Effect of the missense mutation Asp298Asn in *MC4R* on growth and fatness traits in commercial pig crosses in the Czech Republic

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Abstract: The current knowledge of factors regulating voluntary feed intake in pigs is quite limited. The objective of this study was to test the influence of the missense mutation p.Asp298Asn (AF087937:c.746G>A) of the *MC4R* gene on selected production traits in pig crosses. These crosses are commonly used on commercial farms in the Czech Republic. The allele frequencies of c.746G>A were as follows: *G* allele – 0.59 and *A* allele – 0.41. We detected statistically significant differences in the content of intramuscular fat in the *musculus longissimus lumborum et thoracis,* and a similar trend was observed in shoulder and neck. *A* allele correlated with higher values of fatness and *G* allele with a higher percentage of lean meat. However, we did not find any significant influence on either feed intake or growth rate in this study. For another mutation, p.Arg236His (NM_214173.1:c.707G>A), frequencies of alleles were disproportional (*A* allele – 0.02 and *G* allele – 0.98), only two genotypes were observed (*AG* and *GG*) and linkage disequilibrium was not detected. Therefore, we assume that the effect of this polymorphism on growth rate and fatness in the Czech population of pigs is negligible.

Keywords: *MC4R*; growth; fatness; pig

The control of feeding involves a complex network of central neuronal pathways and peripheral physiological feedback mechanisms (Matteri, 2001). Moreover, the current knowledge of factors influencing feed intake in humans and domestic animals is still very limited (factor of environment, social factor, influence of a disease, factor of stress or genetic background), and much of what is known is based on studies in rodents (Barb et al., 2004). It was suggested that the melanocortin system may play an important role in energy homeostasis (Cone, 1999). The receptors for melanocortin-3 and melanocortin-4 are localized within the brain and influence feed intake in rodents (Barb et al., 2004).

Kim et al. (2000b) identified the missense mutation AF087937: c.746G>A in a highly conservative region of *MC4R* which was associated with growth and feed intake in pigs. They noted that the variant of the candidate gene may explain the significant variation in backfat thickness, growth rate, and feed intake in commercial pig crosses. Kim et al. (2004a) performed a functional analysis of the MC4R protein and found differences in cAMP production between the 298Asp and 298Asn variants. The porcine gene melanocortin-4 receptor (*MC4R*) was mapped on chromosome 1q22–q27 (Kim et al., 2000a).

The association of the c.746G>A missense mutation with fatness and growth rate has been confirmed in several populations of pigs with different genetic backgrounds (Kim et al., 2000b; Houston et al., 2004; Jokubka et al., 2006; Óvilo et al., 2006; Van den Maagdenberg et al., 2007; Fan et al., 2009).

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However, some studies did not detect any significant effects of this mutation on production traits (Park et al., 2002; Stachowiak et al., 2005). These varying results were probably caused by differences in genetic background. In this respect Fan et al. (2009) reported that the alleles of the *MC4R* gene had an effect on growth rate and fatness, but these effects of these variants were dependent on the single-nucleotide polymorphism (SNP). Despite these differences, this mutation can be a promising tool for improving daily gain and feed intake in pigs.

The objective of this study was to test the effect of the missense mutation p.Asp298Asn and p.Arg236His in the *MC4R* gene on selected carcass traits in pig crosses in the Czech Republic.

MATERIAL AND METHODS

Animals

The present analyses were based on phenotypic data collected from 483 pigs. This population included seven commercial crosses and one pure breed (Table 1). They were raised and finished at the test station in Ploskov in the Czech Republic. The pigs were fed a commercial diet (wheat, barley, soybean meal, and a premix of supplements of essential elements) ad libitum (22 animals of group 1, all animals of groups 2 to 5) and dosed feed (47 animals of group 1 and all animals of group 6) (Table 1). At the start the complete feed mixtures contained: lysine – 12.2 g/kg, ME – 12.9 MJ/kg, and at the end: lysine: 8.7 g/kg, ME – 12.9 MJ/kg of feed. The groups with dosed feeding had a limited energy intake of 37.4 MJ ME/day since the weight of 90 kg.

Performance traits

The average slaughter weight of pigs was 113 kg (SD ± 10.5). The average daily gain in the test was calculated as the ratio of live weight gained from the beginning to the end of the test to days from the beginning of the test to slaughter. Feed conversion was calculated as the total feed intake divided by the overall number of the total weight gain in the test. Lean meat and muscle depth were measured by the Fat-O-Meter (FOM) method in the *musculus longissimus lumborum et thoracis* (MLLT) and by the two-point method (ZP) in the *musculus gluteus* (MG). The carcass dissection was performed using the method of Walstra and Merkus (1995). Belly 4 is a part of the whole belly after dissection of belly 1, 2 and 3 (Stupka et al., 2004). The muscle depth and area of MLLT were measured by the LUCIA (Laboratory Imaging) programme.

Intramuscular fat content (IMF) in selected samples of the MLLT, shoulder, leg and neck was determined by the Soxhlet method, which uses a gravimetric determination in accordance with the Czech Standard ISO 1443.

Genotyping

Animals were genotyped for SNPs AF087937: c.746G>A (Asp298Asn) and NM_214173.1:c.707G>A (Arg236His) in the MC4R gene according to Kim et al. (2000b) and Meidtner et al. (2006), respectively.

Table 1. Crossbred populations of pigs

Group	Pig crosses	*n*
1	PN × (CZL × CZLW)	69
2	CZLW; CZLW × CZL; Pn × (CZL × CZLW)	70 (23 + 24 + 23)
3	(CZLW × PN) × (CZL × CZLW); PIC × (CZL × CZLW)	71 (35 + 36)
4	FH × PIC	63
5	(D × CZLW) × (CZL × CZLW)	72
6	(PN × H) × (CZL × CZLW)	72
7	PN × (CZL × CZLW)	66

CZLW = Czech Large White; CZL = Czech Landrace; PN = Pietrain; D = Duroc; H = Hampshire; FH = French crosses; PIC = Pig Improvement Company

Association analysis

The association analysis between MC4R genotypes and phenotypic value (PV) was carried out with different numbers of pigs in the groups. (The PVs of carcass traits were measured in a random sample of the population, and the PVs of fattening performance were measured in the entire population, while some extreme values were excluded.)

The effect of the missense mutation p.Asp298Asn of the *MC4R* gene on quantitative and qualitative traits was analysed using the UNIVARIATE, MEANS, GLM (type IV) procedures. The model included the *MC4R* genotype, crossbred combination, sex and type of diet as fixed factors and carcass weight as a regression coefficient (the regression coefficient was not used on average daily gain). The following formula was used:

$$Y_{ijkm} = \mu + a_i + b_j + c_k + \beta x_m + e_{ijkm}$$

where:

Y_{ijkm} = value of the trait
μ = overall mean
a_i = effect of *MC4R* genotype (i = 1, 2, 3)
b_j = combined effect of crossbred combination and diet (j = 1, 2, 3, 4, 5, 6, 7, 8, 9)
c_k = effect of sex (k = 1, 2)
β = regression coefficient on carcass weight
x_m = carcass weight of animal m
e_{ijkm} = random residual

All data were analysed using the SAS statistical programme version 9.1 (SAS, 2001).

RESULTS AND DISCUSSION

The allele frequencies of the missense mutation p.Asp298Asn (c.746G>A) of the *MC4R* gene were 0.59 for 298Asp (or *G* allele) and 0.41 for 298Asn (or *A* allele). The range in individual groups was 0.22 to 0.66 for *A* allele and 0.34 to 0.78 for *G* allele (Table 2).

The present study clearly demonstrated that the porcine *MC4R* missense mutation is significantly associated with intramuscular fat (IMF) in pigs. In this respect, statistically significant differences ($P < 0.0032$) in IMF content in MLLT were found out between the genotypes of the *MC4R* gene. This trend was observed in IMF of the other parts of carcass (Table 3). Stachowiak et al. (2005) detected significant differences in IMF values in Polish Large White, while *A* allele was the most favourable. However, in the same study the authors detected an opposite trend, i.e. *G* allele correlated with higher values of IMF in Polish Landrace. Van den Maagdenberg et al. (2007) reported that the *GG* genotype had a lower IMF content compared with the *AA* genotype in 1155 pigs ($P < 0.05$). These findings were confirmed by our study (Table 3).

With regard to feed conversion, it is clear that there are no conclusive differences associated with the missense mutation Asp298Asn of the *MC4R* gene, although Kim et al. (2000b) showed a significant difference of 0.17 kg between genotypes *AA* and *GG*. Likewise, Houston et al. (2004) demonstrated that the genotypes significantly affected feed conversion and growth rate. However, Hernández-Sánchez et al. (2003) did not confirm this effect, which is in agreement with our results (Table 3).

Table 2. The allele frequencies of p.Asp298Asn and p.Arg236His in the *MC4R* gene

Group	*n*	Asp298Asn		*n*	Arg236His	
		A	*G*		*A*	*G*
2	70	0.39	0.71	54	0.03	0.97
3	71	0.45	0.55	30	0	1
4	63	0.66	0.34	nt		
5	72	0.51	0.49	20	0.02	0.98
6	72	0.44	0.56	67	0.04	0.96
7	66	0.30	0.70	31	0	1
Total	483	0.41	0.59	202	0.02	0.98

nt = not tested

Table 3. The effect of *MC4R* genotypes on several production traits

Traits	*AA* ± SD (*n*)	*AG* ± SD (*n*)	*GG* ± SD (*n*)	*P*-value
Average daily gain (g/day)	907 ± 104.94 (84)	907 ± 107.15 (213)	892 ± 99.20 (117)	0.90
Feed intake (kg/1 kg ADG)	2.82 ± 0.20 (75)	2.85 ± 0.25 (189)	2.87 ± 0.26 (149)	0.80
Lean meat (ZP; %)	57.43 ± 3.89 (88)	57.79 ± 4.45 (216)	58.64 ± 4.27 (172)	0.51
Belly 4 (kg)	$3.47 \pm 0.40^{A,a}$ (45)	3.36 ± 0.49^{a} (139)	$3.24 \pm 0.50^{B,b}$ (95)	**0.05**
Muscle depth of MLLT (mm^2)	67.20 ± 7.32 (46)	68.18 ± 7.16 (138)	70.16 ± 7.52 (94)	0.08
Area of MLLT (mm)	4707 ± 662.04 (46)	4775 ± 647.02 (138)	4986 ± 644.99 (94)	0.11
Intramuscular fat in MLLT (%)	$2.17 \pm 0.56^{A,a}$ (24)	1.89 ± 0.59^{a} (67)	$1.59 \pm 0.51^{B,b}$ (57)	**0.0032**
Intramuscular fat in shoulder (%)	2.70 ± 0.81 (29)	2.57 ± 0.82 (86)	2.13 ± 0.63 (66)	0.0939
Intramuscular fat in ham (%)	3.99 ± 1.86 (29)	4.33 ± 1.89 (48)	2.93 ± 1.16 (38)	0.11
Intramuscular fat in neck (%)	4.36 ± 2.01 (30)	4.17 ± 1.46 (77)	3.90 ±1.71 (58)	0.38

MLLT = *m. longissimus lumborum et thoracis;* SD = standard deviation; ADG = average daily gain
^{A,B}differences among selected traits ($P \leq 0.001$); ^{a,b}differences among selected traits ($P \leq 0.05$)

This study showed that *G* allele was correlated with higher values of lean meat (FOM and ZP methods), muscle depth of MG (ZP method), muscle depth of MLLT ($P < 0.08$) and the area of MLLT ($P < 0.11$) (Table 3; some data not shown). Moreover, *G* allele was correlated with the higher weight of ham, MLLT, shoulder and neck (results not shown).

The highest values of belly 1, 2, 3, 4 and the highest weight of the belly as a whole were detected for genotype *AA* (Table 3; some data are not shown). Although this study did not detect a significant influence on any traits of fatness, in agreement with Park et al. (2002), Meidtner et al. (2006) and Van den Maagdenberg et al. (2007) it can be assumed that the *A* allele corresponds with increased fatness. This assumption was demonstrated by several studies which indicated a significant influence of *A* allele on backfat thickness (Kim et al., 2000b; Hernández-Sánches et al., 2003; Houston et al., 2004; Fan et al., 2009).

As is also evident from the results, this study revealed no significant effect of the *MC4R* missense mutation on growth rate (Table 3). Moreover, we performed separately the association analysis between p.Asp298Asn polymorphisms and growth rate in the crossbred combination PN × (CZL × CZLW) (158 animals) and we did not find any effect on average daily gain (data not shown). Similar conclusions were drawn by Park et al. (2002) and Stachowiak et al. (2005), whereas Fan et al. (2009) reported that the alleles of the *MC4R* gene had an effect on growth rate and fatness, but the effects of these variants were dependent on the SNP. The authors reported that there was probably an interaction between polymorphisms Arg236His and Asp298Asn of the *MC4R* gene. In this context, Fan et al. (2009) indicated that the frequency of Arg236His polymorphism may have an impact on the effect of the *MC4R* gene mutation Asp298Asn. However, only two genotypes of Arg236His polymorphism were found out in this study (*AG* and *GG*) and very low frequencies of *A* allele were detected (Table 2). The two polymorphisms were not in linkage disequilibrium in our populations. Because of low informativeness this polymorphism was not included in the association analysis.

Several authors have reported a similar association of the Asp298Asn polymorphism of the *MC4R* gene with fatness and growth traits in pigs with different backgrounds (Kim et al., 2000b; Houston et al., 2004; Jokubka, 2006; Óvilo et al., 2006; Van den Maagdenberg et al., 2007; Fan et al., 2009). The authors also noted that the mutation c.746G>A of the *MC4R* gene is considered useful for marker-assisted selection.

CONCLUSION

This study showed the impact of the missense mutation c.746G>A of the *MC4R* gene on IMF in the MLLT ($P < 0.0037$), with A allele being the most favourable. We did not find an influence of the p.Asp298Asn mutation on growth rate and feed conversion.

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