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EFFECTS OF MELANOCORTIN-4 RECEPTOR GENE ON GROWTH AND MEAT TRAITS IN PIGS RAISED IN RUSSIA

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ABSTRACT

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 polymorphism (Asp298Asn) and growth and meat productive traits of pigs. The aim of this work was to determine frequencies of the MC4R/Taq I genotypes and alleles in Danish Landrace (LD), Canadian Landrace (LC) and crossbred pigs Danish Landrace × Canadian Landrace (LD × LC) in breeding Cent "Plemzavod Jubilee" (Tyumen) Russia and to estimate their associations with some growth and meat traits. The polymorphism was identified by PCR-RFLP method. The traits studied were: Number of days to 100-kg, Average Daily Gain (ADG), Backfat Thickness (BFT), average daily Feed Intake (FI) and Length of Body (LB). In the all test groups we found a higher frequency of G allele (0.71) compared with the A allele (0.29). The results of present study suggest that the MC4R gene contributes to Days to 100 kg, ADG and BF. The heterozygous genotype AG was favorable in LD breed. The significant effects of-1.43 (LD male) and -2.81(LD female) for Days to 100 kg and of+61.17 (LD male) and +26.3 (LD female) for ADG were calculated. The homozygous genotype GG was favorable in crossbred LD × LC. The significant effects of +74.2 for ADG and-1.5 for BF were calculated. Therefore, the data support a role for the MC4R Asp298Asn polymorphism in the genetic basis of economically important traits in pigs of Russia. Further research is needed to determine the effect of this gene on growth, meat and reproduction traits in pigs of different breeds and crosses.

Keywords: Gene, MC4R, Pigs, Growth, Meat Productive Traits

1. INTRODUCTION

Genetic studies in animals have contributed to the identification of the main genetic causes of obesity (Tenesa *et al*., 2009; Hinney *et al*., 2010; Hebebrand *et al*., 2013; Kalogeropoulos, 2013; Khan *et al*., 2014). Particular interest among the candidates of signaling molecules involved in the regulation of energy homeostasis is Melanocortin-4 Receptor (MC4R). MC4R is responsible for the leptin signal between food intake and body weight (Benoit *et al*., 2000; Loos *et al*., 2008; Wan *et al*., 2012; Kesmanee, 2013; Lisyova *et al*., 2014). Molecular studies in the departments of the paraventricular nucleus of the hypothalamus revealed gene expression of MC4R, which encodes a second type of neuronal melanocortin receptors (Gantz *et al*., 1993). These studies led to the assumption that the participation of the melanocortin-4 receptor in the regulation of hypothalamo-pituitary axis (Mountjoy *et al*., 1994).

The porcine gene Melanocortin-4 Receptor (MC4R) was mapped on chromosome 1q22-q27. Kim *et al*.

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(2000) identified the missense mutation p.Asp298Asn (AF087937: C.746G A) in a highly conservative region of MC4R which was associated with feed intake and carcass fatness traits in pigs. The effect of MC4R polymorphism Asp298Asn on feed intake, growth rate and meat traits has been confirmed in several populations of pigs with different genetic backgrounds (Kim *et al*., 2000; Houston *et al*., 2004; Jokubka *et al*., 2006; Ovilo *et al*., 2006; Piorkowska *et al*., 2010; Switonski *et al*., 2010). However, some studies did not detect any significant effects of this gene polymorphism on production traits (Park *et al*., 2002; Maagdenberg *et al*., 2007). Perhaps these results are related to genetic features associated with the breed of the animals.

The aim of our work was to determine frequencies of the MC4R/Taq I genotypes and alleles in Danish Landrace, Canadian Landrace and crossbred pigs Danish Landrace x Canadian Landrace from Russia and to estimate their associations with some growth and meat traits.

2. MATERIALS AND METHODS

2.1. Animals

A total of 398 pigs were included in the analysis. There were 186 Danish Landrace pigs (LD) (66 meal (♂) and 120 female (♀)); 80 Canadian Landrace (LC) (56 meal (♂) and 24 female (♀)) and 132 female crossbred pigs Danish Landrace × Canadian Landrace (LD × LC). All the pigs were from breeding center Yubileiny, Tyumen, Russia. All individuals were reared in the same conditions and fed with standard feed.

2.2. Studied Traits

The animals were recorded for the following traits: The number of days to 100-kg (days to 100 kg), Average Daily Gain (ADG), Feed Intake (FI) and Backfat Thickness (BFT). All traits were obtained according to the results of growing up to 100 kg.

2.3. Genotyping

DNA was taken from samples of animal tissue (hair) of animals. Genotyping was performed by PCR-RFLP method described by Kim *et al*. (2000) with subsequent primers:

FOR: 5′-TAC CCT GAC CAT CTT GAT TG-3′
5′-ATA GCA ACA GAT GAT CTC TTT G-3′

After amplification, the PCR product was digested with Taq1.

2.4. Statistical Analysis

Frequencies of the MC4R genotypes were calculated as a genotype percentage in the population. Analysis of the gene effect to observed traits were analyzed using a linear model. The model was:

$$Yijkl = \mu + Gi + Sexj + MC4Rk + eijkl$$

Where:
Yijkl = The phenotypic record
μ = The general mean
Gi = The effect of genetic group of sow (i = LD, LC, LD×LC)
Sexj = The effect of sex (j = F, M)
MC4Rk = The effect of MC4R genetype (k = AA, AG, GG)
Eijkl = The random error

3. RESULTS

We tested 186 pigs of Danish Landrace, 80 pigs of Canadian Landrace and 132 crossbred pigs (Danish Landrace × Canadian Landrace) for the Taq1 polymorphism in the MC4R gene by PCR-RFLP. The frequencies of alleles and genetic structure in the MC4R gene are shown in **Table 1**. Lower frequencies of the AA genotype were found in tested pig. We did not find any AA genotype in the group female LC. In the all test groups we found a higher frequency of G allele (0.71) compared with the A allele (0.29).

Effects of the MC4R genotypes on Days to 100 kg, ADG, feed intake, backfat thickness and length of body are presented in **Table 2**.

Table 1. The genetic structure and frequencies of alleles at polymorphism within MC4R gene in pigs of Danish Landrace, Canadian Landrace and crossbred LD×LC

	Genotypes (%)			Allele	
Breed	AA	AG	GG	A	G
♂LD (n = 66)	6.2	49.2	44.6	0.31	0.70
♀LD (n = 120)	10.8	41.7	47.5	0.32	0.69
♂LC (n = 56)	8.9	41.1	50.0	0.30	0.71
♀LC (n = 24)	0.0	50.0	50.0	0.25	0.75
LD×LC (n = 132)	6.3	40.6	53.1	0.26	0.74
N = 398	6.4	44.5	49.1	0.29	0.71

Table 2. Effect of the MC4R genotypes (least square means ± standard error) on the number of days to 100-kg (days to 100 kg), Average Daily Gain (ADG), Feed Intake (FI), backfat thickness (BF) and length of body (LB) in LD, LC and LD×LC pigs

	Days of 100 kg (days)		
MC4R	AA	AG	GG
Danish Landrace (male)			
SM	144.14	142.94*	144.59
SE	1.55	0.78	0.75
Danish Landrace (female)			
SM	163.23	161.30***	165.00
SE	0.62	1.11	1.00
Canadian Landrace (male)			
SM	141.32	141.40	141.30
SE	0.53	0.90	0.81
Canadian Landrace (female)			
SM		164.30	160.21
SE		2.18	2.19
Danish Landrace x Canadian Landrace (female)			
SM		163.40	159.30
SE		2.28	3.25
ADG (g)			
Danish Landrace (male)			
SM	1067.57	1140.16**	1090.41
SE	14.89	18.74	16.15
Danish Landrace (female)			
SM	860.69	865.44**	817.51
SE	14.09	12.30	12.41
Canadian Landrace (male)			
SM	1112.88	1097.41	1114.91
SE	11.98	17.31	15.91
Canadian Landrace (female)			
SM		874.91	844.21
SE		24.06	28.99
Danish Landrace x Canadian Landrace (female)			
SM		768.40	842.60*
SE		20.70	21.50
FI (kg)			
Danish Landrace (male)			
SM	2.57	2.60	2.62
SE	0.06	0.05	0.05
Canadian Landrace (male)			
SM	2.55	2.58	2.50
SE	0.03	0.04	0.04
BF (mm)			
Danish Landrace (male)			
SM	10.29	10.81	10.69
SE	0.61	0.47	0.40
Danish Landrace (female)			
SM	11.54	11.10	11.00
SE	0.85	0.41	0.30
Canadian Landrace (male)			
SM	10.55	11.00	9.75*
SE	0.27	0.40	0.28
Canadian Landrace (female)			
SM		9.25	9.08
SE		0.64	0.54
Danish Landrace x Canadian Landrace (female)			
SM		14.30	12.80*
SE		0.54	0.52
LB (cm)			
Danish Landrace (male)			
SM	127.86	127.06	126.00
SE	1.53	0.76	0.57
Danish Landrace (female)			
SM	126.00	125.50	125.60
SE	1.17	0.70	0.60
Canadian Landrace (male)			
SM	125.29	125.40	125.30
SE	0.44	0.70	0.70
Canadian Landrace (female)			
SM		126.90	126.60
SE		1.28	1.11
Danish Landrace x Canadian Landrace (female)			
SM		124.80	124.30
SE		0.74	0.58

Our results indicate that the MC4R gene contributes to Days to 100 kg, ADG and BF. The heterozygous genotype AG was favorable in LD breed. The significant effects of -1.43 (LD male) and -2.81 (LD female) for Days to 100 kg and of +61.17 (LD male) and +26.3 (LD female) for ADG were calculated.

The significant effects of the MC4R genotypes in LC breed were observed only for male LC. The difference of 0.8±0.38 ($p \leq 0.05$) for BF between genotypes AA and GG of LC male was calculated. We also found that the ADG and FI better in genotype GG (LC male) compared with genotypes AA and AG, but these differences were not significant. Effects of the GG genotypes on Days to 100 kg, feed intake and backfat thickness were observed for female LC but they were not significant. The homozygous genotype GG was favorable in crossbred LD×LC. The significant effects of +74.2 for ADG and -1.5 for BF were calculated.

No significant effects of the MC4R genotypes on length body were observed for all breeds.

4. DISCUSSION

The identification of genes or genetic markers associated with growth, fat deposition and feed intake in pigs could have a great economic impact on pork production. Many polymorphic genes have been analyzed up to date including those for key hormones and their receptors (Hongyu *et al*., 2010; Yu *et al*., 2013; Oczkowicz *et al*., 2013; Huang and Wang, 2013; Ghaly and Al-Sowayan, 2014; Nanuwong and Bodhisuwan, 2014). The Melanocortin-4 Receptor (MC4R) is one of the most important signaling molecules involved in the conduct of the leptin signal and regulates energy homeostasis. Polymorphism of the MC4R gene leads to replacement of the amino acid sequence of the melanocortin-4 receptor. If the missense mutation p.Asp298Asn formed asparagine (Asn), this leads to blocking the leptin signal. Kim *et al*. (2000) were the first to publish information of the MC4R Asp298Asn polymorphism A/G and found association with growth, fat deposition and feed intake in pigs.

Further was demonstrated by several studies a similar association of the Asp298Asn polymorphism of the MC4R gene with fatness and growth traits in pigs with different back-fat thickness and growth rate (Fan *et al*., 2009; Dvorakova *et al*., 2011; Munoz *et al*., 2011).

Our results indicate that heterozygous genotype AG (MC4R gene) was favorable in LD breed for Days to 100 kg, ADG and BF. Thus, the partial blocking of leptin signal increases the rate of growth traits. On the other hand, the homozygous genotype GG could be more favorable for selection to improve the meat traits. According to the results shown above, GG genotype is associated with reduced backfat thickness in commercial crossbreds (LD×LC).

Therefore, can be noted that polymorphism Asp298Asn of the MC4R gene is associated with the growth traits and backfat thickness. Allele G, representing Asp298, conserved amino acids in the other subtypes of MCR, due to the smaller backfat thickness. Allele A, which is Asn298, is associated with more fat and best growth rate. Although allele A blocks the interaction the leptin receptor, melanocortin-4 receptor, while links with other hypothalamic neurons supported. As a result, the secretion of gonadotropins is not disturbed and at an elevated secretion leptin may even be activated. It is possible that the allele A may be associated with reproductive characteristics in pigs. Future studies should pay attention to this issue.

5. CONCLUSION

The results of present study suggest that the MC4R gene contributes to Days to 100 kg, ADG and BF. The heterozygous genotype AG was favorable in LD breed. The significant effects of -1.43 (LD male) and -2.81 (LD female) for Days to 100 kg and of +61.17 (LD male) and +26.3 (LD female) for ADG were calculated. The homozygous genotype GG was favorable in crossbred LD × LC. The significant effects of +74.2 for ADG and -1.5 for BF were calculated.

Therefore, the data support a role for the MC4R Asp298Asn polymorphism in the genetic basis of economically important traits in pigs of Russia. Further research is needed to determine the effect of this gene on growth, meat and reproduction traits in pigs of different breeds and crosses.

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