

THE ASSOCIATIONS OF LEPR AND H-FABP GENE POLYMORPHISMS WITH CARCASS TRAITS IN PIGS

Anna TRAKOVICKÁ ¹, Nina MORAVČÍKOVÁ ², Veronika KUKUČKOVÁ ³, Rudolf NÁDASKÝ ⁴, Radovan KASARDA ⁵

ABSTRACT

The aim of this study was to assess the impact of polymorphisms in porcine LEPR and H-FABP genes on carcass and meat quality traits in population of Large White x Landrace crossbreeds. The genomic DNA samples obtained from in total of 180 animals (86 boars and 94 sows) were genotyped using PCR-RFLP methods. The association analyses were carried out in relation to evaluate back-fat thickness, proportion of valuable meat parts, MLT area and proportion of thigh using One-way ANOVA procedure. The allele frequencies were as follows: H-FABP/HinfI H 0.31 and h 0.69, H-FABP/HaeII 0.65 and 0.35, and LEPR/HpaII 0.61 and 0.39. The average values of heterozygosity (0.37) and FIS index (0.17) indicated in population the prevalence of homozygous genotype across all evaluated loci. The statistical analyses showed significant effects on selected production traits only for H-FABP/HinfI ($p < 0.001$) and LEPR/HpaII ($p < 0.0001$) loci. The obtained results indicated the positive effect of H-FABP/HinfIH and LEPR/HpaIIA alleles in order to improvement of the economically important parameters for breeders.

Key words: pigs, breeds, Landrace, Large White, genetic diversity, SNP genotyping, production traits, meat, carcass, quality

1 INTRODUCTION

For many years, a major objective of the swine industry has been to increase the carcass meat percentage. Carcass traits of pigs – such as back-fat thickness, fat meat weight, skin weight, loin eye area, lean meat weight, ratio of lean meat to fat meat, and ratio of leg and butt to carcass – are very important economic traits for pork production (Zhang *et al.*, 2007). Genetic selection has enabled dramatic improvements in the carcass composition of pigs (Jiang *et al.*, 2012). Modern intensive swine production uses improved breeds with a high percentage of lean meat in the carcass. However, the complex conditions of handling and feeding lead to an appreciable decrease of the meat quality mainly due to

the improvement of growth rate and lean percentage and reduction of intramuscular fat content values (Han *et al.*, 2012; Temperan *et al.*, 2014). Recently, the molecular technology advances have allowed the determination of many autosomal regions containing SNPs that affected pork meat traits (Dekkers *et al.*, 2011). The analysis of structural genetic changes in candidate genes can make possible the selection footprint and deepen the understanding of the genetic basis of complex traits (Cuong *et al.*, 2012). The estimation of the frequency of genetic polymorphisms in different pig breeds allows identification of both males and females carrying beneficial alleles early in life, thereby improving accuracy of selection, reducing the generation interval and accelerating the rate

¹ Slovak University of Agriculture in Nitra, Faculty of Agrobiolgy and Food Resources, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, 94976 Nitra, Slovakia, e-mail: anna.trakovicka@uniag.sk

² Same address as 1, e-mail: nina.moravcikova@uniag.sk

³ Same address as 1, e-mail: veron.sidlova@gmail.com

⁴ Same address as 1, e-mail: qnadasky@is.uniag.sk

⁵ Same address as 1, e-mail: radovan.kasarda@uniag.sk

of genetic improvement of the trait (Pérez-Montarelo *et al.*, 2015).

The H-FABP gene, a member of the fatty acid binding protein family that plays a critical role in intracellular fatty acid transport by binding lipids and regulating metabolic homeostasis, has been mapped to the QTL region on SSC 6 (Gerbens *et al.* 1997; Grindflek *et al.*, 2002). Moreover, the H-FABP gene may also regulate the concentration and lipid metabolism, as well as other processes connected with cell metabolism (Jankowiak *et al.*, 2010). The impact of this gene on IMF has been analysed by many association studies (Gerbens *et al.*, 1999; Sieczkowska *et al.*, 2006; Pang *et al.*, 2006). Currently, 3 types of H-FABP restriction fragment length polymorphisms defined as *HinfI*, *MspI* and *HaeIII* loci have been determined for many pig populations (Chen *et al.*, 2014).

The porcine leptin and its receptor genes are involved in food intake and energy homeostasis, and polymorphisms associated to growth and fatness traits have been detected in both genes (Pérez-Montarelo *et al.* 2015). The *LEPR* (leptin receptor) gene is considered a candidate gene for fatness traits. It is located on SSC 6 in a region in which quantitative trait loci for back-fat thickness, fat area ratios, and serum leptin concentration have previously been detected (Uemoto *et al.*, 2012). Stratil *et al.* (1998) identified the *HpaII* polymorphism located in the four intron of the *LEPR* gene. Several studies suggested that the polymorphisms of *LEPR* gene are significantly associated with production traits including feed efficiency and back-fat thickness.

The aim of present study was to analyse the effect of polymorphisms in *H-FABP* and *LEPR* genes on carcass traits in population of Large White x Landrace cross-breeds. Economically important parameters, including back-fat thickness, proportion of valuable meat parts, MLT area and proportion of thigh, have been evaluated to assess the importance of selected loci for the potentially improving accuracy of selection in analysed population.

2 MATERIAL AND METHODS

Totally, 180 crossbreeds of Large White x Landrace (86 boars and 94 sows) from the Experimental Centre of Farm Animals (Department of Animal Husbandry, Slovak University of Agriculture in Nitra) have been included in present study. All selected animals were farmed in the same conditions, fed with standard feed mixtures and selected based on stratification method.

The genomic DNA samples were extracted from blood samples according to Miller *et al.* (1988). Subsequently, the concentration of DNA were tested by spec-

trophotometry by the optical density at wave length of 260 nm. The genotyping of animals was performed using PCR-RFLP methods. The genotypes of *HinfI* and *HaeIII* polymorphisms in *H-FABP* gene have been identified according to the protocols described by Gerbens *et al.* (1997). The genotyping of *HpaII* loci located in the *LEPR* gene has been performed according to method by Stratil *et al.* (1998). The PCR products and restriction fragments were separated and visualised by horizontal electrophoresis in 2.5 % agarose gels in 0.5 x TBE (130 V for 50 min) stained with dye GelRed.

The allele and genotype frequencies were estimated in order to determine the population structure by using PowerMarker v3.25 software (Liu and Muse, 2005). The significance of differences between observed and expected genotype frequencies were tested based on Chi-square (χ^2) analysis. To estimate the state of genetic diversity in population the basic parameters, including observed (H_o) and expected heterozygosity (H_e), effective allele numbers (N_e), polymorphic information content (PIC), and FIS index has been calculated.

The selected carcass and meat quality traits – back fat thickness (BFT), lean meat percentage (LM), thigh percentage (TP) and MLT area were measured by standard technical norm STN 466164. The One-way ANOVA procedure adopted in SAS software (SAS Institute Inc., 2009) has been used to assess the impact of loci in *H-FABP* (*HinfI*, *HaeIII*), and *LEPR* (*HpaII*) genes on the selected production traits.

3 RESULTS AND DISCUSSION

Across all analysed loci the prevalence of homozygous genotypes was found (Table 1). For the *H-FABP* gene the lowest proportion was observed for homozygous HH (*HinfI*) and dd (*HaeIII*) animals. In case of *LEPR/HpaII* loci the lowest proportion has been found for animals with BB genotype. The high distribution of homozygous animals was reflected in the increase of frequency of alleles h (*H-FABP/HinfI*), D (*H-FABP/HaeIII*) and A (*LEPR/HpaII*). The non-significant differences ($p > 0.05$) between observed and expected genotype frequencies were found only for the *H-FABP/HaeIII* polymorphisms. In two other cases the differences caused the bias from the Hardy-Weinberg equilibrium.

In accordance with observed alleles frequency several studies revealed the prevalence of h (*HinfI*) and D (*HaeIII*) *H-FABP* allele in different pig breeds (Gerbens *et al.*, 1997; Kováčik *et al.* 2011; Chalupová *et al.*, 2012). On the contrary, the obtained frequency of *LEPR/HpaII* allele was much smaller compared for example to Stratil *et al.* (1998) and Mindeková *et al.* (2006). This stud-

Table 1: Summary of basic genetic diversity parameters in analysed population

Locus	Genotypes frequency			Alleles frequency		χ^2 test	H _o	H _e	N _e	PIC	F _{IS}
<i>H-FABP/HinfI</i>	HH	Hh	hh	H	h	**	0.33	0.43	1.75	0.35	0.22
	0.14	0.33	0.53	0.31	0.69						
<i>H-FABP/HaeIII</i>	DD	Dd	dd	D	d	ns	0.41	0.46	1.84	0.29	0.11
	0.44	0.41	0.15	0.65	0.35						
<i>LEPR/HpaII</i>	AA	AB	BB	A	B	*	0.39	0.48	1.91	0.31	0.18
	0.42	0.39	0.19	0.61	0.39						

H_o – observed homozygosity, H_e – observed heterozygosity, N_e – effective allele number, PIC – polymorphic information content, F_{IS} – fixation index; ns – not significant, * $p < 0.05$, ** $p < 0.01$

ies showed that in many pigs breeds including Landrace, Meishan, Large White and Czech meat pig can be expected the prevalence of B allele. However, Cuong *et al.* (2012) revealed that in spite of the similar genetic background for IMF content of several pig breeds the selection pressure on 1 locus should not influence the allelic frequencies of the other loci.

Table 1 shows the summary of basic genetic diversity parameters observed in analysed population. The values of observed heterozygosity and also Wright's F_{IS} fixation index across all loci indicated the deficiency of heterozygous animals with comparison to the Hardy-Weinberg expectations. The F_{IS} index is also considered as molecular equivalent of individual inbreeding coefficient with respect to the population, thus its value reflected in population the increase of homozygosity resulting from the high animal's relatedness. The analysed loci showed median level of polymorphisms according to the classification of Botstein *et al.* (1980). The effectiveness of alleles was tested also by effective allele numbers and in loci comparison the highest N_e was found for the *LEPR/HpaII* polymorphism. Generally, the value of N_e indicated relative good level of genetic variability in analysed population.

The statistical analysis of *H-FABP/HinfI* locus effect on selected production traits showed the significant impact of its genotypes on back-fat thickness and MLT area ($p < 0.01$), with H as desirable allele. In case of lean meat and thigh percentage based on different *H-FABP/HinfI* only slight tendency to improvement of this traits was found (Table 2). Similarly the genotypes of *H-FABP/HaeIII* showed only non-significant associations ($p > 0.05$) with all analysed traits. The evaluation of *LEPR/HpaII* genotype effect on carcass and meat quality traits showed its significant influence on all evaluated production traits. The results indicated that the animals with A allele in genotype can be perspective for the increase of production parameters in selected group of animals.

In the production of pork there are many genes involved in the metabolic processes that control growth, fat reduction, back-fat thickness, intramuscular fat content, stress resistance and other meat quality parameters (Kováčik *et al.*, 2013). The intramuscular fat content (IMF) as a major determinant of pork quality is closely related with the tenderness, palatability, juiciness, and special flavor of meat (Wang *et al.*, 2015). The IMF deposition is affected by genetic, physiological and biochemical factors. With respect to genetic factors, 4 to 13 quantitative trait loci (QTLs) for IMF content have been identified in different pig populations. These QTLs for IMF were found on 11 autosomes (Gao and Zhao, 2009; Cuong *et al.*, 2012). In addition, many SNPs of candidate genes have been analysed in order to identify their associations with pork quality (Dekkers *et al.*, 2011; Kováčik *et al.*, 2013; Tempfli *et al.*, 2015). Among them, a confirmed effect on carcass and meat quality has been found for well-known polymorphisms of the *H-FABP* (heart fatty acid-binding protein) and *LEPR* (leptin receptor) genes (Cuong *et al.*, 2012; Pérez-Montarelo *et al.* 2012).

The results of association analysis of the *H-FABP* gene polymorphisms impact on pig production traits remained inconsistent. The *H-FABP* gene is mostly described as candidate gene for IMF content and its significant effect has been found in many studies (Árnyasi *et al.*, 2006; Jankowiak *et al.*, 2010; Han *et al.*, 2012). These studies indicated that the ordering of IMF *H-FABP* genotypes is HH > Hh > hh (*HinfI*) and DD < Dd < dd (*HaeIII*) and that porcine meat quality might be improved by increasing the frequency of genotype dd-HH in pig breeds (Chen *et al.*, 2014). But other studies have found no significant associations with IMF content (Nechtberger *et al.*, 2001; Chmurzyńska, 2006; Chalupová *et al.*, 2012). On the other hand Wei-Jun *et al.* (2006) found the associations between the *H-FABP* gene polymorphisms and fat deposition, when the HH and dd genotypes showed significantly higher deposition of fat in adipocytes. Similarly Hanna *et al.* (2010) found that

Table 2: Average values of analysed carcass and meat quality traits in relation to different *H-FABP/HinfI*, *H-FABP/HaeIII* and *LEPR/HpaII* genotypes

Genotypes	N	Traits (in average)							
		BFT (mm)	P	LP (%)	P	MLT area (cm ²)	P	TP (%)	P
<i>H-FABP/HinfI</i>									
HH	26	18.00	**	55.47	ns	45.48	**	22.80	ns
Hh	60	18.09	**	54.95	ns	44.61	**	22.61	ns
hh	94	16.58	**	55.44	ns	43.21	**	22.61	ns
<i>H-FABP/HaeIII</i>									
DD	80	17.17	ns	55.36	ns	44.17	ns	22.73	ns
Dd	73	17.37	ns	55.20	ns	43.85	ns	22.54	ns
dd	27	17.40	ns	55.27	ns	43.94	ns	22.62	ns
<i>LEPR/HpaII</i>									
AA	75	18.74	***	54.92	**	45.14	**	22.63	*
AB	70	16.18	***	55.33	**	43.26	**	22.45	*
BB	35	16.39	***	55.94	**	43.06	**	23.03	*

BFT – back fat thickness, LP – lean meat percentage, MLT area – area of musculus longissimus thoracis, TP – thigh percentage (%)
 ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

in Polish native Złotnicka Spotted pigs the meat with genotype hh was marked by a higher intramuscular fat content compared with the HH group.

4 CONCLUSION

The statistical analyses showed significant effects of *H-FABP/HinfI* and *LEPR/HpaII* loci on selected production traits. The obtained results indicated the positive effect of *H-FABP/HinfI^H* and *LEPR/HpaII^A* alleles in order to improvement of the economically important parameters for breeders. It is generally accepted that both of the presented genes has important role in intracellular fatty acid transport, fat deposition and are involved in food intake and energy homeostasis. Because of this they can be considered as candidate genes mainly for economically important traits in different pig breeds. Further research on the association between candidate genes and both meat quality and diversity could clarify its exact role in regulation of carcass and meat quality traits in pigs.

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