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**IMPACT OF CXCL SIGNALING NETWORK GENE POLYMORPHISMS  
ON RESISTANCE/SUSCEPTIBILITY TO BOVINE LEUKEMIA VIRUS (BLV)**

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**Summary.** Bovine leukemia virus (BLV) is an infectious agent that has taken enormous economic toll on cattle breeding worldwide. Effective breeding requires the search of molecular and genetic markers of susceptibility to this infectious disease. CXCL chemokines and their receptors play an important role during different stages of LV infection.

Was to analyze the correlation between gene polymorphisms of CXCL2,3 (GROX, GRO3) chemokines as well as their receptors CXCR2 (IL8B) and susceptibility to bovine leucosis. We also evaluated the possible functional contribution of the polymorphisms to disease pathogenesis.

The genotype study of cattle with different BLV status showed that C-allele presence in gene *IL8RB* (AH6–3 SNP) causes leukemia predisposition in viral carriers diagnosed as BLV-infected (AGID+, PCR-). The pattern was demonstrated on animals with leucosis (AGID+, PCR+) and healthy animals (AGID-, PCR-) (OR=1.51; P=95 %).

SNP markers of *GRO X* (AH-3–1, 3,5), *GRO 3* (AH5–3), and *IL8RB* (CXCR2) (AH 6–1,3) are useful to study both bovine lymphocytosis and lymphoid tumors. Further study on how SNP allelic diversity influences BLV susceptibility is required.

Resistance/susceptibility to tumor-associated infectious diseases depends on immune gene polymorphism, in particular on the genes of chemokine network, since chemokines are key messengers of intercellular

communications. Numerous findings confirm the association between gene polymorphism and the development of many virus-induced disease (Susek K. H. et al. 2018).

BLV belongs to genus *Deltaretrovirus* of the family *Retroviridae* and is closely related to human and simian T-cell leukemia virus (HTLV and STLV). These viruses are exogenous, have similar genomic organization and integrate into dispersed sites within the host genome. BLV generally infects mononuclear B cells (HTLV – T -cells) in peripheral blood, but the majority of BLV-infected animals remain clinically healthy. After the first infection virus persists in host for a long time. About one-third of infected cattle develop persistent B-cell lymphocytosis and only a few percent develop lymphoid tumors (Aida Y et al. 2017, Mirsky M.L et al.) This supports the presence of host and viral genetic component and the role of their microevolutionary interactions.

The aim of the present study was to investigate genotype and haplotype distributions of some genes of chemokine network (CXCL (GROX and GRO3) /CXCR2 (IL8)) in populations of Black- and White x Holstein cross-breed cattle and to estimate their impact on resistance/susceptibility to BLV infection.

The blood was collected from Black and White Holstein (n=340) cross breed cattle at two farms, Tulinskoye (with low prevalence BLV infection) and Kremlevskoe (with high prevalence BLV infection) in Novosibirsk region. The DNA blood extraction was performed using the standard technique. The DNA amplification was carried out under standard conditions in the volume of 15–50 µl using DNA-polymerase. Locus-specific PCR for genotyping was performed as it described previously (Heaton M. P. et al. 1999) with some modifications. For RFLP-analysis of signaling network genes CXCL (GROX, GRO3 and CXCR2 (IL8RB)), we used the following restriction endonucleases: HinfI (AH3–1), VspI (AH3–3), Sse9I (AH3–2), MspR9I (AH3–5), Sau 3AI (AH6–1), HpaII (AH6–2) (SibEnzyme, Novosibirsk).

Commercial diagnostic test based on agar gel immunodiffusion (AGID) was used to detect BLV. Additional PCR analysis of BLV infection was described previously (Licursi M. et al. 2002). The following groups of animals have been formed depending on their BLV-status: 1) AGID-PCR-: healthy animals (control group); 2) BLV-infected (AGID+ PCR-) 3) animals with leucosis (AGID+, PCR+). Statistical processing was performed using SPSS software.

The genotype study of cattle with different BLV status

Some of previously identified SNP-markers of cytokine network genes (Heaton M. P. et al. 1999) were chosen for analysis: chemokine GRO family (GROX and GRO3), their common receptor IL8RB. These molecules are believed to be involved in immune protection and tumor angiogenesis. The PCR-RFLP genotyping results for animal groups with different BLV status are presented in Table 1.

Table 1

**Frequency of SNP genotypes in cattle with different BLV status**

SNP	Genotype	AGID-PCR-	AGID+PCR-	AGID+PCR+	Chi <sup>2</sup>	P
1	2	3	4	5	6	7
<i>GROX</i>						
<i>AH3–1</i>	GG	27.59	8.00	7.69	11.056	0.026
	TG	55.17	40.00	51.28		
	TT	17.24	52.00	41.03		
<i>AH3–2</i>	AA	100.00	100.00	100.00		
<i>AH3–3</i>	AA	27.59	8.00	7.69	11.056	0.026
	GA	55.17	40.00	51.28		
	GG	17.24	52.00	41.03		
<i>AH3–5</i>	CC	17.24	52.00	41.03	11.056	0.026
	CG	55.17	40.00	51.28		
	GG	27.59	8.00	7.69		
<i>GRO 3</i>						
<i>AH5–1</i>	AG	100.00	100.00	100.00		
<i>AH5–2</i>	CC	9.09	13.04	8.33	5.169	0.270
	TC	54.55	26.09	30.56		
	TT	36.36	60.87	61.11		

Continuation table 1

1	2	3	4	5	6	7
AH5-3	GG	0.00	0.00	2.56	14.888	0.005
	TG	7.69	56.52	35.90		
	TT	92.31	43.48	61.54		
1	2	3	4	5	6	7
<i>IL8RB (CXCR2)</i>						
AH6-1	AA	0.00	0.00	10.26	7.448	0.114
	CA	10.71	24.00	17.95		
	CC	89.29	76.00	71.79		
AH6-2	AA	0.00	0.00	2.56	9.250	0.055
	CA	3.45	29.17	28.21		
	CC	96.55	70.83	69.23		
AH6-3	CC	0.00	16.00	7.89	15.348	0.053
	CG	0.00	8.00	18.42		
	GG	100.00	76.00	71.05		

Table 1 clearly shows that genotype distribution patterns have locus- and SNP-specificity. Simultaneous genotype frequency comparison revealed the significance of the most of analyzed SNPs (gray line), with the exception of AH5-1, 2, and AH6-1, To better understand the role of SNP in leukemia development we have performed a pairwise comparison of animal groups to look at polymorphism patterns in different BLV statuses (Table 2).

Table 2

**Pairwise comparison of SNP genotype frequency distribution in animal groups with different BLV status.**

SNP	Significance level (P) by Chi <sup>2</sup>		
	AGID-PCR- vs AGID+ PCR-	AGID+PCR+ vs AGID+PCR-	AGID+PCR+ vs AGID-PCR-
AH3-1,3,5	0.108	0.006	0.313
AH5-2	0.589	0.116	0.501
AH5-3	0.046	0.286	0.591
AH6-1	0.471	0.336	0.060
AH6-2	0.037	0.131	0.087
AH6-3	0.308	0.331	0.047

Table shows that differences in AH3 GRO2 (X) SNP marker distribution patterns (gray line) were most significant between animals with leucosis (AGID+PCR+) and animals BLV infected animals (virus carries (AGID+ PCR-). Significant differences were also identified for distribution of AH5-3 GRO3 and AH6-2 IL8R SNP markers for BLV infected animals (AGID+PCR-) vs healthy animals (AGID+PCR-). Comparison of healthy animals and animals with leucosis («disease-susceptible») showed significant differences in AH6-3 IL8RB marker distribution. So, these are the markers of infection predisposition and disease progression.

OR (odds ratio) analysis suggests that C-allele presence in gene IL8RB AH6-3 (CC+CG) SNP marker causes leukemia predisposition. The pattern was observed for both animals with leucosis (AGID+, PCR+) and healthy animals (AGID-, PCR-) (OR=1.51; P=95%). For all the other SNP markers we obtained OR<1.5; P=95%.

Thus, there are clear evidence that suggest various chemokine network genes and their allelotypes are involved in different stages of virus leukemia.

SNP markers of *GRO X* (AH3-1, 3,5), *GRO 3* (AH5-3), *IL8RB (CXCR2)* (AH 6-1,3) will be useful to study bovine lymphocytosis and lymphoid tumors.

The mechanisms of the effect of allelic variability of chemokine and their receptors genes on susceptibility to bovine lymphocytosis require further study.

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## REFERENCES

1. *Mechanisms* of pathogenesis induced by bovine leukemia virus as a model for human T-cell leukemia virus /Aida Y., Murakami H., Takahashi, M., [et al.] //Front Microbiol. 2013; 4: 328 p.
2. *Interleukin-8* haplotype structure from nucleotide sequence variation in commercial populations of U.S. beef cattle. /Heaton M.P., Chitko-McKown C.G., Grosse W.M. [et al.] // Mamm. Genome. – 12. – 2001. P. 219–226
3. *Licursi M, Inoshima Y, Wu D, Yokoyama T, González ET, Sentsui H.* Genetic heterogeneity among bovine leukemia virus genotypes and its relation to humoral responses in hosts. – Virus Res. – 2002. – V. 86. – P. 101–110.
4. The prevalence of proviral bovine leukemia virus in peripheral blood mononuclear cells at two subclinical stages of infection. /Mirsky M.L. Olimstead C.A., Lewin H.A. [et al.] // J. Virol. – 70. – 1996. – P. 2178–2183.
5. *The Role of CXCC chemokine Receptors 1–4 on Immune Cells in the Tumor Microenvironment/* Susek K. H., Karvouni M., Alici E and Lundqvist A. – 2018 – V.9. – P. 1–10.