Journal of Microbiology, Biotechnology and Food Sciences

Kováčik et al. 2012 : 1 (4) 498-506

REGULAR ARTICLE

THE EFFECT OF THE PORCINE MELANOCORTIN-5 RECEPTOR (*MC5R*) GENE ASSOCIATED WITH FEED INTAKE, CARCASS AND PHYSICO-CHEMICAL CHARACTERISTICS

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ABSTRACT

The aim of this paper was to investigate the associations between *MC5R* gene polymorphism (A303G) and feed intake, carcass and physicochemical traits in Large White x Landrace crossbred pigs. The experiment was conducted on 106 pigs (LW x L). The polymorphism of *MC5R* gene was analyzed by PCR-RFLP method using the *Bsa*HI restriction enzyme. Two genotypes were identified, *AA* (72.64%) and *AG* (27.36%) genotype.In the test group we found clearly observed a higher frequency of*A* allele(0.8633) compared with the *G* allele (0.1367).In this study, significant differences were observed in *MC5R* (*Bsa*HI) between genotypes for ADG ($p \leq 0.01$), feed intake ($p \leq 0.001$) and feed conversion ($p \leq 0.001$), in favor of the *AG* genotype.We observed a weak association between the A303G polymorphism system and carcass (MLT - $p \leq 0.05$) and physicochemical (pH 1 MLT - $p \leq 0.05$) characteristics. No significant differences were found in other parameters.

Keywords: production traits, *MC5R* gene, polymorphism, pig

INTRODUCTION

Melanocortins (peptide hormones) regulate a variety of important physiological functions such as pigmentation, steroidogenesis in adrenal cortex, body weight, neuronal regeneration, pain, inflammation and sexual behavior. Melanocortin peptides regulation their function by binding to and activating their melanocortinreceptors (**Nijenhuis et al., 2003**).

Five melanocortin receptors are known to exist: melanocyte-stimulating hormone receptor (*MC1R*), adrenocorticotropin hormone receptor (*MC2R*), two neutral receptors (*MC3R, MC4R*) and melanocortin-5 receptor (*MC5R*) (**Haegeman et al., 2000**). Specific binding sites of melanocortin receptors was discovered in the adipose tissue, in the duodenum, in the skin and hypothalamus (**Nijenhuis et al., 2003**).

Ability to binding aguti-related proteins (AgRP) and presence in areas with high expression of *MC3R*/*MC4R* proteins suggests that the effect of AgRP on food intake is regulated by *MCR* proteins (**Nijenhuis et al., 2003**).

Based on these physiological function melanocortin receptors could be consideredan important candidate genes for obesity and meat production traits in pigs (**Haegeman et al., 2000**).

The *MC5R* gene mediates the effects of adrenocorticotropic hormone (ACTH) and melanocortin stimulating hormones (MSH) on exocrine gland functions, including thermoregulation, immunomodulation, and sexual behavior (**van der Kraanet al., 1998**).

The mammalian *MC5R* is expressed in a variety of tissues, notably the skin, muscle, brain, adrenal zonaglomerulosa, esophagus, pancreas, thymus, and circulating lymphocytes (**Griffon et al., 1994; Labbe et al., 1994; Chhajlani, 1996**). Characteristic for the *MC5R* is its expression in and control of exocrine glands, such as the Harderian, preputal, lacrimal, and sebaceous glands (**Griffon et al., 1994; Labbé et al., 1994; Chen et al., 1997**).

Previous studies shown that melanocortin-5 receptor stimulate protein and peroxidase secretion in lacrimal acinar cells (**Leiba et al., 1990**) and in sebaceous and preputial glands *MC5R* stimulate sebum secretion, dermal and preputiallipogenesis (**Thody et al., 1976**).

Kim et al. (2000) mappedporcine*MC5R* gene to the 6th chromosome (6q24-1/2q31). The nucleotide substitution at the position A303Gcauses an amino acid change of alanine for threonine (Ala109Thr). This type of polymorphism using the restriction enzyme *Bsa*HI with digested polymorphic fragments (allele *A* 238 bp; allele *G* 179 bp, 59 bp) **Kim et al. (2000)** first detected.

Kim et al. (2000) found in the observed population clearly higher frequency of allele A in the five breeds (Landrace, Hampshire, Duroc, Chester White, Yorkshire).

Emnett et al. (2001) results indicate that the *MC5R*(*Bsa*HI) PCR-RFLP was polymorphic in Berkshire, Duroc, Landrace and Hampshire populations (the frequency of alelle A was 0.82). The results of the individual breed analysis revealed differences between *MC5R* genotypes with color, Instron tenderness for Berkshire, quality index for Hampshire, all backfat measures and intramuscular fat % for Landrace and for total population analysis showed effects of *MC5R* on 10^{th}rib backfat.

The second type of *MC5R* polymorphism (position C841T) was detected allele-specific PCR (allele *C* 128 bp; allele *T* 118 bp) (**Kim et al. 2000**). Previous studies have not investigated the association between the *MC5R* genotypes (*CC*, *CT*, *TT*) and growth, meat or carcasss quality traits in pigs.

The aim of this paper was to investigate the associations between *MC5R* gene polymorphism (A303G) and feed intake, carcassandphysicochemical traits in Large White x Landrace crossbred pigsand so to provide a general view about the expected effects of the investigated gene on meat traits in pigs.

MATERIAL AND METHODS

Animals

A total of 106 crossbred pigs (Large White x Landrace) were included in the analysis. There were 51 boars and 55 sows. All thepigs were originated from Experimental Centre for Livestock. They were handled under constant feeding conditions and were slaughtered in the same slaughterhouse at a live weight about 100 kg (±5kg).

Studied traits

The animals were recorded for the following traits: average daily gain (ADG), feed intake (FI), feed conversion (FC), half carcass weight (HCW), lean meat content (LM), thigh content (TC), backfat thickness (BFT), *musculuslongisimusthoracis* area (MLT), pH 1 MLT (at 1 h *post mortem*), pH 24 MLT (at 24 h *post mortem*), thigh pH (pH^t), meat pH (pH^m), drip loss (DL) and meat color (MC).

Genotyping

DNA was isolated from blood samples of animals according to **Miller et al. (1988)**. The single nucleotide polymorphisms (SNPs) analyzedin the present study was first detected by**Kim et al. (2000)**. Genetic polymorphism at *MC5R* (*Bsa*HI) was genotyped PCR-RFLP method described by **Kim et al. (2000)** with subsequent primers: forward 5´ TCA GCC TCT TGG AGA ACA TC 3´ and reverse 5´ GCC ACC AAG GAG ATG CAG 3´.

Amplification reaction was conducted in a final volume of 25 µl, containing 1 µl DNA, 5 U/µl Taq polymerase (Fermentas), 10 mMdNTP, 25 mM $MgCl_2$, 1 x Reaction buffer, 0.4 µl of each primer (forward and reverse).

The PCR profile was as follows: first denaturation at 94°C for 3 min, the cycling temperature consisted of 35 cycles of denaturation at 94°C for 10s, annealing at 55°C for 20s, extension at 72°C for 25s with a final extension at 72°C for 10 min.

10 µL of PCR product was digested for 5 min at 37 °C with 1 µl*Bsa*HI (Fermentas, FastDigest) restriction enzyme in a final volume of 25 µl containing 1× enzyme reaction buffer. Restriction fragments were electrophoresed in a 2% agarose gel stained with GelRed (Biotium).

Statistical analysis

Frequencies of the *MC5R* genotypeswere calculated as a genotype percentage in thepopulation. A calculation of allele frequencies wasbased on the direct gene count method.

Statistical analyses to test putative associations among *MC5R* polymorphism and performance traits were carried out using the sas(2010) statistical program. Analysis of the gene effect to observed traits were analyzed using a linear model GLM with fixed and variable effects. The GLM model was: $Y_{ijkl} = \mu \pm g_{ij}MC5R + sex_k + e_{ijkl}$, where Y_{ijkl} observation; μ is general mean; $g_{ij}MC5R$ is fixed effect of genotype (i,j = AA, AG); $sex_k$is fixed effect of sex(k = F, M); e_{ijkl} is random error.

RESULTS

We tested 106 pigs of Large White x Landrace for the *Bsa*HI polymorphism in the *MC5R* gene by PCR-RFLP. The frequencies of alleles and genetic structure in the *MC5R*gene are shown in table 1.Higher frequencies of the *AA*(72.64%)genotype and lower frequencies of

the *AG*(27.36%) genotype were found in tested crossbreeds. We did not find any *GG* genotype. In the test group we found clearly observed a higher frequency of*A* allele(0.8633) compared with the *G* allele (0.1367).

Table 1 The genetic structure and frequencies of alleles at polymorphism within *MC5R* gene in Large White x Landrace pigs

Breed	*MC5R*/*Bsa*HI genotypes (n = 106)			Alleles (n = 212) ± SE		χ^2
LW x L	*AA*	*AG*	*GG*	*A*	*G*	*d.f. = 2*
n = 51	34	17	0	0.8333	0.1667	2.0400$^-$
Boars	35.417	14.167	1.416	±0.036	±0.036	
n = 55	43	12	0	0.8909	0.1091	0.8246$^-$
Sows	43.654	10.691	0.654	±0.029	±0.029	
n = 106	77	29	0	0.8633	0.1367	2.6619$^-$
	78.983	25.033	1.984	±0.023	±0.023	

Legend: P>0,05$^-$

Realization of the variability in the *MC5R* locus was set at 23.83%. PIC (0.2082) and EA (1.3089) values were considerable reduced in comparison with the limit values. The reduced level of the polymorphic information content is caused by significantly higher frequency of *A* allele (table 2).

Table 2 Effectiveness of *MC5R* gene alleles in the study population

Breed LW x L	Alleles	H_e	PIC	C_a	EA	V%
Boars		0.2778	0.2392	0.7222	1.3846	28.06
Sows	*A, G*	0.1944	0.1550	0.8056	1.2413	19.63
Total		0.2360	0.2082	0.7640	1.3089	23.83

Legend: H_e – heterozygosity, PIC – polymorphic information content,C_a – homozygosity coefficient, EA – effectiveness of alleles, V% - variability

Our results indicate that the *MC5R* gene contributes to ADG,feed intake, feed conversion, MLT area and ph 1 MLT. The results are presented in table 3.

Table 3 Least square mean an their standard errors for effects of the genotypes at *MC5R* loci in pork traits of Large White x Landrace pigs

Breed Large White x Landrace	*MC5R*/*Bsa*HI	
	AA (no. = 77)	*AG* (no. = 29)
Trait	LSM ± SE	LSM ± SE
ADG (g)	909.43 ± 107.27**	976.10 ± 94.44**
FI (kg)	2.87 ± 0.25***	2.51 ± 0.12***
FC	0.35 ± 0.03***	0.40 ± 0.02***
HCW (kg)	40.86 ± 1.20	40.52 ± 1.19
LM (%)	55.12 ± 2.01	54.32 ± 2.30
TC (%)	22.42 ± 1.33	22.31 ± 1.27
BFT (mm)	17.88 ± 3.58	18.65 ± 3.42
MLT (cm^2)	44.22 ± 4.35*	42.23 ± 3.56*
pH 1 MLT	6.34 ± 0.25*	6.21 ± 0.29*
pH 24 MLT	5.73 ± 0.20	5.70 ± 0.17
pH^t	6.19 ± 0.28	6.26 ± 0.32
pH^m	5.76 ± 0.37	5.83 ± 0.22
DL (%)	6.78 ± 3.18	7.57 ± 3.49
MC	26.43 ± 2.59	25.72 ± 2.50

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

Legend: feed intake traits - ADG - average daily gain, FI - feed intake, FC - feed conversion; carcass traits - HCW - half carcass weight, LM - lean meat content, TC - thigh content, BFT - backfat thickness, MLT - *musculuslongisimusthoracis* area;physicochemical traits - pH 1 MLT (at 1 h *post mortem*), pH 24 MLT (at 24 h *post mortem*), pH^t - thigh pH, pH^m - meat pH, DL - drip loss, MC - meat color

In this study, significant differences were observed in *MC5R* (*Bsa*HI) between genotypes for ADG ($p \leq 0.01$), feed intake ($p \leq 0.001$) and feed conversion ($p \leq 0.001$), in favor of the *AG* genotype.

Although a significant association was found between MLT area ($p \leq 0.05$) and *MC5R*, we did not find any relationship between *MC5R* genotypes and other carcass traits. While we have found no significant differences, the results showed better carcass characteristics (LM, BFT) for *AA* genotype.

No significant associations between MC5R and physicochemical traitswere found, except pH 1 MLT ($p \leq 0.05$).

DISCUSSION

Kim et al. (2000)were the first to publish information of the *MC5R* gene polymorphism A303G andfound the higher frequencies of the *A* allele in breeds Landrace (0.93) and Duroc (0.75).Authors did not find *G* allele in Hampshire, Chester White and Yorkshire. **Mindeková and Trakovická (2006)** observed a higher percentage of the *AG* genotype (0.652) in the sows, they not detected any *GG* genotype and reported a higher frequency of *A* allele (0.674) compared with the *G* allele (0.326). **Kamińsky et al.(2009)** have reported the frequency 0.570 for *A* allele in the Large White and Landrace boars and frequencies of genotypes was 0.428 for *AA* and 0.284 for *AG* genotype.

Emnett et al. (2001) analyzed population composed of Berkshire, Duroc, Hampshire and Landrace breeds. Statistical analyses were performed within each breed separately, and across breeds for the total population. The authors found effect ($p \leq 0.05$) of *MC5R* on 10^{th} rib backfat, similar differences were also noted for the last rib backfat ($p = 0.14$) (*AA>AG>GG*) and loin muscle area also approached significance for the total population analysis ($p = 0.10$) (*AA<AG<GG*). The results of the individual breed analysis revealed differences ($p \leq 0.05$) between *MC5R* genotypes with color and all backfat measures and intramuscular fat content for Landrace **(Emnett et al., 2001)**.

Our results are not consistent with the observation of our previous study of association of the *MC5R* (*Hsp*92I) with porcine production traits, when we described a significant association between*A* allele and higher BFT and we were indicate an association of the *G* allele with higher ADG and LM **(Kováčik et al., 2010)**.

CONCLUSION

The analysis was carried out to investigate possibleeffects of *MC5R* candidate geneon differentproduction and meat quality traits in pigs. The *G* allele of *MC5R* might have thepotential to accelerate growth of the Large White x Landrace crossbreed byincreasing average daily gain and feed conversion.We observed a weak association between the A303G polymorphism system and carcass and physicochemical characteristics.Together with further studies, these results may contribute as information about the usefulness of this gene for selection on production and meat traits.

A drawback in this study was the low number of animals and therefore research with the larger populationsand other breeds will be neededin order to fully characterize the effects of this marker on traits of interest.

This article is one of the first information on the impact of *MC5R* gene polymorphism (A303G)with the pigs qualitative and production characteristics.

Acknowledgments: This work was financially supported by VEGA scientific grant 1/0790/11 and 1/0061/10.

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