Analysis of polymorphism of MHC class II BuLA DRB3 exon 2 gene in North West Iranian populations of the Water buffalo *(Bubalus bubalis)* through PCR-SSCP

Sheikhmohammadi, R.[1]*; Hashemi, A.[2] and Mardani, K.[3]

[1]Institute of Bioscience and Technology, Urmia University, Urmia, Iran. [2]Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran. [3]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Key Words:
MHC; *BuLA-DRB3*; Asian water buffalo; polymorphism; PCR-SSCP.

Correspondence
Institute of Bioscience and Technology, Urmia University, P. O. Box: 57153-1177, Urmia, Iran.
Tel:+98(441)2972618
Fax:+98(441)2972618
Email: srasol@yahoo.com

Received: 26 November 2009,
Accepted: 6 July 2010

Abstract

The *DRB3* gene is a highly polymorphic major histocompatibility complex (MHC) class II gene and plays an important role in variability of immune responsiveness and disease resistance. In the present study, the MHC class II *DRB3* gene in water buffalo *(Bubalus bubalis)* populations from Northwest regions of Iran was investigated through PCR-SSCP. Genomic DNA was extracted from whole blood samples collected from 50 buffaloes. A 284 bp segment of exon 2 of *BuLa-DRB3* was amplified by standard PCR, using locus-specific primers. The PCR products were subjected to a non-denaturing gel electrophoresis. A number of 11 different SSCP patterns indicating allelic variation were identified. The three most frequent patterns 1, 4 and 10 accounted for 58% of the total patterns. Results indicated that exon 2 of the *BuLA-DRB3* gene is highly polymorphic among the examined animals.

Introduction

Iran maintains a large population of Asian water buffaloes *(Bubalus bubalis)*. According to the Iranian Association of Buffalo Breeders, there are currently 0.5 million buffaloes in Iran, including local breeds known as Khozestani, Azerbaijani and Mazandarani that belong to the subspecies of river buffalo *(B. bubalis bubalis)*.

The major histocompatibility complex (MHC) is an organized cluster of tightly linked genes with immunological and non-immunological functions, and is present in all vertebrates except jawless fish (Tizard, 2004). MHC genes encode cell surface glycoproteins that are classified as class , II and III based on differences in their cellular distribution, molecular weight and function. These glycoproteins have an essential role in the recognition of foreign peptide antigens (Hughes and Nei, 1989). Functional MHC class II glycoproteins are expressed as alpha and beta chains on the surface of antigen-presenting cells and bind to the foreign polypeptides (antigens). They present them to T-helper lymphocytes to initiate an immune response (Rask *et al*., 1985). The high degree of genetic polymorphism in some of MHC class I and II genes has been documented in a number of vertebrate species and is of great interest to evolutionary biologists (Klein, 1975; Zinkernagel and Doherty, 1979). Two different types of MHC class II molecules including the DQ and DR subtypes, which are the most polymorphic both in humans and domestic animal species, have been described. These MHC class II subtypes probably play an important role in the development of MHC-restricted immune responses (Amills *et al*., 1996).

In bovines, three *BuLA-DRB* genes, including *DRB1*, *DRB2* and *DRB3*, have been reported, but among them only DRB3 gene appears to be functional (Lewin *et al*., 1999). The DRB3 gene in river buffaloes is located on chromosome 2 and chromosome 23 in cattle. It has been revealed that polymorphism in the DRB3 gene of African buffaloes is fully consistent with established characteristics of MHC variation (Hedrick, 1994). The second exon sequence from the buffalo *DRB3* locus is homologous to the cattle *DRB3* gene (Iannuzzi, 1993). Few genetic studies have been conducted on functionally important loci in water buffalo so far, unlike in cattle and related bovids. This is especially true for loci related to disease resistance and susceptibility such as MHC class II genes (Aravindakshan, 2000; De *et al*., 2002).

Several methods including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and sequencing have been used to investigate polymorphism in the *BuLA-DRB3* gene in domestic animals (Van Eijk *et al*., 1992; Sena *et al*., 2003; Pipalia *et al*., 2004).

Sequencing is the most reliable and efficient method, but also very expensive. PCR-RFLP on the other hand is faster but requires prior knowledge of the DNA sequence for choosing an appropriate enzyme for DNA digestion. PCR-SSCP is an easier method than sequencing and RFLP and can be adopted for the detection of polymorphisms where sequence information is not available. The aim of this study was to identify the extent of polymorphism in *DRB3* gene exon 2 using PCR-SSCP in water buffalo populations from Northwest Iran.

Materials and Methods

Blood samples collection and genomic DNA extraction

Blood samples (approximately 5 to 10 ml) were obtained from 50 unrelated Azerbaijani buffaloes, belonging to herds located in different regions of the Western Azerbaijan province in Iran. Blood samples were stored in EDTA-coated vacutainer tubes (Kima, Italy). Approximately 10 µg of the genomic DNA was extracted from 0.3 ml blood using the genomic DNA purification kit according to the manufacturer's instructions (Fermentas, EU). The quality and quantity of extracted DNA was measured by 2% agarose gel electrophoresis and by spectrophotometric measurement of the optical density at wavelengths of 260 and 280 nm.

Amplification of BuLA-DRB3 Exon 2

The DNA amplification of the *BuLA-DRB3* gene was achieved through PCR. Two primers, HLO30 (5´-ATCCTCTCTCTGCAGCACATTTCC-3´) and HLO32 (5´-TCGCCGCTGCACAGTGAAACTCTC-3´), targeting a fragment of 284 bp, were employed as described by Van Eijk *et al*. (1992). The PCR reactions were carried out in 50 µl volumes using a PCR master mix kit (Cinnagen, Iran) containing 2.5 units *Taq* DNA polymerase in reaction buffer, 4 mM $MgCl_2$, 0.4 mM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer and 5 µl of extracted DNA. Amplification was performed using 35 cycles of incubation at 94°C for 45 sec, 60°C for 40 sec and 72°C for 25 sec, with a final extension at 72°C for 2 min.

SSCP

PCR products were mixed with 8 µl of denaturing loading dye [95% (w/v) deionized formamide, 0.05% (w/v) xylene cyanol, 0.05% (w/v) bromophenol blue and 0.02 M EDTA] for a total volume of 15 µl. The mixture was denatured at 95ºC for 5 minutes and was instantly chilled on ice (Pipalia *et al*., 2004). The total volume was applied on a 15% polyacrylamide gel, according to the assay protocol (Herring *et al*., 1982). The electrophoresis was performed in TBE buffer (Tris 100 mM, Boric Acid 9 mM, and EDTA 1 mM) at room temperature (10-20ºC) with a constant voltage of 200 V until the sample dyes ran off the bottom of the gel. After electrophoresis, polyacrylamide gels were silver stained according to the protocol described by Herring et al. (1982).

Results

PCR

The amplification of a 284 bp fragment of exon 2 of the *BuLA-DRB3* gene was successful in our first attempt. All DNA samples extracted from buffalo blood which were amplified through PCR yielded a specific single band PCR product without any nonspecific bands (Figure 1). Therefore the obtained PCR products were directly used for SSCP analysis without further purification.

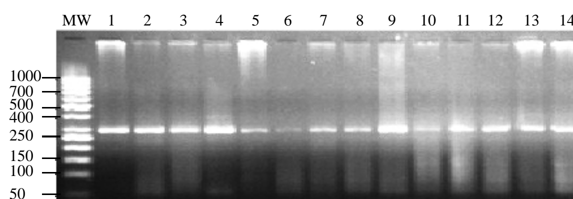

Figure 1: PCR amplified products of DRB3 gene visualized by electrophoresis on a 2% agarose gel. Lane MW: 50 bp DNA ladder (Cinnagen, Iran), lanes 2 to 15: 284 bp PCR products for 14 randomly selected DNA extracts from buffalo blood samples.

SSCP

Polymorphism in the *BuLA-DRB3* gene was analyzed by PCR-SSCP. A total of 11 SSCP patterns were observed in the examined buffaloes and named as I to XI. Six SSCP patterns are shown in Figure 2. The frequency and percentage of observed SSCP patterns of the *BuLA-DRB3* gene of the examined buffaloes are shown in Table 1. The most frequent SSCP patterns for *BuLA-DRB3* were patterns I, IV and X, with frequency percentage of 28%, 18% and 12%, respectively.

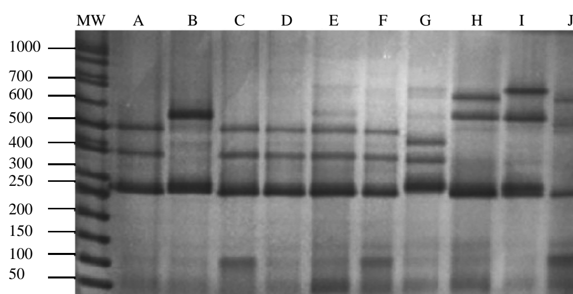

Figure 2: PCR-SSCP patterns of BuLa-DRB3 on non-denaturing polyacrylamide gel (15%) stained with silver nitrate. Lane MW: 50 bp DNA ladder; lanes A, C, D, E and F: SSCP pattern I; lane B: SSCP pattern II; lane G: SSCP pattern III; lane H: SSCP pattern VI; lane I: SSCP pattern V; lane J: SSCP pattern VI.

Discussion

In the present study 11 different SSCP patterns were identified for the *BuLA DRB3* in Iranian water

Table 1: Frequency distribution and percentage of the observed SSCP patterns of the *BuLA-DRB3* gene.

	SSCP patterns	Frequency of the observed patterns in the examined buffaloes	Percentage of the observed patterns in the examined buffaloes (%)
1	I	14	28
2	II	2	4
3	III	4	8
4	IV	6	12
5	V	3	6
6	VI	1	2
7	VII	2	4
8	VIII	4	8
9	IX	2	4
10	X	9	18
11	XI	3	6
Total	11	50	100%

buffaloes, indicating a high degree of polymorphism for this gene. The results obtained from this study confirm the outcomes of previous studies, which found high degrees of polymorphism in *BuLa-DRB3* in different buffalo breeds (De *et al*., 2002; Sena *et al*., 2003; Pipalia *et al*., 2004). The high polymorphism of this region was also explored by the PCR-RFLP technique using RsaI and Hae restriction enzymes (Acharya *et al*., 2002).

According to Ohta, (1998) and Parham, (1989), MHC gene polymorphisms are generated initially by point mutations, and then by gene conversion that shuffles short DNA sequences between alleles or loci. Recombination events, which are essentially random processes, may also contribute to the generation of polymorphisms in MHC genes (Hickford *et al*., 2004). High levels of polymorphisms and heterozygosity within the MHC genes provide the immune system with a selective advantage against the diversity and variability of pathogens and help to increase the fitness against infections of the host and population (Potts, 2002). There is a general assumption that buffalo breeds are more tolerant to many infectious diseases than cattle breeds but no attempt has been made to systematically characterize the different DRB3 alleles that exist in buffalo breeds. To date, no data on *BuLa-DRB3* gene variation and the extent of polymorphism in Iranian water buffaloes have been reported. This report provides a basis for further analysis of this gene, which might also be present in the wild buffalo population. While RFLP patterns respond to a nucleotide change only at a specific point on the DNA chain, SSCP patterns in contrast are generated as a result of nucleotide sequence change at one or several positions together.

In summary, the PCR-SSCP technique used in this study provides evidence of a high degree of polymorphism at the *BuLA-DRB3* locus in Iranian northwest water buffaloes. Further studies are required to associate identified patterns with the performance and susceptibility or resistance to infectious diseases of the animal. In addition, the functional role of the polymorphic sites of the *BuLa-DRB3* gene in buffaloes needs to be analyzed and confirmed by means of gene expression assays. Therefore, the polymorphic nature of exon 2 of *BuLA-DRB3* in buffaloes makes this gene an appropriate genetic marker for breeding purposes.

Acknowledgments

We would like to sincerely thank Mr Farhangpajooh for his technical assistance and Urmia University for funding the project.

References

1. Acharya, C.P.; Pipalia, D.L.; Rank, D.N.; Joshi, C.G.; Brahmkshtri, B.P.; Solanki, J.V. and Shah, R.R. (2002) BOLA-DRB3 gene polymorphism in Jafarabadi and Mehsani buffaloes as revealed by PCR-RFLP. Indian Veterinary Journal 79: 652-656.
2. Amills, M.O.; Sanchez, A. (1996) A PCR-RFLP typing method for the caprine MHC class DRB gene. Veterinary Immunological Immunopathology 55: 255-260.
3. Aravindakshan, T.V.; Nainar, A.M. and Sivasdvam, S.N. (2000) Polymorphism in exon 2 of BOLA-DRB3 gene in Indian buffalo *(Bubalus bubalis)* detected by PCR-RFLP. Animal Science 70: 221-226.
4. De, S.; Singh, R.K. and Butchaiah, G. (2002) MHC-DRB exon 2 allele polymorphism in Indian river buffalo *(Bubalus bubalis)*. Animal Genetics 33: 215-219.
5. Hedrick, P.W. (1994) Evolutionary genetics of the Major Histocomaptibility Complex. The American Naturalist 143: 945-964.
6. Herring, A.J.; Inglis, N.F.; Ojeh, C.K.; Snodgrass, D.R. and Menzies, J.D. (1982) Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. Journal of Clinical Microbiology 16: 473-477.
7. Hickford, J.G.; Zhou, H.; Slow, S. and Fang, Q. (2004) Diversity of the bovine DQA2 gene. Animal Science 82: 1553-1563.
8. Hughes, A.L.; Nei, M. (1989) Nucleotide substitution at major histocompatability complex class II loci: evidence for overdominant selection. Proc Natl Academic Science USA 86: 958-962.
9. Iannuzzi, L.; Gallagher, D.S.; Womack, J.E.; Di Meo, G.P.; Skow, L.C. and Ferrara, L. (1993) Chromosomal localization of the MHC in cattle and river buffalo by FISH. Hereditas 118: 187-190.
10. Klein, J. (1975) Biology of the Mouse Histocompatibility-2 Complex. Springer Verlag, Berlin, 73-75.
11. Lewin, H.A.; Russel, G.C. and Glass, E.J. (1999) Comparative organization and function of the major histocompatibility complex of domesticated cattle. Immunological Reviews 1687: 145-158.
12. Ohta, T. (1998) On the pattern of polymorphisms at major histocompatibility complex loci. Journal of Molecular Evolution 46: 633-638.
13. Parham, P. (1989) MHC protein structure. Getting into

thegroove.Nature342:617-618.

14. Pipalia, D.L.; Joshi, C.G.; Rank, D.N.; Brahmkshtri, B.P. and Solanki, J.V. (2004) PCR-SSCP typing of MHC in cattle and buffaloes. Indian Journal of Animal Science 74: 637-639.
15. Potts, W.K. (2002) Wisdom through immunogenetics. Nature Genetics 30: 130-131.
16. Rask, L.; Gustafsson, K.; Larhammar. D.; Ronne, H. and Peterson, P.A. (1985) Generation of class II antigen polymorphism. Immunological Reviews 84: 123-143.
17. Sena, L.; Schneider, M.P.C.; Brenig, B.; Honeycutt, R.L. and Womak, J.E. (2003) Polymorphism in MHC-DRA and DRB alleles of water buffalo (Bubalus bubalis) reveal different features from cattle dr alleles. Animal Genetics 34: 1-10.
18. Tizard, I.R. (2004) Acquired immunity: antigen-presenting receptors. In: Veterinary immunology: an introduction. Elsevier, Philadelphia, PA, USA 153: 67-77.
19. Van Eijk, M.J.; Stewart, T.; Haynes, J.A. and Lewin, H.A. (1992) Extensive polymorphism of the BOLA0DRB3 gene distinguished by PCR-RFLP. Animal Genetics 23: 483-496.
20. Zinkernagel, R.M.; Doherty, P.C. (1979) MHC restricted T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction- specificity, function and responsiveness. Advances in Immunopathology 27: 221-229.