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SHORT COMMUNICATION

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An association between the G/A single nucleotide polymorphism within intron II of VIP gene and milk performance traits in dairy cattle

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Abstract

Single nucleotide polymorphisms (SNPs), which are present in the encoding part of the genes responsible for important breeding functions, exert an influence on the cattle's phenotype since their function is to regulate the genes expression. In this study a G/A single nucleotide polymorphism within intron II of the vasoactive intestinal peptide (VIP) gene was detected. The study covered a herd of 185 Jersey dairy cows from the Wielkopolska Province in Poland. All possible VIP/*Dra*I genotypes determined by using two alleles (AA, AG, GG) were examined in the herd of cows under study. The AA genotype frequency was 0.48; AG 0.22, and GG 0.30. Allele A frequency was 0.592, whereas allele G was 0.408. Analyzed VIP/*Dra*I gene polymorphism with respect to milk utility traits showed slight statistical differences in the percentage of fat and protein content in milk of the animals with different VIP/*Dra*I genotypes. This study could have a significant influence on dairy cattle breeding programs in the future as improvements in genetic selection methods will continue to be important in milking management.

Additional key words: vasoactive intestinal peptide; lactation; milk utility traits.

Vasoactive intestinal peptide (VIP) acting in paracrinical and autocrinical ways, affects the secretion of hypothalamic hormones and other hormones secreted through the endocrine glands, causes the relaxation of smooth muscle and neurogenesis in foetal life. Furthermore, VIP has an influence on maintaining the physiological homeostasis by immunomodulatory, anti-inflammatory and cytoprotective actions and by inhibiting the apoptosis and proliferation of cancer cells (Sherwood *et al*., 2000; Said *et al*., 2001). VIP falls under the overriding strength of the hypothalamus insofar as it controls the prolactine secretion. The hypothalamus shows a high concentration of VIP-immunoreactive cells (Gozes *et al.,* 1989). El Halawani *et al*. (1997) found that VIP being under the stimulation of dopamine or serotonin is a hypophysiotropic factor in releasing prolactin. In birds, the secretion of VIP is regulated at the level of transcription during their reproductive cycle and is connected with circulating levels of prolactin. This transcription regulation may also be related to the photoperiodic mechanism. Prolactin responses with the participation of VIP to photoperiod, is correlated with an increase of VIP transcription levels in the hypothalamus (Youngren *et al*., 1996). It suggests that VIP and its transcriptional regulation may be connected to the photoperiodism of birds and their seasonal predisposition. In the case of mammals the level of VIP-mRNA in the hypothalamus depends on the circadian rhythms (Okamoto *et al*., 1991) and hormonal conditions of the species (Gozes *et al.,* 1989). VIP-containing fibres have been located with median prominance in the hypothalamus. Further research has acknowledged the role of the hypothalamus VIP in the regulation of hypophysis functioning and its influence on the release of prolactin, adrenocorticotropic hormone, growth hormone and lutenei-

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Abbreviations used: ACRS-PCR (artificially created restriction site–polymerase chain reaction); RFLP (restriction fragment length polymorphism); SNP (single nucleotide polymorphism); VIP (vasoactive intestinal peptide).

sing hormone, and its influence on the concentration of gamma-aminobutyric acid within the hypothalamus. This peptide has also been located in the posterior hypophysis, where it partially participates part in the oxytocin and vasopressin release and thus released to systemic circulation (Youngren *et al*., 1996).

VIP is a 28 amino-acid peptide with a molecular weight of 3326 kD. Bovine VIP gene is 8545 bp in length, located on chromosome 9 and consists of 7 exons separated by 6 introns. Each of these 7 exons codes a specific functional domain of the protein. The expression of the VIP gene is partially controlled by growth factors and neurotransmitters that switch on several cis-active elements within the gene by protein kinases, calcium and signal pathways mediated by cAMP. Further information about gene polymorphisms can be found at the NCBI database. Additional information can be found on the sequencing of the bovine (Hereford) genome to identify single nucleotide polymorphisms (SNPs) for genetic studies, released by the Baylor College of Medicine Human Genome Sequencing Center released as Btau_4.0 in September 2007. The results form the basis of a Bovine HapMap Project (The Bovine Map Consortium, 2009).

For this study the G/A SNP within intron II of the VIP gene was chosen. We decided to conduct studies on mutations located in this intron because of our interests in the functionality of this uncoding part of the gene. Relevant biological effects can be linked to the nucleotide alterations located in introns (Chorev & Carmel, 2012). The after-effects of the described genetic variation can be important for changes in the splicing regulatory sequences, motifs of various readings of codons and for the influence on mRNA secondary structures (Shabalina *et al*., 2006; Parmley & Hurst 2007; Hsu *et al*., 2010; Gingold & Pilpel, 2011).

VIP is a significant subject in the area of breeding cattle because of its function. That is to say, it is used in the modulation of expression of the prolactin gene and its secretion. It is well known that prolactin stimulates the enlargement of mammary glands and lactation, therefore VIP exerts an indirect influence on the functional processes. This is to underline the importance of economic factors which are essentially influenced by various genes and their modifications through various environmental factors. However, no information on the subject can be found in the present field of study regarding the correlation between the VIP genotype and the performance of milk utilization and their influence on bovines. For this reason, the analysis of nucleotide sequence variation in the gene encoding VIP of dairy cattle was chosen as the object of this study. Previously, studies concerning the influence the VIP gene and lactation have been performed on rats. Gozes *et al*. (1989) have conducted studies on their quantitative RNA which show an increase in the VIP-mRNA content in the hypothalamus of lactating rats. Our study may have a significant influence regarding dairy cattle breeding programs, as any improvements in the methods of genetic selection are vital in the milk production management.

In this study we investigated a VIP gene restriction fragment length polymorphism (RFLP) by using an artificially created restriction site (ACRS-PCR) in a herd of 185 Jersey dairy cows from the Wielkopolska Province in Poland and its correlation with milk production.

Blood was drawn from the jugular vein and collected into vacuum test tubes containing K_3-EDTA acting as anticoagulant. The DNA isolation was performed using the Master Pure™ kit (Epicentre®). The DNA sequence using A nucleotide instead of G nucleotide (G/A substitution) gave us the opportunity to create restriction sites for the *Dra*I restricted enzyme. The sequence near the nucleotide mutation of intron II was amplified through the use of a partially mismatched PCR primer: F 5' CAG TTG AAC ATT CTT CCT CCA TTT AA 3'.

The PCR reactions were performed in a total volume of 15μL under the following temperature profile: initial denaturation of DNA templates for 5 min at 95°C followed by 33 cycles of denaturation for 1 min at 95°C, primer annealing for 1 min at 58°C, synthesis of PCR products for 2 min at 72°C, and final synthesis for 10 min at 72°C. Afterwards, 15 μL of the PCR reaction products (178 bp) diluted in 10x Buffer Tango™, were digested with 0.2 μL of *Dra*I at 37°C for 24h.

Digestion products were submitted to electrophoresis in 2.5% agarose gel containing ethidium bromide and 1x TBE buffer with pUC19/*Msp*I™ (Thermo Scientific®) as a DNA fragment size marker. Products of digestion were analyzed and documented using a set of instruments for electrophoresis gel documentation and analysis (Vilber Lourmat®). Visualization of the restriction fragments revealed the existence of 154 bp and 24 bp of AA genotype, 178 bp of GG genotype, and 24 bp, 154 bp, and 178 bp of AG genotype.

The results of the ACRS-PCR-RFLP analysis were statistically analyzed to determine: (i) the frequencies of the VIP/*Dra*I alleles and genotypes and their expected distribution, (ii) the frequencies of homo- and he-

terozygous genotypes and their expected distribution. The statistical analysis concerning the association between milk utility traits in the studied herd of cows and in particular VIP/*Dra*I genotypes was carried out using STATISTICA data analysis software using the following mixed model: $Y_{ijkl} = \mu + a_i + c_j + d_k + f_l + b + e_{ijkl}$, where: Y_{ijkl} = trait; μ = overall mean; a_i = fixed effect of the *VIP/Dra*I genotype; c_j = random effect of the herd; d_k = random effect of year/season; f_l = random effect of the sire; b = regression coefficient of Jersey dairy cow gene share on the trait; and e_{ijkl} = random error.

All possible VIP/*Dra*I genotypes determined by two alleles were found in the herd of 185 Jersey cows: AA, AG, GG. The AA genotype frequency was 0.48; AG 0.22, and GG 0.30. Allele A frequency was 0.592, whereas allele G 0.408.

In the herd of 185 Jersey cows 77.8% of individuals were homozygotes, and 22.2% were heterozygotes. Statistically significant differences were also found in VIP/*Dra*I genotype groups between apparent abundance and the abundance calculated theoretically according to the Hardy-Weinberg law (Edwards, 2008).

We analyzed the associations between VIP/*Dra*I polymorphism and milk, fat and protein yield, the sum of fat and protein yields, as well as the percentage of fat and protein content, and the sum of fat and protein content in milk (detailed results of this analyses is shown in Table 1). Statistically significant differences, between animals with different VIP/*Dra*I genotypes, in the percentage of fat and protein in milk ($p \leq 0.05$) were observed.

Analyzing associations between VIP/*Dra*I genotype and three consecutive lactation milk yields, the yield of the GG genotype cows was found to be the highest in the second and third lactation. However, the resulting differences were not statistically confirmed. Similarly, the analysis of fat yield of the bovines with various VIP/*Dra*I genotypes failed to establish a statistical significance.

The analysis of associations between the VIP/*Dra*I polymorphism and the percentage of fat content in milk showed that in the 1st and 3rd lactations, milk from cows with VIP/*DraI* AA genotype had the highest fat content, but these differences were also not statistically confirmed. Concerning the 2nd lactation, the highest fat content was to be found in milk from cows with AG genotype, and the lowest in cows with genotype GG and these differences were statistically confirmed at a significant level ($p \leq 0.05$).

Table 1. Values of analyzed milk utility traits of Jersey cows depending on VIP genotype. All values are means ± standard deviation

Lactation	VIP genotype	n	Milk yield (kg)	Fat		Protein		F + P (%)	F + P (kg)
				(kg)	(%)	(kg)	(%)		
I	AA	86	4,127 ± 795	234 ± 43	5.71 ± 0.55	161 ± 28	3.92 ± 0.23	9.50 ± 1.12	395 ± 68
	AG	40	4,160 ± 824	236 ± 49	5.69 ± 0.52	161 ± 31	3.88 ± 0.18	9.61 ± 0.63	397 ± 78
	GG	55	4,150 ± 700	229 ± 37	5.56 ± 0.52	159 ± 25	3.85 ± 0.23	9.41 ± 0.68	388 ± 60
	Total	181	4,140 ± 770	233 ± 42	5.66 ± 0.54	160 ± 28	3.89 ± 0.22	9.49 ± 0.91	394 ± 68
	Significance of differences		ns	ns	ns	ns	ns	ns	ns
II	AA	77	4,629 ± 725	268 ± 40	5.77 ± 0.53	188 ± 28	4.03 ± 0.24	9.82 ± 0.69	455 ± 66
	AG	38	4,600 ± 790	269 ± 51	5.86 ± 0.56	184 ± 33	4.02 ± 0.22	9.89 ± 0.71	454 ± 83
	GG	49	4,631 ± 775	253 ± 40	5.50 ± 0.57	181 ± 27	3.91 ± 0.23	9.48 ± 0.88	434 ± 65
	Total	164	4,623 ± 751	264 ± 43	5.71 ± 0.56	185 ± 29	3.99 ± 0.24	9.74 ± 0.77	449 ± 70
	Significance of differences		ns	ns	*	ns	*	ns	ns
III	AA	56	5,149 ± 935	302 ± 55	5.87 ± 0.55	209 ± 39	4.07 ± 0.21	9.94 ± 0.67	511 ± 92
	AG	27	4,760 ± 694	275 ± 46	5.78 ± 0.54	192 ± 30	4.03 ± 0.17	9.81 ± 0.65	467 ± 75
	GG	37	5,194 ± 1,048	282 ± 54	5.47 ± 0.64	203 ± 38	3.93 ± 0.24	9.40 ± 0.81	485 ± 89
	Total	120	5,075 ± 933	290 ± 53	5.72 ± 0.60	204 ± 53	4.02 ± 0.22	9.74 ± 0.75	493 ± 89
	Significance of differences		ns	ns	ns	ns	*	ns	ns

*: differences significant at $p \leq 0.05$. ns: non-significant differences.

The analysis of protein yield in milk showed that in 2nd and 3rd lactations cows with VIP/*DraI* AA genotype had the highest amount of protein, but these differences were not statistically confirmed.

The analysis of associations between the VIP/*Dra*I polymorphism and the percentage protein content clearly proved that in all lactations, milk from the cows with the VIP/*DraI* AA genotype had the highest amount of protein. Furthermore, cows with the GG genotype had the lowest amount of protein in their milk. These differences were confirmed statistically at a significant level ($p \leq 0.05$).

The analysis of the percentage sum of fat and protein content, as well as the sum of fat and protein yields in three consecutive lactations of the tested herd of Jersey cows did not show any statistically significant difference between animals with different VIP/*DraI* genotypes.

In summary, as a result of the conducted ACRS-PCR analysis of the VIP gene fragment we found that two alleles exist in the examined herd of Jersey cows and they control the presence of three genotypes: AA, AG, GG. The AA genotype frequency was 0.48, whereas the AG genotype frequency was 0.22, and the GG genotype frequency was 0.30. The studied herd of cows was not in a genetic equilibrium as the number of VIP/*Dra*I genotypes was significantly different from the number of genotypes calculated according to the Hardy-Weinberg principle. Analyzed VIP/*Dra*I gene polymorphism with respect to milk utility traits showed slight differences in the fat percentage and protein content of milk in animals with different VIP/*Dra*I genotypes. As the number of records was low, further analyses are needed to confirm the results.

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References

Chorev M, Carmel L, 2012. The function of introns. Frontiers in Genetics 3: 1-15.

Edwards AWF, 2008. G. H. Hardy (1908) and Hardy-Weinberg equilibrium. Genetics 179: 1143-1150.

El Halawani ME, Youngren OM, Pitts GR, 1997. Vasoactive intestinal peptide as the avian prolactin releasing factor. In: Perspectives in avian endocrinology (Harvey S, Etches R, eds). The Society for Endocrinology, Bristol. pp: 403-416.

Gingold H, Pilpel Y, 2011. Determinants of translation efficiency and accuracy. Mol Syst Biol 7: 481.

Gozes I, Avidor R, Biegon A, Baldino F Jr, 1989. Lactation elevates vasoactive intestinal peptide messenger ribonucleic acid in rat suprachiasmatic nucleus. Endocinology 124: 81-186.

Hsu FR, Shia WCh, Lo WJ, Lin HCh, Chang HY, 2010. Discovering the relationship between single nucleotide polymorphisms and alternative splicing events. Proc 10th WSEAS Int Conf on Applied Informatics and Communications, and 3rd WSEAS Int Conf on Biomedical Electronics and Biomedical Informatics. Stevens Point. pp: 333-340.

Okamoto S, Okamura H, Miyake M, Takahashi Y, Takagi S, Akagi Y, Okamoto H, Ibata I, 1991. A diurnal variation of VIP mRNA under a daily light-dark cycle in the rat suprachiasmatic nucleus. Histochem 95: 525-528.

Parmley JL, Hurst LD, 2007. How do synonymous mutations affect fitness? Bioessays 29: 515-519.

Said SI, Filippatos GS, Gangopadhyay N, Lalude O, Parameswaran N, Spielman W, Uhal BD, 2001. Regulation of apoptosis by vasoactive peptides. Am J Physiol Lung Cell Mol Physiol 281(4): 749-761.

Shabalina SA, Ogurtsov AY, Spiridonov NA, 2006. A periodic pattern of mRNA secondary structure created by the genetic code. Nucl Acids Res 34: 2428-2437.

Sherwood NM, Kruecki SL, McRory JE, 2000. The origin and function of the pituitary adenylate-cyclase activating polypeptide glucagon superfamily. Endocr Rev 21: 619-670.

The Bovine Map Consortium, 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science 324: 528-532.

Youngren OM, Pitts GR, Phillips RE, El Halawani ME, 1996. Dopaminergic control of prolactin in the turkey. Neuroendocrinology 104: 225-230.