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Major Histocompatibility Complex-DQA Variants Analysis: Rich Repertoire with Selection Advantages and Disadvantages

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A B S T R A C T

Insulin dependent diabetes mellitus, an autoimmune disorder in human being, is associated with MHC class-II, which reacts with auto peptide released by MHC class-II. HLA-DQ alleles (DQA1 and DQB1) are revealed to be linked with type 1 diabetes in humans. The DQ alleles passed from parents to the offspring predisposes them with risk of type I diabetes. In present study, DQ variants (DQA1 and DQA2) were amplified and sequenced with product size 826 bp (DQA1) and 865 bp (DQA2) from sheep's blood. Sequences were *insilico* characterized for percent identity, phylogeny, and protein property. Phylogeny analysis suggests a rich repertoire of DQA alleles in sheep. Ramachandran plot analysis had shown the residues are occurring under the core region of both the DQ loci with 90.8% and 92.1% of residues lie in the favored region in DQA1 and DQA2 loci, respectively.

Introduction

Blood glucose serves as both a source of energy for the body's tissues and cells and a key supply of fuel for the brain. Diabetes is a group of illnesses that help understand how the body responds to blood glucose.

Diabetes mellitus is a combination of the Greek words diabetes, which means to syphon or pass through, and the Latin word mellitus, which means sweet (Sapra and Bhandari, 2023). There are many types of diabetes, but they all have similar effects in that they can increase blood sugar levels, which can cause major problems including kidney damage. Type 1 diabetes and type 2 diabetes are the two kinds of diabetes mellitus. Type-I diabetes is a form of the disease where the immune system destroys ß-cells because it views them as non-self (Rosa *et al.*, 2010).

This kind of diabetes affects both children and young adults, is not age-specific, and may be passed down to the next generation. Diabetes is a chronic disease brought on by inadequate insulin synthesis, which regulates blood sugar levels.. According to a

WHO survey (https://www.who.int/health-topics/diabetes, accessed dated 02.07.2023), over 422 million people worldwide have diabetes, and each year there are approximately 1.5 million deaths related to the disease. One of the major genetic determinants for onset of type I diabetes in humans are polymorphisms in class II MHC (Bruno *et al.,* 2001)

(Noble and Valdes, 2011). Human MHC gene is known as Human leukocyte antigen (HLA) and these molecules play a crucial role in graft rejection and immune response.

HLA class II encodes DQ, DR, and DP genes in human and is located on chromosome 6. Ovine leukocyte antigen (OLA) in sheep is synteny to HLA and located on chromosome 20. The MHC class-II gene in sheep is well characterized and contains DRA, DRB, DQA, DQB, DNA, DOB, DYA, DYB, DMA, and DMB gene types (Dukkipati *et al.,* 2006). It also presents antigenic peptide to CD4 on helper T-cells. DQ is an antigen-presenting cell-presented cell surface receptor protein. Two HLA loci, HLA-DQA1 and HLA-DQB1, which are found on MHC class-II, code for the two hetrodimeric chains on the protein: the alpha chain and beta chain.

Allelic forms the MHC type II gene is playing predisposing cause for onset of type I diabetes in humans (Nejentsev *et al.,* 2007). In the present study, sheep DQA loci has been analyzed to find out diversity in allelic forms of the gene.

Materials and Methods

RNA isolation and cDNA synthesis

RNA was isolated from Malpura sheep's blood through TRIzol based procedure. Briefly, RBCs were lysed using RBC lysis buffer and TRIzol reagent (1 ml) was added to resuspend the WBC pellet. The sample was vortexed and incubated at room temperature for 5 minutes. Chloroform (200 µl) was added to each sample, mixed and was allowed to stand for 15 minutes at room temperature before centrifugation at 12000 x g for 15 minutes at 8°C and the aqueous phase was collected in a DEPC treated centrifuge tube. Isopropanol (500 µl) was added to each sample, and the sample was allowed to stand for 10 minutes at room temperature before centrifugation at 12000 x g for 15 minutes at 8°C. Supernatant was discarded and the RNA pellet was washed with 75 % ethanol. RNA pellet was air-dried and was dissolved in RNase free water (20µl). Complementary DNA (cDNA) was synthesized using cDNA synthesis kit following manufacturer instructions.

PCR amplification, cloning and sequencing of ovine DQA gene

Ovine DQA1 and DQA2 gene was amplified using gene specific primers (table 1) in a thermal cycler (BIORAD) with the steps: 95 °C for 3 minutes and a 40 cycles consisting of denaturation at 94 °C for 30 sec, annealing at 61°C for 30 sec, amplification at 72° C for 45 sec and a final extension at 72°C for 5 minutes. PCR products were gel purified and ligated to pDrive cloning vector (Qiagen) following manufacturer instruction. Competent cells (*E.coli DH5α)* were prepared for transformation of ligated PCR product and transformation of gene-ligated vector was done using transform aid bacterial transformation kit (Thermo Fisher) following manufacturer instruction. Colonies were picked with sterile pipette tips and were placed into conical tubes (15 ml) containing LB broth (6 ml) with Ampicillin (50µg/ml). Tubes were incubated overnight at 37˚C in a shaker incubator and plasmid was isolated using plasmid isolation kit (Thermo Fisher) following manufacturer instructions. True clones were identified using colony PCR and gene were get sequenced from Eurofins Genomic India Pvt Ltd, Begaluru, India.

In-silico analysis

Clustal omega was used to determine the coding sequence of nucleotide sequence (https://www.ebi.ac.uk/Tools/msa/clustalo/) by

multiple alignments and phylogeny tree were prepared. Expasy was used to find the amino acid sequences of given nucleotides. Protein primary structure (ProtParam), secondary structure (PsiPred) and 3D protein model (https://swissmodel.expasy.org/.) was analyzed. Protein visualization and validation was done by Pymol and Ramachandran plot, respectively.

Results and Discussion

RNA quality was evaluated on 2% agarose gel electrophoresis (figure 1) and the quantity was estimated with microvolume spectrophotometer. The quality of cDNA was estimated by PCR using GAPDH primers. An intact band was visualized on agarose gel (figure 1). DQA1 and DQA2 were amplified with product size 826- and 865-bp, respectively as depicted in figure 1.Purified PCR products were ligated with pDrive vector and transformed in *E.coli DH5α* strain. True recombinant colonies (figure 1) were cultured overnight and plasmids were sequenced.

Nucleotide sequences were analyzed and presence of 5' UTR region (30 bp upstream), the coding region (31.798 bp) and 3'UTR region (27 bp downstream) was confirmed in DQA1 sequences. Similarly presence of 5' UTR region (37 bp upstream), the coding region (38.805 bp) and 3'UTR region (60 bp downstream) was confirmed in DQA2 sequence. There were two alleles from DQA1 locus and one allele from DQA2 locus observed in present study. Sequences were submitted to NCBI with accession numbers OR058546, OR058547, and OR058548. The assigned protein IDs of these sequences are WIA07489, WIA07490 and WIA07491, respectively.

There was an ancient interlocus recombination increases the class II MHC-DQA diversity in sheep and other Bovidae (Ballingal *et al.*, 2015). The diversity in DQA loci was also observed in Indian sheep breeds (Gowane *et al.*, 2023). BLAST search with the ovine DQA sequences available in NCBI database yielded a total of twenty six alleles of ovine DQA genes. Percent identity analyses revealed that DQA1 protein (WIA07489) of Malpura sheep is closest to XP_042092950 (99.61%) and farthest to AAA16625 (83.53%). Similarly, DQA2 protein (WIA07491) of Malpura sheep is closest to AFN25707 (99.61%) and farthest to CDL68739 (81.96%).

Percent identity between DQA1 alleles (WIA07489 and WIA07490) of Malpura sheep is 89.02 %. These findings suggest wide sequence diversity in the ovine DQA alleles. Phylogenetic tree analysis of ovine DQA protein sequences revealed 3 major clusters (figure2). Primary structures were analyzed by Expasy's ProtParam and generated information about primary structure (table 2). Furthermore, secondary structures were analyzed by PSIPRED and protein visualized in different forms and Ramachandran plot was generated by PROCHECK (figure3) shown the residues are occurring under the core region of both the DQ loci (DQA1 and DQA2) which shows that the model is stereo chemically stable. The DQA1 model had 0.0% residues in the disallowed region, while 90.8% of residues lied in the favored region, and DQA2 had 92.1% of its residues in the favored region, while no residues were found in the outlier region.

MHC gene has the evolutionary significance in development of a species and has been duplicated by selection pressure and different loci of MHC genes exist in many variant forms in a species. This variant form of a gene is called alleles. Individuals possessing a specific allele have advantages and disadvantages depending upon interaction of genes with environment and also with gene-to-gene interaction. Certain variant of a gene predisposes the individuals to a particular disease condition and other variants of the same gene provide resistance to an ailment and give selective advantages.

Table.1 Forward and reverse primers used for amplification of DQA1 and DQA2 loci

S. No	Gene	Seq 5'-3'	mers	Product size
1.	DQA1	F5'- **AGCAACTGCTGAGACCACCT**-3' R5'- **ACAGTGCACCTTCCTTCTGG**-3'	20 20	826 bp
2.	DQA2	F5'- **TCAGAACAGCCACTGCTGAG**-3' R5'- **AGTGCGTCCACTCTTCTGCT**-3'	20 20	865 bp

Fig.1 Molecular cloning of sheep DQA genes. (i) Quality estimation of extracted RNA from sheep's blood; (ii) Quality check of cDNA synthesized by PCR amplification of GAPDH gene; (iii) PCR amplification of DQA loci and (iv) transformation of vector (pDrive) ligated DQA genes.

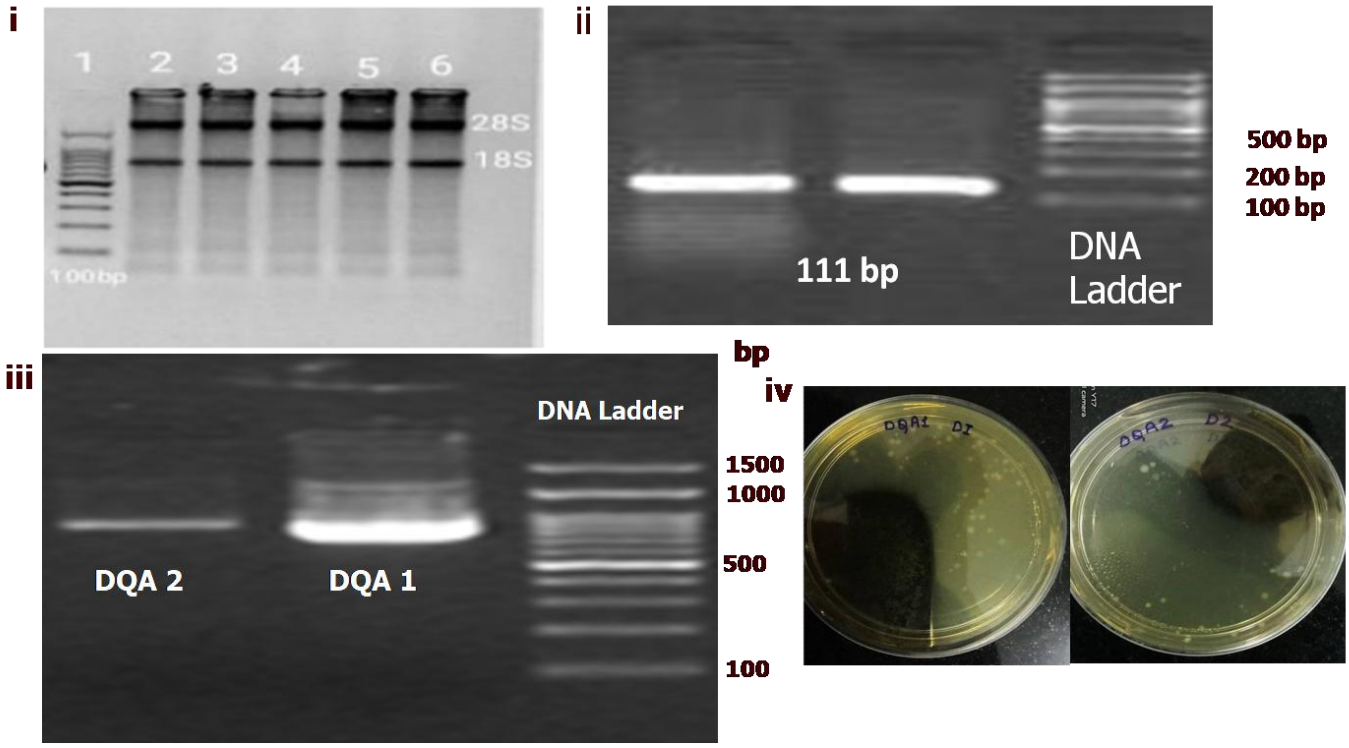


Fig.2 Phylogeny tree with distance corrections (Malpura DQA alleles; WIA07489, WIA07490 and WIA07491 are generated in present study).

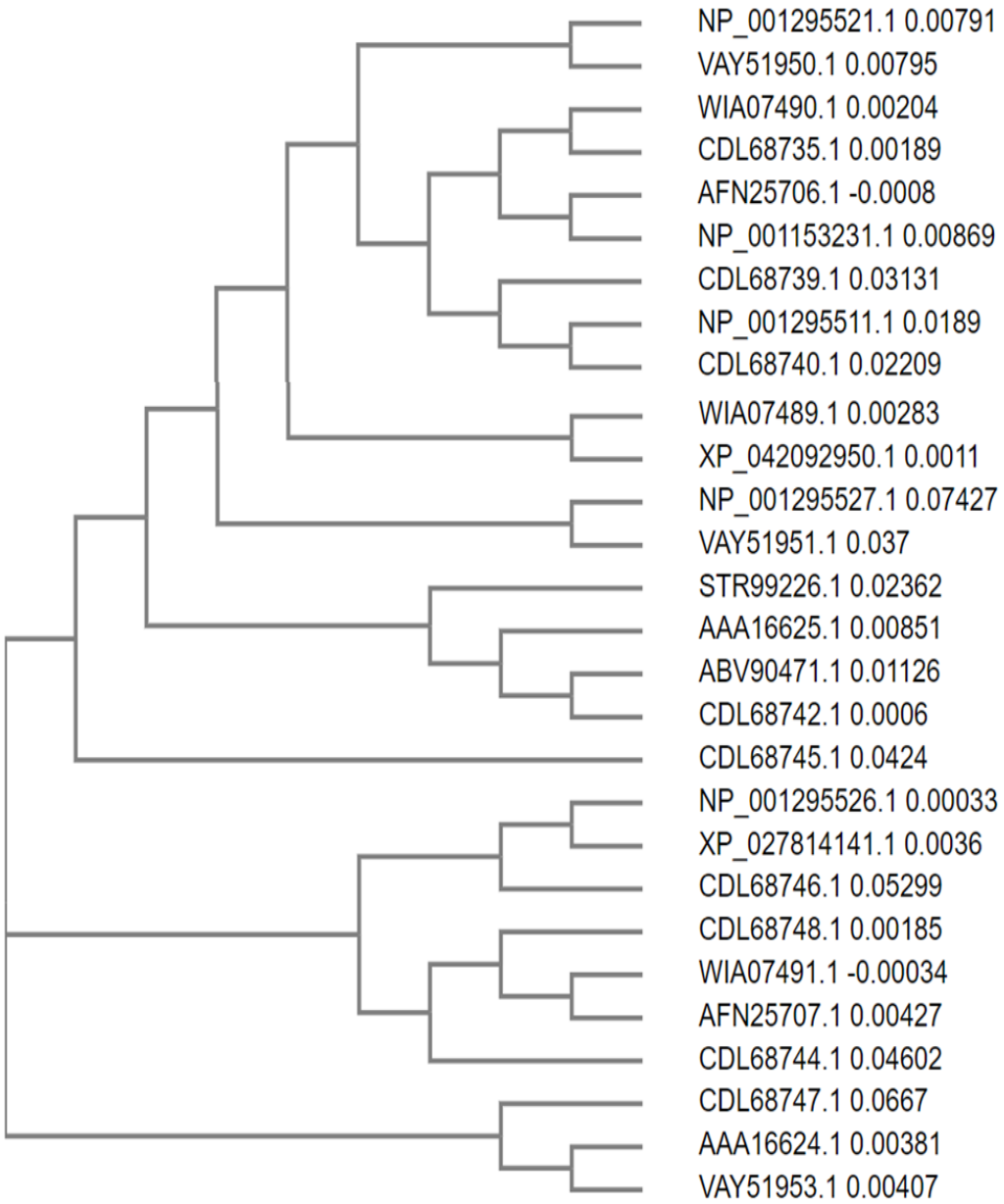
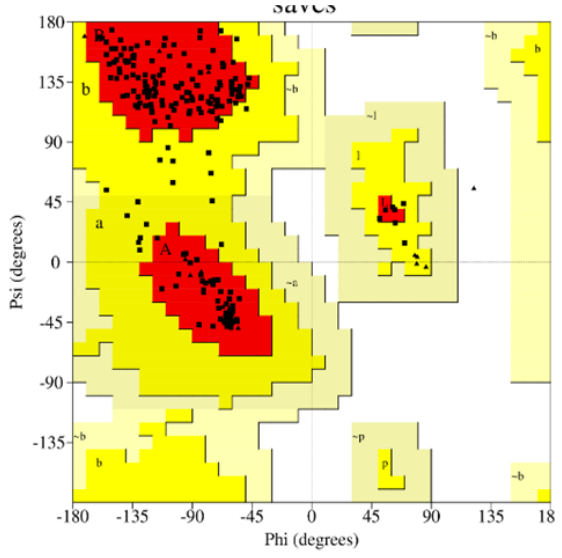


Table.2 Different physico-chemical properties of DQA1and DQA2 predicted protein using Expasy ProtParam tool.

Parameter	DQA1	DQA2
Molecular weight	28140.07	28128.96
Theoretical pI	5.11	4.70
No. of positive residues	18	14
No. of negative residues	30	32
Half-life mammalian reticulocytes (in vitro)	30hrs	30hrs
Half-life yeast	20hrs	20hrs
Half-life *E.coli*	10hrs	10hrs
Extinction coefficient	33920	36440
Aliphatic index	92.08	91.65
Instability index	52.35	44.32
GRAVY index	-0.054	0.009

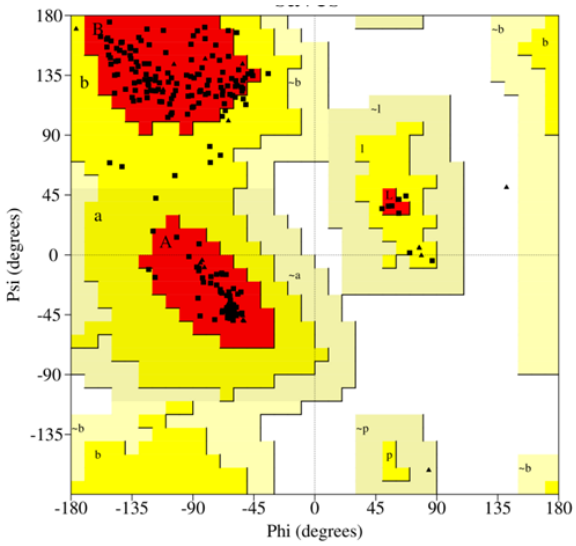
Fig.3 Prediction of possible conformation of the peptides i. e. ovine DQA1 and DQA2 gene by Ramachandran plot.



Plot statistics

Residues in most favoured regions [A,B,L]	197	90.8%
Residues in additional allowed regions [a,b,l,p]	20	9.2%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	217	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	20	
Number of proline residues	16	
Total number of residues	255	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.



Plot statistics

Residues in most favoured regions [A,B,L]	198	92.1%
Residues in additional allowed regions [a,b,l,p]	17	7.9%
Residues in generously allowed regions [~a,~b,~l,~p]	0	0.0%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	215	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	21	
Number of proline residues	17	
Total number of residues	255	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

It was demonstrated that certain variants of DQ loci in human being predisposes to type I diabetes. It has been evidenced that class II HLA haplotypes predisposes the individuals for risk of type I diabetes in human and certain other haplotype confer strong protection from it (Nejentsev *et al.,* 2007). The future prospect of the present work can be suggested that analyzing a large population of sheep, many alleles of MHC-DQA loci can be identified and can be associated with a particular type of ailment.

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References

Ballingal K T, Steele P, Lantier I, Cotelli M, Todd H, Lopez G, Martin E and Lantier F. 2015. An ancient interlocus recombination increases class II MHC DQA diversity in sheep and other Bovidae. *Animal Genetics*, 46 (3): 333-336.

Dukkipati V S, Blair H T, Garrick D J, Murray A. 2006. 'Ovar-Mhc' - ovine major histocompatibility complex: structure and gene polymorphisms. *Genetics and Molecular Research*, 5(4):581-608. PMID:17183471.

Gowane G R, Sharma P, Kumar R, Misra S S, Alex R, Vohra V, Chhotaray S, Sharma Nikita, Chopra R, Kandalkar Y, Choudhary A and Mangotra A. 2023. Population-wide genetic analysis of Ovar-DQA-1 and DQA-2 loci across sheep breeds in India revealed their evolutionary importance and fitness of sheep in a tropical climate. *Animal Biotechnology*, https://doi.org/10.1080/10495398.2023.2180010.

Nejentsev S, Howson J M, Walker N M, Szeszko J, Field S F, Stevens H E, Reynolds P, Hardy M, King E, Masters J, Hulme J, Maier L M, Smyth D, Bailey R, Cooper J D, Ribas G, Campbell R D, Clayton D G, Todd J A. 2007. Wellcome Trust Case Control Consortium. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. Nature, 450(7171):887-892, PMID: 18004301; PMCID: PMC2703779. https://doi.org/10.1038/nature06406.

Noble J A, Valdes A M. 2011. Genetics of the HLA region in the prediction of type 1 diabetes. *Current Diabetes Reports*, 11(6):533-542. PMID: 21912932; PMCID: PMC3233362. https://doi.org/10.1007/s11892-011-0223-x.

Rosa, D.S., Ribeiro, S.P. & Cunha-Neto, E. 2010. CD4+ T Cell Epitope Discovery and Rational Vaccine Design. Arch. Immunol. Ther. Exp. 58, 121–130. https://doi.org/10.1007/s00005-010-0067-0

Sapra A, Bhandari P. Diabetes. [Updated 2023 May 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- available from: https://www.ncbi.nlm.nih.gov/books/NBK551501

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