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Research Article

MHC-DRB1/DQB1 Genes Polymorphism and Its Association with Resistance to *Cystic Echinococcosis* in Chinese Merino Sheep

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Abstract

In this study, we used the single strand conformation polymorphism (SSCP) method to analyze the associations between polymorphisms of exon 2 of the DRB1 and DQB1 genes and Cystic *Echinococcosis* (CE) in Chinese Merino sheep. We examined 96 CE positive sheep and 115 negative sheep in this study. The results showed thatthere were 53 genotypes controlled by 22 alleles in the DRB1 gene and 47 genotypes controlled by 17 alleles in the DQB1 gene. The comparison of allele frequencies in the CE positive and negative animals revealed that DRB1 alleles k ($P<0.05$), q , t ($P<0.01$) and alleles a , l ($P<0.01$). These results indicate a strong association between these alleles k,q,t and CE resistance, the alleles a,l are associated with CE susceptibility. We analyzed the DRB1 genotype frequencies and found that the genotypes KK and TT ($P<0.05$) are associated with CE resistance, while AA, LL ($P<0.01$), and AL ($P<0.05$) were related to CE susceptibility. In DQB1 exon 2 the d and e alleles ($P<0.01$) were related to CE resistance, while c and k ($P<0.01$) were significantly related to CE susceptibility. We analyzed the DQB1 genotype frequencies and found that DD and EG ($P<0.01$) were associated with CE resistance, while genotypes CC, KK, and CK ($P<0.01$) were associated with CE susceptibility. Using haplotype analysis and artificially infection tests with *Echinococcus granulosus* (EG), we found that the DRB1-TT/DQB1-EE was a CE resistant haplotype in Chinese Merino sheep.

Keywords: *DRB1, DQB1Echinococcosis Resistance, Susceptibility*

MHC-DRB1/DQB1 Gen Polimorfizmi ve Çin Merinos Koyununda Kistik Echinococcosise Dirençle İlişkisi

Özet

Bu çalışmada tek sarmal konformasyon polimorfizm (SSCP) metodu kullanılarak DRB1 ve DQB1 genlerinin ekzon 2'leri ile Çin Merinos koyununda Kistik *Echinococcosis* (CE) polimorfizmleri arasındaki ilişki araştırıldı. Çalışmada 96 CE pozitif ve 115 negatif koyun kullanıldı. DRB1 geninde 22 allel tarafından kontrol edilen 53 genotip ile DQB1 geninde 17 allel tarafından kontrol edilen 47 genotip bulunduğu tespit edildi. CE pozitif ve negatif hayvanlarda allel frekansları karşılaştırıldığında DRB1 allel k ($P<0.05$), q, t ($P<0.01$) ve a, l ($P<0.01$). Elde edilen bulgular doğrultusunda CE direnci ile k, q, t allelleri arasında güçlü ilişki olduğu ve a, 1 allellerinin CE duyarlılığı ile ilgili olduğu belirlendi. DRB1 genotip frekansları analiz edildiğinde KK ve TT genotiplerinin ($P<0.05$) CE direnci ile ilişkili olduğu, AA, LL ($P<0.01$) ve AL ($P<0.05$)'nin ise CE duyarlılığı ile ilişkili olduğu tespit edildi. DQB1 ekzon 2'de d ve e allelleri ($P<0.01$) CE direnci ile ilişkili iken c ve k ($P<0.01$) anlamlı derecede CE duyarlılığı ile ilişkiliydi. DQB1 genotip frekansları analiz edildiğinde DD ve EG ($P<0.01$) CE direnci ile ilişkili iken CC, KK ve CK ($P<0.01$) genotipleri CE duyarlılığı ile ilişkili bulundu. Haplotip analizi ve *Echinococcus granulosus* (EG) ile deneysel enfeksiyon testi kullanılarak DRB1-TT/DQB1-EE Çin Merinos koyununda CE dirençli haplotip olarak belirlendi.

Anahtar sözcükler: *DRB1, DQB1Echinococcosis Direnç, Duyarlılık*

INTRODUCTION

The major histocompatibility complex (MHC) is a tightly linked cluster of genes and is the most highly polymorphic set of genes in vertebrate genomes. In 2001, it was proposed that MHC genes may be candidate genetic markers for disease resistance [1]. MHC genes play a central role in vertebrate immunity because they code for proteins that present peptides to T cells [2]. The immune response is triggered when MHC non-self-peptide complexes are recognized by T cells [3]. MHC class I molecules present epitopes of proteins synthesized inside the cell and initiate

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CD8 cell responses [4]. The class II molecules present foreign peptides that are obtained by phagocytosis and processed within the host cells. The class II molecules initiate responses by CD4+ cells [5,6].

As in other vertebrate species, the MHC genes of *Ovis aries* (Ovar) include two major subfamilies: class I and class II genes. There is a high degree of polymorphism in the class II genes and most of the class II gene polymorphic sites are located in exon 2. The interaction between host and parasite drives a variety of biological processes [7]. Co-evolution may be mediated at the genetic level via the host recognition of parasite antigens and the consequent alteration of virulence genes [8]. In terms of immune recognition and reaction, MHC is the most important genetic element of the mammalian immune system [9,10]. A variety of studies have been performed in many fields as a result of the highly polymorphic character of the MHC genes. Currently, the research for correlation between Ovar polymorphism and disease resistance/susceptibility is mainly concentrated on class II genes [11-17]. However, many studies examining the associations between MHC and hydatidosis have focused on humans [18-22]. This experimental research content mainly investigating the associations between Ovar polymorphism and resistance or susceptibility to the hydatidosis.

Cystic *Echinococcosis* (CE) is also called hydatidosis and is a cosmopolitan zoonotic parasitic disease caused by the larval stage (metacestode stage) of the tapeworm Echinococcus granulosus (EG). The parasite cycles between canines as definitive hosts and various herbivores as intermediate hosts. In the intermediate hosts and humans, the larvae develop into hydatid cysts in various organs, including the liver and lungs. CE is associated with severe morbidity and disability, especially in pastoral areas of Northwestern China, where the overall prevalence rate of hydatidosis is 38.89-61.25% [23]. The prevalence of hydatidosis decreases livestock production and reduces human lifequality. Chinese Merino sheep is well known sheep breed for wool production, which is beneficial to local sheep husbandry. However, thisbreed is more susceptible to the hydatidosis. Thus, we investigated the causes of disease susceptibility.

In this study, we examined the association between Ovar polymorphisms and resistance or susceptibility to CE by using single strand conformation polymorphism (SSCP). This research mainly explore the correlation between genetic markers and the resistance to CE.

MATERIAL and METHODS

Ethics Statement: This research was approved by the Ethical Committee of Animal Experiments of the Institute of Zoology, Chinese Academy of Sciences. This committee does not issue a number to any animal study. All sheep care and use were conducted in strict accordance with the Animal Research Committee guidelines of the Institute of Zoology, Chinese Academy of Sciences. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

Study Areas and Sample Preparation: All of the animals included in this study were adult Chinese Merino sheep. The samples were obtained from the Yili district in Xinjiang Uygur Autonomous Region, China. This region has CE disease incidence. We used ELISA kits to divide the Chinese Merino sheep into CE negative and CE positive groups. The livers and lungs were macroscopically evaluated to confirm the presence of lesions characteristic of CE disease. Of the 211 sheep selected for this study, 96 had lesions from CE, and 115 had no lesions. Genomic DNA was extracted from 211 blood samples and stored at -20°C until analysis.

Primer Design: DRB1 gene primer synthesis references reported sequences of primers [24,25], by the Shanghai sangon biotech synthesis, the primer sequence were as follows:

OLA-ERB1 (GC): 5′-CCG GAA TTC CCG TCT CTG CAG CAC ATT TCT T-3′; HL031: 5′-TTT AAA TTC GCG CTC ACC TCG CCG CT-3′; OLA-XRBI: 5′-AGC TCG AGC GCT GCA CAG TGA AAC TC-3′.

With reference to the goat reported a DQBI MHC gene exon 2 of the results of the study [26,27]. According to the Genbank database of sheep MHC-DQB sequence accession NO. Z28523, synthetic primers DQB/FW and DQB/REV, the specific primer sequences are as follows:

DQB/FW: 5′-CCC CGC AGA GGA TTT CGT G-3′; DQB/REV: 5′-ACC TCG CCG CTG CCA GGT-3′.

Polymerase Chain Reaction Amplification: Exon 2 of DRB1 was amplified by PCR in two stages. The first round of PCR was performed with primers ERB1 and HL031. Genomic DNA (100 ng) was amplified in a total volume of 20 µl and included 1.5 mmol MgCl and 120 µmol dNTPs. Primers were added at a concentration of 0.2 mmol, and 1.5 U of Taq polymerase was used in each reaction. The reactions were performed in a thermocycler using the following conditions: one cycle of incubation for 5 min at 94°C, followed by 15 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 60 s. A final extension was performed at 72°C for 10 min. We used 3 µl of the resulting mixture and the ERB1 and XRBI primers for the second round of PCR [24]. The conditions for the second round of PCR were as follows: one cycle for 5 min at 94°C, followed by 30 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 60 s. A final extension was performed at 72°C for 10 min.

DQB1 exon 2 was amplified with primers FW and REV [25]. The PCR was performed in 50 µl reaction volumes with 150 ng DNA, 1.5 mmol MgCl, 100 µmol dNTPs, 0.2 mmol of each primer, and 2 U Taq polymerase. The PCR

was conducted using the following cycling conditions: 5 min at 95°C, followed by 33 cycles of 94°C for 30 s, 67°C for 30 s and 72°C for 45 s; a final extension was performed at 72°C for 10 min.

Single Stranded Conformation Polymorphism: One microliter aliquots of the PCR products were mixed with 7 µl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue) and then incubated at 98°C for 10 min and chilled on ice for 5 min. The denatured DNA was loaded on an 8% PAGE gel in 0.5× TBE buffer and the DNA was separated at a constant voltage of 180 V for 4 h. The gel was then stained with 0.1% silver nitrate solution. The configuration of the bands was visualized by silver staining. Samples with similar banding patterns were rearranged and run again on neighboring lanes to enable genotyping.

Cloning and Sequencing: We selected resistant susceptible alleles of the DRB1 and DQB1 genes to clone and sequence. The purified PCR products were cloned in the pGEM-T vector. The inserts were amplified and chosen for sequencing by running the amplifications on an SSCP gel. Only those clones that presented exactly the same SSCP patterns as the genomic SSCP were sequenced. At least three subclones from each individual were sequenced.

Artificial Infection Experiment: Sixteen two year-old Chinese Merino sheep tested as negative for hydatidosis by ELISA were chosen to conduct artificial infection experiments with EG. Eight sheep with the resistant haplotype were chosen as a test group. The other eight animals had haplotypes that were not associated with either CE resistance or CE susceptibility. Each sheep was fed on ten adult cestodes with fertilized egg proglottides by mouth. These sixteen sheep were bred under the same conditions.

Statistical Analysis: The allelic and genotypic frequencies were estimated with t-tests to evaluate the relationship between genotypic polymorphisms and CE infection. The chi-square test was used to analyze the relationship between the different haplotypes and CE resistance. The CE infection rates of the test group and the control group after artificial infection were compared using Fisher's exact test. The sequence alignments were performed using DNAman and Mega4 software [28].

RESULTS

PCR Amplification and SSCP Analysis: We used the DRB1 and DQB1 primers, and the amplified fragment lengths were 296 bp and 280 bp, respectively. The SSCP analysis of the amplified products was performed. Under the established conditions, 53 different SSCP patterns were detected in the DRB1 gene, which was controlled by 22 alleles. The alleles were named 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h', 'i', 'j', 'k', 'l', 'm', 'n', 'o', 'p', 'q', 'r', 's', 't', 'x', and 'y' *(Fig. 1)*. There were 47 genotypes detected in the DQB1 gene, which was controlled by 17 alleles. The alleles were named 'a', 'b', 'c', 'd', 'e', 'f', 'g', 'h', 'i', 'j', 'k', 'l', 'm', 'n', 'o', 'p', and 'q' *(Fig. 2)*.

Association between MHC Polymorphism and CE Resistance/Susceptibility: The allele frequency of exon 2 of the DRB1 gene in CE positive and CE negative animals was analyzed. The results showed that the k ($P<0.05$), q and t ($P<0.01$) alleles were significantly more common in the CE negative animals than in the CE positive animals. These results indicated that k, q and t were related to CE resistance. The frequencies of the a and l ($P<0.01$) alleles in the CE positive animals were significantly higher than in the CE negative animals. These results suggested that a and l were related to CE susceptibility *(Table1)*.

Additional analysis of the genotype frequencies found that KK and TT ($P<0.05$) were resistant to CE, while AA, LL ($P<0.01$), and AL ($P<0.05$) were susceptible to CE *(Table 2)*. The results for DQB1 exon 2 showed that d and e ($P<0.01$) were CE resistant alleles, and the c and k ($P<0.01$) alleles were susceptible to CE *(Table 3)*.

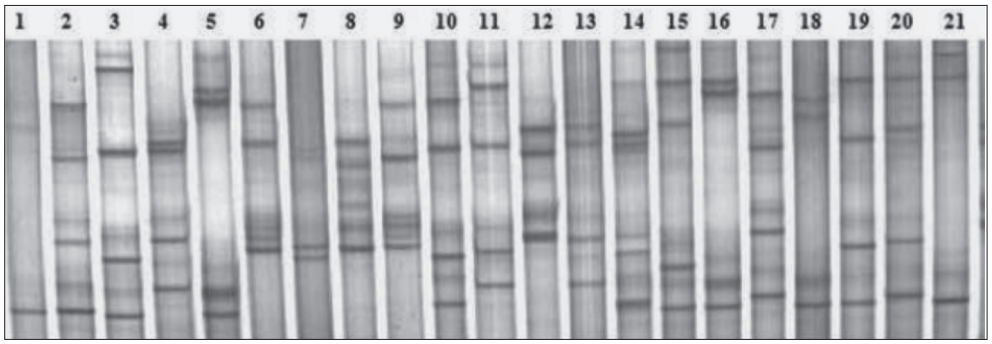

Fig 1. SSCP patterns of DRB1 exon 2 after silver staining in Chinese merino sheep. The alleles are 'a' (lane 1), 'b' (lane 2), 'l' (lane 3), 'i' (lane 4), 'q' (lane 5), 'm' (lane 6), '0' (lane 7), 'n' (lane 8), 'x' (lane 9), 'x' (lane 9), 'c' (lane 10), 'd' (lane 11), 'k' (lane 12/22), 'e' (lane 13), 'j' (lane 14), 'f' (lane 15), 'g' (lane 16), 'h' (lane 17), 's' (lane 18), 'r' (lane 19), 'y' (lane 20), 'p' (lane 21), and 't' (lane 23). The alleles k, q and t were significantly associated with CE resistance, while alleles a and l were associated with CE susceptibility

Şekil 1. Çin merinos koyununda gümüş boyama sonrasında DRB1 ekzon 2'nin SSCP görüntüsü. Alleller; 'a' (şerit 1), 'b' (şerit 2), 'l' (şerit 3), 'i' (şerit 4), 'q' (şerit 5), 'm' (şerit 6), '0' (şerit 7), 'n' (şerit 8), 'x' (şerit 9), 'x' (şerit 9), 'c' (şerit 10), 'd' (şerit 11), 'k' (şerit 12/22), 'e' (şerit 13), 'j' (şerit 14), 'f' (şerit 15), 'g' (şerit 16), 'h' (şerit 17), 's' (şerit 18), 'r' (şerit 19), 'y' (şerit 20), 'p' (şerit 21) ve 't' (şerit 23). k, q ve t CE direnci ile ilişkili iken a ve l allelleri CE duyarlılığı ile ilişkilidir

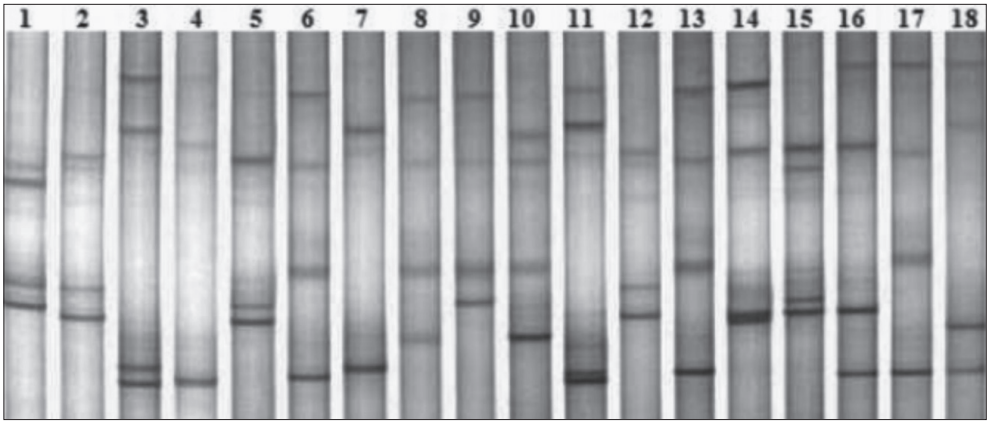

Fig 2. SSCP patterns of DQB1 exon 2 after silver staining in Chinese merino sheep. The alleles are 'd' (lane 1), 'e' (lane 2), 'a' (lane 3), 'h' (lane 4), 'l' (lane 5), 'k' (lane 6/13), 'i' (lane 7), 'p' (lane 8), 'q' (lane 9), 'o' (lane 10), 'c' (lane 11), 'n' (lane 12), 'b' (lane 14), 'j (lane 15), 'm' (lane 16), 'f' (lane 17), and 'g' (lane 18). The alleles d and e were related to CE resistance, while c and k were significantly susceptible to CE

Şekil 2. Çin merinos koyununda gümüş boyama sonrasında DQB1 ekzon 2'nin SSCP görüntüsü. Alleller; 'd' (şerit 1), 'e' (şerit 2), 'a' (şerit 3), 'h' (şerit 4), 'l' (şerit 5), 'k' (şerit 6/13), 'i' (şerit 7), 'p' (şerit 8), 'q' (şerit 9), 'o' (şerit 10), 'c' (şerit 11), 'n' (şerit 12), 'b' (şerit 14), 'j (şerit 15), 'm' (şerit 16), 'f' (şerit 17) ve 'g' (şerit 18). d ve e CE direnci ile ilişkili iken c ve k anlamlı derecede CE duyarlılığı ile ilişkilidir

Table 1. *The allele frequency of DRB1 exon 2 in CE negative and positive sheep*
Tablo 1. *CE negatif ve pozitif koyunlarda DRB1 ekzon 2'nin allel frekansları*

CE Negative (N = 104)			CE Positive (N = 93)		
Allele	**Number**	**Frequency**	**Allele**	**Number**	**Frequency**
a	5	0.024038	a	36	0.193548**
b	10	0.048076	b	9	0.048387
c	10	0.048076	c	15	0.080645
d	11	0.052885	d	9	0.048387
e	5	0.024038	e	6	0.032258
f	3	0.014423	f	4	0.021505
g	2	0.009615	g	4	0.021505
h	4	0.019231	h	3	0.016129
i	6	0.028846	i	2	0.010753
j	12	0.057692	j	8	0.043011
k	29	0.139423*	k	12	0.064516
l	7	0.033654	l	28	0.150538**
m	7	0.033654	m	9	0.048387
n	6	0.028846	n	2	0.010753
o	3	0.014423	o	8	0.043011
p	6	0.028846	p	2	0.010753
q	27	0.129808**	q	10	0.053763
r	8	0.038462	r	2	0.010753
s	14	0.067308	s	6	0.032258
t	23	0.110577**	t	3	0.016129
x	4	0.019231	x	4	0.021505
y	6	0.028846	y	4	0.021505

** represents P<0.05, ** represents P<0.01*

The genotypes DD and EG (P<0.01) were associated with resistance to C.E, while CC, KK, and CK (P<0.01) were associated with CE susceptibility *(Table 4)*. To efficiently analyze the polymorphisms of the DRB1/DQB1 genes and CE the relationships between different haplotypes and CE resistance/susceptibility were examined. The results found that haplotypes DRB1-TT/DQB1-EE (P<0.05) were resistant to CE, while DRB1-LL/DQB1-CK and DRB1-AA/DQB1-CC (P<0.01) were susceptible to CE *(Table 5)*.

Sequence Comparison of Resistant and Susceptible Alleles: The sequence analysis revealed that the resistant and susceptible alleles were typical multiple mutations. We compared the resistant DRB1 exon 2 alleles k, q, and t to the susceptible alleles a and l and found the alleles to be significantly different *(Fig. 3)*. To detect whether the nucleotide mutation resulted in amino acid variation, we also examined the amino acid sequence. These data are shown in *Fig. 4*.

We also found the resistant and susceptible DQB1 alleles typically included multiple mutations *(Fig. 5)*. Many of the nucleotide mutations resulted in amino acid variations *(Fig. 6),* which may be a reason for the different alleles in CE resistance or susceptibility, as amino acid sequence changes mightlead to changes in the function of the encoded protein. It is unclear how the functional protein controls resistance or susceptibility, and further studies are required to elucidate the mechanism.

Artificial Infection Experiment: We found the DRB1-TT/DQB1-EE haplotype to be resistant to infection and this result was verified using artificial infection tests. We sacrificed 16 sheep that were artificially infected with mature EG for pathological autopsy 60 days after infection. A positive infection was determined based on visible protruding EG cysts on the liver or lung surface and the presence of hydatid sand by microscopic examination of the cyst fluid. The results showed 3 positive infections in the test group and 6 positive infections in the control group. Thus, the infection rate in the test group was significantly lower than in the control group (P<0.05). These data confirmed that DRB1-TT/DQB1-EE was a resistant haplotype in Chinese Merino sheep.

DISCUSSION

The extensive diversity at many MHC loci provides a valuable source of genetic markers for examining the relationship between host and disease resistance or

Table 2. *The genotype frequency of DRB1 exon 2 in CE negative and positive sheep*

Tablo 2. *CE negatif ve pozitif koyunlarda DRB1 ekzon 2'nin genotip frekansları*

CE Negative (N = 104)			CE Positive (N = 93)		
Genotype	**Number**	**Frequency**	**Genotype**	**Number**	**Frequency**
AA	1	0.009615	AA	12	0.129032**
BB	3	0.028846	BB	3	0.032258
CC	3	0.028846	CC	5	0.053763
DD	2	0.019231	DD	2	0.021505
EE	1	0.009615	EE	2	0.021505
FF	1	0.009615	FF	2	0.021505
GG	1	0.009615	GG	2	0.021505
HH	2	0.019231	HH	1	0.010753
II	3	0.028846	II	1	0.010753
JJ	3	0.028846	JJ	2	0.021505
KK	8	0.076923*	KK	1	0.010753
LL	1	0.009615	LL	8	0.086022**
MM	3	0.028846	MM	4	0.043011
NN	2	0.019231	NN	1	0.010753
OO	1	0.009615	OO	4	0.043011
PP	3	0.028846	PP	1	0.010753
QQ	9	0.086538	QQ	3	0.032258
RR	3	0.028846	RR	1	0.010753
SS	7	0.067308	SS	3	0.032258
TT	9	0.086538*	TT	1	0.010753
XX	2	0.019231	XX	1	0.010753
YY	3	0.028846	YY	2	0.021505
AB	1	0.009615	AB	1	0.010753
AC	1	0.009615	AC	2	0.021505
AL	1	0.009615	AL	7	0.075269*
AJ	0	0	AJ	1	0.010753
AK	0	0	AK	1	0.010753
BD	1	0.009615	BD	0	0
BK	2	0.019231	BK	2	0.021505
CD	2	0.019231	CD	0	0
CE	1	0.009615	CE	0	0
CL	0	0	CL	1	0.010753
CK	0	0	CK	2	0.021505
DL	0	0	DL	2	0.021505
DJ	0	0	DJ	1	0.010753
DK	2	0.019231	DK	2	0.021505
EK	0	0	EK	1	0.010753
ER	1	0.009615	ER	0	0
EQ	0	0	EQ	1	0.010753
FJ	1	0.009615	FJ	0	0
FR	1	0.009615	FR	0	0
KQ	4	0.038462	KQ	0	0
HQ	0	0	HQ	1	0.010753
KT	2	0.019231	KT	1	0.010753
LK	3	0.028846	LK	1	0.010753
LJ	0	0	LJ	1	0.010753
LQ	1	0.009615	LQ	0	0
MJ	1	0.009615	MJ	1	0.010753
XQ	0	0	XQ	2	0.021505
NJ	1	0.009615	NJ	0	0
NQ	1	0.009615	NQ	0	0
QJ	1	0.009615	QJ	0	0
QT	3	0.028846	QT	0	0

** represents P<0.05, ** represents P<0.01*

Table 3. *The allele frequency of DQB1 exon 2 in CE negative and positive sheep*

Tablo 3. *CE negatif ve pozitif koyunlarda DQB1 ekzon 2'nin allel frekansları*

CE Negative (N = 115)			CE Positive (N = 96)		
Allele	**Number**	**Frequency**	**Allele**	**Number**	**Frequency**
a	17	0.073913	a	13	0.067708
b	8	0.034783	b	5	0.026042
c	6	0.026087	c	45	0.234375**
d	47	0.204348**	d	18	0.093750
e	54	0.234783**	e	15	0.078125
f	10	0.043478	f	6	0.031250
g	27	0.117391	g	11	0.057292
h	13	0.056522	h	8	0.041667
i	14	0.060870	i	7	0.036458
j	4	0.017391	j	3	0.015625
k	6	0.026087	k	34	0.177083**
l	6	0.026087	l	10	0.052083
m	5	0.021739	m	6	0.031250
n	6	0.026087	n	7	0.036458
o	3	0.013043	o	2	0.010417
p	2	0.008696	p	0	0
q	2	0.008696	q	2	0.010417

*** represents P<0.01*

susceptibility [26]. Many researchers have examined genetic markers associated with resistance or susceptibility to parasites. These prior studies have improved the diagnosis and selection of desirable genotypes. Many studies have examined hydatidosis resistance/susceptibility and MHC polymorphisms. However, these studies focused on humans [19,20,29,30] and mice [31-33]. For example, Al-Ghoury determined that HLA-DR1, 8 and DR-52 are associated with resistance and that HLA-DR 16 is associated with susceptibility to EG infection in Yemeni patients [34]. Li reported that susceptibility to alveolar *Echinococcosis* (A.E) was significantly associated with HLA-DR4 and that the DR7 allele might confer protection against A.E in humans [35,36].

In this study, we found that there were many polymorphisms in DRB1 exon 2 and in DQB1 by screening genetic markers of CE resistance in Chinese Merino sheep. We also confirmed that the DRB1-TT/DQB1-EE haplotype was resistant to CE by artificial infection. Li investigated the association between the polymorphism of DRB1 exon 2 and CE resistance in Hazakh sheep. They found a strong association between DRB1 polymorphisms and CE resistance and confirmed that MvaIbc-SacIlab-Hin1lab was the resistant haplotype of CE in Hazakh sheep [37]. These results were similar to our findings in this study. We also found that several alleles and genotypes of DRB1 exon 2 were associated with CE resistance or susceptibility in Hazakh sheep. These results indicated that alleles H and F and genotypes FF and GH exhibited a correlation with CE resistance. However, alleles K and G and the genotype KK had a significant predisposition to CE infection. Shen reported that the DQB1 gene had a significant association with resistance to CE in Dolang sheep and Chinese Merino sheep [38,39]. Similarly, Yu suggested that the DRB1 gene was associated with CE resistance in Dolang sheep [40]. These results proved that Ovar polymorphisms were associated with resistance/susceptibility to CE in sheep. However, there has been a discrepancy among different populations that might be attributable to difficulties associated with the MHC typing methods used by the majority of these investigations. Additionally, there are ethnic differences in the distribution of MHC alleles in different populations.

In vertebrates, MHC plays a central role in foreign antigen recognition and immune response to pathogens [41].

Table 4. *The genotype frequency of DQB1 exon 2 in CE negative and positive sheep*

Tablo 4. *CE negatif ve pozitif koyunlarda DQB1 ekzon 2'nin genotip frekansları*

CE Negative (N = 115)			CE Positive (N = 96)		
Genotype	**Number**	**Frequency**	**Genotype**	**Number**	**Frequency**
AA	4	0.034783	AA	3	0.031250
BB	2	0.017391	BB	1	0.010417
CC	1	0.008696	CC	12	0.125000**
DD	18	0.156522**	DD	4	0.041667
EE	11	0.095652*	EE	4	0.041667
FF	2	0.017391	FF	1	0.010417
GG	7	0.060870	GG	4	0.041667
HH	3	0.026087	HH	3	0.031250
II	3	0.026087	II	1	0.010417
JJ	2	0.017391	JJ	1	0.010417
KK	1	0.008696	KK	9	0.093750**
LL	3	0.026087	LL	4	0.041667
MM	2	0.017391	MM	3	0.031250
NN	3	0.026087	NN	3	0.031250
OO	1	0.008696	OO	1	0.010417
PP	1	0.008696	PP	0	0
QQ	1	0.008696	QQ	1	0.010417
AB	1	0.008696	AB	0	0
AC	1	0.008696	AC	4	0.041667
AD	1	0.008696**	AD	0	0
AE	4	0.034783	AE	0	0
AK	1	0.008696	AK	1	0.010417
AI	1	0.008696	AI	1	0.010417
AH	0	0	AH	1	0.010417
BC	1	0.008696	BC	2	0.020833
BE	2	0.017391	BE	0	0
BL	0	0	BL	1	0.010417
CD	0	0	CD	3	0.031250
CE	0	0	CE	2	0.020833
CF	1	0.008696	CF	1	0.010417
CK	1	0.008696	CK	9	0.093750**
DE	2	0.017391	DE	1	0.010417
DF	2	0.017391	DF	0	0
DG	1	0.008696	DG	0	0
DH	2	0.017391	DH	0	0
DI	2	0.017391	DI	2	0.020833
DM	1	0.008696	DM	0	0
DK	0	0	DK	4	0.010417
EF	3	0.026087	EF	3	0.031250
EG	11	0.095652**	EG	1	0.010417
EH	5	0.043478	EH	0	0
EI	5	0.043478	EI	0	0
GK	1	0.008696	GK	2	0.020833
OK	1	0.008696	OK	0	0
HI	0	0	HI	1	0.010417
IJ	0	0	IJ	1	0.010417
LN	0	0	LN	1	0.010417

** represents P<0.05, ** represents P<0.01*

There may be several MHC alleles that are better suited to display antigens to certain diseases and thus generate better immunity through an improved T-cell response repertoire. However, there were many other unknown host genetic factors that could play roles in initial CE infection.

To verify whether the DRB1-TT/DQB1-EE genotypes were resistant genetic markers we used artificial infection tests. Zheng collected the cyst vesicle fluid from the diseased livers of the artificially infected sheep and then injected the fluid into healthy sheep in the peritoneum [42]. An infection model using EG was established using this method. In our study, adult cestodes with fertilized egg proglottides were fed orally to sheep. The objective was to imitate natural infection with hydatids. The result indicated that the haplotype DRB1-TT/DQB1-EE was resistant to CE Therefore, the DRB1-TT/DQB1-EE haplotype could be used as a genetic marker of CE resistance. Haplotype analysis may lead to the identification of more significant associations and improve our understanding of the role of MHC and antigens in CE resistance.In future breeding and treatment studies, greater consideration should be given to genetic markers of resistance/susceptibility.

In conclusion, the results of this study suggest that MHC polymorphisms maybe used in linkage and association research on CE resistance in Chinese Merino sheep. The identification of MHC haplotypes composed of such polymorphisms is a powerful tool for analysing the associations between MHC and immunity to infectious diseases.

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Table 5. *The part haplotypes in CE negative and positive sheep*
Tablo 5. *CE negatif ve pozitif koyunlarda parça haplotipler*

Haplotype	CE Negative (104)		CE Positive (93)		χ^2
	Number	Frequency	Number	Frequency	
DRB1-TT/DQB1-EE	8	0.076923	0.010753	1	4.523688*
DRB1-KK/DQB1-EG	5	0.048077	0.010753	1	1.521913
DRB1-SS/DQB1-DD	6	0.057692	0.032258	3	0.853513
DRB1-QQ/DQB1-GG	7	0.067308	0.032258	3	1.650508
DRB1-KQ/DQB1-EH	3	0.028846	0	0	1.138309
DRB1-LL/DQ1-CK	0	0	0.075269	7	6.848920**
DRB1-AA/DQB1-CC	1	0.009615	0.086022	8	6.764010**
DRB1-AL/DQB1-KK	1	0.09615	0.043011	4	1.487790
DRB1-CC/DQB1-DK	0	0	0.032258	3	1.845730

*①$\chi^2>\chi^2_{0.01\text{-}1}$ = 6.63, P<0.01, $\chi^2>\chi^2_{0.05\text{-}1}$ = 3.84, P<0.05, $\chi^2<\chi^2_{0.05\text{-}1}$ = 3.84, P>0.05; * P<0.05, ** P<0.01. ②There are only part of the haplotypes,the others because of no significant difference, so there is no list*

Fig 3. Nucleotide sequences of DRB1 exon2 in Chinese Merino sheep. BLAST results of resistant and susceptible alleles. The alleles k, q and t are resistant to CE, while the alleles a and l are susceptible to CE. Their nucleotide sequences exhibit typical multiple mutations

Şekil 3. Çin Merinos koyununda DRB1 ekzon 2'nin nükleotid sekansı. Dirençli ve duyarlı allellerin BLAST sonuçları. k, q ve t alleleri CE'ye dirençli iken a ve l allelleri CE'ye duyarlıdır. Nükleotid sekansları tipik çoklu mutasyonlar göstermektedir

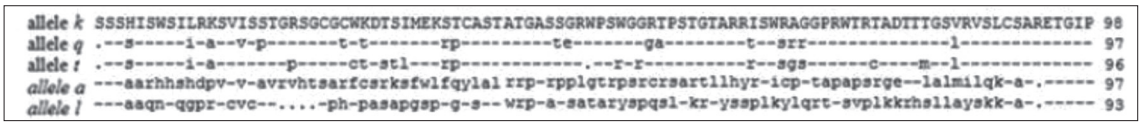

Fig 4. Amino acid sequences encoded by DRB1 exon 2 in Chinese Merino sheep. Comparison result of resistant and susceptible alleles. The alleles k, q and t are resistant to CE, while a and l are susceptible to CE. The amino acid variations resulting from nucleotide mutations are shown

Şekil 4. Çin Merinos koyununda DRB1 ekzon 2 tarafından kodlanan amino asit sekansları. Dirençli ve duyarlı allellerin karşılaştırma sonuçları. k, q ve t allelleri CE'ye dirençli iken a ve l CE'ye duyarlıdır. Nükleotid mutasyonlarından kaynaklanan amino asit varyasyonları gösterilmektedir

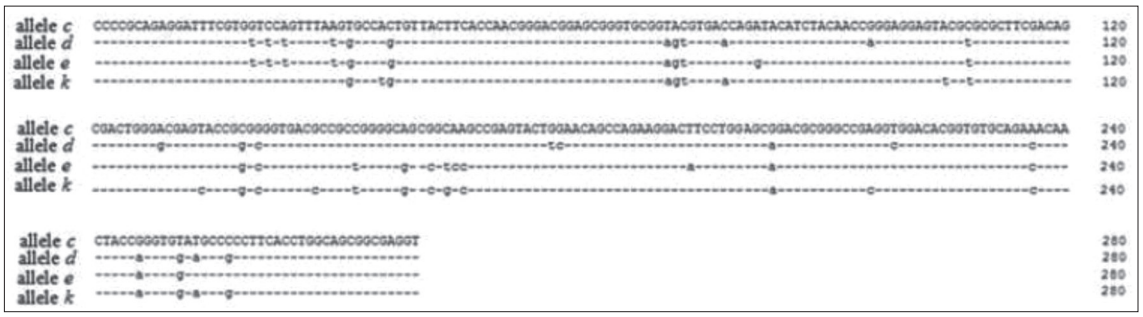

Fig 5. Nucleotide sequences of DQB1 exon 2 in Chinese Merino sheep. BLAST result of resistant and susceptible alleles. The alleles d and e are related to CE resistance, while c and k are susceptible to CE. These nucleotide sequences also exhibit typical multiple mutations

Şekil 5. Çin Merinos koyununda DQB1 ekzon 2'nin nükleotid sekansı. Dirençli ve duyarlı allellerin BLAST sonuçları. d ve e alleleri CE'ye dirençli iken c ve k allelleri CE'ye duyarlıdır. Nükleotid sekansları tipik çoklu mutasyonlar göstermektedir

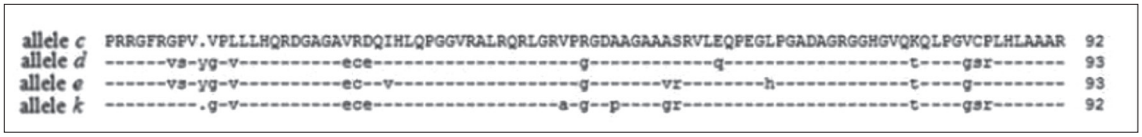

Fig 6. Amino acid sequences encoded by DQB1 exon2 in Chinese Merino sheep. Comparison result of resistant and susceptible alleles. The alleles d and e are related to CE resistance, while c and k are susceptible to CE. The nucleotide mutations change the amino acid composition

Şekil 6. Çin Merinos koyununda DQB1 ekzon 2 tarafından kodlanan amino asit sekansları. Dirençli ve duyarlı allellerin karşılaştırma sonuçları. d ve e allelleri CE'ye dirençli iken c ve k CE'ye duyarlıdır. Nükleotid mutasyonları amino asit kompozisyonunu değiştirmektedir

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