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Short Communication

A study of the association between chronic superficial keratitis and polymorphisms in the upstream regulatory regions of DLA-DRB1, DLA-DQB1 and DLA-DQA1

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ABSTRACT

Canine chronic superficial keratitis (CSK) is an inflammatory corneal disease that primarily occurs in German shepherd dogs (GSDs). Several studies support the hypothesis that CSK is an immune-mediated disease. To investigate the genetic factors associated with CSK development, the upstream regulatory regions (URRs) of the DLA-DRB, -DQA and -DQB genes were genotyped in 60 dogs, including 32 CSK animals. LD analysis identified two blocks ($r^2 \leq 45$), with two DLA-DRB1 and five DLA-DQB1 haplotypes. Analysis of DLA-URR alleles/haplotypes showed a significant association between DQB1*-154 [C/T] ($p = 0.016$) and CSK, suggesting that the T variant may increase the risk for developing CSK disease (OR = 3, 95% CI = 1.25–7.68). When haplotype associations were performed, the URR-DQB*CATT haplotype was significantly associated with CSK ($p = 0.016$), increasing the risk of develop this disease over two-fold (OR = 3, 95%, CI = 1.25–7.68). These results showed that dogs homozygous at DRB1*69 [C/T] had a risk for developing CSK disease that was over four times the risk for heterozygotes. This genetic association supports the previous clinical, histological and pharmacological studies that suggest that CSK is an immune-mediated disease, and this association could potentially be used to identify susceptible animals.

1. Introduction

Canine chronic superficial keratitis (CSK) or pannus is a bilateral inflammatory corneal disease that leads to blindness if left untreated. Canine CSK occurs most frequently in German shepherd dogs (GSDs) (Bedford and Longstaffe, 1979). The average age of CSK development is 5.50 years (Chavkin et al., 1994). Breed predisposition, clinical signs and lesions are usually sufficient for a clinical diagnosis of CSK (Williams, 1999). CSK is characterised in its acute stage by subepithelial cell infiltration associated with neovascularisation. In the chronic phase of CSK, the development of fibrous tissue and pigmentation in the anterior stroma is observed (Bedford and Longstaffe, 1981). Previous work has described the histopathology and inflammatory characteristics of CSK (Gelatt, 1999; Chavkin et al., 1994) as well as the predominance of CD4+ T cells within the lesions (Williams, 1999). The response observed following treatment with topical steroids and other immunosuppressive

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drugs lend support to the hypothesis that CSK is an immune-mediated disease (Gelatt, 1999; Nell et al., 2005).

The normal central cornea has low expression of Major Histocompatibility Complex class II (MHC). Conversely, in CSK, there is an overexpression of MHC class II molecules, and this process is associated with secretion of IFN-gamma by infiltrating CD4+ T cells. Although this change is likely to be a secondary feature of the CSK lesion, increased MHC class II expression may play a part in perpetuating the corneal inflammation observed in this disease (Williams, 2005). A comparison of MHC class II expression between normal and CSK-affected canine corneas showed that incubating the canine cornea with gamma interferon results in aberrant expression of MHC class II molecules compared to normal corneas, which supports that upregulation of MHC class II gene expression in CSK is associated with an increased secretion of IFN-gamma by infiltrating CD4+ T cells (Williams, 2005).

The Dog Leucocyte Antigen system (DLA) class II has been associated with a number of canine autoimmune diseases including SLE-related diseases (Wilbe et al., 2009), pancreatic acinar atrophy (Tsai et al., 2013), hypoadrenocorticism (Addison's disease) (Massey et al., 2013), necrotising meningoencephalitis (NME) (Greer et al., 2010) and anal furunculosis (Barnes et al., 2009). For this reason, in addition to the histopathological studies mentioned above, DLA class II genes are candidates for studying the molecular genetic factors that may be associated with CSK development. The aim of this study was to analyse DLA-DRB, -DQA and -DQB upstream regulatory region (URR) polymorphisms in dogs affected with CSK compared to normal controls.

2. Materials and methods

2.1. Population studied and sample collection

This study analysed dogs that presented with bilateral loss of corneal transparency in the ventral-temporal region that progressed to the centre of the cornea. Patients showed no signs of ocular pain during any stage of the disease, and the owners reported no history of eyelid inflammation. We assessed patients' eyes by slit-lamp biomicroscopy (Kowa SL-14, Japan). To rule out the presence of keratoconjunctivitis sicca, tear production was measured using the Schirmer test (Showa Yakuhin Kako, Tokyo, Japan). The Schirmer test was completed with indirect ophthalmoscopy (indirect ophthalmoscope, All Pupil, Keeler, England and magnifier Volk 20) to evaluate the posterior segment in those patients with corneal opacity.

The healthy control group included GSDs that were over seven years old and had no prior signs of corneal injury.

Blood samples from 60 GSDs were collected into tubes containing 6% EDTA (ethylenediaminetetraacetic acid), which served as an anticoagulant.

After ophthalmologic diagnosis, animals were classified into a case group ($N = 32$), which included all dogs with CSK, or a control group ($N = 28$). This classification resulted in a ratio of 1.14:1 for sick and healthy dogs, respectively, and a ratio of females to males of 1.39:1. The control group dogs were at least eight years old with an average age of 10.43 years (SD ± 1.92), while the case group had an average age of 5.67 years (SD ± 1.97).

2.2. DNA extraction and genotyping

Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's recommendations. DLA-DRB1, -DQA1 and -DQB1 upstream regulatory regions (URRs) were PCR amplified using the previously described primers and PCR conditions (Berggren and Seddon, 2005). PCR products were genotyped by direct DNA sequencing in a DNA sequencer MEGABACE 1000 (GE Healthcare) using the DYEnamic ET Terminator Kit (GE Healthcare), and the sequencing primers were previously reported in the literature (Berggren and Seddon, 2005). Raw nucleotide sequences were edited using MegaBACE Sequence Analyzer (GE Healthcare) and aligned and compared to DLA-DRB1, -DQA1 and -DQB1 upstream regulatory regions reported on GenBank database (http://www.ncbi.nlm.nih.gov/, Berggren and Seddon, 2005) using DNAMAN version 4.15 (Lynnon BioSoft, Quebec, Canada) and Chromas version 2.4 (Technelysium Pty Ltd).

2.3. Statistical analysis

Allele and genotype frequencies and Hardy-Weinberg equilibrium (HWE) for each polymorphism were estimated using GENEPOP 4 software (Rouset, 2008). The unbiased expected (h_e) and observed heterozygosity (h_o) were calculated using the ARLEQUIN 3.1 program (Schneider et al., 2000). The phases were reconstructed and visualised using the Haploview v3.31 software (Barrett et al., 2005).

The association between CSK and DLA-URR alleles/haplotypes was evaluated using a classical case–control study. Fisher's exact test, odds ratio (OR) and its confidence intervals (CI) were calculated by comparing allele frequencies (in number of alleles) between the cases and the controls. P-values of less than 0.05 were considered statistically significant. Statistical analysis was performed using the Epitools package of R statistical computing software (http://www.r-project.org/).

3. Results and discussion

To investigate the role of MHC class II genes in CSK, we collected 60 dog samples, including 32 samples from CSK-affected animals. The ratio of female to male CSK-affected dogs was 1.39:1. This result was not in agreement with previous reports that indicated a sex-bias towards females being more susceptible for developing CSK than male dogs (Bedford and Longstaffe, 1979; Jokinen et al., 2011).

DNA sequencing of the promoter region of the three analysed DLA class II genes revealed two SNPs in the DLA-DRB1 gene (one in the URR and one in exon 1), two SNPs in the DLA-DQA1 gene (one in the URR and one in exon 1) and six SNPs in the DLA-DQB1 gene (five in the URR and one in exon 1). Furthermore, one *indel* in the URR was detected in the DLA-DQB1 gene (Table S1). The wolf

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Table 1
Gene frequencies, observed (h_o) and expected (h_e) heterozygosities, Hardy–Weinberg equilibrium (HWE) calculated for each polymorphism detected for DLA-DRB1, DLA-DQA1, and DLA-DQB1.

Polymorphism ID	Gene frequency		h_o	h_e	HWE F_{IS}–p
DRB1*-128 [C/T]	0.63	0.37	0.542	0.474	0.16–0.65
DRB1*69 [C/T]	0.63	0.37	0.542	0.474	0.16–0.65
DQA1*-85 [A/T]	0.02	0.98	0.033	0.033	−0.02–1.00
DQA1* 38 [C/T]	1	0	0	0	ND
DQB1*-272 [TAAC/−]	0.07	0.93	0	0.135	1.00–0.003
DQB1*-256 [G/T]	1	0	0	0	ND
DQB1*-170 [G/A]	0.43	0.57	0.61	0.495	−0.23–0.39
DQB1*-154 [C/T]	0.59	0.41	0.535	0.488	−0.08–1.00
DQB1*-105 [A/T]	1	0	0	0	ND
DQB1-87 [C/T]	0.36	0.64	0.535	0.466	−0.10–1
DQB1*-71[C/T]	0.72	0.28	0.302	0.396	0.07–1
DQB1*46 [G/A]	1	0	0	0	ND

specific A/G non-synonymous mutation at position 46 in exon 1 of DLA-DQB1 was not detected in our dog samples. No new polymorphisms were observed. These results are in accordance with data previously reported (Berggren and Seddon, 2005).

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For each detected polymorphism, gene and genotype frequencies were calculated for the total sample as well as for the case and control groups. In our total sample, both DLA-DRB1 SNPs were detected at appreciable gene frequencies (Table 1). Previous work showed that DRBp*1 (in the present work the C variant of the DRB1*-128 [C/T]) is the most common allele in dogs and is fixed in several breeds (*e.g.*, Rottweiler, Airedale terrier, Irish setter, Cocker spaniel and Boston terrier). However, in agreement with our data (Table 1), the previous work showed that the GSD alleles occur at considerable gene frequencies ($C = 0.58$ and $T = 0.42$; Berggren and Seddon, 2005). Two SNPs were reported in the DLA-DQA1 gene in dogs and wolves. The first SNP corresponded to an A-T transversion located within the T box at position -85 of the URR, while the second SNP was a synonymous substitution at position 69 in exon 1 of the gene. The allele T of DQA1*-85 [A/T] and C of DQA1* 38 [C/T] were the most common variants in dogs, while the allele A of DQA1*-85 [A/T] and T of DQA1* 38 [C/T] were only identified in Doberman pinscher, English springer spaniel and Saluki and were absent in GSDs (Berggren and Seddon, 2005). In our population, the allele C of DQA1* 38 [C/T] was fixed, while the allele T of DQA1*-85 [A/T] was almost fixed. The variant A of DQA1*-85 [A/T] was only observed in two heterozygote animals (gene frequency = 0.02). Berggren and Seddon (2005) reported seven SNPs and two *indels* in the DLA-DQB1 gene in different dog breeds and wolves, with the DQB1*46 [*G*/*A*] SNP being specific for wolves. In the present work, we detected the same polymorphisms with similar gene frequencies, with the exception of DQB1*-272 [TAAC/-] because DQB1*71 [indel] was not analysed (Table 1).

LD analysis allowed us to identify two blocks with an $r^2 \leq 45$ (Fig. 1). The first block included two DLA-DRB1 haplotypes, while the second block was comprised of five DLA-DQB1 haplotypes. These results are not unexpected because previous reports indicated that LD reaches a high r^2 at very small distances (<100 kb; Goddard and Hayes, 2012; Axelsson et al., 2012), and DLA-DRB1 and DLA-DQA1 are approximately 70 kb apart. These haplotypes may correspond to the haplotypes of DRB1*1, DRB1*2, DQBp*2, DQBp*3, DQBp*6 and DQBp*7 that were previously reported (Berggren and Seddon, 2005). The remaining DQB haplotype, which was observed at a low frequency, were detected for the first time in the German shepherd breed. The gametic phase disequilibrium of DLA-DQA cannot be analysed because almost all of the animals were homozygous for both DLA-DQA SNPs. The haplotype frequencies are shown in Table 3.

The association between CSK and DLA-URR alleles/haplotypes were evaluated using a classical case–control study design. For this purpose, gene frequencies as well as haplotype frequencies within the case and control groups were estimated (Tables 2 and 3). The results showed a significant association between DQB1*-154 [C/T] ($p = 0.016$), which suggests that the T variant may increase the risk for developing CSK (OR = 3, 95% CI = 1.25–7.68). This result was confirmed by haplotype association analysis. To this end, the URR-DQB*CATT haplotype, with only the T variant at DQB1*-154 [C/T] SNP, was significantly associated with CSK ($p = 0.016$) and increased the risk of developing CSK over two-fold (OR = 3, 95% CI = 1.25–7.68, calculated using the Fisher's exact test).

Expression of MHC class II molecules is primarily regulated at the level of transcription by a complex process that involves highly conserved DNA sequences located in the URR of all classical and non-classical MHC class II genes. This regulatory unit comprises six DNA sequences (S, X, X2, Y, CCAAT and TATA boxes) that are highly conserved across vertebrates (Handunnetthi et al., 2010). These sequences represent the interaction sites for nuclear transcription factors such as NF-YB, NF-YC, RFX5, RFXANK and CREB-1, which form a 'transcriptome' by interacting with the class II transactivator (CIITA) and mediate transcriptional control (Ting and Trowsdale, 2002). Furthermore, these regulatory DNA sequences are necessary for optimal constitutive and cytokine-induced gene expression. Nucleotide polymorphisms in the URR of class II genes have specific functional

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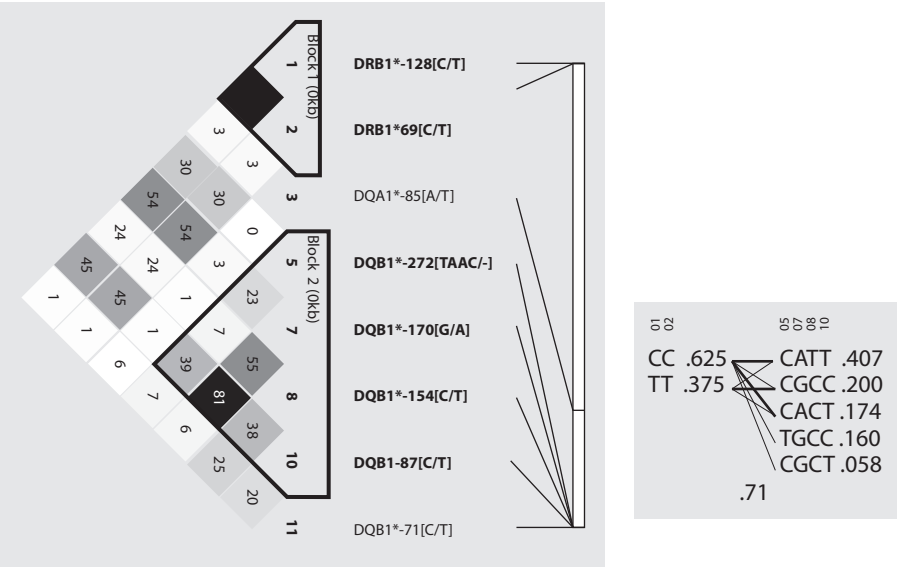

Fig. 1. Haplotypes and linkage disequilibrium in the Upstream Regulatory Region (URR) of DLA-DRB1, -DQA1 and -DQB1 genes are shown. (a) Block 1 and 2 haplotypes; (b) A linkage disequilibrium (r^2) plot obtained using HAPLOVIEW 3.31 (Barrett et al., 2005). The solid lines mark the two blocks identified. In (b), the r^2 values were shown within the block.

consequences on the transcriptional activities of these genes (Louis et al., 1994; Singal and Qiu, 1994, 1996; Janitz et al., 1997).

Overexpression of MHC class II genes occurs in CSK and is regulated by the secretion of IFN-gamma by infiltrating CD4+ T cells, which recognise the URR through a complex of transcription factors (Andersen et al., 1991; Williams, 2005). Considering both features of CSK and the role of the URR in transcriptional control, we hypothesised that the associated polymorphism DQB1*-154 [C/T], which is located in the 3′-end of the X2 regulatory box, could affect the level and/or the pattern of DLA class II gene expression

Table 2
Gene frequencies estimated in case and control groups for DLA-DRB1, DLA-DQA1 and DLA-DQB1 genes. Frequency of each allele within each groups were compared using Fisher's exact test and odds ratio (OR). CI: confidence interval. ND: not determined.

Polymorphism ID	Gene frequency		Fisher's exact test *p* value	OR	95% CI
	Case	Control			
DRB1*-128 [C/T]	0.73/0.27	0.6/0.4	0.19	0.54	0.22–1.3
DRB1*69 [C/T]	0.69/0.31	0.56/O.44	0.33	0.58	0.22–1.53
DQA1*-85 [A/T]	0/1	0.06/0.94	0.38	inf	0.04–inf
DQA1* 38 [C/T]	1/0	1/0	1	ND	ND
DQB1*-272 [TAAC/−]	0.1/0.9	0.18/0.82	0.66	0.47	0.05–2.89
DQB1*-256 [G/T]	1/0	1/0	1	ND	ND
DQB1*-170 [G/A]	0.38/0.66	0.52/0.48	0.2	1.8	0.76–4.4
DQB1*-154 [C/T]	0.45/0.55	0.72/0.28	0.016	3	1.25–7.68
DQB1*-105 [A/T]	0.98/0.02	1/0	0.47	ND	ND
DQB1-87 [C/T]	0.28/0.72	0.44/0.56	0.17	2	0.81–5.1
DQB1*-71[C/T]	0.8/0.2	0.68/0.32	0.22	0.53	0.19–1.4
DQB1*46 [G/A]	1/0	1/0	1	ND	ND

Table 3
Haplotype frequencies estimated in case and control groups for DLA-DRB1, and DLA-DQB1 genes. Frequency of each haplotype within each groups were compared using Fisher's exact test and odds ratio (OR). CI: confidence interval. ND: not determined.

Haplotype	Gene frequency		Fisher's exact test *p* value	OR	95% CI
	Case	Control			
URR-DRB1*CC	35	25	0.67	0.79	0.34–1.83
URR-DRB1*TT	19	17	0.67	0.79	0.34–1.83
URR-DQB*CATT	22	13	0.016	3	1.25– 7.68
URR-DQB*CGCC	6.5	10.7	0.41	0.57	0.17– 1.7
URR-DQB*CACT	5	10	0.39	0.53	0.15–1.66
URR-DQB*TGCC	4.5	9.3	0.25	0.47	0.11–1.61
URR-DQB*CGCT	2	3	1	0.77	0.09–5.34

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Table 4
Analysis of Hardy-Weinberg equilibrium (HWE) for each polymorphism of DLA-DRB1, DLA-DQA1 and DLA-DQB1 within case and control groups were performed using F_{IS} index. The proportions of homozygotes/heterozygotes between both groups were compared through Fisher's exact test and odds ratio (OR). CI: confidence interval. ND: not determined.

Polymorphism ID	HWEF_{IS}–p case	HWEF_{IS}–p control	Fisher's exact test p value	OR	95% CI
DRB1*-128 [C/T]	0.16–0.65	−0.39–0.09	0.14	2.83	0.86–9.96
DRB1*69 [C/T]	0.16–0.65	−0.39–0.09	0.048	4.2	1.07–18.21
DQA1*-85 [A/T]	−0.02–1	ND	1.00	1.21	0.01–100.77
DQA1* 38 [C/T]	ND	ND	ND	ND	ND
DQB1*-272 [TAAC/-]	1–0.033	ND	ND	ND	ND
DQB1*-256 [G/T]	ND	ND	1	0	0–inf
DQB1*-170 [G/A]	−0.23–0.4	−0.27–0.36	0.76	1.26	0.36–4.42
DQB1*-154 [C/T]	−0.07–1	0.1–1	0.07	3.48	0.99–13.47
DQB1*-105 [A/T]	ND	ND	0.47	0	0–35.6
DQB1-87 [C/T]	−0.1–1	−0.18–0.63	0.76	1.32	0.4–4.57
DQB1*-71[C/T]	0.07–1	0.47–0.06	0.5	0.58	0.12–2.4
DQB1*46 [G/A]	ND	ND	ND	ND	ND

in animals with CSK. However, the functional role of this polymorphic site still needs to be analysed and confirmed by means of gene expression assays.

In humans, a high level of LD between HLA class II promoters and their exons has made it impossible to assess the significance of promoter variation in diseases (Howson et al., 2013). For this reason, we cannot exclude the possibility that the DQB1*-154 [C/T] T allele SNP could be in LD with exon 2 alleles and that this LD could explain the observed association with CSK. In accordance with this alternative hypothesis, Jokinen et al. (2011) genotyped the second exon of all DLA-D genes and reported that dogs carrying the DLA-DRB1*01501/DQA1*00601/DQB1*00301 haplotype had a 2.7 times higher risk for developing CSK than dogs carrying other haplotypes.

Finally, we evaluated the hypothesis that homozygosis in the MHC genes increases the risk of susceptibility to autoimmune diseases. To confirm this assumption, h_o, h_e and HWE were estimated in the entire sample as well as within the case and control groups. In addition, OR were calculated to compare the ratio of homozygote/heterozygote genotypes in both groups. The results obtained are summarised in Table 4. Several studies have shown that heterozygosity confers a selective advantage against infectious and autoimmune diseases (heterozygote advantage or homozygote disadvantage) in different mammal species, such as humans and cows (Thursz et al., 1997; Hraber et al., 2007; Takeshima et al., 2008). Despite all of the studied SNPs being in HWE in both the case and control groups, the results showed that dogs homozygous at DRB1*69 [C/T] had a four times higher risk for developing CSK than dogs that were heterozygous ($p = 0.048$; OR = 4.2, 95% CI = 1.07–18.21, Table 4). These results are in agreement with a previous report that showed that homozygosis in MHC class II genes increases the risk for developing CSK (Jokinen et al., 2011).

In conclusion, this is the first study that examined the role of the URR of class DLA gene polymorphisms in the pathogenesis of CSK in dogs. The findings of this study validate previously reported associations found in an independent population, which provides additional support for the association between MHC class II genes and the development of CSK in dogs. This genetic association also supports the previous clinical, histological and pharmacological studies demonstrating that CSK is an immune-mediated disease (Bedford and Longstaffe, 1979; Williams, 1999, 2005). Therefore, it is likely that this MHC association can primarily explain the genetic basis of CSK, although other susceptibility genes might also contribute to the development of this disease and will need to be further examined. These results could potentially be used to identify susceptible animals that require monitoring for early pathological changes.

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References

Andersen, L.C., Beaty, J.S., Nettles, J.W., Seyfried, C.E., Nepom, G.T., Nepom, B.S., 1991. Allelic polymorphism in transcriptional regulatory regions of HLADQB genes. J. Exp. Med. 173 (1), 181–192.
Axelsson, E., Webster, M.T., Abhirami, R., The LUPA Consortium, Ponting, C.P., Kerstin, L-T., 2012. Death of PRDM9 coincides with stabilization of the recombination landscape in the dog genome. Genome Res. 22, 51–63.
Barnes, A., O'Neill, T., Kennedy, L.J., Short, A.D., Catchpole, B., House, A., Binns, M., Fretwell, N., Day, M.J., Ollier, W.E., 2009. Association of canine anal furunculosis with TNFA is secondary to linkage disequilibrium with DLA-DRB1*. Tissue Antigens 73, 218–224.
Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.
Bedford, P.G.C., Longstaffe, J.A., 1979. Corneal pannus (chronic superficial keratitis) in the German shepherd dog. J. Small Anim. Pract. 20, 41–56.
Bedford, P.G.C., Longstaffe, J.A., 1981. Corneal pannus (chronic superficial keratitis) in the German shepherd dog. J. Small Anim. Pract. 20, 41–56.
Berggren, K., Seddon, J., 2005. MHC promoter polymorphism in grey wolves and domestic dogs. Immunogenetics 57, 267–272.
Chavkin, M.J., Roberts, S.M., Salman, M.D., Severin, G.A., Scholten, N.J., 1994. Risk factors for development of chronic superficial keratitis in dogs. J. Am. Vet. Med. Assoc. 204 (10), 1630–1634.

Gelatt, K.N., 1999. Veterinary Ophthalmology, 3ª ed. Lippincott, Williams & Willkins, Philadelphia, pp. 355–425, 635–673 (Chap. 9).
Goddard, M.E., Hayes, B.J., 2012. Genome-wide association studies of linkage disequilibrium in cattle. In: Bovine Genomics. John Wiley & Sons, Ames, Iowa, USA, pp. 192–211.
Greer, K.A., Wong, A.K., Liu, H., Famula, T.R., Pedersen, N.C., Ruhe, A., Wallace, M., Neff, M.W., 2010. Necrotizing meningoencephalitis of Pug dogs associates with dog leukocyte antigen class II and resembles acute variant forms of multiple sclerosis. Tissue Antigens 76, 110–118.
Handunnetthi, L., Ramagopalan, S.V., Ebers, G.C., Knight, J.C., 2010. Regulation of MHC class II gene expression, genetic variation and disease. Genes Immun. 11 (2), 99–112.
Howson, J.M., Roy, M.S., Zeitels, L., Stevens, H., Todd, J.A., 2013. HLA class II gene associations in African American Type 1 diabetes reveal a protective HLA-DRB1*03 haplotype. Diabet. Med. 30 (6), 710–716.
Hraber, P., Kuiken, C., Yusim, K., 2007. Evidence for human leukocyte antigen heterozygote advantage against hepatitis C virus infection. Hepatology 46, 1713–1721.
Janitz, M., Mitchison, A., Reiners-Schramm, L., Lauster, R., 1997. Polymorphic MHC class II promoters exhibit distinct expression pattern in various antigen-presenting cell lines. Tissue Antigens 49, 99–106.
Jokinen, P., Rusanen, E., Kennedy, L., Lohi, H., 2011. MHC class II risk haplotype associated with Canine chronic superficial keratitis in German Shepherd dogs. Vet. Immunol. Immunopathol. 140 (1–2), 37–41.
Louis, P., Pinet, V., Cavadore, P., Kerlan-Candon, S., Clot, J., Eliaou, J.F., 1994. Differential expression of HLA-DRB genes according to the polymorphism of their regulatory region. C. R. Acad. Sci. 137, 161–166.
Massey, J., Boag, A., Short, A.D., Scholey, R.A., Henthorn, P.S., Littman, M.P., Husebye, E., Catchpole, B., Pedersen, N., Mellersh, C.S., Ollier, W.E., Kennedy, L.J., 2013. MHC class II association study in eight breeds of dog with hypoadrenocorticism. Immunogenetics 65, 291–297.
Nell, B., Walde, I., Billich, A., Vit, P., Meingassner, J.G., 2005. The effect of topical pimecrolimus on keratoconjunctivitis sicca and chronic superficial keratitis in dogs: results from an exploratory study. Vet. Ophthalmol. 8 (1), 39–46.
Rouset, F., 2008. GENEPOP'007, a complete reimplementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.
Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin, A software for population genetics data analysis. Ver 2.0. Genetics and Biometry Lab. Department of Anthropology, University of Geneva.
Singal, D.P., Qiu, X., 1994. Polymorphism in the upstream regulatory regions and level of expression of HLA-DRB genes. Mol. Immunol. 31, 1117–1120.
Singal, D.P., Qiu, X., 1996. Polymorphism in both X and Y motifs controls level of expression of HLA-DRB1 genes. Immunogenetics 43, 50–56.
Takeshima, S.N., Matsumoto, Y., Chen, J., Yoshida, T., Mukoyama, H., Aida, Y., 2008. Evidence for cattle major histocompatibility complex (BoLA) class II DQA1 gene heterozygote advantage against clinical mastitis caused by *Streptococci* and *Escherichia* species. Tissue Antigens 72, 525–531.
Ting, J.P.Y., Trowsdale, J., 2002. Genetic control of MHC class II expression. Cell 109, 121.
Thursz, M.R., Thomas, H.C., Greenwood, B.M., Hill, A.V., 1997. Heterozygote advantage for HLA class-II type in hepatitis B virus infection. Nat. Genet. 17, 11–12.
Tsai, K.L., Starr-Moss, A.N., Venkataraman, G.M., Robinson, C., Kennedy, L.J., Steiner, J.M., Clark, L.A., 2013. Alleles of the major histocompatibility complex play a role in the pathogenesis of pancreatic acinar atrophy in dogs. Immunogenetics 65, 501–509.
Williams, D.L., 1999. Histological and immunohistochemical evaluation of canine chronic superficial keratitis. Res. Vet. Sci. 67 (2), 191–195.
Williams, D.L., 2005. Major histocompatibility class II expression in the normal canine cornea and in canine chronic superficial keratitis. Vet. Ophthalmol. 8 (6), 395–400.
Wilbe, M., Jokinen, P., Hermanrud, C., Kennedy, L.J., Strandberg, E., Hansson-Hamlin, H., Hannes, L., Andersson, G., 2009. MHC class II polymorphism is associated with a canine SLE-related disease complex. Immunogenetics 61, 557–564.

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