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Research Article

Evaluation of Presence of MHC Class II Susceptible and Resistant Alleles to Oncovirus in Seropositive and Seronegative Holstein- Frisian Dairy Cattle in Iran

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Abstract

Bovine leukemia virus (BLV) is an exogenous C- type oncovirus in the Retroviridae family. This virus is an important model for human T-cell leukemia virus type 1 infection because they shared molecular and biological features. The BOLA- DRB3.2 gene is a part of the major histocompatibility complex (MHC) class II in cattle and its association with susceptibility and/or resistance to development of different forms of BLV infection has been confirmed. One hundred and ninety six blood samples from Holstein- Frisian cattle of two different dairy farms were taken in this research. The alleles' typing was carried out using polymerase chain reaction and restriction fragment length polymorphism (PCR- RFLP). The association between the BOLA- DRB 3.2 alleles and oncovirus infection profile was determined by odds ratio. In native population the frequency of BOLA- DRB3.2 alleles 11 and 28 (resistant alleles to oncovirus infection) were 2%. The frequency of BOLA- DRB 3.2* alleles 22, 24 and 8 (susceptible alleles to oncovirus infection) were 8%, 6% and 6%, respectively. In Holstein dairy cows the frequency of BOLA- DRB3.2* alleles 6 and 12 showed significant association with susceptibility to oncovirus infection (OR= 1.034; P< 0.02 and OR= 1.028; P< 0037).

Keywords: MHC class II; Oncovirus; BOLA- DBR3.2; PCR-RFLP

Introduction

Major histocompatibility complex (MHC) has a key role in disease susceptibility/resistance in humans. In animals such investigations are much less carried out, but preliminary research works showed the relationship of MHC with resistance to diseases. The major histocompatibility complex genes (MHC) encoded MHC class I and class II proteins are responsible for present peptide fragments to T cells. Thereby, these polymorphic presenting peptide fragments derived from antigens to T cells. Thereby, these polymorphic proteins critically influence susceptibility to infectious diseases.

The study of the genetic diversity of native breeds is necessary to maintain the genetic resources in livestock. It has also been reported that genetic variability exists with regard to resistance to infectious diseases. Some animals are more susceptible than others and the background of this mechanism is strongly associated with the major histocompatibility complex (MHC) genes [1,2]. The genetic structure of MHC system in different mammalian species is similar. MHC genes in human are located on chromosome 6 and have about 4 Mbp (Mega base pair). Histocompatibility complex in cattle called BoLA and is located on autosomal 23 and spans about 2.5 Mbp [3].

The bovine leukaemia virus (BLV), the aetiological agent of bovine lymphosarcoma, is the prototype of the genus Delta retrovirus, which belongs to the family Retroviridae [4]. BLV-infected cattle carry the virus for all the life. Proviral DNA persists by integrating into the target lymphocytes and thus blocks the detection of viral antigens. Mainly, BLV disseminates in the host as integrated proviral DNA, expanding along with the infected lymphocytes. About 30% of BLV-infected cattle develop severe proliferation of lymphocyte populations. This essentially benign condition is named persistent lymphocytosis (PL) [5]. BLV-induced bovine leukaemia or lymphosarcoma is a fatal neoplastic disease. Its frequency is about one-third higher among cattle with PL than among BLV carriers without lymphocytosis. Total frequency is estimated to be below 10%. It has been shown that genetic makeup of animals is involved in their resistance to infectious diseases. This means some animals are more susceptible to these diseases than others and this is strongly associated with the major histocompatibility complex (MHC) genes [6].

Early studies on the relationship between BLV infection and bovine MHC (BOLA) revealed association between serologically determined BOLAA class I Ag and resistance and susceptibility to B-cell lymphocytosis in Shorthorn, Holstein and other breeds [7]. Subsequently, it has been showed that resistance and susceptibility to PL map more closely to the MHC class II BOLA-DRB3.2 gene than to the BOLA A locus. A peptide motif named ER present in BOLA-DRB3.2 alleles *11, *23 and *28, was associated with resistance to PL in BLV-infected cattle. Resistance appears to be dependent upon the presence of the polar amino acids Glu–Arg at positions 70–71 within a highly polymorphic segment of the peptide binding region. This contributes to the putative peptide binding specificity of the molecule at the b1 domain [8].

The present study has been conducted in order to detect BOLA-DRB3.2 susceptible and resistant alleles to bovine leukemia virus in two big dairy farms in Tehran district.

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Materials and Methods

Sample preparation

Total of 120 blood samples (with EDTA coated vacutainers) were taken from Holstein- Frisian cattle which were ≥ 3 years old from two different dairy farms (one herd with high seropositive to BLV and in the other herd all the dairy cows were seronegative to BLV)in Shahriar and Karaj, Tehran suburb. Confirmation of seropositivity and/ or seronegativity in acquired blood samples of two dairy farms was carried out by ELISA. ELISA Bovine Leukosis Serum Blocking (Instituue Pourquire, Montpellier, France) was used for detection of antibody against gp51 antigen of BLV.

PCR- RFLP

Extraction of DNA from blood mononuclear cells was done using i- genomic Blood DNA Extraction Mining Kit (Intron Co, Korea). The proposed method of Van Eijk et al. (1992) for amplification and genotyping of the second exon of BOLA-DRB3 with PCR-RFLP were used in this study. Primers HL030 (5'-ATCCTCTCTCTGCAGCACATTTCC-3') and HL031 (5'-TTTAATTCGCGCTCACCTCGCCGCT-3') were used in the first amplified round. The amplification reaction was carried out with 100 ng of DNA (5 µl) in a total volume of 25 µl containing 1 x PCR buffer; 2.5 mM $MgCl_2$; 100 µM dNTPs; 0.5 µM of each primer and 1 unit of Taq DNA polymerase. First thermal round of amplification was carried out at 94°C for 3 min followed by 10 cycles 25 s at 94°C, 30 s at 60°C, 30 s at 72°C and final extension step of 5 min at 72°C.The second step PCR reaction was done with 3 µl of first-step product into one new tube containing the same volume and concentration as described above except primers HL030 and HL032 (5'-TCGCCGCTGCACAGTGAAACTCTC-3') were used. Primer HL032 is internal to the sequence of the amplified product of the first-round PCR. The thermal cycling profile for the second round was 25 cycle of 40 s at 94°C and 30 s at 65°C, followed by a final extension step of 5 min at 72°C. PCR products were analysed by electrophoresis on 2% agarose gels in 1X TBE buffer and visualized by ethidium bromide staining. PCR products were digested with *Rsa*I, *Hae* III and *Psu* l enzymes. The restriction fragment was analysed by gel electrophoresis on 12% acrylamide gel and visualized with silver staining.

Results

The age and parturition number of sampled cattle in this study are shown in the Table 1. ELISA examination of 120 taken serum samples revealed that 66 samples (belonging to one dairy farm) were seropositive to BLV (diagnosed as the seropositive farm) and 54 samples were seronegative to BLV (diagnosed as the seronegative farm).

The frequencies of BOLA- DRB3.2 in sampled cattle of two dairy farms are shown in Table 2.

Chi-square statistical analysis showed that frequencies of resistance alleles to BLV in dairy cows of seronegative farm were significantly higher than cows in seropositive farm (P< 0.05). Statistical analysis also showed a significant difference in frequency of presence of sensitive alleles in seropositive dairy farm as compared with seronegative dairy farm (P< 0.05).

Table 1: Mean values of age (year) and parturition number of sampled cattle from two dairy farms (Mean ± SE).

Group	*No*	*Age*	*Parturition Number*
Seropositive farm	66	5.23 ± 0.21	3 ± 0.18
Seronegative farm	54	4.74 ± 0.19	2.7 ± 0.18

Table 2: Frequencies of BoLA-DRB 3.2 alleles detected by PCR-RFLP in Holstein cattle from two different dairy farms.

Allel	*Seropsitive farm*	*Seronegative farm*	*Odds ratio*	*type*
DRB3.2*2	0.044	0	1.006	N
DRB3.2*6	0.023	0	1.055	S
DRB3.2*7	0.068	0	1.032	N
DRB3.2*8	0.035	0.055	1.35	N
DRB3.2*9	0.0047	0	1.022	N
DRB3.2*10	0.063	0.035	1.034	N
DRB3.2*11	0.074	0.048	0.93	N
DRB3.2*12	0.044	0	1.09	S
DRB3.2*13	0.086	0.034	1.34	N
DRB3.2*15	0.0024	0.0320	0.17	N
DRB3.2*16	0.034	0.2051	1.043	N
DRB3.2*18	0.0165	0	1.064	N
DRB3.2*22	0.085	0.0705	0.76	N
DRB3.2*23	0.034	0.066	1.012	N
DRB3.2*24	0.054	0.250	.0456	R
DRB3.2*25	0	0.0256	0.974	R
DRB3.2*26	0.026	0.035	0.336	N
DRB3.2*27	0.027	0.045	1.017	N
DRB3.2*28	0.024	0	1.016	N
DRB3.2*32	0	0.0028	0.876	N
DRB3.2*37	0.018	0.0054	1.6	N
DRB3.2*40	0	0.014	0.825	N
DRB3.2*42	0.004	0	1.006	N
DRB3.2*43	0	0.007	0.994	N
DRB3.2*51	0.039	0.025	0.679	N

S: Susceptible; R: Resistant; N: Neutral

Discussion

Major histocompatibility complex (MHC) of cattle was explained in 1978 [9], and was named as BOLA (Bovine lymphocyte antigens). The BOLA complex is a multiallelic 2.5 Mb genetic- region located on chromosome 23 at band 22, and it is classified into class I, II and III genes, which encode proteins with different tissue distributions and functions [10-12]. The nomenclatures for factors of the BOLA system are based on the amino acid sequences and the format is gene. exon* allele. The BOLA-DRB3.2 gene is the most polymorphic and more than 115 alleles have been identified. The objective of this study was to describe the gene frequency distribution of the *BoLA-DRB3.2* locus in Iranian dairy cattle and to compare their correlation with the previously reported allele for resistance/susceptibility to EBL.

The determination of BoLA-DRB3.2 allele pattern and comparison of allele frequency in Iran in two subpopulations of Holstein- Friesian cow was carried out by Nassiry et al. (2008) [13].

An association between BOLA and disease in cattle was reported by Stear et al. (1988) and Juliarena et al. (2008) [14], in which a panel of 19 antigens was evaluated in a ¾ Brahman and ¼ Shorthorn for tick resistance. BLV is the prototype of the Delta retrovirus genus of the family Retroviridae. Other members of this genus are viruses of the HTLV and STLV series that infect humans and various simian species, respectively. HTLV-I is the etiologic agent of adult T cell leukemia in endemic areas, and some of the simian T-cell lymphotropic viruses have been implicated in the induction of malignant lymphomas in their hosts. Several new Deltaretrovirus isolates have been found in various primates recently. Two newly identified HTLV-like viruses in humans named HTLV-III and HTLV-IV [15] have been detected. In the present study the frequency of presence of DRB3.2 alleles susceptible, resistance and neutral alleles to BLV were investigated in two different dairy farms in Iran, in which one farm had high seropositivity to BLV and dairy cows in another farm were seronegative to BLV. Results of PCR-RFLP test on blood samples of dairy cattle of two farms showed that there was a highly significant presence of susceptible alleles to BLV in population of dairy cattle in the seropositive farm ($P < 0.05$) and on the contrary there was a high prevalence of resistance alleles in dairy cattle of the seronegative dairy farm ($P < 0.05$).

Conclusion

From the results of the present study it can be concluded that MHC class II susceptible and resistant alleles to oncovirus are present in Holstein- Frisian cattle of dairy farms in a Tehran suburb and there was a very significant difference between the genetic makeup's of dairy cattle in a farms with high seropositivity to BLV as compared with seronegative dairy farms.

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