Molecular characterization of sheep major histocompatibility complex (OVAR-MHC) Class II DQA1 gene in two Egyptian breeds

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Abstract

The major histocompatibility complex (MHC) is a large locus on vertebrate DNA that contains a set of closely related polymorphic genes coding for cell surface proteins essential for an adaptive immune system. MHC is thought to be under strong selection pressure because of its main role in infectious disease resistance. Thus, the pattern of different MHC genes variation should reflect this selection pressure in animal's natural populations. To test this hypothesis, we investigate the nature and frequency of polymorphism of the sheep MHC class II DQA1 gene in two different common local Egyptian sheep breeds (Ossimi and Saidi). Ovar-DQA1 gene is considered one of the most important response genes in the MHC region. In the current study, blood samples of 50 Egyptian sheep from two breeds (32 Ossimi and 18 Saidi) with different ages and sex and from seven different farms in Qena governorate were collected for DNA extraction and PCR amplification. The PCR products of 18 DQA1 alleles were examined using direct sequencing, for the detection of polymorphism in a functionally relevant domain of the DQA1 gene. Among the investigated samples the phylogenetic tree reconstruction and sequence comparison revealed different DQA1 alleles. Thirty-two sheep samples lacking DQA1, five different alleles were identified for the two breeds. The multiple sequence alignment illustrates nucleotide polymorphism and conserved regions in the identified sequences. This variation leads to a better understanding of the genetic makeup of Ovar-MHC and facilitates the selective breeding programs to determine the resistance and susceptibility to disease in sheep.

Keywords: DQA1, Ovar-MHC, Ovis aries, Ossimi sheep, Saidi sheep.

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Introduction

Recent breeding and genetic selection are highly dependent on the protection of genetic diversity to meet current production needs under different environmental conditions (Abd El Gawad et al., 2019). Assessing the genetic diversity of domestic sheep is the first step in breeding strategy for conservation in the management of genetic resources (Ghazy et al., 2013).

In the last two decades, Egyptian sheep breeds are shown an increasing number with about 5.69 million heads, exceeding the numbers of other farm animals such as cattle, buffaloes, and goats (Elshazly and Youngs, 2019). The Nile Delta, Upper Egypt, and desert rangelands represent the main region where Egyptian sheep breeds are distributed (Iñiguez, 2005). In Upper Egypt sheep, breeds are mainly domestic animals, in mixed herds, with about 1.5 million heads of Egyptian breeds (Othman et al., 2016).

Ossimi Egyptian breed is primarily from Giza province in northern Upper Egypt, derived its name from the town of Ossim near Cairo. It is a major breed inhabiting the Nile valley, central and southern deltas of Egypt. Their numbers exceed the other breeds and represent the most reproductive breed in the Middle East. The Saidi sheep is considered a minor type of Egyptian sheep breed, it is distributed in Upper Egypt, and its place of origin is near Bani-Adi in the province of Assiut (Othman et al., 2018; Elshazly and Youngs, 2019). Fuller et al, 2011 indicated that the Egyptian livestock industry has provided detailed deep information and insight into the historical phenomena affecting livestock biodiversity because the country is strategically located at the gateway for most domestic species in Africa.

The genetic makeup of the current sheep breeds considers the main factor that increases sheep production. Therefore, it is necessary to understand the sheep breed resources in Egypt and how to use them to increase the production of different breeds of sheep (Elshazly and Youngs, 2019). Compared to European sheep breeds and other native African sheep populations, studying the genetic diversity and structure of Egyptian breeds can show how anthropological and demographic events affect the complexity of the genome structure of domesticated sheep species (Gizaw et al., 2007 and Lawson Handley et al., 2007).

The sheep Major Histocompatibility Complex (Ovar-MHC) also known as ovine lymphocyte antigen (OLA), contains immune gene clusters related to the general resistance/susceptibility of animals to infectious diseases (Flajnik and Kasahara, 2001; Kaufman, 2002; Danchin et al., 2004). The MHC genes are closely related to many agricultural traits, especially genes related to disease resistance of various pathogenic viruses, bacteria, and parasites (Buitkamp et al., 1996; Escayg et al., 1997; Konnai et al., 2003). In addition, the loci are arranged in the MHC to form different functional clusters such as Class I, Class II, and Class III, indicating that there is a significant level of conservation among mammalian species (Beck et al., 1999 and Elsik et al., 2009). The importance of sheep MHC molecules in disease resistance and the related structure features in mammals have led to increased studies on the sheep MHC (Gao et al., 2010). The DNA variations encoding the peptide binding domains of classical class I and class II MHC loci in sheep have been reported in different studies (Ballingall and McKeever, 2005; Stear et al., 2007; Ballingall, et al., 2008 & 2010; Ballingall and Tassi, 2010; Stirling and Stear, 2010).

Herrmann-Hoesing et al., 2008 and Wright & Ballingall, 1994 revealed the existence of two DQ loci in ovine MHC class II, each containing two DQA and two DQB genes, organized in a tail-to-tail orientation. A linear track of 130 kb and 160 kb of DNA linked the two loci which are 22-25 kb apart.
Two DQA genes in all sheep studied haplotypes, most of which have DQA1 and a DQA2 locus and are polymorphic (Scott, 1991). However, the DQA1 loci don’t appear to exist in some haplotypes (DQA1 null) (Fabb et al., 1993 and Ballingall et al., 2015). Snibson et al., 1998 assumed that 10-18% of sheep are reported to be completely lacking the DQA1 gene (DQA1 null). In these haplotypes, the DQA2 locus is detected in combination with a second locus. According to the phylogenetic analysis of the second exon, this locus seems to be more related to DQA2 than to DQA1, therefore the characterization of the locus is DQA2-like. In resistant animals, the Ovar-DQA1 gene was 8.4 times more highly expressed. This was related to the fact that susceptible animals had a higher frequency of DQA1 null alleles (Keane et al., 2007). Kostia et al., 1998 indicated that the MHC of sheep is poorly characterized among domesticated species. In this study, we used sequence-based genotyping to investigate the DQA1 gene polymorphism to increase knowledge on the DQA1 genetic diversity in local Egyptian breeds such as Ossimi and Saidi sheep. Evolutionary relationships by phylogenetic tree construction in DQA1 alleles in sheep and other species were investigated, which will improve the definition of MHC class II genes in sheep.

Materials and methods

1. Sample’s collection:

A total of 50 sheep blood samples (Table 1) (34 Ossimi and 16 Saidi breeds of sheep) were collected from different localities in Qena governorate, Egypt from smallholders and farms. The animal sex was 19 males and 31 females, aged from 4 to 60 months. Three milliliters of blood were collected in glass tubes containing the anticoagulant disodium EDTA by jugular vein puncture, the tubes were centrifuged at 4000 rpm for 10 minutes and plasma was separated into a 1.5 ml Eppendorf tube for DNA extraction.

Table 1: The study samples, breed, alleles name and GenBank accession numbers.

| Sample | Breed  | Allele  | Accession number |
|--------|--------|---------|------------------|
| 1      | Ossimi | Null    |                  |
| 2      | Saidi  | DQA1*2  | MW672099         |
| 3      | Ossimi | DQA1*3  | MW683492         |
| 4      | Ossimi | Null    |                  |
| 5      | Saidi  | Null    |                  |
| 6      | Ossimi | DQA1*6  | MW683493         |
| 7      | Saidi  | DQA1*7  | MW683494         |
| 8      | Ossimi | Null    |                  |
| 9      | Saidi  | DQA1*9  | MW683495         |
| 10     | Ossimi | DQA1*10 | MW683496         |
| 11     | Ossimi | Null    |                  |
| 12     | Saidi  | Null    |                  |
| 13     | Ossimi | Null    |                  |
| 14     | Ossimi | Null    |                  |
| 15     | Ossimi | Null    |                  |
| 16     | Saidi  | Null    |                  |
| 17     | Ossimi | DQA1*17 | MW683497         |
| 18     | Saidi  | Null    |                  |
| 19     | Ossimi | DQA1*19 | MW683498         |
| 20     | Ossimi | Null    |                  |
| 21     | Saidi  | Null    |                  |
| 22     | Ossimi | Null    |                  |
| 23     | Saidi  | Null    |                  |
| 24     | Ossimi | Null    |                  |
| 25     | Ossimi | Null    |                  |
| 26     | Saidi  | DQA1*26 | MW683499         |
| 27     | Saidi  | DQA1*27 | MW683500         |
| 28     | Saidi  | Null    |                  |
| 29     | Ossimi | DQA1*29 | MW683501         |
| 30     | Ossimi | Null    |                  |
| 31     | Ossimi | Null    |                  |
| 32     | Ossimi | Null    |                  |
| 33     | Saidi  | DQA1*33 | MW683502         |
| 34     | Ossimi | DQA1*34 | MW683503         |
| 35     | Ossimi | DQA1*35 | MW683504         |
| 36     | Ossimi | Null    |                  |
| 37     | Ossimi | Null    |                  |
| 38     | Ossimi | Null    |                  |
| 39     | Ossimi | Null    |                  |
| 40     | Ossimi | Null    |                  |
| 41     | Saidi  | Null    |                  |
| 42     | Ossimi | Null    |                  |
| 43     | Saidi  | DQA1*43 | MW683505         |
| 44     | Ossimi | Null    |                  |
| 45     | Ossimi | Null    |                  |
| 46     | Saidi  | DQA1*46 | MW683506         |
| 47     | Saidi  | DQA1*47 | MW683507         |
| 48     | Ossimi | Null    |                  |
| 49     | Saidi  | DQA1*49 | MW683508         |
| 50     | Ossimi | Null    |                  |

MW683492 - SVU-IJVS, 4 (3): 24-37
2- DNA extraction:

QIAamp Blood Mini Kit (Qiagen) was used to extract DNA from whole blood using the procedures of the Spine protocol, and according to the manufacturer’s instructions. The UV-Vis NanoDrop Spectrophotometer (Nano Drop 2000, Thermo) was used to estimate the DNA concentration and purity using the ratio between the absorbance readings at 260 nm and 280 nm, to use pure samples in the PCR reaction.

3- PCR amplification and sequencing:

The specific PCR primers used to amplify the genomic DNA were the forward primer DQA1_F, 5’-ACCTGACTCACCTGACCACA-3’, and the reverse primer DQA2_R, 5’-AACACATACTGTTGGTAGCAGCA- 3’ (Scott et al., 1991).

PCR amplification was carried out in PCR tubes on ice with a total volume of 25 μL using PCR Master Mix Kit (Qiagen). Amplification was performed in a thermal cycler (Longe gene A200 radiant thermal cycler, Longe gene Scientific Instrument, Japan). The PCR conditions were, denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30s, 72°C for 30 s; this was followed by an extension step at 72°C for 5 min. The PCR products were visualized by electrophoresis tray Seakem LE agar (Sci-Plas, LTD, England) in 1.5% agarose (Biotech, Bio Basic Canada Inc.) using 1X TAE buffer (Thermo Scientific) containing ethidium bromide (50 µg/ml).

The PCR products were visualized under a UV transilluminator and photographed using a documentation system (MicroDoc Cleaver Scientific Ltd, United Kingdom). PCR products were purified using the EnzSAP™ PCR Clean-up Reagent (Edge Biosystems) and according to the manufacturer’s instructions. Sequencing reactions were carried out in the MJ Research PTC-225 Peltier Thermal Cycler using ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the manufacturer’s protocol. The sequencing process was performed by Macrogen Inc., Seoul, Korea.

Data analyses

The sequences were edited and trimmed by the Chromas program version 2.6.6. In addition, the polymorphic sites in the sequences were visually recognized and assigned a letter representing the appropriate substitution according to the (NC-IUB) rules. The resulting sequences of the different alleles of the DQA1 gene were submitted to the GeneBank using Bankit submission tool and given the accession numbers as shown in (Table 1).

The DQA1 sequences used to construct the phylogenetic trees (Figures 1, 2) were retrieved from the NCBI GeneBank using the BLAST (Basic Local Alignment Search Tool) and their accession numbers are; Sheep (Ovis aries): AF317616.2, AY230209.1, AY230210.1, AY265308.1, HQ728652.1, L49463.1, LN736359.1, LN827891.1, LN827892.1, LN827893.1, LN827894.1, LN827895.1, LN868265.1, Z28418.1, and Z28420.1; Goat (Capra hircus): AY464656.1, AY464657.1, AY665664.1, AY665665.1, AY665666.1; Cattle (Bos taurus): LR797960.1, LR797961.1, LR797962.1, LR797964.1, LR797966.1; Water buffalo (Bubalus bubalis): DQ822573.1, KP966639.1, KT428711.1, KX789011.1, KX789012.1, and Human HLA-DQA1 L34082.1 as an outgroup.

BioEdit program version 7.2.5 was used for the multiple sequence alignment (Clustal W) and identifying the conserved
regions and percentages of sequence similarity (Table 2). Phylogenetic trees (Figures 1, 2) were constructed for the aligned sequences by Clustal W alignment using the MEGA X program (version 10.2.2) (Kumar et al., 2018) based on the Kimura-2 parameter (Kimura, 1980).

A model with branch relative support was applied to make bootstrap analysis with 1000 replicants (Felsenstein, 1985) by the neighbor-joining method (Saitou and Nei, 1987). DnaSP V6 (Rozas et al., 2017) was used in the study of the genetic diversity of breeds, nucleotide diversity, and allele diversity were identified (Table 3).

Table 2: Sequence Identity Matrix of Ovar-DQA1 sequences of this study using BioEdit program.

| Sequence     | DQA1*2 | DQA1*3 | DQA1*6 | DQA1*7 | DQA1*9 | DQA1*10 | DQA1*17 | DQA1*19 | DQA1*26 | DQA1*27 | DQA1*29 | DQA1*33 | DQA1*34 | DQA1*35 | DQA1*43 | DQA1*46 | DQA1*47 | DQA1*49 |
|--------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| DQA1*2       | ID     | 0.99   | 1.00   | 0.99   | 0.90   | 0.99   | 0.99   | 0.99   | 0.90   | 0.99   | 0.99   | 0.90   | 0.99   | 0.94   | 0.98   | 0.99   | 0.99   | 0.99   |
| DQA1*3       | 0.99   | ID     | 0.99   | 1.00   | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 0.10   | 1.00   | 0.89   | 0.94   | 0.99   | 0.99   | 0.99   | 0.99   |
| DQA1*6       | 1.00   | 0.99   | ID     | 0.99   | 0.89   | 0.99   | 0.99   | 0.99   | 0.90   | 0.99   | 0.98   | 0.98   | 0.89   | 0.94   | 0.98   | 0.99   | 0.99   | 0.99   |
| DQA1*7       | 0.99   | 1.00   | 0.99   | ID     | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 1.00   | 1.00   | 0.89   | 0.94   | 0.99   | 0.99   | 1.00   | 1.00   |
| DQA1*9       | 0.90   | 0.89   | 0.89   | 0.89   | ID     | 0.89   | 0.89   | 0.90   | 0.84   | 0.89   | 0.89   | 0.88   | 1.00   | 0.89   | 0.87   | 0.88   | 0.89   | 0.89   |
| DQA1*10      | 0.99   | 0.99   | 0.99   | 0.99   | 0.89   | ID     | 0.98   | 0.90   | 0.99   | 0.99   | 0.99   | 0.99   | 0.89   | 1.00   | 0.94   | 0.98   | 0.99   | 0.99   |
| DQA1*17      | 0.99   | 0.98   | 0.99   | 0.98   | 0.89   | 0.98   | ID     | 0.99   | 0.89   | 0.98   | 0.98   | 0.89   | 0.98   | 0.94   | 0.99   | 0.98   | 0.98   | 0.98   |
| DQA1*19      | 1.00   | 0.99   | 1.00   | 0.99   | 0.90   | 0.99   | 0.99   | ID     | 0.90   | 0.99   | 0.99   | 0.99   | 0.90   | 0.99   | 0.94   | 0.98   | 0.99   | 0.99   |
| DQA1*26      | 0.90   | 0.90   | 0.90   | 0.90   | 0.84   | 0.90   | 0.89   | 0.90   | ID     | 0.90   | 0.90   | 0.90   | 0.84   | 0.90   | 0.88   | 0.89   | 0.90   | 0.90   |
| DQA1*27      | 0.99   | 1.00   | 0.99   | 1.00   | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 1.00   | 1.00   | 0.89   | 0.94   | 0.99   | 0.99   | 1.00   | 1.00   |
| DQA1*29      | 0.99   | 1.00   | 0.98   | 1.00   | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 0.99   | 0.99   | 0.89   | 0.94   | 0.99   | 0.99   | 0.99   | 1.00   |
| DQA1*33      | 0.99   | 1.00   | 0.98   | 1.00   | 0.88   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 0.99   | 0.99   | 0.88   | 0.99   | 0.94   | 0.99   | 0.99   | 1.00   |
| DQA1*34      | 0.90   | 0.89   | 0.89   | 0.89   | 1.00   | 0.89   | 0.89   | 0.90   | 0.84   | 0.89   | 0.89   | 0.88   | ID     | 0.89   | 0.87   | 0.88   | 0.89   | 0.89   |
| DQA1*35      | 0.99   | 0.99   | 0.99   | 0.99   | 0.89   | 1.00   | 0.98   | 0.99   | 0.90   | 0.99   | 0.99   | 0.99   | 0.89   | ID     | 0.94   | 0.98   | 0.99   | 0.99   |
| DQA1*43      | 0.94   | 0.94   | 0.94   | 0.94   | 0.87   | 0.94   | 0.94   | 0.94   | 0.94   | 0.94   | 0.87   | 0.94   | ID     | 0.93   | 0.94   | 0.94   | 0.94   | 0.94   |
| DQA1*46      | 0.98   | 0.99   | 0.98   | 0.98   | 0.98   | 0.98   | 0.99   | 0.98   | 0.99   | 0.99   | 0.99   | 0.98   | 0.98   | ID     | 0.99   | 0.99   | 0.99   | 0.99   |
| DQA1*47      | 0.99   | 1.00   | 0.99   | 1.00   | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 1.00   | 0.89   | 0.99   | 0.94   | 0.99   | 0.99   | 1.00   |
| DQA1*49      | 0.99   | 1.00   | 0.99   | 1.00   | 0.89   | 0.99   | 0.98   | 0.99   | 0.90   | 1.00   | 1.00   | 1.00   | 0.89   | 0.99   | 0.94   | 0.99   | 1.00   | 1.00   |

Table 3: The genetic variation of Ovar-DQA1 gene of the studied Ossimi and Saidi breeds.

| Breed      | Number of sequences (N) | Number of polymorphic sites (S) | Avg. no. of nucleotide differences (K) | Nucleotide diversity Per site (π) | Number of mutations |
|------------|-------------------------|---------------------------------|----------------------------------------|----------------------------------|---------------------|
| Ossimi sheep | 8                       | 24                              | 6.343                                  | 0.03075                          | 24                  |
| Saidi sheep  | 10                      | 31                              | 7.578                                  | 0.03508                          | 35                  |
| Two breeds  | 18                      | 31                              | 6.863                                  | 0.03177                          | 35                  |
Figure 1: Neighbour-joining tree of sheep DQA1 sequences, constructed using the second exon nucleotide sequences of sheep of this study and GenBank published sheep DQA1 sequences. The tree was rooted to human DQA1 (L34082.1) as an out-group.
Figure 2: Neighbor-joining tree of Ovar-DQA1 sequences, constructed using the second exon nucleotide sequences of sheep, buffalo, cattle and goats illustrated in Materials and Methods. The tree was rooted to human DQA1 (L34082.1) sequences as out-groups. The numbers at the branch indicate the bootstrap confidence value, and branch lengths are proportional to genetic distance.
Results

We have investigated 18 different DQA1 sequences by sequence-based typing of genomic DNA of two groups (Ossimi and Saidi sheep) while 32 sheep are DQA1 null (64%) (Table 1). Of the 18 sequences (36%), 8 sequences were identified in Ossimi breed (16%) and 10 sequences were identified in Saidi breed (20%). The similarity matrix (Table 2) was ranged from 87% of three alleles DQA1*43, DQA1*9, and DQA1*34 to 100%. Different alleles are identical and have a similarity matrix of 100%; DQA1*2 alleles are identical to DQA1*19 and DQA1*6 while DQA1*3 allele is identical to DQA1*7, DQA1*27, DQA1*29, DQA1*33, DQA1*47, and DQA1*49, both of the alleles DQA1*9 and DQA1*34 are identical and DQA1*10 and DQA1*35 are identical. The multiple alignment ClustalW of sequences (Figure 3) indicate the polymorphic sites along with the sequences, there are three conserved regions with a minimum length of 15 bp were found, the first region with a length of 26 bp from position 18 to 43 with length 26 bp the second region with a length of 15 bp from position 127 to 141 with length 15 bp and the third region with a length of 17 bp from position 182 to 198.

The sequence analysis by DnaSP V6 software (Table 3) showed that these two breeds produced five different alleles; the first has four sequences DQA1*2 (MW672099), DQA1*6 (MW683493), DQA1*17 (MW683497), and DQA1*19 (MW683498), the second; include the ten sequences DQA1*3 (MW683492) DQA1*7 (MW683494), DQA1*10 (MW683496), DQA1*27 (MW683500), DQA1*29 (MW683501), DQA1*33 (MW683502), DQA1*35 (MW683504), DQA1*46 (MW683506), DQA1*47 (MW683507) and DQA1*49 (MW683508), the third; include the two sequences DQA1*9 (MW683495) and DQA1*34 (MW683503), the fourth and fifth; include only one sequence DQA1*26 (MW683499) and DQA1*43 (MW683505) respectively. The result indicated that the Saidi breed sequences have more polymorphic sites (S) with 31 sites from the Ossimi breed with 24 sites with nucleotide diversity (π) 0.03508 and 0.03075 for the two breeds Saidi and Ossimi respectively.

The phylogenetic tree (Figure 1) illustrated that there are two main groups in the constructed tree, the first main group consists of two clusters, one contains the alleles, DQA1*34 and DQA1*9 clustered with the three sequences LN827893.1, L49463.1, and AY266306.1 with bootstrap support 73% and the two sequences AF317616.2 and LN827893.1 in a separated clade, while the second cluster contains the allele DQA1*26 as separated clade and a separated clade also contains the sequence LN827892 clustered with a subcluster contains the allele DQA*43 clustered with the clade containing the sequences LN736359.1 and Z28420.1 with bootstrap support 96%.

The second cluster contains two subclusters, one contains the alleles DQA1*17, DQA1*2, DQA1*6, and DQA1*19 with bootstrap support 51% and the other group contains the two sequences LN868265.1 and AY230209.1 with bootstrap support 42%. The second main cluster with bootstrap support 24%, compresses the sequences DQA1*35, DQA1*10, and DQA1*46 as a separated clad clustered with two clades, one contains the sequences LN827894.1, Z8418.1, and AY230210.1 with bootstrap support 44%, and the other contains the seven alleles of this study DQA1*49, DQA1*47, DQA1*3, DQA1*7, DQA1*27, DQA1*29, and DQA1*33 with bootstrap support 29%. The sequence HQ728652.1 represents a separated clad clustered with the tree with bootstrap support of 82% and the sequence L34082.1 rooted the tree as a human (HLA) outgroup sequence.

The phylogenetic tree constructed from the sequence data is compared with the published GenBank sequences of other
sheep-related Bovidae species (Figure 2). The different goat (Capra hircus) DQA1 sequences appeared much related and clustered with the sheep DQA1 sequences, the most related sequence was AY464656.1 clustered with the Saidi breed sequence DQA1*43 with bootstrap support of 39%.

The five GenBank DQA1 sequences of the water buffalo (Bubalus bubalis) clustered with the main cluster comprise the sheep species with bootstrap support of 30%, and the cattle (Bos taurus) DQA1 isolates are clustered together form a separated group related to the sheep species.

Figure 3: Multiple sequence alignment of the Ovar-DQA1 exon 2 nucleotide sequences after trimming the ends, a dot indicates identity with the top sequence.
Discussion

Polymorphism at the DNA level is often used as a genetic marker in domestic animal studies of herds. The genetic markers and the variation found at these loci reflect the level of variation in the genome of a studied herd (Mahfouz et al., 2008). The results of this study revealed 31 polymorphic sites in the DQA1 gene of these two breeds, these polymorphic MHC loci are commonly used as genetic markers in the study of disease resistance or susceptibility and in the case of where variations are determined in responses to infection or vaccination (Hill et al., 1991; Eckels, 2000 and Messaoudi et al., 2002).

Several studies have been performed to investigate genetic variants within Ovar-MHC and their relationship with infectious disease resistance (Dukkipati et al., 2006). Our results showed that 32 sheep samples lack DQA1 (Table 1), which was supported by Snibson et al., 1998 who assumed that 10-18% of sheep are reported to completely lack the DQA1 gene (DQA1 null). They found that the DQA2 locus was detected in these haplotypes combined with the second locus. Based on phylogenetic analysis of the second exon this second loci appeared to be more closely related to DQA2 than to DQA1. Thus, the characteristic of this locus is DQA2-like.

The multiple sequence alignment indicated that there was a nucleotide polymorphism in the sequences and only three conserved regions were found along the sequence (Figure 3). This finding is supported by the results of the recent study by Ali et al., 2017, on the haplotype diversity of the MHC class II sheep region. Additional duplications and deletions of the DQA and B loci were detected in the haplotype’s diversity of certain class II of sheep.

In this study, the analysis of the DQA1 exon 2 of 18 sequences revealed five different alleles. Based on sequence variation and PCR amplification of exon 2, Scott et al. (1991) and Fabb et al. (1993) identified several alleles of Ovar- DQA1; (one allele) by Snibson et al. (1998); (two alleles) by Wright & Ballingall (1994), (three alleles) by Zhou & Hickford (2001); (six alleles) by Zhou & Hickford (2004). In 2001, Zhou & Hickford used the same PCR-SSCP technique to conduct several studies on 300 sheep belonging to different breeds. They investigated the DQA1 gene and found up to 14 alleles suggested an extensive polymorphism in the exon 2 sequence.

The evolutionary history was inferred using the Neighbor-Joining method for the second exon of the Ovar-DQA1 revealed that these studied alleles are clustered with other sheep DQA1 sequence published by GenBank (Figure 1) and the other phylogenetic tree (Figure 2) show similarities between the DQA1 genes of sheep, goat, cattle, and water buffalo sequences. Compared with other sheep sequences, goat (Capra hircus) alleles seem to be more similar or related to some sheep sequences than other Capra hircus sequences as the results showed that the most related sequence was AY464656.1 which clustered with the single allele DQA1*43 with bootstrap support of 40%.

Our results revealed multiple clusters of DQA1 sequence alleles of the sheep, and when comparing the sequence of the DQA1 exon 2 sequence of the sheep was more similar to cattle alleles than the other sheep alleles. This is supported by the study of Zhou and Hickford, 2001, who found multiple clusters of ovine DQA1 sequence alleles were more similar to cattle alleles than the other sheep alleles. They referred to this trans-species polymorphism to be the result of balanced selection at the DQA1 locus. In another study conducted by Zhou and Hickford, 2004, they argued these similarities are due to pathogen detection which could provide selection pressure to maintain specific MHC alleles which is necessary to introduce a specific immune response to a common pathogen. This leads to seeing polymorphic similarities not only between individuals of the same species but
also between genera. (Hughes and Yeager, 1998).

The description of MHC Class I and II polymorphism on different sheep breeds will help to explain the relationship between host and pathogen and improve the selection of disease-resistant animals (Nikbakht et al., 2012).

**Conclusion**

In this study, we performed sequence-based genotyping in exon 2 of the Ovar-MHC DQA1 gene, to study the DQA1 allelic polymorphism of 50 heads of two local Egyptian sheep breeds. The results of the current study help to identify the allelic polymorphism of MHC, which is one of the most variable regions of DNA, encodes polypeptides which aid in the recognition of different antigens and their associations with disease resistance.

**Conflicts of interest**

The authors declare that there is no conflict of interest.

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