ASSOCIATION ANALYSIS OF GENES CSRP3, EDG4 A PRKAG3 WITH MEAT QUALITY IN CZECH LARGE WHITE PIGS

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ABSTRACT

In this part of our project we focused on pH and meat colour and three actual candidate genes (*CSPR3, EDG4* and *PRKAG3*). Their mutations were chosen for investigating the effect and influence of these three markers on pork quality in commercial breed population of Czech Large White pigs.

The blood and tissue samples (*m.longissimus lumborum et thoracis*) were collected from a purebred population of 86 Czech Large White sows. All animals were from one breed, fed using standard commercial protocol and slaughtered at average 91.2 kg of live weight in the same abattoir. Genotypes in actual candidate genes for meat quality *EDG4*, *CSPR3* and *PRKAG3* were detremined by previously reported PCR-RFLP assays. The measured phenotypes were pH ultimate and meat colour characteristics L* (lightness), a* (redness), b* (yellowness). The statistical analysis was performed by the general linear model (GLM) by SAS for Windows 9.1.4.

All markers were polymorphic in our Czech large White population but for *CSRP3* and *PRKAG3* markers only two genotypes were observed, homozygous animals *TT* and *GG* in *CSRP3* and *PRKAG3*, respectively, were not observed. We revealed no significant associations between *EDG4, CSPR3, PRKAG3* and meat colour or pH ultimate. These results can be influenced by limited number of animals included in this preliminary study. In next part of our project we focus on another meat quality traits in larger number of animals to confirm previously reported asscociation of mentioned genes.

Key words: Czech Large White pigs, *CSPR3, EDG4, PRKAG3*

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INTRODUCTION

The pig, a representative of the artiodactyla clade, is one of the first animals domesticated, and has become an important agriculture animal as one of the major human nutritional sources of animal based protein (Chen *et al*., 2007). Meat production and quality traits in pigs are largely affected by genetic factors that have been the matter of an increasing number of studies aimed at identifying the causative mutations responsible for their intra and inter line or breed variability (Rothschild, Hu and Jiang, 2007). In this part of our project we focused on pH and meat colour and three actual candidate genes (*CSPR3, EDG4* and *PRKAG3*). Their mutations were chosen for investigating the effect and influence of these three markers to pork quality in commercial breed population of Czech Large White pigs.

EDG4 (endothelial cell diferentiation gene 4) is identified as cellular receptors for lysophosphatidic acid (LPA), belonging to the endothelial cell differentiation gene (EDG) family of G protein-coupled receptors (GPCR) which play an important role in the function of LPA. *EDG4* couples with three types of G proteins to mediate LPA-induced cellular signaling (Contos, Ishii, and Chun, 2000). Radiation hybrid mapping data indicated that *EDG4* gene maps to q2.1 of pig chromosome 2 (SSC2). This gene contains three exons and two introns and cDNA consists of 1,621 bp that contains an open reading frame (ORF) of 1,056 bp encoding a protein of 351 residues (Shan *et al*., 2009). In this study the single nucleotide polymorphism C/T in exon 2 of *EDG4* gene was analysed.

CSRP3 (cysteine and glycine-rich protein 3) is the muscle-specific form of the cysteine and glycine-rich protein family and plays an important role in myofiber differentiation. The cytoplasmic CSRP3 plays as a scaffold protein, interacts and co-localizes with alphaactinin, beta-spectrin and telethonin (T-cap) overlying the Z- and M-lines of myofibrils (Xu *et al*., 2010). *CSRP3* is expressed only in strited muscle and its expression coincides with myogenic differentiation (Arber, Halder and Caroni, 1994). Porcine *CSRP3* gene was assigned to SSC2p14-17 and consisted of six exons and five introns (Xu *et al*., 2010). In this study the single nucleotide polymorphism C1924T in exon 4 of *CSRP3* gene was analysed.

PRKAG3 (protein kinase adenosine monophosphate-activated, gamma 3 subunit) gene encodes a muscle specific isoform of the regulatory γ-subunit of the adenosine monophosphate-activated protein kinase, an enzyme that has a key role in regulating energy metabolism (Škrlep *et al*., 2009). Further analysis of the PRKAG3 signaling pathway may provide insights into muscle physiology as well as the pathogenesis of noninsulin-dependent diabetes mellitus in humans, a metabolic disorder associated with impaired glycogen synthesis (Milan *et al*., 2000). Porcine *PRKAG3* gene affects the glykogen content in muscle and in general the meat quality of pigs that include ultimate pH and

colour measures and that are correlated with water-holding capacity, drip loss, tenderness and cooking loss (Ciobanu *et al*., 2001). In this study the single nucleotide polymorphism A/G which changes amino acid in protein (Ile199Val) of *PRKAG3* gene was analysed.

MATERIALS AND METHODS

Animals and samples

A purebred population of 86 Czech Large White sows was used in this study. All animals were from one breed, fed using standard commercial protocol with the same diet and slaughtered at average 91.2 kg of live weight in the same abattoir. The blood and tissue samples (*m.longissimus lumborum et thoracis*) from each individual were colleceted immediately after slaughter and during the cutting, respectively. The blood was stored with EDTA at 8°C until automated purification.

Genotypes detection

DNA purification was realised by QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany) on QIAcube® (QIAGEN, Hilden, Germany). Purified DNA was stored at -20°C until SNPs genotyping.

Genotypes in actual candidate genes for meat quality *EDG4*, *CSPR3* and *PRKAG3* were determined by PCR-RFLP as described by Shan *et al.* (2009), Xu *et al. (*2010) and Ciobanu *et al.,* (2001), respectively. Restriction enzymes, primer sequences and PCR-RFLP patterns are listed in Table 1. DNA fragments after digestion were separated on 2-3% agarose gels after the electrophoresis and visualised by ethidium bromide.

Tab. 1 Detailed information about 3 SNPs in EDG4, CSPR3 and *PRKAG3 genes*

Gene	SNP	Loc.	Primer sequence (5′-3′)	Tm (°C)	Restr. enzyme	PCR-RFLP pattern (bp)	Ref.
EDG4 (LPAR2)	c.236C>T	exon 2	GCCAGTGCTACTACAATGAG CCCAGAATGATGACAACAG	59	*Mbo*I	729 509/220	Shan *et al.*(2009)
CSRP3	c.1924 C>T	exon 4	GGTACTGTTCGCCAAGGAGA TCCAGGAAAGTGGGTGAAGA	60	*Taq*I	344 138/206	Xu *et al.*, (2009)
PRKAG3	A>G	Ile199 Val	GGAGCAAATGTGCAGACAAG CCCACGAAGCTCTGCTTCTT	57	*Bsa*HI	49/ 91/118 91/167	Ciobanu *et al.*, (2001)

Analysed traits and statistical analysis

The following traits were analysed: meat colour and pH ultimate. Meat colour was determined by spectrophotometry on CM-3500d (KONICA MINOLTA) according to CIELAB (L*, a*, b*) and pH ultimate by Portamess® 911 pH (KNICK).

The statistical analysis was performed by the general linear model (GLM) by SAS for Windows 9.1.4. The genotypes of relevant genes (G_{i-k}) were used as fixed effects. The model used to analyze the data was assumed to be: $y_{ijkl} = \mu + G_i + G_j + G_k + e_{ijkl}$, where y_{ijkl} is the observation or the trait, μ is the population mean, $G_{i, j, k}$ is the effect of i, j, k-th genotype, e_{ijkl} is the random residue.

RESULTS AND DISCUSSION

In Table 2 the frequency of genotypes and alleles for three markers (*EDG4*, *CSPR3* and *PRKAG3* **)** are shown. For *CSRP3* and *PRKAG3* markers only two genotypes were observed, homozygous animals *TT* and *GG* in *CSRP3* and *PRKAG3*, respectively, were not observed.

Tab. 2 Frequency of genotypes, number of observations and the allele frequency for different markers used in this study

Gene	Genotype frequency (no. of animals)			Allele frequency	
EDG4	CC	CT	TT	C	T
	60.47 (52)	36.05 (31)	3.49 (3)	0.78	0.22
CSRP3	CC	CT	TT	C	T
	79.31 (69)	20.69 (17)	0	0.90	0.1
PRKAG3	AA	AG	GG	A	G
	65.52 (56)	34.48 (30)	0	0.83	0.17

Results of association analysis between meat quality traits (pH ultimate and meat colour) and polymorphisms in actual candidate genes are listed in Table 3.

Shan *et al*. (2009) reported new polymorphism c.236C>T in exon 2 of porcine *EDG4* gene. This polymorphism is in coding region but it does not alter the amino acid sequence of the protein. Shan *et al*. (2009) genotyped this SNP in 7 breeds (5 Chinese indigenous and 2 commeracial breeds) and showed great variation in allele frequency, in 2 Chinese breeds allele *T* is dominant and on the other hand allele *C* is dominant in introduced commercial breeds (0.62 – 0.65). Our results obtained from Czech Large White pigs are in accordance with Shan *et al*. (2009), SNP in *EDG4* gene were polymorphic in our population with dominance of allele *C* (0.78). The exact function and influnce over the phenotypic variation of *EDG4* is not obvious, to these days Shan *et al*. (2009) revealed that porcine *EDG4* is mainly expressed in brain, liver, spleen, lung, kidney, intestine, but absent in muscle tissue of pigs and Contos *et al*. (2002) that targeted deletion of the *EDG4* gene in mice does not affect reproductive function. However Shan *et al*. (2009) published association between polymorphism c.236C>T and drip loss and carcass lenght in experimental population consisting of Tongcheng, Landrace, Yorkshire and two crossbred porcine populations. Our preliminary study of *EDG4* effect revealed no association between c.236C>T and meat colour or pH in Czech Large White pigs.

Tab. 3 Association analysis between EDG4, CSPR3, PRKAG3 genes and meat colour and pH ultimate

Marker/ Trait	Genotype (Least square mean value ± SE)		
EDG4	CC (n = 52)	CT (n = 31)	TT (n = 3)
L*	57.385 ± 0.676	57.864 ± 0.916	57.495 ± 2.576
a	3.807 ± 0.541	2.497 ± 0.732	1.462 ± 2.061
b	12.873 ± 0.395	12.038 ± 0.535	10.407 ± 1.506
pHul	5.661 ± 0.024	5.673 ± 0.032	5.581 ± 0.091
CSRP3	CC (69)	CT (17)	
L	56.843 ± 0.911	58.319 ± 1.422	
a	2.3 ± 0.729	2.878 ± 1.138	
b	11.308 ± 0.532	12.237 ± 0.831	
pHul	5.661 ± 0.032	5.615 ± 0.049	
PRKAG3	AA (56)	AG (30)	
L	58.093 ± 0.990	57.069 ± 1.282	
a	1.964 ± 0.792	3.213 ± 1.025	
b	11.616 ± 0.579	11.928 ± 0.749	
pHul	5.637 ± 0.035	5.640 ± 0.045	

* meat colour characteristic L (lightness), a (redness), b (yellowness), pHul = pH ultimate
n – number of animals with mentioned genotype

The *PRKAG3* 200Q mutation responsible for the RN^- allele has been identified only in the Hampshire breed or in pig lines with Hampshire blood (Fontanesi *et al*., 2008). But also other mutation, e.g. I199V, have been suggested to affect muscle glycogen content, glycolytic potential, pH, meat colour and drip loss (Ciobanu *et al*., 2001; Fontanesi *et al*., 2008). Our results are different to previously reported frequencies of Ciobanu *et al*. (2001) or Fontanesi *et al*. (2008) where allele 199I occured in frequency 0.22 or 0.17, respectively in Bershire x Yorkshire and Italian Large White pigs. Fontanesi *et al*. (2008) revealed significant association between polymorphism I199V and pH_1 (2 hours post-mortem), on the other hand Otto *et al*. (2007) confirmed that initial pH value did not differ between genotypes of this SNP in commercial lines of PIC pigs whereas pigs of the homozygous genotype *II* showed significantly higher ultimate pH value compared with those of genotype *VV* and pH_{24} measured in the ham was significantly different between all genotypes. Unfortunately in our preliminary study no significant associations of I199V of *PRKAG3* gene were observed for any traits.

Xu *et al*. (2010) identified synonymous mutation C1924T substitution recognized by *Taq*I in exon 4 of porcine *CSRP3* gene by comparative sequencing. Genotyping of this SNP by Xu *et al*. (2010) revealed that Chinese pig breeds had higher frequencies for the allele *T*, whereas the western breeds appeared to have lower frequencies and most individuals were *CC* or *CT* genotypes and only few *TT* homozygous individuals had been detected. Our results suggested analogous tendency, because we detected no *TT* homozygous animal and allele *C* was in majority (Tab. 2). Association study of

Xu *et al*. (2010) performed on Chinese pig breeds, Landrace, Duroc and Berkshire x Yorkshire F_2 population indicated that animals of *CC* genotype had much more desirable meat quality and it was revealed that the substitution of C1924T had significant associations with firmness, pH, flavor score and water holding capacity so Xu *et al.* (2010) suggested that *CSRP3* is a functional candidate gene affecting pig meat quality. This gene was probably involved in regulation of muscle development and regulation of myofiber distribution and the investigation of the porcine *CSRP3* gene will probably provide some evidence about its influnce on meat quality. However we revealed no association of C1924T polymorphism and pH or meat colour in Czech Large White pigs.

CONCLUSION

For the present study, we analysed previously reported polymorphisms of *EDG4, CSPR3, PRKAG3* actual candidate genes in population of Czech Large White pigs. We revealed no significant associations between *EDG4, CSPR3, PRKAG3* and meat colour or pH ultimate. These results can be influenced by limited number of animals included in this preliminary study. In next part of our project we focus on another meat quality traits in larger number of animals to confirm previously reported asscociation of mentioned genes.

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