ASSOCIATION OF *SLC11A1* GENE AND PRODUCTION TRAITS USING LOGISTIC REGRESSION

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SlC11A1 gene is a potential candidate gene related to susceptibility to a wide range of intracellular pathogens. In this research, we used logistic regression analysis in a case-control study to find the relationship between *SLC11A1* gene and estimated breeding values of production traits (estimated breeding values of protein and fat yield, and protein and fat percentage) in Holstein dairy cattle in a selective genotyping method. Samples have been genotyped based on clinical mastitis data using PCR-SSCP method, previously. The contrast between heterozygous genotype and both homozygous genotypes showed that despite of significant relationship between clinical mastitis and *SLC11A1*, there was no negative effect of the gene of interest on production traits. Hence, considering the unfavorable relationship between clinical mastitis and production traits, selection of *SLC11A1* gene will have no unfavorable effect on production traits, and it will not disorganize selection breeding programs based on health data.

Keywords: *SLC11A1* gene, protein percentage, fat yield, logistic regression, mastitis.

INTRODUCTION

Solute Carrier family 11 members 1 (*SlC11A1*) gene, which has been previously nominated as Natural Resistance Associated Macrophage Protein-1 (*NRAMP1*) gene, is related

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to susceptibility to lots of intracellular pathogens. It has been mapped on chromosome 2 and is composed of 39 exons and 38 introns. *SlC11A1* gene encodes a divalent action transporter that has a key role in innate immunity which promotes bacterial killing by macrophages (VIDAL *et al.*, 1995). This gene can be expressed in reticuloendothelial and CD11c+ dendritic cells, and it has pleiotropic effects on cytokine transcription, major histocompatibility complex (MHC) class II molecule expression and presentation of protein antigens to T lymphocytes (STOBER *et al.*, 2007). Hence, the host can be resistant to specific pathogens. The multi-pass membrane protein encoded by this gene, functions as a divalent transition metal (iron and manganese) transporter involved in iron metabolism.

Several studies have shown that mutations in *SlC11A1* gene are related to susceptibility to infectious diseases such as tuberculosis, leprosy, and inflammatory diseases. Bovine *SLC11A1* gene is associated with natural resistance against brucellosis in cattle and buffalo (CAPPARELLI *et al.*, 2007; MARTINEZ *et al.*, 2008). A moderate variation in the coding sequence of *SlC11A1* gene in cows has been found and it was shown that it would be a sign of selection pressure on this gene (GERNAND *et al.*, 2012). BAGHERI *et al.* (2016) have shown that *SLC11A1* gene plays a critical role in mastitis resistance. Since, there is an antagonistic relation between udder health and production traits in dairy cattle, production traits should also be considered in selection programs for health traits, as well. The main objective of this short report was to identify the relationship between bovine *SLC11A1* gene and estimated breeding values (EBVs) of milk production traits i.e. fat yield and protein and fat percentage (which are known as important economic traits and determining factors of milk economic value) based on clinical mastitis data following our previous study, in order to find whether or not selection programs based on health traits can unfavorably affect the production traits.

MATERIALS AND METHODS

Data structure has been described in BAGHERI *et al.* (2013) comprehensively. Concisely, 1,647 clinical mastitis (CM) cases of Holstein dairy cows of two populations (from two different areas) were recorded as the pool of cows for selective genotyping, i.e. the most resistant and the most susceptible groups for CM was extracted based on values for clinical mastitis residuals (CMR). The number of CM cases per lactation was analyzed by applying GLM procedure in SAS version 9.2 for analysis of variance. Two extreme groups including 135 cows per group based on values for CMR were extracted for selective genotyping. After genotyping samples for *SLC11A1* gene (NM_174652.2), association analysis was carried out using SAS Glimmix macro (SCHABENBERGER, 2007) logistic analysis for estimation of SNP effects.

The statistical model (1) for estimation of probability of genotype GC versus genotype CC or GC versus genotype GG was defined as follow:

$$logit\ (\pi_r) = \log[\frac{\pi_r}{1-\pi_r}] = a + bY_r \tag{1}$$

where, π_r is the probability of genotype GC of cow$_r$; a is the intercept; Yr is the EBVs for production traits and b is the linear regression coefficient of genotype GC on EBVs.

The significance test of linear regression coefficient (b) was at first based on sum of squares type I tests as implemented in Glimmix macro (KÖNIG *et al.*, 2005). Then, for the second step, the contrast (α) of the heterozygous genotype to the homozygous genotypes, i.e. the effect of

genotype GC in contrast to genotype CC or genotype GC in contrast to genotype GG, were estimated as described by SHARMA *et al*. (2006) using below equation

$$\alpha = \frac{-1 + \sqrt{1 = b^2 \sigma_X^2}}{b} \tag{2}$$

which, σ_X^2 demonstrates the variance of EBVs in the unselected base population.

The identified locus was bi-allelic, with three different genotypes (GC, GG and CC). Both homozygous genotypes (GG and CC) were contrasted to the heterozygous genotype GC in two consecutive runs, i.e. first contrasting GC to GG, and second, contrasting GC to CC.

RESULTS AND DISCUSSION

Polymerase chain reaction–mediated single strand conformation polymorphism (PCR-SSCP) results showed a polymorphism due to a mutation at position 1139 (NM_174652.2:c.1139 C > G). More information on SSCP variants has been presented in BAGHERI *et al*. (2016). Differences in EBVs for production traits when comparing the heterozygous genotype to both homozygous genotypes of *SLC11A1* SNP are given in Table 1. In spite of the positive significant effect of *SLC11A1* gene on clinical mastitis resistance, which has been previously shown (BAGHERI *et al*., 2016), no significant relation was found between this gene and production traits (protein and fat yield and protein and fat percentage).

Table 1. *Differences in EBVs of production traits when comparing the heterozygous genotype versus both homozygous genotypes for SLC11A1 gene.*

	Difference CG vs. GG		b- value	Difference CG vs. CC		b-value
	General units	SD units		General units	SD units	
Protein yield EBV [1]	10.9	0.4	0.16 [ns]	0.46	0.02	0.0006[ns]
Protein % EBV	0.1	0.14	0.02 [ns]	-0.04	-0.05	-0.07 [ns]
fat yield EBV	-0.79	-0.04	-0.002 [ns]	-0.54	-0.03	-.001 [ns]
fat% EBV	-0.06	-0.41	-3.19 [ns]	-0.03	-0.22	-1.62 [ns]

[ns] non-significant ($P > 0.05$)

[1] Protein yield data are resulted from Bagheri *et al*., 2016.

It should be kept in mind that selection of specific alleles of important genes may improve udder health; however, correlated response for other economic traits should not be ignored. Selection for health traits could be disturb improvement of the production traits, so, in the selection strategy, selection of genes of interest should be based on their relation. Based on quantitative genetics methodology, the genetic correlation between milk production traits and resistance to clinical mastitis are antagonistic (GERNAND *et al*., 2012). Fifty percent of selection emphasis belongs to production traits when used in combination with other breeding goals in most breeding programs for dairy cattle (MIGLIOR *et al*., 2005). Hence, it is suggested that both

health and production traits to be considered simultaneously in selection breeding programs. There are several genes that simultaneously have positive effects on production traits and health traits such as clinical mastitis resistance (BAGHERI *et al*., 2013; BAGHERI *et al*., 2016), which can be simply used in selection strategies for dairy cattle programs. On the other hand, some genes have positive effects on production traits, while increasing the incidence of clinical mastitis cases (HERINGSTAD *et al*., 2007). In spite of significant relationship between the interested single nucleotide polymorphism (SNP) and clinical mastitis residuals, results showed that there was no relationship between this SNP and production traits in cow.

CONCLUSION

Overall, based on our findings and considering the well-known unfavorable relationship between clinical mastitis and production traits, selection of SLC11A1 gene will have no unfavorable effect on production traits, and it will not disorganize selection breeding programs based on health data. Therefore, we can insert this gene as selection criteria for improving mastitis resistance without interrupting in production traits.

REFERENCES

BAGHERI, M., R., MIRAIE-ASHTIANI, M., MORADI-SHARHRBABAK, A., NEJATI-JAVAREMI, A., PAKDEL, U.U., VON BORSTEL, E.C.G., PIMENTEL, S., KÖNIG (2013): Selective genotyping and   logistic regression analyses to identify favorable SNP-genotypes for clinical mastitis and production traits in Holstein dairy cattle. Livest. Sci., *151*: 140-151.

BAGHERI, M., M., MORADI-SHARHRBABAK, R., MIRAIE-ASHTIANI, M., SAFDARI-SHAHROUDI, R., ABDOLLAHI-ARPANAHI (2016): Case–control approach application for finding a relationship between candidate genes and clinical mastitis in Holsteindairy cattle. J. Appl. Genetics., *57*: 107-112.

CAPPARELLI, R., F., ALFANO, M.G., AMOROSO, G., BORRIELLO, D., FENIZIA, A., BIANCO, S., ROPERTO, F., ROPERTO, D., IANNELLI (2007): Protective Effect of the Nramp1 BB Genotype against Brucella abortus in the Water Buffalo (*Bubalus bubalis*). Infect. Immun.*, 75*: 988-996.

GERNAND, E., P., REHBEIN, U.U., VON BORSTEL, S., KÖNIG (2012): Incidences of and genetic parameters for mastitis, claw disorders, and common health traits recorded in dairy cattle contract herds. J. Dairy Sci., *95*: 2144-2156.

HERINGSTAD, B., G., KLEMETSDAL, T., STEINE (2007): Selection responses for disease resistance in two selection experiments with Norwegian Red cows. J. Dairy Sci., *90*: 2419-2426.

KÖNIG, S., A.R., SHARIFI, H., WENTROT, D., LANDMANN, M., EISE, H., SIMIANER (2005):   Genetic parameters of claw and foot disorders estimated with logistic models. J.   Dairy Sci., *88*: 3316-3325.

MARTINEZ, R., S., DUNNER, G., BARRERA1, J., CANON (2008): Novel variants within the coding regions of the *SLC11A1* gene identified in *Bos taurus* and *Bos indicus* breeds. J. Anim. Breed. Genet., *125*: 57–62.

MIGLIOR, F., B.L., MUIR, B.J., VAN DORMAAL (2005): Selection indices in Holstein cattle of various countries. J. Dairy Sci., *88*: 1255-1263.

SCHABENBERGER, O. (2007): Growing up fast: SAS 9.2 enhancements to the GLIMMIX procedure SAS Global Forum Citeseer. SAS Institute Inc. Cary. NC (US).

SHARMA, B.S., G.B., JANSEN, N.A., KARROW, D., KELTON, Z., JLANG (2006): Detection and characterization of amplified length polymorphism markers for clinical mastitis in Canadian Holsteins. J. Dairy Sci., *89*: 3653-3663.

STOBER, C.B., S., BRODE, J.K., WHITE, J.F., POPOFF, J.M., BLACKWELL (2007): Slc11a1, Formerly Nramp1, is expressed in dendritic cells and influences major histocompatibility complex class II expression and antigen-presenting cell function. Infect. Immun., *75 (10)*: 5059–5067.

VIDAL, S.M., M.L., TREMBLAY, G., GOVONI, S., GAUTHIER, G., SEBASTIANI, D., MALO (1995): The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. J. Exp. Med., *182*: 655-666.

ASOCIACIJA *SLC11A1* GENA I PROIZVODNIH OSOBINA UPOTREBOM LOGISTIČKE REGRESIJE

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Izvod

SlC11A1 je gen potencijalnih kandidata koji se odnosi na podložnost širokom opsegu intracelularnih patogena. U ovom istraživanju koristili smo logističku regresionu analizu u proučavanju slučaj-kontrola kako bismo pronašli vezu između gena *SLC11A1* i procenjene oplemenjivačke vrednosti proizvodnih osobina (procenjene oplemenjivačke vrednosti belančevina i masti, kao i procenat proteina i masti) u Holstein mlečnim govedima metodom selektivne genotipizacije. Uzorci su prethodno genotipizovani na osnovu kliničkih podataka o mastitisu pomoću metode PCR-SSCP. Kontrast između heterozigotnog genotipa i oba homozigotna genotipa pokazao je da, uprkos značajnoj vezi između kliničkog mastitisa i *SLC11A1*, nije bilo negativnog uticaja gena od interesa na proizvodne osobine. Stoga, imajući u vidu nepovoljan odnos između kliničkog mastitisa i proizvodnih osobina, selekcija gena *SLC11A1* neće imati nepovoljan efekat na proizvodne osobine i neće dezorganizovati selekcione programe zasnovane na zdravstvenim podacima.