TWO MAJOR GENE POLYMORPHISMS AFFECTING SKATOLE AND ANDROSTERONE LEVELS IN FOUR COMMERCIAL AND ONE INDIGENOUS PIG BREED

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Abstract

Skatole and androstenone and their accumulation in fat are the main molecular compounds causing the unpleasant boar taint of porcine meat. This research focused on the variability of genotypes in two genetic markers associated with skatole (*CYP2E1*) and androstenone (*TEAD3*) levels in boars of five breeds in the Czech Republic. Four wide-spread commercial breeds and one autochthonous breed (genetic resource) – Large White, Landrace, Duroc, Pietrain and Prestice Black-Pied pig – were chosen for analysis. Fifty breeding boars from each were genotyped by PCR-RFLP method. Differences in genotypes and allele frequencies provide important information and the possibility of genomic selection of makers associated with boar taint compounds. The allele and genotype frequency results indicate a higher incidence of preferred alleles of both genes in Prestice Black-Pied pig and Pietrain compared with Large White, Landrace and Duroc.

Key words: boar taint, skatole, androstenone, CYP2E1, TEAD3, pig breeds

Skatole and its accumulation in adipose tissue is the main molecular cause of faecal-like odour in porcine meat (BABOL et al., 1998). The main enzyme in skatole metabolism is the cytochrome P450 II E1 enzyme (CYP2E1) from the cytochrome P450 family (DIAZ and SQUIRES, 2000). Many studies describe polymorphisms in the *CYP2E1* gene (e. g. MOE et al., 2009) and evaluate their relationship to gene expression (e. g. LIN et al., 2006). MÖRLEIN et al. (2012) investigated SNPs in the *CYP2E1* promoter (according to SKINNER et al., 2005) and the impact of SNP substitution g.2412 C>T on skatole level of two commercial pig populations. Genotype *CC* was associated with higher skatole level significantly compared to skatole level in individuals with *CT* and *TT* genotypes. Androstenone is considered to be one of the main compounds of urine-like odour of pork meat. The *TEAD3* gene appeared to be a candidate gene for androstenone metabolism (ROBIC et al., 2011). A g.726 C>T substitution is present in the *TEAD3* promoter. This SNP caused a higher level of androstenone associated with the *CC* genotype in LW. The *T* allele appears to be associated with lower androstenone level at least in LW (ROBIC et al., 2012).

Material and Methods

Fifty breeding boars were chosen from each of the most frequent commercial pig breeds and one local breed in the Czech Republic. The research focused on genotyping of purebred boars of Large White (LW), Landrace (LA), Duroc (D) and Pietrain (PN) and Czech native breed and genetic resource Prestice Black-Pied pig (PC). Genotyping in specific SNPs within two genes – CYP2E1 and TEAD3 – was carried out by PCR-RFLP method.

Genomic DNA was extracted from blood (Genomic DNA Mini Kit (Blood/Cultured Cell), Geneaid) and hair samples (NucleoSpin®Tissue, Macherey-Nagel GmbH & co. KG, Germany) according to the manufacturer´s instructions. The PCR reaction was performed with a specific set of primers and amplification condition according to Mörlein et al. (2012) for CYP2E1 and Robic et al. (2012) for TEAD3. The PCR products lengths are 209 bp (CYP2E1) and 312 bp (TEAD3). The restriction enzymes BtsMutI (for CYP2E1) and HaeIII (TEAD3) were used for RFLP analysis and yielded fragments of 109 and 100 bp (CYP2E1) and 225, 160, 65, 48, 39 bp (TEAD3). Agarose gel electrophoresis was used to separate restriction fragments separation and visualize genotypes.

Results and Discussion

The results in *CYP2E1* showed genotype variability among breeds. The *CC* genotype was absent in both PC and PN (Tab. 1). The highest frequency was found in LA (0.40), then in LW (0.22) and D (0.16). The frequency of *TT* genotype was higher in D (0.34) than in LW (0.28) and LA (0.12) and the highest was found in PN (0.86) and PC (0.42). MÖRLEIN et al. (2012) described the frequency of *CC* genotype in Duroc hybrids as 0.25, the frequency of *CT* genotype 0.52 and *TT* 0.23. Genotype frequencies *CC* 0.21, *CT* 0.11, *TT* 0.06 were found in the production population of the Landrace/Yorkshire/Duroc hybrids (SKINNER et al., 2005).

The more advantageous *T* allele is most common in the Pietrain breed and subsequently in the Prestice Black-Pied breed.

For the *TEAD3* marker, the lowest frequency of the *CC* genotype was found in both PC and PN (0.34) and the highest in LW (0.82) (Tab. 2). The highest frequency of *TT* genotype was detected in PC (0.16), similarly in LA and PN (0.12), almost absent in D (0.04) and missing in LW. The allele frequencies are again remarkable: although the *C* allele frequency is higher than the frequency of *T* allele (preferred) in PC (and in all other breeds), the *T* allele frequency is still the highest in PC compared to other breeds. The frequency of *T* allele is again close in PC and PN. ROBIC et al. (2012) described the frequency of *T* allele in LA 0.37 and D 0.52 and the frequency of *TT* genotype in LW 0.48.

Comparison of allele frequencies suggests important information and possible perspectives for genomic selection. In the case of both genetic markers, a higher frequency of preferred genotypes and alleles was recorded in PC and PN breeds than in the remaining commercial breeds. A possible reason is the process of breeding the Prestice Black-Pied pig with the Pietrain in its breeding history, when 32 genealogical lines spread along the boars of the PN breed (VÁCLAVKOVÁ & BĚLKOVÁ, 2019).

Table 1. Variability of CYP2E1 gene in five pig breeds

	CYP2E1				
Breed	Genotype frequency			Allele frequency	
	CC	*CT*	*TT*	*C*	*T*
PC	0.00	0.58	0.42	0.29	0.71
LW	0.22	0.50	0.28	0.47	0.53
LA	0.40	0.48	0.12	0.64	0.36
D	0.16	0.50	0.34	0.41	0.59
PN	0.00	0.14	0.86	0.07	0.93

Table 2. Variability of TEAD3 gene in five pig breeds

Breed	*TEAD3* Genotype frequency			Allele frequency	
	CC	*TC*	*TT*	*C*	*T*
PC	0.34	0.50	0.16	0.59	0.41
LW	0.82	0.18	0.00	0.91	0.09
LA	0.50	0.38	0.12	0.69	0.31
D	0.44	0.52	0.04	0.70	0.30
PN	0.34	0.54	0.12	0.61	0.39

Conclusion

In this study, a total of 250 individuals of five breeds of pigs – LW, LA, D, PN, PC – were genotyped in two genetic markers (*CYP2E1* and *TEAD3*) associated with boar taint. Differences among breeds in genotype and allele frequencies were detected. More favorable genotypes and alleles were found in both markers in the Prestice Black Pied and the Pietrain breeds compared to the Large White, Landrace and Duroc breeds.

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