GENETIC POLYMORPHISM OF MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS II, *DQB1* GENE IN MALABARI AND ATTAPPADY BLACK GOATS

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Abstract

MHC is a family of highly polymorphic genes implicated in immunity. Polymorphism of MHC class II DQB1 gene in two breeds of goats of Kerala is studied using PCR-RFLP technique with enzyme Hae III, giving 2 alleles (3 genotypes) in Malabari breed, and one allele (one genotype) in Attappady Black breed.

Key words: *MHC, polymorphism, DQB1, immunity, Malabari, Attappady Black*

The Major Histocompatibility Complex (MHC) is a multigene family that encodes key receptor molecules that recognize and bind foreign peptides for presentation to specialist immune cells, and the subsequent initiation of immune response. The particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual's TH and TC cells can respond. Therefore, the MHC genes have been implicated in susceptibility to disease as well as in development of autoimmunity.

According to Piertney and Oliver (2006), the MHC contains the most variable functional genes described in vertebrates, with nearly a hundred alleles identified at certain human MHC loci. A pressure to maintain high allelic diversity at MHC loci appears intuitively obvious, given that individuals or populations with higher sequence variation at the MHC loci can identify and process a larger number of pathogenic antigens, and as such combat a wider range of immune insults.

The present study on the second exon of MHC class II *DQB1* gene was taken up in Malabari and Attappady Black goats of Kerala, to study its polymorphism with PCR-RFLP technique using restriction enzyme *Hae III*.

Materials and Methods

5ml of venous blood sample each were collected from a total of 100 goats from Kerala Veterinary and Animal Sciences University Goat and Sheep Farm, and Malabari goats from field units of All India Co-ordinated Research Project (AICRP) on Malabari goat improvement. Of these, 71 were of Malabari breed, and 29 of Attapady Black breed.

Genomic DNA was extracted from the blood samples using standard phenol chloroform extraction procedure (Sambrook *et al.,* 1989). The quality and molecular weight of DNA were assured electrophoretically using 0.7 per cent agarose in 1 X TAE buffer in a horizontal submarine electrophoresis unit. The purity and concentration of DNA samples were estimated by UV spectrophotometry by estimating the ratio between the readings at 260nm and 280nm wavelengths. DNA stock solutions having OD260/ OD280 between 1.7 and 1.9

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were diluted in sterile triple distilled water to a concentration of 100ng/µl to give template DNA for PCR, and was stored at -20^{0}C.

The set of primers used by Amills *et al.* (2004) for amplification of second exon of Cahi-DQB1 gene, which is given below, were used for the amplification of the second exon of MHC class II DQB1 gene by polymerase chain reaction (PCR).

Forward: 5' CCC CGC AGA GGA TTT CGT G- 3'

Reverse: 5' ACC TCG CCG CTG CCA GGT- 3'

A master mix was prepared just before setting up the PCR assay combining 10x PCR buffer, 50mM $MgCl_2$, dNTP mix, primer pairs, Taq DNA polymerase and sterile distilled water in such a way to get a final concentration of 1X PCR buffer, 1.5mM $MgCl_2$, 100µM of each dNTP, 0.5µM of each primer and 1.25 unit Taq DNA polymerase in a total volume of 50µl in 200µl reaction tubes.

To each reaction tube 48µl of master mix and 2µl (200ng) of template DNA were added. The tubes were spun briefly and placed in the thermal cycler. The standardized thermal cycling profile consisted of an initial denaturation of 5min at 94^{0}C followed by 33 cycles of 30 seconds at 94^{0}C, 30 seconds at 65^{0}C and 45 seconds at 72^{0}C. This was followed by a final extension of 5 min at 72^{0}C. The mix after PCR was checked for amplification by agarose gel electrophoresis on 2% agarose gel, with 100 base pair DNA ladder as the molecular size marker (fig. 1). The PCR amplified products were stored at -20^{0}C till analysed.

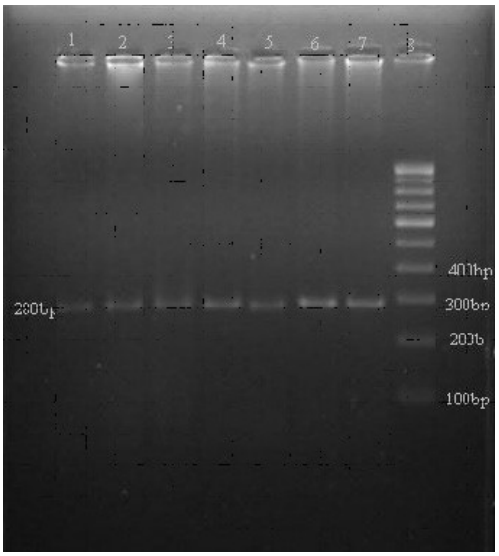
The amplified product was digested using restriction enzyme *Hae III*. All the following ingredients were added in order to make a reaction mixture of 15µl.

Distilled water	-	2.5µl
10X assay buffer	-	1.5µl
PCR product	-	10.0µl
Hae III	-	1.0µl

Digestion was carried out at 37ºC for 4 hours in a final volume of 15µl in a dry bath. Following the digestion, the enzyme was inactivated by incubating the tubes at 65^{o}C for 20 min and the digested products were stored at 4^{o}C till analysed. The pattern of restriction digestion was analysed using Poly Acrylamide Gel Electrophoresis (PAGE) on 12% acrylamide-bisacrylamide gel.

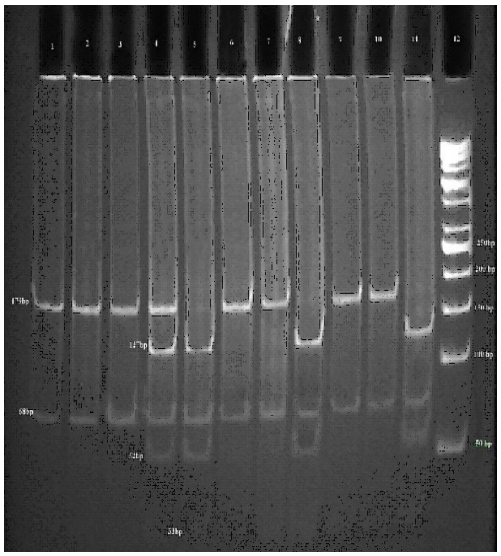
Results and Discussion

The mean yield of DNA from 5ml samples



Lane 1 to 7: PCR products
Lane 8: 100bp + DNA ladder

Fig.1. Amplified products of *DQB1* gene after electrophoresis on 1.5% agarose gel along with 100 bp ladder and stained with ethidium bromide



Lanes 1, 2, 3, 6, 7, 9 and 10: genotype AA
Lanes 5, 8 and 11: genotype BB Lane 4: genotype AB
Lane 12: 50bp+ DNA ladder

Fig. 2. PCR-RFLP fragments of *DQB1* gene on Polyacrylamide gel stained with ethidium bromide indicating the three genotypes

of goat blood extracted by phenol-chloroform method was 282.81±52.32µg. Chithra (2002) and Remya (2010) reported slightly lower yield of 231.097±11.65µg and 245.762±26.593µg of DNA from 5 ml of goat blood. Mathew (2004) and Seena (2006) reported higher yields of 350.05±10.05µg and 357±23.057µg of DNA, respectively from 5ml of blood of Malabari goats.

Beckman *et al*. (1986) and Senthil *et al*. (1996) reported 300-500µg and 444.58±21.54 µg, respectively per 10 ml of whole blood of cattle using the phenol chloroform method. The yield of DNA per unit volume of blood was high in goat as compared to that of cattle. The ratios of optical density at 260 and 280 nm were between 1.7 and 1.9, indicating good deproteinisation. Chithra (2002) and Remya (2010) reported similar ratio in goats.

Amplification of exon2 by PCR

The DNA isolated from blood samples of goat was amplified by PCR at the *DQB1* locus without any major non-specific amplified products using primers cited by Amills *et al.* (2004), and Xing *et al.* (2008) (Fig.1.). The concentration of reagents and the time-temperature combinations used were similar to that used for amplification of the same gene in Saanen goats by Amills *et al.* (2004), and in Laiwu Black goats, Lubo goats and Boer goats by Xing *et al.* (2008).

The size of the amplified product was approximately 280bp in both Attapady Black and Malabari goats, formed by 8bp from intron1, the whole of exon 2 spanning 270bp, and 2bp from intron 2. The same product size was reported by Amills *et al.* (2004) in Saanen goats, and by Xing *et al.* (2008), who used the same primer sequences.

RFLP analysis and polymorphism

On restriction enzyme digestion with *Hae III* enzyme and resolution of restriction fragments by non-denaturing PAGE, three restriction digestion patterns were observed in Malabari goats and one pattern in Attapady Black goats, indicating the presence of 3 *Hae III* genotypes in Malabari goats, and a single *Hae III* genotype in the latter (Fig.2). Genotype AA of Malabari goats showed pattern 179/68/33. Of the four sequences described by Amills *et al.* (2004) for the same gene in Saanen goats, one sequence would give a similar pattern of digestion. In genotype BB, the 179bp fragment was further cut into a 127bp fragment and a 52bp fragment leading to a pattern of 127/68/52/33. The other three of the four sequences of the same gene in Saanen goats, described by Amills *et al.* (2004) would give a similar pattern of digestion as genotype BB of Malabari goats. Genotype AB had bands from digestion patterns of AA and BB genotypes, giving 5 bands corresponding to 179, 127, 68, 52 and 33 base pairs.

However, PCR-RFLP analysis carried out by Xing *et al.* (2008) in Laiwu Black goats, Lubo goats and Boer goats using the same primer set for PCR, and *Hae III* enzyme for RFLP analysis, gave entirely different digestion patterns. They reported 7 genotypes showing 7 band patterns *viz.* 179/77/24bp, 127/77/52/24bp, 203/77bp, 151/77/52bp, 179/127/77/52/24bp, 203/179/77/24bp and 203/127/77/52/24bp. None of these band patterns were observed in any of the Malabari or Attapady Black goats under study.

MHC is said to be one of the most polymorphic locations in vertebrate genome (Piertney and Oliver, 2006). The observed absence of polymorphism in Attapady Black goats could be due to the small sample size of animals of this breed under study, which were also closely related to each other and therefore with low genetic diversity. Under such condition, polymorphism could go undetected if the alternate genotypes have a very low frequency.

Polymorphism could go undetected also due to the limitation of PCR-RFLP technique in detecting polymorphisms. The PCR-RFLP technique is used to identify variations in sequences at specific locations in the amplified product, the difference being shown as presence or absence of a cutting site for the particular restriction enzyme being used. The technique can detect a difference only at cutting site locations. This difference need not necessarily imply the presence or absence of variations at other locations. In this study, the enzyme used - *Hae III* - is identifying base sequence at 8 or 12 nucleotide positions altogether along the 280bp-long sequence. Therefore, the absence of polymorphism at the *Hae III* cutting sites in Attapady Black goats does not mean that the gene is not polymorphic, as it could vary in sequence at other nucleotide positions not detected by this enzyme. Further studies using techniques that reveal all sequence differences may be needed to assess the actual

polymorphism status in Attapady Black goats.

The aforesaid limitation of the PCR-RFLP technique would also mean that the two alleles of Malabari goat may in fact represent two classes of alleles, each consisting of numerous alleles, which are similar in sequence regarding *Hae III* restriction sites, but different otherwise. For instance, if the four exon 2 sequences of Saanen goat described by Amills *et al.* (2004) were subjected to RFLP with *Hae III*, they would also give restriction patterns corresponding to 2 alleles A and B alone, but the B allele is in fact formed by 3 different sequences representing three alleles. The sequences obtained in Malabari and Attapady Black are therefore inadequate in describing the *Cahi DQB1* exon2 alleles present in these goat populations. Further studies using techniques that reveal all sequence differences may be needed to assess the actual polymorphism status in Malabari goats too.

If such an elaborate study also gives a similar result of low variability at this locus, it would indicate a population bottleneck during the evolution of these goat populations. A population bottleneck is an evolutionary event in which a significant percentage of a population or species is killed or otherwise prevented from reproducing. A similar low MHC variability was reported in a study on three mountain goat populations in Canada by Mainguy *et al.* in 2007. Their study on class II *DRB* genes revealed the presence of two alleles alone, one of which was fixed in one of these populations. The possibility of past population bottlenecks during Pleistocene glaciations was cited as the main factor contributing to the low levels of MHC diversity in those populations.

The PCR-RFLP technique can be used efficiently in populations where sequence differences are already known, so that the choice of restriction enzymes can be made in such a way as to reveal these differences. Sequence data was not available for *DQB* gene of Malabari or Attapady Black goats. So the choice of enzyme was made based on published literature describing the use of *Hae III* enzyme in other goat populations to identify 7 *DQB1* genotypes (Xing *et al.,* 2004). However, on sequencing of the PCR products, it was clear that the *DQB1* sequence pattern in Malabari and Attapady Black goats were different from those in the goat populations analyzed by Xing *et al.* (2004) and so didn't give similar degree of polymorphism information.

References

Amills, M., Sulas, C., Sanchez, A., Bertoni, G., Zanoni, R. and Obexer-Ruff, G. 2004. Structural characterization of the Caprine Major Histocompatibility Complex Class II *DQB1* (Cahi *DQB1*) gene. *Mol. Immun*. **41**: 843-846.

Beckmann, J. S., Kashi, Y., Hallerman, E. M., Nave, A. and Soller, M. 1986. Restriction fragment length polymorphism among Israeli Holstein Friesian dairy bulls. *Anim. Genet*. **17**: 25-38.

Chithra, 2002. Polymorphism of growth hormone gene in Malabari goats (*Capra hircus*). *MVSc thesis*. Kerala Agricultural University, Thrissur, 79p.

Mainguy, J., Worley, K., Coté, S. D. and Coltman, D. W. 2007. Low MHC *DRB* class II diversity in the mountain goat: past bottlenecks and possible role of pathogens and parasites. *Conserv. Genet*. **8**: 885-891.

Piertney S. B. and Oliver M. K., 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity (Edinb)*. **96**: 7-21.

Remya, J. V. 2010. Genetic polymorphism of Major Histocompatibility Complex Class II genes in goats. *MVSc thesis*. Kerala Agricultural University, Thrissur. 98p.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual.* 2nd ed. Cold spring Harbor Laboratory Press. New York. pp.1147-1886.

Senthil, M., Nainar, A. M., Ramadass, P. and Nachimuthu, K. 1996. A simple procedure for isolation of DNA from cattle white blood cells for RFLP studies. *Int. J. Anim. Sci*. **11**: 413-414.

Xing, F., Li, P. P., Li, J., Li, C. B. and Wang, J. M. 2008. Association of polymorphism of exon2 of GoLA *DQB1* gene with immune traits in goats. *Yi Chuan.* **30**: 870-876.

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