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Il1b Gene Expression and Association With Scs in Guilan Native and Holstein Cow Populations and Their Reciprocal Crosses

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Abstract

IL1B gene, as one of the interleukine-1 (IL-1) members, involves in inflammatory and immune responses, also the expression of this gene will increase due to the immune response in milk somatic cells. In the current study, the IL1B gene expression was compared among four different genotypes including native and purebred cows and their crosses. Real-time PCR technique was used to evaluate the expression of the IL1B target gene by applying two internal control genes (GAPDH and RPLP0). The highest and lowest IL1B gene expression was obtained in Guilan native and Holstein-sired crossbred cows, respectively. Statistical analysis revealed that there are significant expression differences of IL1B gene amongst studied populations ($p<0.05$). The highest (14.18) and lowest (13.27) SCS was observed in the Guilan native-sired crossbred cows and Guilan native cows, respectively. A significant negative correlation (-0.34) was obtained for IL1B gene expression and SCS of studied populations ($p < 0.05$). A negative correlation between IL1B gene expression and SCS may show the importance of higher expression of IL1B gene in immune responses related to cow udder infection. The higher fold-change ratio of IL1B gene in the robust native cows to the other studied groups showed that the importance of this gene in resistance and tolerance.

Keywords: IL1B gene, crossbreeding, SCS, native cow, gene expression.

INTRODUCTION

The negative association of disease resistance and production traits and the polygenic nature of such traits are some of the animal breeding main problems. One of the most important goals of crossbreeding programs is to use positive traits in both purebreds to obtain high production in line with stabilizing resistance to diseases and harsh environmental conditions, but usually crossbred animals are more vulnerable to diseases and environmental stresses compare to native cows. So, it's necessary to investigate the genetic resistance and immune capacity of crossbreds like their pure parents. To reach this goal, biological markers (biomarkers) detection could help to evaluate genetic changes in different populations [1]. Studying genes expression in different pure and cross breeds can help to understand the effect of crossbreeding in phenotype and genotype variations and evaluate correct biomarkers in crossbreeding programs.

Interleukin-1 (IL-1) family includes 11 members which are involved in local and systemic inflammation, also protect against infections and immune responses. Interleukin-1 beta (IL1B) is one of the IL-1 family members and produced by activated macrophages as a pro-protein, which is proteolytically processed to its active form by caspase-1. This cytokine is one of the most important mediators of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [2]. During udder infection and in the initial steps of immune response the level of IL1B gene expression increased in milk somatic cells [3]. Association of IL1B gene and SCS (Somatic Cell Score) due to mastitis inoculation has been investigated in several studies. Griesbeck-Zilch et al., [4] first selected high and low resistant cows to mastitis base on the level of SCS, then they evaluated the IL1B gene expression after 1, 6 and 24 hours after mastitis infection. Their results showed the level of IL1B gene expression increased in selected animals due to mastitis and reaches to maximum level after 6 hour of infection. Brand et al., [5] using *Escherichia coli* and *Staphylococcus aureus* inoculation induces mastitis and profiled the different genes expression in udder epithelial cells. They revealed that the IL1B gene expression increased during cells maturation and acute phase responses. Also the level of IL1B gene expression increased during different infectious agents such as *Mycoplasma bovis* and *Klebsiella pneumoniae* [6, 7].

The goals of the current study are to exploring the IL1B gene expression variations amongst Guilan native and Holstein purebred cattle population and their crossbreds, also studying the effect of IL1B gene expression on the number of milk somatic cells under normal environmental condition of breeding of these animals. Guilan native and Holstein cow have indicine (*Bos indicus*) and taurine (*Bos taurus*) origins, respectively; so understanding the biomarker variations in these two populations and their crosses willingly giving us valuable information about biological variations of them.

MATERIALS AND METHODS

The tail vein blood samples of 44 cattle were collected using heparinized venoject tubes from Guilan native cow protection station located in Hossein-koh of Foman city (Guilan province, Iran) and immediately (less than one hour) were transferred on ice (4 ^{0}C) to the laboratory for RNA extraction. Sampling were performed from 14 Guilan native (N), 12 Holstein (H), 8 Holstein-sired crossbred cows (CSH), and 10 Guilan native-sired crossbred cows (CSN). Animals were kept in the same food, environment, and management conditions and all samples were collected in the same season (spring 2014). All the experimental animals were tested for mastitis during samples collection, including measuring milk SCC (Somatic Cell Count). We used Ecomilk scan device (SEL ESKAN-290) for SCC measurement. The results of SCC tests were transferred to SCS (Somatic Cell Score) using $SCS=\log_2 (SCC/100) + 3$ equation to achieve normalized distribution of the collected data [8].

After separation of blood leukocytes from at least 4 ml of fresh blood with 2000 g at 4 ^{0}C for 10 minute and two-step RBC's (Red Blood Cells) and centrifugation with 2000 g at 4 ^{0}C for 10 minute, RNA extraction were performed using TRIzol (Invitrogen, USA) method [9]. The quality and quantity of extracted RNA's were performed using NanoDrop-2000 spectrophotometer device (Thermo Scientific), the 260 to 280 ratio and 0.8 percent agarose gel electrophoresis (80 voltages for 30 minute). Based on extracted RNA's quantity or concentration, the cDNA synthesis of all samples was performed using cDNA synthesis kit (Thermo Scientific) following manufacturer instruction. The forward and reverse primers of the target (IL1B) and two internal control genes (GAPDH and RPLP0) were designed (Table 1). To obtain optimum temperature for annealing of primers, we run Real-time PCR at 12.5 microliter (6.25 microliter of SYBR green master mix, 0.4 microliter of 10 mill molar forward and reverse primers and 4.45 microliter of nuclease-free ddH$_2$O) using gradient temperature procedure, including 5-minute initial annealing at 95 ºC, 40 cycles with three steps of 30 second at 95 ºC, 30 second at annealing temperature between 55 to 65 ºC for 8 different temperatures and 30 second at 72 ºC. To obtain amplification curve, plate reads were performed after these cycles and then 60 cycles for 5 second with increasing temperature to 0.5 ºC at each cycle were performed from 65 to 95 ºC to obtain melt curve of each sample.

Table 1. Specifications of all primers used for studying of IL1B gene expression

Name	Sequence	Melting temperature (ºC)	Product size (bp)	Annealing temperature (ºC)
IL1B-F	5' TCTTCCTGGGACATTTCG 3'	55.6	118	60
IL1B-R	5' CAGTCCTCGGGGTTATTCA 3'	55.4		
GAPDH-F	5' CATTGCCCTCAACGACCA 3'	57.8	77	60
GAPDH-R	5' CCACCACCCTGTTGCTGTAG 3'	58.5		
RPLP0-F	5' CAACCCTGAAGTGCTTGACAT 3'	57.5	189	60
RPLP0-R	5' GCAAGTGGGAAGGTGTAATCA 3'	57.6		

The Real-time PCR reactions of each sample for all primer were done the same as gradient temperature procedure except the annealing temperature of 40 cycles which was run at the annealing temperature of each primer. For all Real-time PCR reactions, we used Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific) and CFX96 device (Bio-Rad).

LinRegPCR software was used to obtain the amplification efficiency of each sample [10]. We applied the raw dataset of Real-time PCR outputs to LinRegPCR software and using the amplification curve of each sample for all primers the relative normalized expression of all samples were calculated. Using geNorm software the two internal control genes (GAPDH and RPLP0) were evaluated and the normalization factor for each sample was obtained [11]. The geNorm software using the relative expression of genes provided the geometric average of each gene and M-value for each sample. The M-value's lower than 1.5 for genes are acceptable as trustable genes as internal control genes. In this experiment, M-value was 1.35 for both internal control genes (GAPDH and RPLP0). The relative normalized expression of the target gene for all samples was calculated with the dividing the relative expression (from LinRegPCR software) on normalization factor (from geNorm software). Therefore, the relative expression of each gene for each sample was corrected and normalized using the geometric mean of both genes and this could impact on the reduction of genes expression variations [11].

To test significant expression of the studied gene, we used GLMMIX procedure of SAS 9.2 software (SAS institute, Cary, NC). Tukey's test was performed to compare the mean expression of IL1B gene expression in different populations. Moreover, Sigmaplot 11.2.0.5 (Systat Software, Inc, Germany) was used to assess the association of the IL1B gene with milk SCS ($p < 0.05$).

RESULTS AND DISCUSSION

The IL1B gene expression in blood leukocytes was statistically different amongst four studied groups ($p < 0.05$). The highest and lowest relative normalized expression of IL1B gene was in N and CSH, respectively (Figure 1). The IL1B gene revealed higher significant expression in Guilan native population in comparison with CSH, but the results did not show any statistically significant difference between N and H and also CSN.

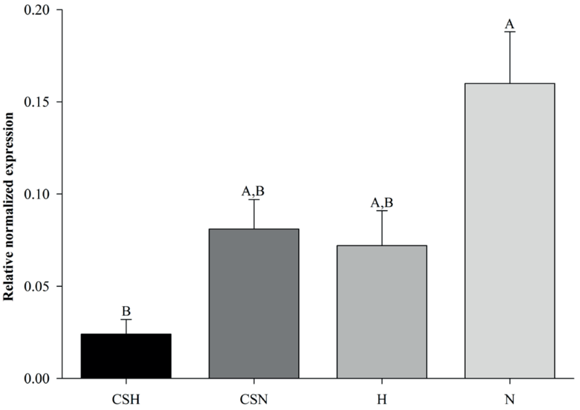

Figure 1. The normalized expression of IL1B gene in the four studied groups. Bars represent standard errors and words on the bars show the results of Tukey test ($p < 0.05$). (CSH = sire Holstein and dam Guilan native crossbred, CSN = Guilan native and dam Holstein crossbred, H = Holstein, and N = Guilan native cattle)

Table 2 represents the fold change ratio for the IL1B gene. The IL1B gene displayed a higher fold change ratio for N compare to all other populations. However, CSH showed a lower fold change ratio compare to the other populations. The highest fold change ratio belongs to N compare to CSH (6.56 times) and the lowest was CSH to N (0.15 times).

Table 2. Fold change ratio of IL1B gene in the different cow populations *

	N	H	CSN	CSH
N	1.00	0.46	0.52	0.15
H	2.18	1.00	1.13	0.33
CSN	1.94	0.89	1.00	0.30
CSH	6.56	3.01	3.38	1.00

* Reading of numbers is column-based, the amount of normalized expression of columns were divided on rows

While the CSH population had SCS higher than 14 (200,000 cells per milliliter), the other populations had SCS lower than 14. Usually, in Iran SCS higher than 14 is considered as subclinical cases of mastitis. None of the studied cows showed clinical mastitis. The highest and lowest average of SCS was observed in CSH and N cow populations, respectively (Table 3).

Table 3. Average of SCS in the four studied populations

Population	SCS Mean	Standard deviation	Max	Min
CSH	13.96	0.83	14.76	12.81
CSN	14.18	1.31	16.87	12.92
H	13.62	0.71	14.84	12.81
N	13.27	0.56	14.52	12.17

The relative normalized expression of IL1B gene revealed significant negative coefficient correlation (-0.34) with SCS in the studied populations ($p < 0.05$, Figure 2).

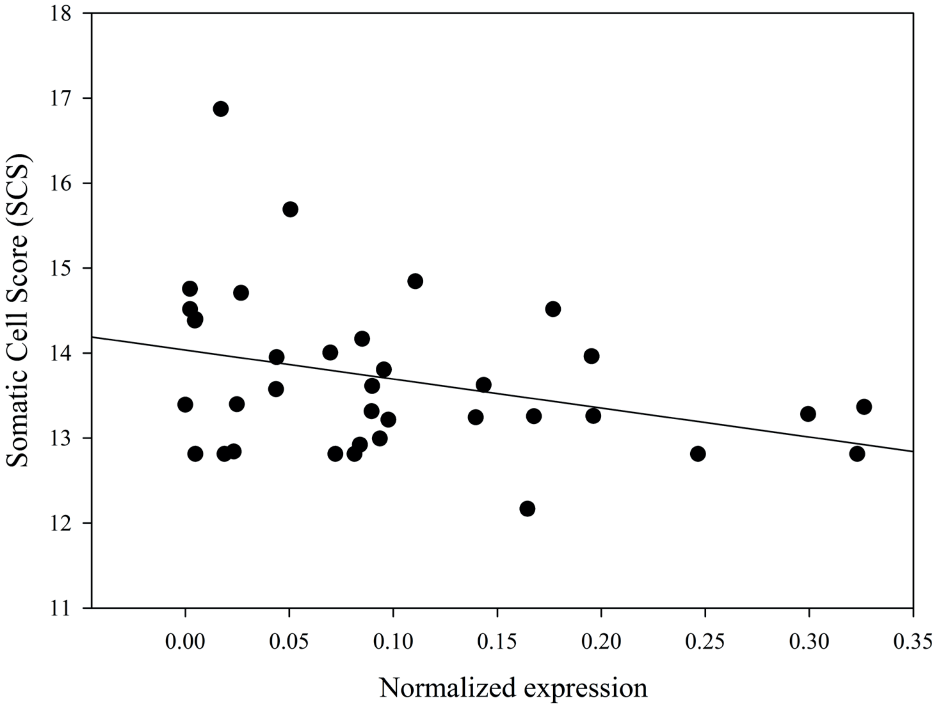

Figure 2. Simple scattered regression plots of relative normalized expression of IL1B gene vs. SCS

The results displayed a higher expression of IL1B gene in Guilan native (*Bos indicus*) (Figure 1). The IL-1 family has an indirect effect on immune functions. IL-1β induces gene expression and synthesis of cyclooxygenase type 2 (COX-2), type 2 phospholipase A, inducible nitric oxide synthase (iNOS), platelet-activating factor and nitric oxide (NO) production which is related to immune responses [2, 12-14]. Higher expression of IL1B gene in native cow implied that the resistance and tolerance in this kind of animals are related to higher expression of some immune-related genes.

Another pro-inflammatory characteristic of IL-1β is its ability to increase the expression of adhesion molecules such as intercellular adhesion molecule-1 on mesenchymal cells and vascular cell adhesion molecule−1 on endothelial cells. Together with the induction of chemokine's, these properties of IL-1β promote the infiltration of inflammatory and immunocompetent cells from the circulation into the extravascular space and then into tissues where tissue remodeling is the end result of chronic IL-1-induced inflammation [2].

The expression of IL1B gene in CSH cows was completely lower than N, H and CSN cows. Usually, crossbred cows dispersed in different rural and forest areas in the Caspian Sea coast are sire Holstein and dam Guilan native, like CSH cows. These crossbred cows are produced by artificial insemination of Holstein semen. The result of this study showed that the crossbred cows had lower expression of IL1B gene. Huang et al., [15] reported higher expression of IL1B gene in *Bos indicus* (Cholistani breed) compare to *Bos taurus* (Holstein and Jersey breeds) which is in accordance with our findings. Glass and Jensen [16] showed that there are no significant differences between Sahiwal (*Bos indicus*) and Holstein (*Bos taurus*) breeds for resistance to parasites ($p<0.05$). In addition, Baranwal et al., [17] investigated the TLR2 activity in monocyte-derived macrophages of zebu (Tharparkar) and crossbred (Holstein-Friesian × Jersey × Brown Swiss × Hariana) cattle. They did not found significant different activity of TLR2 in Tharparkar compare to crossbred cattle.

In this study, we observed significant the coefficient correlation of IL1B gene expression and SCS ($p < 0.05$). The negative correlation between IL1B gene expression and SCS may show the importance of higher expression of IL1B gene in immune responses related to cow udder infections like *Escherichia coli* and *Staphylococcus aureus* [5, 18]. Griesbeck-Zilch et al., [19] reported a positive correlation of mastitis incidence and increases in IL1B gene expression. Domingues et al., [20] found that the kind of up-regulated genes in resistant cows group was different from susceptible group. Regarding the observed IL1B gene expression differences between purebred and crossbred populations, the IL1B gene could be used as a biomarker in crossbreeding programs. Moreover, the reciprocal crossing or changing the parental lines had a major effect on IL1B gene expression. It showed heterosis and dominant effects may change by applying resistant or susceptible breed as a sire. The CSN cows had an intermediate gene expression and also the SCS indicator (Figure 1).

CONCLUSION

Guilan crossbred cattle population are steeply increasing [21]; if there would be no control on the crossbreeding programs, certainly we will lose most of Guilan native cattle as a genetic resource adapted to harsh environment, diseases, and food scarce of mountain and plains regions of Caspian Sea coast in Iran. However, the alternative program can be considered such as applying native cow as a sire after we establish an intelligent system for controlling of the level purebred blood shares.

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