The relationship between MHC-DRB1 gene second exon polymorphism and hydatidosis resistance of Chinese merino (Sinkiang Junken type), Kazakh and Duolang sheep


Summary:
The present study aimed at detecting the association of ovine major histocompatibility complex class II (Ovar II) DRB1 gene second exon and susceptibility or resistance to hydatidosis in three sheep breeds of Sinkiang. The MHC-DRB1 second exon was amplified by polymerase chain reaction (PCR) from DNA samples of healthy sheep and sheep with hydatidosis. PCR products were characterized by the restriction fragment length polymorphism (RFLP) technique. Five restriction enzymes, MvaI, HaeIII, SacI, SacII, HinII, were used, yielding 14 alleles and 31 restriction patterns. Frequencies of patterns MvaIbc, HinIIab, HaeIIIld, HaeIIldf (P < 0.01) in Kazakh sheep, SacIab (P < 0.05) in Duolang sheep, and HaeIIld, HaeIIldf, HaeIIld (P < 0.01) in Chinese Merino (Sinkiang Junken type) sheep, were significantly higher in healthy sheep compared with infected sheep. These results indicated a strong association between these patterns and hydatidosis resistance. In contrast, the frequencies of MvaIbb, SacIIab, HinIIbb, HaeIIlf (P < 0.01) and HaeIIld (P < 0.05) in Kazakh sheep, SacIbb, HaeIlla, HaeIIlf (P < 0.05) in Duolang sheep, SacIIab (P < 0.05) and HaeIIldf, HinIIbb, HaeIIlf (P < 0.01) in Chinese Merino sheep (Sinkiang Junken type) were significantly lower in healthy sheep compared with infected sheep. This indicated a strong association between these patterns and hydatidosis susceptibility. In addition, sheep with the pattern of HaeIIlf demonstrated a high hydatidosis susceptibility (P < 0.01) in all three breeds, while sheep with the pattern HaeIIld demonstrated significant hydatidosis resistance (P < 0.01) in Kazakh and Chinese Merino sheep (Sinkiang Junken type). These results suggest that the Ovar-DRB1 gene plays a role in resistance to hydatidosis infection in the three sheep breeds.

KEY WORDS: Chinese merino sheep (Sinkiang Junken type), Kazakh sheep, Duolang sheep, Ovar-DRB1 exon 2, PCR-RFLP, hydatidosis.

INTRODUCTION

Hydatidosis (Echinococcus granulosus) is recognized as one of the world major zoonoses, and is found all over the world (Rausch, 1995; Andersen et al., 1997; Dalimi et al., 2002; Eckert & Deplazes, 2004; Jenkins et al., 2005). Sinkiang Autonomous Region of China is a prevalent area of hydatidosis. In sheep, the overall prevalence rate for hydatidosis cysts is 38.89 % to 61.25 % (Li et al., 2005). Kazakh sheep and Duolang sheep, the local sheep of Sinkiang, are bred for meat and fat. Chinese Merino sheep (Sinkiang Junken type) produce excellent wool. In Sinkiang, hydatidosis in farm animals causes considerable economic problems due to the loss of meat and edible liver, as well as the value of the fleece from infected sheep. Therefore it also affects the life quality of herdsmen.
In recent years, many animal breeding studies have focused on MHC genes as candidate genes for disease resistance and susceptibility. The MHC is a multigene family that controls immunological self/non-self recognition. They include genes for cell surface glycoproteins that present peptides of foreign and self proteins to T cells, thereby controlling both cell- and antibody-mediated immune responses (Klein, 1986). A striking characteristic of MHC genes is their extreme polymorphism.

Diversity driven by pathogens implies a strong association between MHC alleles and patterns of resistance to specific autoimmune or infectious diseases. Such a link was first shown for chickens, in which the B21 haplotype (MHC class II) confers the strongest resistance to the viruses responsible for Marek’s disease (Briles et al., 1977; Longeoneker & Gallatin, 1978). Equally well known is the role of the chicken class I MHC in providing resistance to the Rous sarcoma virus (Schierman & Collins, 1987; Kaufman & Venugopal, 1998). The polymorphism of Ovar-DRB1 plays an important role in resistance to nematode infection in the Suffolk breed (Sayers et al., 2005).

MHC Class II Ovar-DRB1 was chosen as the immune response gene in this study because it is highly polymorphic, transcribed, and there are over 100 different DRB1 alleles reported in Genbank based upon either restriction fragment length polymorphisms (RFLP) or the deduced amino acid sequence for the β1 domain encoded by exon 2 (Dutia et al., 1994; Schwaigger et al., 1996; Jugo & Vicario, 2001; Konnai et al., 2003; Herrmann et al., 2005). Others have shown specific MHC-DRB1 alleles associate with resistance and/or less severe clinical signs in human hydatidosis (Gottstein et al., 1996; Li et al., 2005). Konnai et al. (2003) detected the Ovar-DRB1 exon 2 polymorphisms of Suffolk sheep by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

In the present study, the polymorphism of the class II Ovar-DRB1 exon 2 was detected by PCR-RFLP analysis in three sheep breeds. We characterized the relationship between the Ovar-DRB1 exon 2 polymorphism and hydatidosis resistance, and screened the genotypes associated with hydatidosis resistance and susceptibility in each sheep breed. Results of the present study may play an important role in developing new sheep breeds that are resistant to hydatidosis.

**MATERIALS AND METHODS**

**Animals sampling and sample preparation**

Blood samples of Chinese Merino sheep (Sinkiang Junken type; 604 healthy animals and 425 animals with hydatidosis) were donated by agricultural construction division 9 in Sinkiang. Blood samples of Duolang sheep (122 healthy sheep and 70 sheep with hydatidosis) were donated by agricultural construction division 3. Blood samples of Kazakh sheep (400 healthy sheep and 302 sheep with hydatidosis) were donated by agricultural construction division 4. The sheep with hydatidosis were distinguished from healthy sheep by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Shenzhen Combined Biotech Co., Ltd, Shanghai, China). Genomic DNA were obtained from whole blood by phenol-chloroform method (Liu et al., 1997), and stored in a -20 °C freezer until analysis.

**Design of Ovar-DRB1 exon 2-specific primers and PCR amplification**

The second exon of Ovar-DRB1 was amplified by PCR in two rounds. The first round of PCR was performed with primers OLA-ERB1 (5′-CCG GAA TTC CCG TCT CTG CAC CAG CAC ATTTCT T-3′) and HL031 (5′-TTT AAA TTC GCG CTC ACCTCG CCG CT-3′) (adopted from Konnai et al., 2003). We subjected 100 ng of genomic DNA to PCR amplification in a total volume of 20 µl, containing 1.5 mM MgCl2, 120 µM dNTP, 0.2 mM each primer, and 1.5 U Taq polymerase (TIANGEN Biological Engineering Technology And Service Company, Beijing, China). Reactions were performed in a thermocycler (Bio RAD, Germany) under the following conditions: a single cycle of 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, with a final extension at 72 °C for 10 min. We used 3 µl of the resulting mixture and primers OLA-ERB1and OLA-XRBI (5′-AGC TCG AGC GCT GCA CAG TTC GCG CTC ACCTCG CCG CT-3′) (adopted from Konnai et al., 2003) for the second round of PCR. The cycling conditions for the second round were: a single cycle of 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 with a final extension at 72 °C for 10 min.

**Polymorphism detection by RFLP**

PCR products (10 µl) from the second round were digested for 4 h at 37 °C with 5 U of MvaI, HaeIII, SacI, SacII, or HinII (Shanghai Sangon Biological Engineering Technology And Service Co., Ltd., Shanghai, China) in a total volume of 20 µl. The products of enzyme digestion were analyzed by a 2.5 % or 3 % agarose gel electrophoresis.

**Statistical analysis**

Hardy-Weinberg equilibrium of Ovar-DRB1 genotypes was analyzed by χ² test. The distribution of genotypes was analyzed in healthy sheep and sheep with hydatidosis within a breed was analyzed by χ² test. SPSS version 13.0 was used for statistical analysis.
Restriction enzymes | The genotypes of each restriction enzyme
---|---
SacI | aa (296bp) | ab (296bp/208bp/88bp) | bb (208bp/88bp)
HinII | aa (296bp) | ab (296bp/178bp/118bp) | bb (178bp/118bp)
MvaI | bb (123bp/87bp/86bp) | bc (210bp/123bp/87bp/86bp) | cc (210bp/86bp)
SacII | aa (296bp) | ab (296bp/229bp/69bp) | bb (229bp/69bp)

Table I. – PCR-RFLP genotypes of the second exon of the MHC-DRB1 gene.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Restriction fragments (bp)</th>
<th>Genotypes</th>
<th>Restriction fragments (bp)</th>
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<tbody>
<tr>
<td>HaeIII aa</td>
<td>173/71/48/4</td>
<td>HaeIII af</td>
<td>173/159/71/52/48/14/4</td>
</tr>
<tr>
<td>HaeIII bb</td>
<td>173/123</td>
<td>HaeIII bd</td>
<td>173/159/123/14</td>
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<tr>
<td>HaeIII cc</td>
<td>159/137</td>
<td>HaeIII be</td>
<td>173/159/123/71/66</td>
</tr>
<tr>
<td>HaeIII dd</td>
<td>159/123/14</td>
<td>HaeIII cd</td>
<td>159/137/123/14</td>
</tr>
<tr>
<td>HaeIII ee</td>
<td>159/71/66</td>
<td>HaeIII ce</td>
<td>159/137/71/66</td>
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<tr>
<td>HaeIII ff</td>
<td>159/71/52/14</td>
<td>HaeIII cf</td>
<td>159/137/71/52/14</td>
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<tr>
<td>HaeIII ab</td>
<td>173/123/71/48/4</td>
<td>HaeIII de</td>
<td>159/123/71/66/14</td>
</tr>
<tr>
<td>HaeIII ac</td>
<td>173/159/137/71/48/4</td>
<td>HaeIII df</td>
<td>159/123/71/52/14</td>
</tr>
<tr>
<td>HaeIII ad</td>
<td>173/159/123/71/48/4</td>
<td>HaeIII ef</td>
<td>159/71/66/52/14</td>
</tr>
<tr>
<td>HaeIII ae</td>
<td>173/159/71/66/48/4</td>
<td></td>
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</tbody>
</table>

Table II. – The genotypes of PCR-RFLP by restriction enzyme HaeIII in the second exon of the Ovar-DRB1 gene.

Fig 1 – Electrophoretic patterns of PCR product of the second exon of Ovar-DRB1 in Kazakh sheep, M: PUC19 DNA marker.

Fig 2 – Electrophoretic patterns of the second exon of Ovar-DRB1 digested with SacI in Kazakh sheep, M: puc19 DNA marker.

Fig 3 – Electrophoretic patterns of the second exon of Ovar-DRB1 digested with HincII in Kazakh sheep, M: puc19 DNA marker.

Fig 4 – Electrophoretic patterns of the second exon of Ovar-DRB1 digested with MvaI in Kazakh sheep, M: puc19 DNA marker.

Fig 5 – Electrophoretic patterns of the second exon of Ovar-DRB1 digested with SacII in Kazakh sheep, M: puc19 DNA marker.

Fig 6 – Electrophoretic patterns of the second exon of Ovar-DRB1 digested with HaeIII in Kazakh sheep, M: puc19 DNA marker.
RESULTS

PCR AMPLIFICATION

Ovar-DRB1 exon 2 was amplified by PCR with primers OLA-ERB1, OLA-HL031, and OLA-XRBI. A 296-bp band corresponding to the expected size of exon 2 was observed by 1.5% agarose gel electrophoresis (Fig. 1).

PCR-RFLP

Restriction enzyme analysis with SacI, HinfI, MvaI, SacII and HaeIII produced restriction patterns and allele frequencies in accordance with that reported by Konnai et al. (2003). Restriction patterns are shown in Table I (SacI, HinfI, MvaI, SacII) and Table II (HaeIII); fragments the genotypic restriction map is shown in Figs 2-6; and a diagram of this exonic region, the cleavage sites is shown in Fig. 7. The Ovar-DRB1 exon 2 of Chinese Merino, Duolang and Kazakh sheep was analyzed by PCR-RFLP using restriction enzymes SacII (two alleles, three genotypes), MvaI (two alleles, three genotypes), SacI (two alleles, three genotypes), HinfI (two alleles, three genotypes), and HaeIII (six restriction profiles, 19 patterns). Polymorphisms were detected at base pairs 229, 225, 208, 210, 178, 175, 159, 87.

CHI-SQUARE ANALYSIS

The Ovar-DRB1 exon 2 alleles of three breeds were analyzed by $\chi^2$ test to determine whether they were consistent with the Hardy-Weinberg distribution, using data shown in Table 3. The $\chi^2$ value of the patterns in Kazakh sheep were 173.85 (MvaI; 2 degrees of freedom [df]; $P < 0.01$), 9.24 (SacI; 2 df; $P < 0.01$), 0.33 (SacII; 2 df; $P > 0.05$), and 5.84 (HinfI; 2 df; $P > 0.05$). These results indicated that patterns produced with restriction enzymes SacII and HinfI were in Hardy-Weinberg equilibrium, while patterns produced by restriction enzymes MvaI and SacI were not.

The $\chi^2$ value of patterns in Duolang sheep were 1.25 (MvaI; $P > 0.05$), 13.63 (SacI; $P < 0.01$), 7.44 (SacII; $P < 0.05$), and 16.11 (HinfI; $P < 0.01$). These results suggested that patterns produced by MvaI were in Hardy-Weinberg equilibrium, while patterns produced by SacI, SacII and HinfI were not.

The $\chi^2$ value of patterns in Chinese Merino sheep were 0.03 (MvaI; $P > 0.05$), 1.62 (SacI; $P > 0.05$), 0.38 (SacII; $P > 0.05$), 0.35 (HaeII; $P > 0.05$), 7.44 (HinfI; $P < 0.05$), and 16.11 (HinfI; $P < 0.01$). These results suggested that patterns produced by MvaI were in Hardy-Weinberg equilibrium, while patterns produced by SacI, SacII and HinfI were not.
Comparison of genotypes in sheep with hydatidosis and healthy controls is shown in Table III. Analysis revealed a higher frequency of patterns MvaIbb, HinIab, SacIa, HaeIIIc, and HaeIIIId (P < 0.01) in Kazakh sheep, SacIab (P < 0.05) in Duolang sheep, and HaeIIab, HaeIIice, HaeIIId, and HaeIIIee (P < 0.01) in Chinese Merino sheep (Sinkiang Junken type) in healthy sheep compared with infected sheep, indicating a strong association between these patterns and hydatidosis resistance. Frequencies of patterns MvaIbb, SacIIa, HinIab, HaeIIIe (P < 0.01) and HaeIIab (P < 0.05) in Kazakh sheep, SacIbb, HaeIIIc, HinIab (P < 0.05) and HaeIIla, HaeIIIbe, HaeIIle (P < 0.01) in Duolang sheep, SacIaa (P < 0.05) and HaeIIlb, HinIbb, HaeIIicf, HaeIIle (P < 0.01) in Chinese Merino sheep (Sinkiang Junken type) were lower in healthy sheep compared with infected sheep, indicating a strong association between these patterns and hydatidosis susceptibility.

### DISCUSSION AND CONCLUSION

At present, both domestic and international studies have indicated that MHC genes show extensive polymorphism in humans, mice, cattle (Blattman et al., 1993; Xu et al., 1995), sheep, goats (Amills & Francino, 1995, 1996; Yang et al., 2006; 2011).
Sun et al., 2004), and chickens (Xu et al., 2005). Using PCR-RFLP, Yang et al. (2006) and Sun et al. (2004) investigated the MHC-DRB5 polymorphism in sheep and goats. Konnai et al. (2003) determined the Ovar-DRB1 exon 2 polymorphisms of 52 Suffolk sheep by PCR-RFLP with restriction enzymes SacI (two alleles), SacII (two alleles), HinII (two alleles), and HaeIII (six alleles), which was consistent with our results. Peng et al. (2007) determined Ovar-DRB1 exon 2 polymorphisms of 211 Chinese Merino sheep (Sinkiang Junken type) by PCR-RFLP with restriction enzymes SacI (two alleles, three genotypes) and HinII (two alleles, three genotypes), which was also consistent with our results. However, six alleles and 15 patterns were found using the restriction enzyme HaeIII in their study, while six alleles and 19 patterns were found using this enzyme in the present study. This difference may be due to different numbers and species of sheep. Last but not the least, the genotypes of HaeIII bc and bf weren’t appearance in the population which we chosen. Perhaps they will be found in a larger population.

Ovar-DRB1 is a principal member of MHC class II DRB in sheep (Deverson et al., 1991; Scott et al., 1991; Ballingall et al., 1992). It is often used as a genetic marker in disease association studies (reviewed by Dukkipati et al., 2006). Azab et al. (2004) demonstrated that people who carry human leukocyte antigen (HLA)-DR3 and HLA-DR11 were at high risk for cystic echinococcosis (CE), and those with HLA-DR3 were more susceptible to complications. Shcherbakov & Monje-Barredo (1989) showed that people with HLA-B5 and B18 of HLA I antigen were at high risk for CE, while those with HLA-B14 and B27 had resistance to CE. In addition, Schaiger et al. (1995) and Sayers et al., (2005) found that MHC-DRB1 was related to nematode resistance. Taken together, these results show that MHC polymorphisms are closely associated with parasite resistance or susceptibility. In the present study, the Ovar-DRB1 exon 2 polymorphisms in three breeds of sheep were also shown to be associated with hydatidosis resistance and susceptibility (Table III).

Analysis of the restriction patterns revealed that Chinese Merino sheep (Sinkiang Junken type), Duolang sheep, and Kazakh sheep with the pattern HaeIIIef all had high hydatidosis susceptibility. Chinese Merino (Sinkiang Junken type) and Kazakh sheep with the pattern HaeIllde had strong hydatidosis resistance; in Duolang sheep, the HaeIIIdc appeared to confer some hydatidosis resistance, but the association was not statistically significant. In addition, restriction analysis using enzyme HaeIII produced 19 patterns in Chinese Merino sheep (Sinkiang Junken type) in the present study; however, four of these patterns (HaeIIIb, HaeIIId, HaeIIibe, HaeIIIdc) were not detected in Kazakh sheep, and three of these patterns (HaeIIIdc, HaeIIId, HaeIIIdc) were not detected in Duolang sheep. Kazakh sheep are the local sheep of Sinkiang Province. The Duolang sheep is a cross between Feitun sheep from Afghanistan and local sheep from Kashi City of Sinkiang (Jiang et al., 2006). The Chinese Merino sheep (Sinkiang Junken type) is a cross between a Merino ram from Australia and Sinkiang Junken sheep. The difference among the three breeds in HaeII patterns of the Ovar-DRB1 second exon may be due to different breeding histories. In the present research, we screened restriction patterns associated with hydatidosis resistance and susceptibility in three sheep breeds by PCR-RFLP. Additional research is needed to determine whether these patterns could serve as genetic markers for hydatidosis.

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Original contribution

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