Genotyping of OLA-DRB1 in Fashandi Sheep for effective Vaccination and Disease Management Strategies

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Abstract

Introduction: The major histocompatibility complex (MHC) in large animals, known as Ovine Leukocyte Antigen (OLA) in sheep, is crucial for understanding genetic diversity, disease susceptibility, reproductive traits, and vaccine responsiveness. This sequenced OLA-DRB gene Fashandi sheep breeds to explore their role in disease resistance and vaccine response.

Material and Methods: Fifty blood samples were collected from Fashandi breed using EDTA tubes. Forty samples were stored at −20°C for DNA extraction using the i-genomic Blood DNA Extraction Mini Kit. Amplified PCR products were electrophoresed and sequenced. BLASTn (NCBI) was used for sequence identification. DRB sequences were aligned and compared with reference sequences from the Immuno Poly morphism Database (IPD) using BioEdit software. Allelic/genotypic frequencies were determined via direct counting, and genetic diversity metrics (homozygosity, heterozygosity, allele number) were calculated using Levene's test and Nei's formulas via PopGene 1.32 and GenAleXA. For bioinformatics, sequences were aligned with ClustalX and analyzed using BioEdit and CLC Sequence Viewer. Shannon entropy

measured amino acid variability, and phylogenetic trees were constructed with MEGA5.3 using neighbor joining.

Results: Eight Ovar-DRB1 exon 2 alleles were identified: *08, *19, *07, *24, *30, *16, *03, and *14. The most frequent were *08 (30%) and *07 (25%), while *30 and *03 were least common (5%). The predominant genotypes were *08/*08 and *07/*07 (each 20%). Homozygosity and heterozygosity were equal at 50%.

Conclusion: The presence of multiple alleles suggests genetic diversity influencing disease susceptibility and resistance and also vaccine response in Iranian Fashandi sheep breeds. Hopefully, understanding these genetic markers can guide better breeding, vaccination and disease management strategies.

Keywords: Fashandi Sheep breed, OLA-DRB1, Genotyping, PCR , Sequencing

1. Introduction

The major histocompatibility complex (MHC) is a locus in the genome of large livestock that provides unparalleled evidence of their genetic map and racial diversity. It is also associated with resistance and susceptibility to diseases, production and reproductive traits, breeding, livestock immunity, and identification of sheep populations. MHC proteins in sheep and goats are generally called Ovine Leukocyte Antigen (OLA) or Ovar. Over the last two decades, a lot of research has been conducted on OLA due to its key role in immune responses, its association with infectious diseases, and its genetic diversity about evolutionary history. [1-6] Numerous studies have linked OLA to immunological properties of resistance or susceptibility to various infectious diseases, mammary tumors, foot-and-mouth disease, etc. [7-12] This study aims to sequence OLA-DRB and Ovar-A genes in five major Iranian sheep breeds in order to understand the cause of susceptibility and resistance to diseases and the cause of livestock's poor response to vaccines, and to suggest appropriate strategies for vaccination, prevention, and treatment of diseases. The objectives of the study include access to immunogenetic reserve and frequency patterns and allelic genotypes of the OLA-DRB1 locus involved in immune responses in the Iranian Fashandi sheep population. To investigate the frequency of alleles identified as agents for susceptibility. To make resistance to infectious diseases through

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64 sequencing and analysis of the OLA-DRB1 locus, providing solutions for optimizing existing
65 vaccines such as agalaxia based on the frequency and genotype of identified OLA-DRB1
66 alleles, and designing new vaccines for greater adaptability and responsiveness in the target
67 population.
68

69 ## 2. Materials and Methods

70 **2.1 Blood Sampling from Fashandi Sheep**
71 Blood samples were randomly collected from the jugular vein of 50 Fashandi sheep (males and
72 females of various ages), in Razi Vaccine and Serum Research Institute and research stations
73 of the National Animal Science Research Institute in Iran. Collection was performed using 2-
74 ml venoject tubes containing EDTA anticoagulant. The blood samples were subsequently
75 stored at -20°C until DNA extraction, for immunogenetic, genotyping studies, and analysis of
76 MHC regions (OLA-DRB1). It is noteworthy that these genes are autosomal; therefore, gender
77 does not play a role in the evaluation of alleles. Similarly, age does not play a role in the genetic
78 evaluation of the animals.
79

80 **2.2 DNA extraction for PCR of OLA-DRB1.** DNA can be extracted from blood in sheep with
81 different methods. In this study, the commercial i-genomic Blood DNA Extraction Mini Kit
82 (iNtRON, South Korea) was used in the R&D laboratory of Razi Vaccine and Serum Research
83 Institute to extract DNA from blood samples. The extraction steps are as follows, based on the
84 manufacturer's instructions: First, 200 µL of whole blood was poured into a 1.5-mL microtube,
85 20 µL of proteinase K and 1 µL of RNase were added, and gently mixed. Then, 200 µL of lysis
86 buffer (containing chaotropic salts) was added to the above solution, vortexed vigorously until
87 no cell clumps remained, and the lysate was incubated at 65 °C for 10 min. During this time,
88 the tube was inverted 3-4 times for complete lysis. After complete lysis, the color of the lysate
89 changed from red to dark green. The tube was centrifuged briefly. Then, 200 µL of binding
90 buffer was added to the lysate and mixed well by gently inverting 5-6 times. After mixing, the
91 tube was centrifuged briefly and the solution was carefully transferred to the column (placed in
92 a 2-mL microtube) and centrifuged for one minute at 16,000 g with Pars Azma microcentrifuge.
93 The eluate from the column and the tube were discarded. If the lysate was not completely
94 extracted from the column membrane, the solution was recentrifuged at high speed until it was
95 completely extracted, and then the column was placed in another 2-ml tube. In the washing

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step, 700 μL of wash buffer A was added to the column and centrifuged for one minute at 16,000 g and the eluate was discarded. Then, 700 μL of wash buffer B (to which 40 mL of pure ethanol had been previously added) was added to the column and centrifuged for one minute at 16,000 g and the eluate was discarded, and centrifuged again for one minute to dry the column membrane. The eluate and tube were discarded, and the column was placed in another 1.5-mL tube. Finally, 100 μl of wash buffer was poured directly into the column membrane and incubated for five minutes at room temperature. The column was then centrifuged for one minute at 16,000 g to separate the DNA from the column membrane.

2.3 Determining the quantity and quality of the extracted DNA. After the extraction, the quality and quantity of the DNA were measured to determine if it is appropriate and pure enough for the experiment. The quality of the samples was measured using spectrophotometry and by calculating the ratio of light absorption at a wavelength of 260 nm to 280 nm. The appropriate ratio for DNA samples should fall within the range of 1.7-2 and inappropriate samples should undergo re-extraction. DNA concentration was also calculated based on light absorption at a wavelength of 260 nm, and finally all DNA samples were prepared with a concentration of 100 ng/μl. Also, to further ensure the quality of the DNA samples, 5 microliters of each sample was loaded onto a 1% agarose gel and electrophoresed. After the samples were 4 cm away from the wells, electrophoresis was terminated and the gel was stained with ethidium bromide. Then, the gel was examined under an ultraviolet transilluminator and the intensity of the bands related to the samples were compared with standard DNA. The quality of the DNA was also determined by observing the shape of the bands. The presence of completely sharp bands without the slightest stretch indicates the best quality. A stretch between the well and the band indicates protein contamination or salt residue in the samples. The presence of an additional band at the bottom of the gel and at a great distance from the main band indicates the presence of RNA in the samples. The extracted DNA samples were stored at -70 ºC until the experiment time.

2.4 Direct sequencing of Ovar-DRB1 gene exon 2 from samples

A separate primer pairs named OLA-ERB1-F1 and HL031-R was used for the first step of the semi-nested PCR. For the second step, the OLA-ERB1-F1 primer from the previous pair and the new OLA-XRB1 primer were used (Table 1). Also, 20 μl of the PCR products that showed

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128 bands in electrophoresis were sent to Noorgen Company with the forward primer (F) for
129 sequencing. PCR for other primers was run in a final volume of 50 μL, including 3 mM $MgCl_2$,
130 400 μM dNTP, 1 mM primer, 2 units of Taq DNA Polymerase (Cinnagen Company), and 2 μL
131 DNA. Then, after electrophoresis and confirming the presence of the desired band, the products
132 were sent to the company in a 1.5-mL microtube for sequencing.

133

134 **Table 1.** The primer used for semi-nested PCR analysis of the Ovar-DRB1 gene alleles, along
135 with the sequence and length of the resulting fragment.

136

Primer name	Sequence	Fragment length	Program
OLA-ERB1-F1 HL031-R	5-CCGGAATTCCCGTCTCTGCAGCACATTTCTT-3 5-TTTAAATTCGCGCTCACCTCGCCGCT-3	300 bp~	1 cycle: Initial denaturation for 5 min at 94°C 36 cycles: 94°C for 30 s, 50°C for 30 s, 1 min at 72°C 1 cycle: final extension at 72°C for 10 min
OLA-ERB1 OLA-XRB1	5--CCGGAATTCCCGTCTCTGCAGCACATTTCTT-3 5-GCTCGAGCGCTGCACAGTGAAACTC-3		1 cycle: Initial denaturation for 5 min at 94°C 38 cycles: 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min 1 cycle final extension: 72°C for 10 min

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140 **2.5 Sanger sequencing**

141 Sequencing was performed with an automated DNA sequencer by Noorgen Co., and sequences
142 were identified using BLASTn on the NCBI website (http://www.ncbi.nlm.nih.gov/pmc).
143 DRB3.2 sequences obtained by BioEdit software (www.mbio.ncsu.edu/bioedit/bioedit.html)
144 were compared with reference sequences registered on IPD website
145 (http://www.ebi.ac.uk/ipd/mhc/bola/index.html).

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147 **2.6 Statistical analysis**

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148 Allelic and genotypic frequencies were determined by direct counting. The expected
149 homozygosity and heterozygosity were also calculated using Levene's (1949) formula.
150 Deviation from Hardy-Weinberg equilibrium with Fis variables was estimated based on the
151 formula proposed by Weir and Cockerham (1984).[13] Gene diversity was also obtained with the
152 number of alleles and expected heterozygosity based on Nei's formula (1973).[14] PopGene 1.32
153 software was used to calculate the expressed variables.
154

155 **2.7 Bioinformatics analysis**

156 **2. 7.1 Recovery and classification of required protein/nucleotide sequences**

157 In addition to the sequences obtained in this study, the protein/nucleotide sequence of exon 2
158 of the Ovar-DRB1 gene in sheep and its equivalent sequences in some other large livestock
159 (cattle, buffalo, goats, and horses) and dogs and cats were extracted from the NCBI reference
160 databases (http://ncbi.nlm.nih.gov/), UniProtKB/Swiss-Prot (www.expasy.org/uniprot), and
161 Immuno Polymorphism Database (IPD).
162

163 **2.7.2 Sequence alignment and analysis of variability and conservation using a hybrid**
164 **method**

165 The allelic sequences obtained from the sequenced samples of the present study on the Fashandi
166 sheep, along with the sequences retrieved from the Immuno Polymorphism Database (IPD)
167 related to sheep, were aligned using the ClustalX algorithm and evaluated using BioEdit 7.7.9
168 and CLC Sequence Viewer. Subsequently, short sequences and regions with ambiguous
169 alignment were removed. The amino acid variability (diversity) and conservation of the allelic
170 sequences were analyzed separately using three different methods. Shannon entropy analysis
171 was used to examine the degree of variation, diversity, and conservation among the amino acids
172 of the sequences corresponding to different alleles. Swiss-PdbViewer software
173 (http://spdbv.vital_it.ch/) was used to visualize variable regions within the three-dimensional
174 structure of the protein.
175

176 **2.7.3 Phylogenetic analysis**

177 To construct the phylogenetic tree, the Neighbor Joining method was used in MEGA.8
178 software. To ensure the reliability and reproducibility of the generated trees, bootstrapping with
179 1,000 replicates was performed, and an appropriate outgroup was also included.

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180

181 **2.7.4 Calculation of the mean pairwise sequence distance and analysis of their similarity**

182 Also, the mean sequence distance and their similarity for sheep, cattle, buffalo, goat, and horse
183 were calculated using the MEGA.8 software and matrix plotting.

184

185 **2.7.5 Prediction of 3D structure using Homology modeling**

186 Basic Local Alignment Search Tool (BLASTP) and PSI-BLAST were used in the Protein Data
187 Bank (PDB) to select the best template (homologous sequence) and to design the 3D structure
188 of the allele by homology modeling. This approach was applied because the allele shared more
189 than 25% similarity with data from PDB. Template selection criteria included a resolution lower
190 than 3 Å from X-ray crystallography, an R-value below 0.3, template similarity greater than
191 35% to the target sequence, and a low E-value. Additionally, the target sequence (allele
192 sequence) and the selected template were required to have appropriate structural alignment with
193 low RMSD (Root Mean Square Deviation) values. Applying these criteria increases the
194 reliability and validity of the modeling process. After aligning the closest template sequence
195 identified, this sequence was aligned with the sequence analyzed in the present study. Modeller
196 9v8 software was then used to build the 3D structure based on the template. To visualize the
197 3D protein structure, Swiss-PdbViewer software (http://spdbv.vital_it.ch/) was utilized.

198

199 **2.7.6 Validation and quality assessment of the constructed 3D model**

200 After energy optimization, the next step is the quality assessment of the model, which is
201 considered a critical phase in homology modeling. To assess the validity of the constructed
202 model structure, a Ramachandran plot was used to evaluate the stereochemical quality and rigor
203 by calculating the dihedral angles of the modeled protein. This analysis was run in RAMPAGE
204 server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

205

206

207 ## 3. Results

208 **3.1 Results of blood sampling from sheep**

209 Preliminary studies, coordination for sample collection, and the actual sampling of flocks took
210 four months. Forty samples were used for DNA extraction and immunogenetic and genotyping

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studies of Fashandi sheep, including both males and females of different ages. The remaining samples (10 samples) were preserved as genetic reserves for emergency purposes.

3.2 DNA extraction with a commercial kit

DNA extraction was successfully performed using the commercial i-Genomic Blood DNA Extraction Mini Kit (iNtRON, South Korea) at the Research and Development Laboratory of Razi Vaccine and Serum Research Institute. The extracted DNA was aliquoted into 2-mL microtubes, labeled for each sample, and stored at –70 °C. Additionally, a portion of the extracted DNA was taken for quantitative and qualitative assessment.

3.3 Results of quantitative and qualitative assessment of extracted DNA

To assess the quality and quantify the extracted DNA, a NanoDrop spectrophotometer was used. Additionally, the quality of the extracted DNA was visually examined using agarose gel electrophoresis. Fig. 1 shows the Nanodrop results for the samples, demonstrating quantitative evaluation and the absence of possible contamination. NanoDrop operates based on light absorption at specific wavelengths (spectrophotometry) from varying concentrations of substances. It determines the concentration and quality of DNA by measuring the wavelength of light passing through the sample. The device is connected to a computer and presents the results numerically and visually. In this method, the quality of DNA is assessed by reading the A260/A280 ratio, where light absorption at 260 nm is measured to estimate the DNA concentration. The ideal OD260/OD280 ratio ranges between 1.7 and 2. Values below 1.7 indicate protein contamination and other ultraviolet-absorbing impurities, while ratios above 2 suggest a high RNA content in the sample. The extraction results in the present study indicate an appropriate DNA quality with an average ratio of 1.7. Additionally, the extracted samples showed no contamination with proteins, salts, or other impurities.

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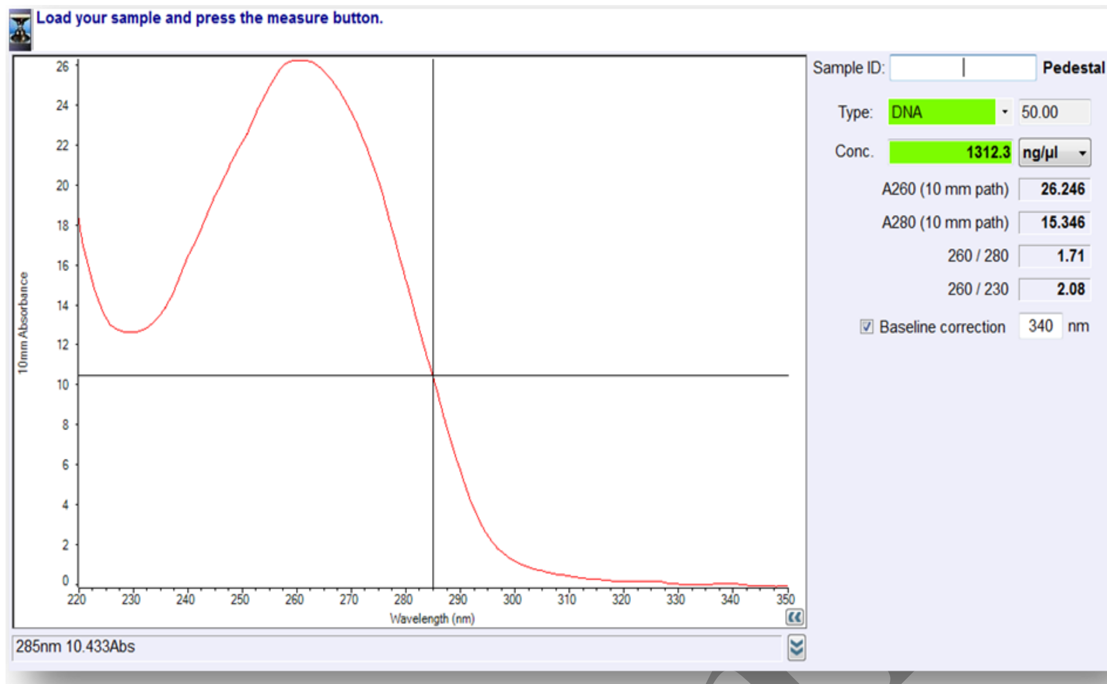

237

238 **Fig. 1.** Quantitative and qualitative assessment of extracted DNA using NanoDrop

239

240 **3.4 Results of genomic DNA extraction and electrophoresis**

241 After extracting DNA from blood samples using the commercial kit, the presence and quality
242 of the DNA content were assessed by electrophoresis on a 1% agarose gel. Fig. 2 shows the
243 genomic DNA bands obtained from four blood samples of sheep, for which genomic DNA
244 extraction was performed, visualized through electrophoresis. A summary of this process is also
245 presented in Fig. 3.

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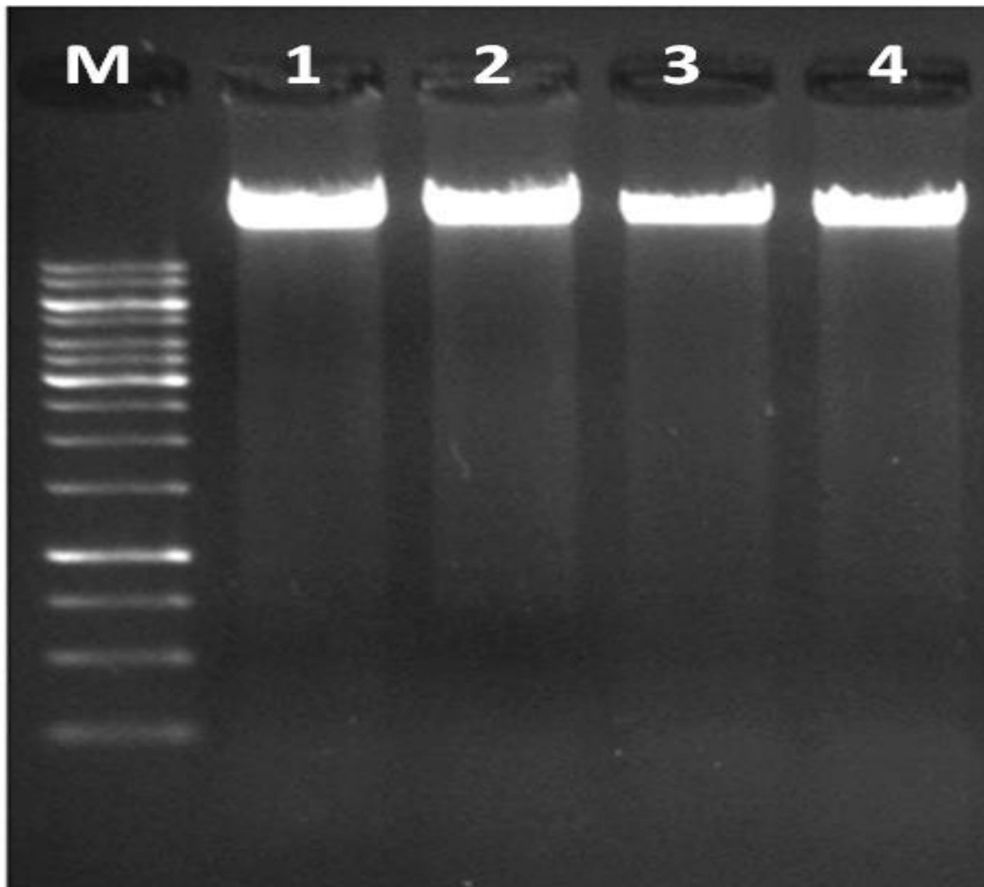

249 **Fig. 2.** Electrophoresis of genomic DNA extracted from sheep blood samples. Numbers 1 to 4
250 represent individual samples, and M indicates the DNA marker**.**

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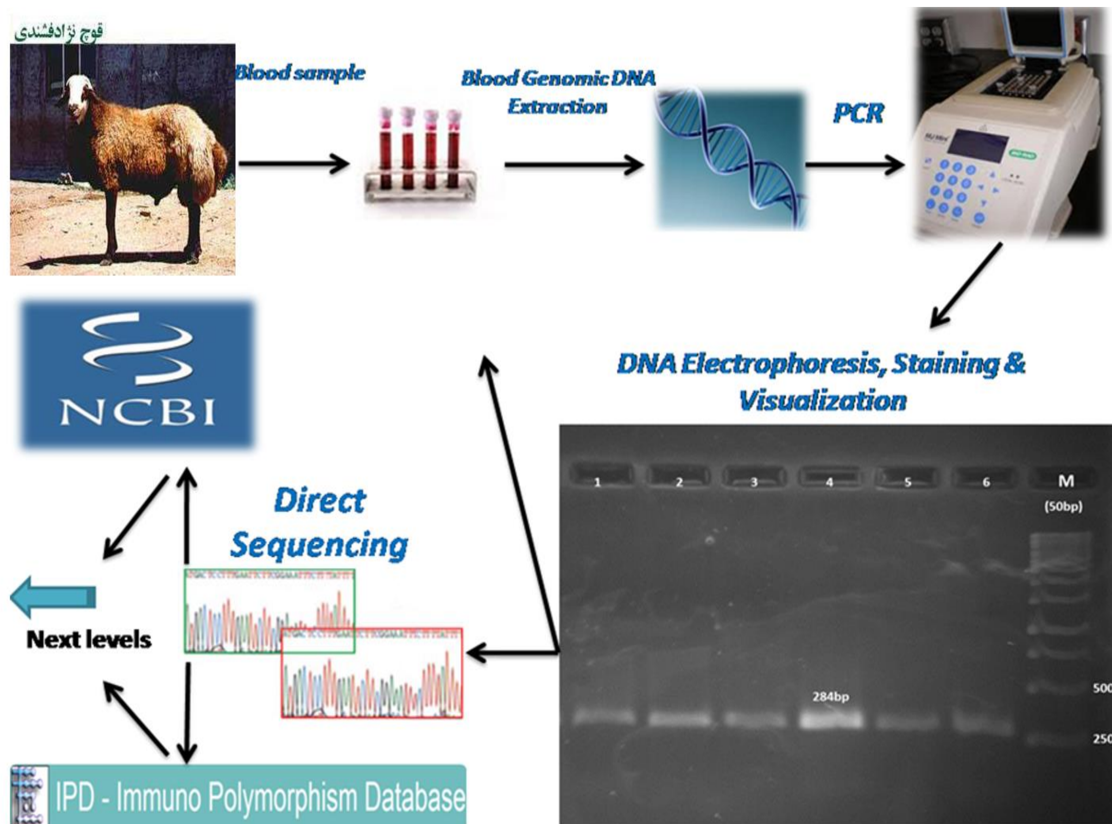

Fig. 3. Summary of the initial steps of the research process and electrophoresis results of PCR products for amplification of the Ovar-DRB1.2 gene on 1.5% agarose gel in Fashandi sheep .samples. Lanes 1 to 6 represent the samples; M: 50 bp marker**.**

3.5 Results of amplification of Ovar-DRB1 gene exon 2. Amplification of Ovar-DRB1.2 gene was performed using the semi-nested PCR method. Fig. 4 demonstrates an example of the PCR product electrophoresis results, along with the steps carried out in this study for analyzing Fashandi sheep samples. Subsequently, the genetic diversity of the Ovar-DRB1.2 gene in Fashandi sheep samples was analyzed using nested-PCR direct sequencing. The nested-PCR products were sent to Noorgen Company for sequencing.

3.6 Sequencing results in Fashandi sheep breed

3.6.1 Sequencing, allele identification, and alignment of sequenced samples in Fashandi sheep breed

In the present study, the study populations were identified using direct sequencing. The resulting sequences were then curated, analyzed for homozygosity/heterozygosity, and alleles were

identified manually using FinchTV, BioEdit 7.7.9, and NCBI software, alongside datasets from IPD and NCBI. An example of the sequencing chromatogram is presented in Fig. 4.

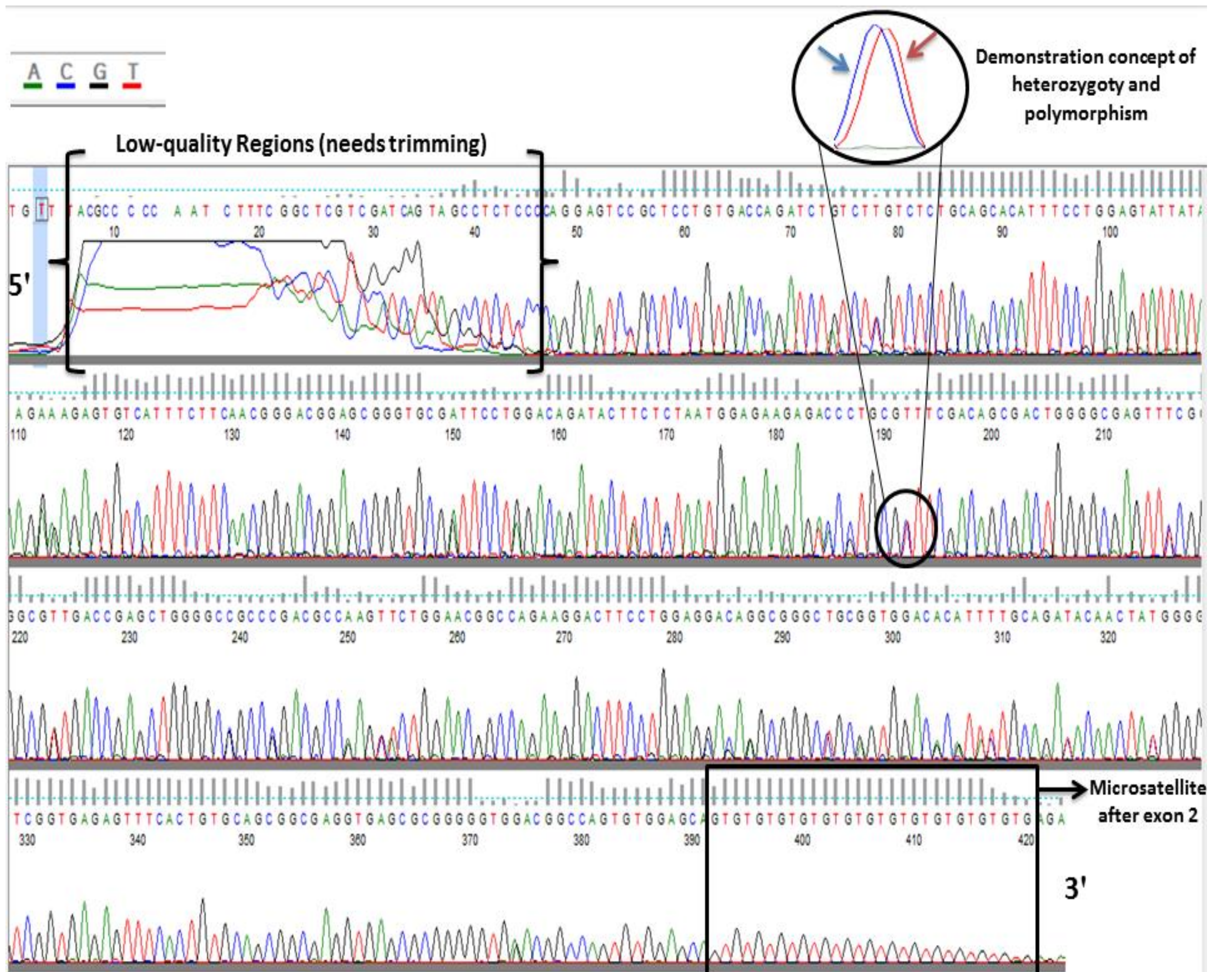

Fig. 4. Chromatogram of a representative sequence obtained by direct sequencing using FinchTV software. The chromatogram displays multiple regions with overlapping peaks, indicating heterozygosity at the allele level.

The arrangement of nucleotide sequences and the identified allele patterns in this study were aligned in order to examine visible variations (Fig. 5). The alignment of nucleotide and protein sequences indicate significant differences and variations among the sequences (alleles). Furthermore, the nucleotide alignment and subsequent translation of Ovar-DRB1 gene exon 2 in Iranian Fashandi sheep revealed that the rate of synonymous substitutions is intriguingly higher than that of non-synonymous substitutions in non-peptide-binding sites (PBS). Another interesting observation was that in homozygous samples, in most cases, two similar alleles differed by at least one, two, or three amino acids and were considered subtypes of each other.

286

287

288 **Fig. 5.** Some of the similarities and differences in the nucleotide sequences of the DRB3.2
289 BoLA alleles identified in the study population were analyzed through alignment using BioEdit
290 version 7.7.9. Identical residues are marked with a dot ('.'), and gaps are shown with a dash ('-
291 '). The lower part of the figure highlights alleles with nucleotide sequences that significantly
292 diverge from other samples.

293

294

295 **3.7 Statistical Analysis**

296 In the samples from Fashandi sheep breed, eight distinct and unique alleles were identified.
297 Their descriptions, allele frequencies, and genotypic frequencies are presented in Tables 2 and
298 3, respectively. One sample was suspected to be a new allele, for which no equivalent was found
299 during similarity assessment with other alleles reported in IPD (this allele is listed separately
300 from the other results). The asterisk (*) in the immunogenetics indicates an allele to distinguish
301 it from the standard numbering.

302 **Table 2.** The identified unique alleles and the frequency of observed alleles in sequenced
303 samples of the Fashandi sheep breed.
304

Alleles number	Alleles name with subtypes	Frequency (%)
1	**Ovar-DRB1*08:02 Ovar-DRB1*08:01 Ovar-DRB1*08:06**	**30% (n=12)**
2	**Ovar-DRB1*19:04**	**5% (n=2)**
3	**Ovar-DRB1*07:02 Ovar-DRB1*07:04**	**25% (n=10)**
4	**Ovar-DRB1*24:01**	**10% (n=4)**
5	**Ovar-DRB1*30:01**	**5% (n=2)**
6	**Ovar-DRB1*14:02**	**10% (n=4)**
7	**Ovar-DRB1*16:05 Ovar-DRB1*16:02:02**	**10% (n=4)**
8	**Ovar-DRB1*03:03**	**5% (n=2)**

305

306 **Table 3.** Observed alleles and their frequencies in sequenced samples of the Fashandi sheep
307 breed.
308

Genotype	Number of observed cases	Frequency (%)
1	*08/*08	20% (n=8)
2	*07/*07	20% (n=8)
3	*19/*08	10% (n=4)
4	*30/*24	10% (n=4)

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5	*16/*08	10% (n=4)
6	*03/*24	10% (n=4)
7	*14/*14	10% (n=4)
8	*16/*07	10% (n=4)

309

310

311 Overall, eight allele types were observed in Ovar-DRB1 gene exon 2, including *08, *19, 312 *07, *24, *30, *16, *03, and *14. Among these, the most frequent allele (including its sub-313 alleles) was *08, with a frequency of 30% (12 cases), followed by *07 with a frequency of 25% 314 (10 cases). The lowest frequencies were seen for alleles *30, *03, and *19, each at 5% 315 frequency (2 cases each). Regarding genotypes, the most commonly observed were *08/*08 316 and 07/07, each accounting for 20% of the population. The frequencies of other genotypes were 317 equal, each at 10%. In total, eight different genotype combinations were identified in the 318 population. Additionally, half of the samples (50%) were homozygous, and the other half (50%) 319 were heterozygous, indicating equal levels of homozygosity and heterozygosity (Table 4).

320

321 **Table 4.** Pairwise similarity analysis of allele *24 with selected homologous sequences in large 322 livestock and carnivores (dog and cat), and independent comparison of their mutual similarities.
323

	BoLA-DRB3*2402	Buffaloe	Sheep	Goat	Horse	Dog	Cat
BoLA-DRB3*2402	1	0.77	0.82	0.83	0.74	0.74	0.63
Buffaloe	0.78	1	0.78	0.74	0.64	0.65	0.64
Sheep	0.81	0.77	1	0.87	0.69	0.73	0.65
Goat	0.81	0.1	0.86	1	0.68	0.72	0.58
Horse	0.72	0.61	0.67	0.67	1	0.71	0.57
Dog	0.72	0.62	0.71	0.71	0.71	1	0.68
Cat	0.62	0.63	0.64	0.59	0.58	0.69	1

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4. Discussion

Numerous studies have indicated an association between OLA and immunological traits related to resistance or susceptibility to various infectious diseases, mastitis, foot-and-mouth disease, and others. The aim of this study was to sequence the OLA-DRB and Ovar-A genes in populations of five major Iranian sheep breeds in order to better understand the basis of disease resistance and susceptibility, as well as the poor response to vaccination in livestock. The ultimate goal was to propose appropriate strategies for vaccination, disease prevention, and treatment.

MHC comprises molecules responsible for binding antigenic peptides and presenting them to T lymphocytes, which initiate the immune response upon recognizing these peptides. The function of MHC molecules is highly dependent on their molecular structure.[15] In all animal species, MHC genes are among the most diverse. Additionally, MHC genes exhibit codominant expression in each individual, thereby increasing the number of MHC molecules available to bind to peptide antigens and present them to T cells.[15]

MHC class II molecules are formed through non-covalent bonding of α1 and β1 chains, which are encoded by specific genes within the MHC. The α1 and β1 domains form the antigen-binding groove of the class II MHC molecule, and this region exhibits genetic polymorphism. Furthermore, the pockets that bind to the amino acid chains of antigens are located within this groove.[15] The antigenic peptide that binds to these pockets is determined by the amino acid motifs at the binding site. Therefore, an antigenic peptide with a specific amino acid sequence binds selectively to the MHC molecule of a given allele. Exon 2 of OVAR class II DRB3 locus, which displays extensive polymorphism, plays a significant functional role. Many DRB3 alleles have been identified in the β1 domain sequence, which constitutes the antigen-binding groove.[16] The selective binding of MHC molecules to antigenic peptides appears to determine the specificity and magnitude of the immune response to an antigen, as well as susceptibility or resistance to diseases.[16]

It has been over 20 years since Van Eijk et al. (1992)[17] first identified 30 BoLA alleles using PCR product digestion with three restriction enzymes. Since then, more than 130 alleles have been identified, some of which have been associated with susceptibility and resistance to diseases, as well as with productive and physical traits in livestock.[18]

Studies suggest that homozygosity reduces the ability to mount an effective response against infectious diseases. In the Iranian Fashandi sheep population, the levels of homozygosity and

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heterozygosity were found to be equal. Moreover, based on the alleles observed in Iran, certain alleles previously identified worldwide as being associated with susceptibility to infectious diseases were also detected in the Fashandi sheep population. Additionally, earlier studies using direct sequencing, PCR-RFLP, and other methods have reported between 8 and 16 alleles in different sheep breeds across the world.

In previous studies, allele *07 has been associated with lower proviral loads in ovine progressive pneumonia. Additionally, allele *03 has been linked to susceptibility to Visna/maedi (VM) disease. Allele *03 is also reported to influence sheep growth and ewe longevity.

Our findings indicate that the Fashandi sheep population, based on the allele and genotype frequencies reported in this study, likely carries multiple alleles associated with susceptibility to infectious diseases. These alleles may play a significant role in determining the success of vaccination and the level of antibody titers produced against the vaccine strain.

5. Conclusion

The present study demonstrates a moderate level of genetic polymorphism at the OLA‑DRB1 exon 2 locus in Iranian Fashandi sheep breed. Also, we identified of eight alleles and balanced levels of homozygosity and heterozygosity. The predominance of alleles *08 and *07 as well as the presence of alleles previously associated with susceptibility to infectious diseases, underscores the functional relevance of MHC class II diversity in shaping immune responsiveness and variability in vaccine-induced protection within this native population.

These findings strongly suggest that OLA‑DRB1 polymorphism may contribute to differential disease susceptibility and vaccination outcomes in Fashandi sheep breed. Incorporating immunogenetic information into breeding programs and vaccination strategies could therefore enhance disease control and herd health management. Further studies integrating DRB1 genotypes with clinical, immunological, and vaccine response outcomes are warranted to establish robust genotype–phenotype associations and to support precision approaches in sheep health management.

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401 ## Authors' Contribution (Abbreviated names)
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404 ## Conflict of interests
405 The authors declare that they have no conflict of interest.

406

407
408 ## Ethics approval
409 We hereby declare all ethical standards have been respected in preparation of the submitted article.

410

411
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413 The authors confirm that they did not receive any financial assistance for the research, authorship, and/or
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415

416
417 ## Data Availability
418 The data that support the findings of this study are available on request from the corresponding author.

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420

421

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