The *visfatin* (*NAMPT; PBEF1*) gene polymorphisms and associations with meat performance traits in three pig breeds kept in the Czech Republic

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ABSTRACT: Visfatin encoded by the *NAMPT* gene (*nicotinamide phosphoribosyltransferase*, formerly also known as *PBEF – pre-B cell colony-enhancing factor*) is suggested to play a role in lipid metabolism and pathophysiology of diabetes mellitus type 2. A new microsatellite *SCZ004* was detected within intron 9 of the *NAMPT* gene. In Czech Large White (n = 95) frequencies of alleles *282*, *286*, *287*, *299*, and *304* were 0.02, 0.39, 0.07, 0.04, and 0.48, respectively. Allele *286* was predominant also in Landrace (n = 11) and Black Pied Prestice (n = 11) breeds. Association analysis was carried out between previously reported SNP AM999341: g.669T>C in intron 9 of the *NAMPT* gene and backfat thickness, average daily gain and lean meat content in Czech Large White (n = 215), Black Pied Prestice (n = 96) and Landrace (n = 105). The *CC* genotype was associated with higher backfat thickness ($P \leq 0.01$) in Black Pied Prestice, however in Czech Large White *CC* was associated with lower backfat thickness when compared to *TT* and *CT* genotypes ($P \leq 0.05$). In Czech Large White, *CC* genotype was associated with higher lean meat content when compared to *CT* (not significant) and *TT* genotype ($P \leq 0.05$) while in Landrace *CC* had the lowest lean meat content when compared to *CT* and *TT* genotypes but only the difference between *CC* and *CT* was statistically significant ($P \leq 0.05$). In Black Pied Prestice no association with lean meat content was found. Average daily gain was not associated with the SNP in any breed.

Keywords: *visfatin*; *NAMPT*; *PBEF*; SNP; microsatellite; pig; association analysis

Growth, carcass composition and reproductive performance (Eliáš et al., 2007; Humpolíček et al., 2007) are crucial for efficient pork production. Identification of genes underlying important performance traits and use of this knowledge through marker-assisted selection can make the production of pork more efficient.

In our work we focused on visfatin, an adipocytokine highly enriched in the visceral fat, which has insulin-like metabolic effects and can also lower plasma glucose in mice (Fukuhara et al., 2005; Garten et al., 2009). This protein was previously described as pre-B cell colony-enhancing factor (PBEF), which acts on early B-lineage precursor cells (Samal et al., 1994). Later PBEF was identified as a nicotinamide phosphoribosyltranferase (NAMPT), an enzyme involved in nicotinamide adenine dinucleotide (NAD) biosynthesis (Rongvaux et al., 2002).

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In humans, variation in *visfatin* gene was found to be associated with fat metabolism (Jian et al., 2006; Tokunaga et al., 2008). Sun et al. (2007) found that serum triacylglycerols are strongly correlated with fasting serum visfatin in men.

The human *visfatin* gene consists of 11 exons and 10 introns and is located on chromosome 7q22.2 (Samal et al., 1994). Chen et al. (2007) and Palin et al. (2008) described the porcine *visfatin* mRNA sequence. The porcine *visfatin* gene is composed of 11 exons at least and has exactly the same exon/intron structure as the human ortholog (Chen et al., 2007). Palin et al. (2008) revealed 6 porcine *visfatin* transcript variants, resulting from alternate polyadenylation or alternate splicing of exons. Variant 1 is the predominant form among species, which contains an open reading frame of 1 473 bp encoding a 52-kDa protein of 491 amino acids (Chen et al., 2007). Čepica et al. (2008) mapped the porcine *visfatin* gene on chromosome SSC9.

On the basis of comparative sequencing of wild boar and Meishan PCR fragments, Čepica et al. (2008) detected a SNP AM999341:g.669T>C in intron 9 of the porcine gene. They studied associations between the SNP, carcass and meat quality traits in two populations of Meishan crosses.

The aim of this work was to perform an association study between the AM999341:g.669T>C and backfat thickness, lean meat content and average daily gain in Czech Large White, Landrace and Black Pied Prestice breeds. Another goal was to investigate a new microsatellite detected within the *NAMPT* gene.

MATERIAL AND METHODS

For this study the gilts of Czech Large White, Landrace and Black Pied Prestice from purebred herds were used. Czech Large White and Landrace are highly prolific breeds with very good parameters of meat efficiency. They are both used as maternal lines in the Czech Republic and they are crossed to produce F1 gilts. On the other hand, Black Pied Prestice has been a local Czech autochthonous breed maintained as a genetic resource since 1992.

In this study, 215 gilts of Czech Large White (CLW) obtained from 3 herds, 105 gilts of Landrace (L) from 2 herds and 96 gilts of Black Pied Prestice (BPP) from 11 herds kept in Western Bohemia were used for association study between SNP AM999341: g.669T>C and meat performance traits. Records for meat performance traits obtained in a field test were as follows: average daily gain calculated from birth to 90 kg of live weight (g), backfat thickness (cm) and lean meat content (%) measured by ultrasound (Piglog 105, SFK, Soborg, Denmark). Records were taken around the live weight of 90 kg and adjusted to the accurate live weight of 90 kg using a regression curve.

*Hpa*II PCR-RFLP assay was used for genotyping AM999341:g.669T>C polymorphism within the *visfatin* gene (Čepica et al., 2008). The PCR was performed in 25 µl reaction volume using 50 ng of porcine genomic DNA, 0.2µM of each primer (F: 5´-GGGTCATAACTTGACTTTGGAGAA-3´ and R: 5´-TCTAGAGAACCTGAAGAGAGCAGAA-3´ designed on the basis of AM999341), 200µM of each dNTP and 1 U of LA polymerase in complete PCR buffer. Amplification conditions were 95°C (2 min) followed by 30 cycles of 95°C (20 s), 54°C (30 s), 68°C (50 s), with a final extension at 68°C (7 min). The length of PCR product was 524 bp. The amplified fragment was digested with 1 U of restriction enzyme *Hpa*II at 37°C overnight. The obtained fragments were separated in 3% agarose gel. The identity of the 524 bp amplicon of the Czech Large White, Black Pied Prestice and Landrace was verified by the direct sequencing of the PCR product.

The microsatellite was revealed within intron 9 of the *visfatin* gene after the sequencing of cloned 402 bp long PCR product. The PCR was performed in 25 µl reaction volume using 50 ng of porcine genomic DNA, 0.2µM of each primer (F: 5´-ATAGCCATCATTAGCTGCCTCC-3´ and R: 5´-TTAAAGTGTCTCCTTCTGGTGGG-3´ designed on the basis of AM999341), 200µM of each dNTP and 1 U of LA polymerase in complete PCR buffer. Amplification conditions were 95°C (2 min) followed by 30 cycles of 95°C (20 s), 56°C (30 s), 68°C (30 s), with a final extension at 68°C (7 min). The PCR product was cloned in pDrive Cloning Vector and QIAGEN EZ Competent Cells (QIAGEN, Hilden, Germany). According to the revealed sequence another set of primers was designed to amplify the labelled PCR product (304 bp) containing a microsatellite (F: FAM labelled 5´-TTGCCTCATCTGCTTCTCCTTC-3´ and R: 5´-ATGCCTTGCCCTTAAACTGATCTA-3´). The PCR was performed in 25 µl reaction volume of 12.5 µl HotStar Mix (Qiagen), 50 ng of porcine genomic DNA and 0.2µM of each primer under the following conditions: 95°C (15 min) followed by 35

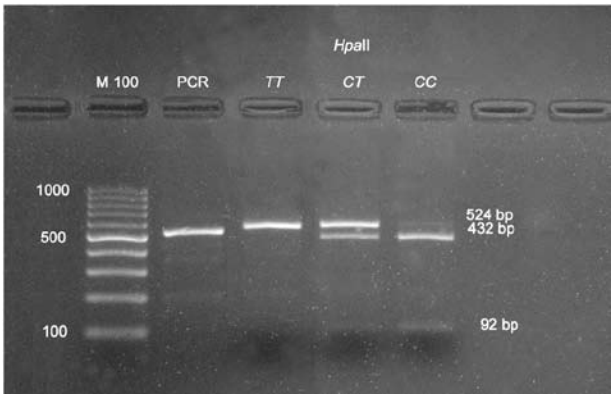

Figure 1. Agarose gel (3%) showing the polymorphism AM999341:g.669T>C in the *visfatin* gene after digestion of the 524 bp fragment with *Hpa*II. M 100 – M 100 DNA ladder (GeneRuler™ Fermentas); PCR – undigested PCR product; genotypes *TT*, *CT* and *CC*

cycles of 95°C (30 s), 55°C (30 s), 72°C (30 s), with a final extension at 72°C (10 min). The FAM labelled PCR product was further analysed by fragment analysis on an ABI PRISM 3100 Avant genetic analyzer (Applied Biosystems Inc., Foster City, CA, USA).

Association analysis was performed using a Mixed Linear Model (MLM Procedure) in SAS for Windows 9.1.4 using the equation:

$$y_{ijklmno} = \mu + NAMPT_i + breed_j + NAMPT \times breed_k + Ybir_l + F_m + M_n + e_{ijklmno}$$

where:

$y_{ijklmno}$ = the phenotypic value of the analysed trait

μ = the population mean

$NAMPT_i$ = the fixed effect of the i^{th} genotype of the *visfatin* gene (i = *TT*, *CT* and *CC*)

$breed_j$ = the fixed effect of the j^{th} breed (j = CLW, L and BPP)

$NAMPT \times breed_k$ = the fixed effect of the k^{th} interaction between *visfatin* genotype and breed

$Ybir_l$ = the fixed effect of the l^{th} year of birth (l = 1992, 1993, 1994, 1995, 1996, and 1997)

F_m = the random effect of m^{th} father

M_n = the random effect of n^{th} mother

e_{ij} = the random error effect of each observation

RESULTS AND DISCUSSION

The polymorphism AM999341:g.669T>C (Čepica et al., 2008) detected in intron 9 was genotyped in Czech Large White, Black Pied Prestice and Landrace breeds. Allele *C* was characterised by 432 bp and 92 bp fragments after *Hpa*II digestion of the 524 bp PCR product, while allele *T* had no restriction site for *Hpa*II (Figure 1). Frequencies of allele *C* were 0.51, 0.23 and 0.33 in Czech Large White, Landrace and Black Pied Prestice, respectively.

Association analysis performed with the SNP revealed that in Czech Large White *CC* genotype was associated with lower backfat thickness when compared to *CT* and *TT* genotypes ($P \leq 0.05$). Furthermore, *CC* genotype was also associated with higher lean meat content when compared to *TT* genotype ($P \leq 0.05$) and *CT* (not significant). The *CC* genotype had higher average daily gain compared to *CT* and *TT* genotypes but differences were not statistically significant (Table 1).

In Landrace *CC* genotype was associated only with lower lean meat content compared to *CT* ($P \leq 0.05$) and *TT* genotypes (not significant). No associations with backfat thickness or average daily gain were revealed in this breed (Table 1).

In Black Pied Prestice *CC* genotype was associated with higher backfat thickness compared to *CT* and *TT* genotypes ($P \leq 0.01$). Moreover, animals with *CC* genotype had a higher growth rate and lower lean meat content than *CT* and *TT* animals, but these differences were not statistically significant (Table 1).

No statistically significant differences were detected between the individual genotypes of the SNP and meat performance traits when all three breeds were merged together.

The newly detected microsatellite *SCZ004* is composed of an irregular motif with predominant T repetition. The length of 3 alleles 286, 299 and 304 was verified by sequencing and the length of other two alleles 282 and 287 was estimated by fragment analysis using capillary electrophoresis. Allele frequencies in the analysed breeds Czech Large White, Landrace and Black Pied Prestice are shown in Table 2.

The associations between SNP, backfat thickness and lean meat content described here had opposite effects in Czech Large White and Black Pied Prestice breeds. The opposite effects on fat accretion were previously observed also in Wild Boar × Meishan F_2 family and commercial Landrace × Chinese-European synthetic population (Čepica et al., 2008). In agreement with the previous study (Čepica et al., 2008), no associations were observed between SNP and average daily gain. These results indicate that the SNP is just a marker that is in

Table 1. Association between the polymorphism AM999341:g.669T>C in the *visfatin* gene and the meat performance traits in different pig breeds (for each genotype LSM ± SE; least square mean ± standard error are given)

Breed	Trait	Genotype		
		CC (*n* = 51)	*CT* (*n* = 118)	*TT* (*n* = 46)
CLW (*n* = 215)	BF	1.00 ± 0.04^{ab}	1.09 ± 0.03^{a}	1.11 ± 0.04^{b}
	ADG	532.31 ± 8.18	530.51 ± 7.15	527.79 ± 8.83
	LM	59.26 ± 0.44^{a}	58.67 ± 0.39	58.14 ± 0.48^{a}
		CC (*n* = 5)	*CT* (*n* = 39)	*TT* (*n* = 61)
L (*n* = 105)	BF	1.05 ± 0.09	1.04 ± 0.04	1.05 ± 0.04
	ADG	494.48 ± 9.22	508.47 ± 8.12	521.68 ± 7.78
	LM	54.73 ± 2.21^{a}	59.79 ± 0.99^{a}	58.79 ± 1.15
		CC (*n* = 9)	*CT* (*n* = 45)	*TT* (*n* = 42)
BPP (*n* = 96)	BF	1.63 ± 0.07^{A,B}	1.43 ± 0.04^{A}	1.40 ± 0.04^{B}
	ADG	545.50 ± 15.26	534.81 ± 8.19	531.57 ± 8.45
	LM	53.43 ± 0.79	54.76 ± 0.40	54.44 ± 0.43

CLW – Czech Large White; L – Landrace; BPP – Black Pied Prestice; traits: BF – backfat thickness (cm); ADG – average daily gain (g); LM – lean meat content (%); the same superscripts in a line show significant differences $^{A,B}P \leq 0.01$; $^{a,b}P \leq 0.05$

Table 2. Allele frequencies of the microsatellite *SCZ004* in different pig breeds

Breed	Number of animals	Alleles				
		282	286	287	299	*304*
Czech Large White	97	0.02	0.39	0.07	0.04	0.48
Landrace	11	0.05	0.76	0.05	0	0.14
Black Pied Prestice	11	0	0.41	0	0.18	0.41

linkage disequilibrium with an unknown causative mutation affecting fatness and muscling and linkage phase of the SNP and causative mutation differs in different breeds. Further research is needed on the possible effect of *NAMPT* gene variation on meat performance in pigs.

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