) was included as a cofactor to decrease the residual error variance and thereby increasing the statistical power in this study. An analysis of variance was carried out using the GLM procedure of SAS (version 6.12, 1997). Interval mapping as described by Haley *et al.* (1994) was also employed.

Single marker analysis revealed no significant effects of *MC4R* on the total phenotypic variance of average backfat thickness ($P = 0.42$) or abdominal fat content ($P = 0.11$). Furthermore, the test statistic obtained using interval mapping was far from significant at the position of *MC4R* (Fig. 1). The interval mapping gave some indication for two

QTL located on each side of *MC4R* but the results did not even reach the 5% chromosome-wide significance threshold (Fig. 1, Table 1). Furthermore, the QTL analysis did not reveal any significant effects of *MC4R* on growth traits (data not shown).

A comparison of the estimated effects associated with different *MC4R* genotypes showed (Table 2) that the non-significant trend in this study was in the opposite direction compared with the data reported by Kim *et al.* (2000). A similar non-significant trend in the opposite direction was also reported for a rather small sample from a Meishan/Large White synthetic line (Kim *et al.* 2000). There are several possible explanations for this discrepancy. Firstly, the previously reported association may not be a direct effect of the Asp298Asn mutation but rather the result of linkage disequilibrium. However, the fact that this non-conservative substitution occurs at a residue that is conserved among all melanocortin receptors reported so far suggests that it may very well be functionally important. Secondly, the effect of the mutation may be too small to be detected in our pedigree. We carried out a power calculation for QTL analysis (Lynch & Walsh 1997) to assess this possibility. Based on the results reported by Kim *et al.* (2000) we assumed that *MC4R* acted as a codominant QTL and that the additive effect on backfat thickness was 0.45 mm. This indicated that *MC4R* was expected to only explain about 1% of the residual variance in our F_2 generation and that the power to detect this effect was only about 30%. Thus, we cannot exclude the possibility that we failed to detect an effect of this locus because of the lack of power. Thirdly, a QTL analysis tests for a chromosome substitution effect and two QTLs in the repulsive phase may cancel out each other (Zeng 1993). This possibility gains some support by the observation of weak indications for the presence of QTLs on each side of *MC4R* (Fig. 1; Table 1). The limited size of this pedigree prohibited a careful evaluation of this possibility.

The results of this study did not provide additional support for an association between the *MC4R* Asp298Asn

Trait ¹	<i>n</i>	<i>F</i> -ratio	Map-position ²	Additive effect ³	Dominance effect ³	Percentage of F_2 variance ⁴
BF (mm)	190	4.4	113	0.49 ± 0.33	2.09 ± 0.65	5.3
AF (%)	190	3.9	105	-0.08 ± 0.05	0.26 ± 0.09	4.8

¹ BF, average back-fat; AF, percentage of abdominal fat in relation to carcass weight.

² The map position is the one giving the highest *F*-ratio on that chromosome estimated in cM from the proximal end.

³ Estimates are given as mean ± SE. The additive and dominance effects were estimated as the deviation of animals homozygous for the Wild Boar allele or heterozygous, respectively, from the mean of the two homozygotes.

⁴ The reduction in the residual variance in the F_2 population obtained when including a QTL at the given position.

Table 1. Quantitative trait loci (QTL) analysis by least-squares analysis for pig chromosome 1 in a Wild Boar × Large White intercross.

Table 2 Estimated effects (mean \pm SE) of MC4R genotypes on fatness traits in the pig.

Trait and study	Genotype			
	1/1	1/2	2/2	n
<i>Backfat thickness (mm)</i>				
This study	26.1 \pm 0.6	26.5 \pm 0.4	25.7 \pm 0.5	190
Kim <i>et al.</i> (2000)	11.1 \pm 0.2	11.6 \pm 0.2	12.0 \pm 0.2	1,120
<i>Abdominal fat (%)</i>				
This study	2.15 \pm 0.09	2.34 \pm 0.06	2.38 \pm 0.08	190

mutation and fatness traits as previously reported by Kim *et al.* (2000) but this negative result may simply reflect a lack of power in the present study. We estimated the 95% confidence interval (according to Sokal & Rohlf 1981) for the difference between the MC4R 1/1 and 2/2 genotypes at -1.11 to $+2.01$ mm which thus included the estimate of -0.9 mm reported by Kim *et al.* (2000). Furthermore, an F_2 intercross is not ideal for detecting minor effects of candidate genes. This is partly because the effect of a candidate locus fixed for different alleles may be completely confounded with the effect of linked QTLs with large effects. Furthermore, the segregation at multiple QTL, some with major effects, gives a high phenotypic variance that reduces the power to detect minor loci. We tried to reduce the latter problem in this study by including the major QTL for fatness on pig chromosome 4 as a cofactor. In conclusion, further genetic and functional studies are required to evaluate the significance of the MC4R Asp298Asn substitution.

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