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Association of BoLA-DRB3.2 gene polymorphism with Tuberculosis susceptibility of dairy cattle in Chinese Holstein cattle

Xiaolin Zheng[1]; Bo Qin[1]; Qinglu Wang L[1]; Youtao Wang[1]; Qishun Liao[2]; Jinling Zheng[2]; Guodong Dong[3]; Yifang Zhang[1]; Xianwei Shi[1]*; Jun Chai[1]*

[1]Department of Animal Medicine, Yunnan Agricultural University, Kunming, China

[2]Yunnan Agricultural Vocational and Technical College, Kunming, China

[3]Yunnan Prevention and Control Center of Animal Disease, Kunming, China

***Corresponding Author(s): Jun Chai & Xianwei Shi**

Department of Animal Medicine, Yunnan Agricultural University, Kunming, China

E-mail: 1726377102@qq.com & xwshi5@163.com

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Abstract

Purpose: Bovine Lymphocyte Antigen-DRB3.2 (BoLA-DRB3.2), plays a critical role in immunologic function. This study focuses on the variations of BoLA-DRB3.2 Gene in Chinese Holstein of dairy cattle and their relationship to tuberculosis susceptibility.

Methods: Case-control design was used in this research. The case group included 106 cows with PPD positive and IFN-γ test. They all came from the same stable. There is a total of 198 anticoagulant blood samples, taken from Yuxi, Dali, Baoshan and other regions of Yunnan Province, including 106 positive samples and 92 negative control samples of tuberculosis, The variation and genotype of the BoLA-DRB3.2 were conducted by PCR amplification and direct sequencing. The association between genotypes/haplotypes and the susceptibility to tuberculosis in cows was further analyzed by comparing the differences of the allele's frequency and genotype frequency in tuberculosis group and control group.

Results: There are 38 SNP-sites found in the DRB3.2 gene, of which 19 are newly discovered, and five of those would cause amino acid variation; There is an extremely significant relationship between 3 sites and the tuberculosis susceptibility of dairy cows ($P<0.01$), and a significant relationship between 16 sites and the tuberculosis susceptibility of dairy cows ($0.01 < P<0.05$); Through analysis, there are 19 haplotypes (frequency greater than 0.01) obtained. Among these types, the frequency of haplotype 2,4, and 5 was found to be significant higher in the positive tuberculosis group than in the negative group; The frequency of haplotype 13 was significant higher in the control group than the case group, and the haplotype 15, 18, and 19 were specific to the tuberculous group. The haplotype 17 was unique to the tuberculous negative group.

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Conclusion: With above studies, we find that bovine BoLA-DRB3.2 gene plays an important role in the development of TB susceptibility/resistance in dairy cows. The five susceptibility genotypes and several resistant haplotypes revealed in this study may be crucial in the evolution and screening of TB in dairy cows. Therefore, we need to further deepen the study of this gene.

Introduction

Tuberculosis is a chronic infectious disease caused by branch Bacillus that both humans and animals suffer from. The pathogens that cause bovine tuberculosis are bovine branch Bacillus and tuberculosis branch Bacillus. Studies have shown that about one-third of the world's population has infected or infected with Mycobacterium tuberculosis, but the affected population accounts for only one-tenth of the infected population, which suggests that genetic factors play an important role in the susceptibility of tuberculosis [1]. Kieran et al. found that there is a link between bovine tuberculosis and polymorphism of multiple congenital immune-associated gene [2]. We investigated the associations between SLC11A1 polymorphisms to tuberculosis (TB) and single-nucleotide polymorphism (SNPs) in the CARD15 gene with susceptibility to BTB in Chinese Holstein cattle [3,4]. The scientists also found that the gene exon 2 mutation of BoLA-DRB3was associated with bovine accessory tuberculosis infection [5]. Therefore, in this thesis, we will focus on the relationship between BoLA-DRB3.2 Gene Polymorphism with Tuberculosis Susceptibility of Dairy Cattle.

BoLA the Bovine Leukocyte Antigen plays an important role in the body's immune system. The study found that its different haplotypes are closely related to the individual's resistance to disease [6]. Domestic and international Studies show that the BoLA gene is related to a variety of diseases, and the current research focus is mainly on the susceptibility and resistance genes of dairy cow mastitis. The main studies are now focused on the incidence and susceptibility of dairy cow mastitis. Mejdell studied on the correlation between BoLA-A and Norwegian bull mastitis and found that allele A 2 of BoLA-A was significantly related to the relative resistance of mastitis, and the allele A7 was significantly related to the relative susceptibility of mastitis [7]. Dietz et al. performed a genotype analysis of the BoLA-DRB3.2 loci of American Holstein cows and found that the DRB3.2 * 16 allele was related to the high SCC, and the DRB3 .2 * 8 allele was related to the high SCC of the first-time lactating cows. The DRB3.2* 16 allele is related to the high SCC of the second-time lactating cow and the DRB3.2* 22 allele is related to the low SCC of the second-time lactating cow, and the DRB3.2* 23 allele is related to the high SCC of the third-time lactating cow [8] Zanotti et al. found that DQA * 3A, DQB * 3A, DRB2 * 2A, and DRB2 .2 * 11 were related to Persistent lymphomatosis [9]. Alizadeh et al. found that the BoLA-DRB3 * 2703 allele may be related to foot-and-mouth disease [10]. Mallard et al. found that the encoded amino acids of DRB3.2*0301, DRB3.2*0302, DRB3.2*0902 and DRB3.2*1202 are highly correlated with Dermatophilosis [11]. However, there is no relevant report on the association between BoLA gene and cow tuberculosis susceptibility.

This study focuses on clarifying the polymorphism distribution characteristics of BoLA-DRB3 gene and the correlation between gene variation and bovine tuberculosis infection of the plateau cows in Yunnan Province, based on analyzing the polymorphism of exon 2 of BoLA-DRB3 gene (BoLA-DRB3.2), to further understand the immune mechanism of the body to bovine tuberculosis, which would lay a scientific foundation for the breeding and purification of tuberculosis resistant cattle, and completely eliminate bovine tuberculosis in the future.

Materials and methods

Experimental sampling

Based on the survey of dairy cow tuberculosis in Yuxi, Dali, Baoshan and other regions of Yunnan Province, a total of 198 whole blood samples were taken by using two methods--- PPD and IFN-γ-ELISA, including 106 whole blood samples that were tested positive for tuberculosis and 92 whole blood samples that were tested negative for tuberculosis by using both methods, which were temporarily preserved at -20 °C and later sent to the laboratory in an ice box, with long-term preservation at -80 °C.

DNA retracted and purification

The extraction of Bovine leukocyte DNA was based on the specification of the extraction reagent kit of whole blood gold DNA. The eluted DNA is stored at -20 °C. 5μL DNA extraction was detected by 1 % AGE (agarose gel electrophoresis) to check the extraction effect and DNA concentration.

Primer Design Synthesis

The design refers to the complete gene sequence of Genbank BoLA-DRB3--- NW_003085994.1, using the primer design software of Primer Premier 5.0 and the primers of Oligo software design 2, and the amplification area includes all exon 2 sequences and some introns 1 and 2 sequences, synthesized by Dalian Takara Company. Primers information is shown in Table 1.

Table 1: Primers information

Name of Primers	Sequence (5'-3')	Length	Size (bp)
BoLA-DRB3-2 F1	TTCAAACTAATGGTTCGGTGTG	22	565
BoLA-DRB3-2 R1	TGGTGTAGGGAGAGGAGACACT	22	
BoLA-DRB3-2 F2	CTCCCAGGGTCAATCAGTAAGA	22	753
BoLA-DRB3-2 R2	GAGCAAATGATCACATGGTGTAG	23	

Gene amplification

The mixing system of PCR reaction shows as follows: EsyTaqase 0.5μl, 10xBuffer 4μl, dNTPs 2.5μl, positive and negative primers 0.5μl for each, template DNA 2μl, ddH_2O 20μl, a total of 30μl. PCR amplification conditions are as follows: 95°C pre degeneration 3min, 95°C denaturation 30s, 56-58 °C reconversion 30s, 72°C extension 1min, 35 cycles, 72°C extension 8min, preservation at 4 °C. The PCR product was taken out after the reaction, and 1% agarose gel electrophoresis was used to detect the effect of PCR amplification. Those samples with bright, non-specific amplified strips can be directly sequenced by Takara.

Sequence comparison and SNP discovery

The sequencing results were compared and analyzed by using the SeqMan program in DNA star software and the sequence published in Genbank as a reference to find all SNP-sites, and the resulting data would be kept in Excel for analysis.

Statistical methods

The alleles and difference of genotypes frequency are tested by using $\chi 2$. If $P<0.05$, it will indicate a significant difference. The relevance of each SNP-site and tuberculosis is analyzed by-

usingtheonline analysis software SNPstats (Http://bioinfo.iconcologia.net/snpstats).

The correlation between a single SNP and tuberculosis is analyzed under five different genetic modes (dominant, recessive, codominant, super-dominant, and additive), and the ratio (Odds Ratio, OR) and 95 % confidence interval (Confidence intervals, CI) are calculated, by using the logical regression method. Then, the most suitable genetic model would be determined by using the Akaike Information Criterion(AIC) and Bayesian Information Criterion(BIC) [12] .If P<0.05, it will indicate the significance of the statistics. The correlation between haplotype and tuberculosis was analyzed by using online analysis software SNP stats.

Results

SNP Analysis

The sequencing sample results were compared with the corresponding sequence downloaded in the NCBI to find the SNP site, and analyze the changes of amino acids caused by the variation of the basic group for each site, by using the SeqMan software in the series of DNA Star software. A total of 38 SNP sites were found in the BoLA-DRB3.2 gene of dairy cows in Yunnan Province, including 19 sites that were landed in the dbSNP database and 19 new sites that were not landed. Seven SNP sites are located in exon 2, where site 672 leads to an amino acid mutation, which mutates from isoleucine to valine. Details of the base mutations, mutation positions, and amino acid changes at the variant sites are shown in Table 2.

Table 2: SNP sites and Amino Acid Change

Polymorphic Site	Nucleotide Variation	dbSNP	Location	Amino Acid Change
E2 (-57)	T/C	rs136459469	Intron1	
E2 (-55)	G/A	rs136995386	Intron1	
E2 (-54)	G/A		Intron1	
E2 (-40)	A/T		Intron1	
E2 (-21)	T/A		Intron1	
E2 (-16)-E2(-15)	-/T	rs135166003	Intron1	
E2 (-14)-E2(-13)	-----/TGTGC	rs136590554	Intron1	
E2 (-1)	T/A		Intron1	
463	G/A		Exon2	
478	G/A	rs134864742	Exon2	
489	G/A		Exon2	
494	T/C		Exon2	
496	T/G	rs137718343	Exon2	
520	A/G		Exon2	
531	T/C	rs133626884	Exon2	
537	C/G		Exon2	
563	C/T		Exon2	
581	G/A	rs208413488	Exon2	E
586	T/C	rs135099999	Exon2	I-T
611	C/T	rs132865043	Exon2	T
653	C/T	rs133898231	Exon2	N
669	C/A	rs209853925	Exon2	H-N
672	A/G		Exon2	I-V
695	T/C	rs135025732	Exon2	N
821	G/T	rs42312242	Exon2	E-D
831	C/A	rs137139902	Exon2	R
832	G/A	rs209467115	Exon2	R-Q
836	A/G	rs135923061	Exon2	T
851	G/A	rs109010468	Exon2	V

E2 (+6)-E2 (+8)	AAG/---		Intron2	
E2 (+11)	C/T		Intron2	
E2 (+14)-E2 (+15)	-/G		Intron2	
E2 (+22)-E2 (+23)	-/A		Intron2	
E2 (+23)	C/-		Intron2	
E2 (+24)	A/G	rs209777727	Intron2	
E2 (+26)	T/C		Intron2	
E2 (+37)	C/T		Intron2	
E2 (+38)	G/A		Intron2	

Note:

The Frequencies of Allele and Genotype in BoLA-DRB3.2 Gene

Through PCR sequencing, 38 SNP-sites were found in the BoLA-DRB3.2 gene of 198 subjects, and polymorphisms of each site were analyzed to obtain alleles and genotypes of each SNP-site in the population. The frequency distribution in both positive group and control group was also measured. Details are shown in Table 3.

Table 3: Genotype and allele frequencies of polymorphic variants of the DRB3.2 gene in patients with TB (n=106) and healthy Controls (n=92) Genotype and allele frequencies of polymorphic variants of the DRB3.2 gene in patients with TB (n=106) and healthy Controls (n=92)

SNPs	Group	Genotype Count(Frequencies)			Allele Count(Frequencies)	
		C/C	C/T	T/T	C	T
E2(-57)	Case	70 (0.66)	3(0.03)	33(0.31)	143(0.67)	69(0.33)
	Control	72(0.78)	4(0.04)	16(0.17)	148(0.8)	36(0.2)
		A/A	A/G	G/G	A	G
E2(-55)	Case	77(0.73)	2(0.02)	27(0.25)	156(0.74)	56(0.26)
	Control	76(0.83)	1(0.01)	15(0.16)	153(0.83)	31(0.17)
		A/A	A/G	G/G	A	G
E2(-54)	Case	6(0.06)	10(0.09)	90(0.85)	22(0.1)	190(0.9)
	Control	12(0.13)	19(0.21)	61(0.66)	43(0.23)	141(0.77)
		A/A	A/T	T/T	A	T
E2(-40)	Case	100(0.94)	4(0.04)	2(0.02)	204(0.96)	8(0.04)
	Control	81(0.88)	10(0.11)	1(0.01)	172(0.93)	12(0.07)
		A /A	A/T	T/T	A	T
E2(-21)	Case	10(0.09)	6(0.06)	90(0.85)	26(0.12)	186(0.88)
	Control	10(0.11)	4(0.04)	78(0.85)	24(0.13)	160(0.87)
		T/T	T/-	-/-	T	-
E2(-16)-E2(-15)	Case	47(0.44)	0(0)	59(0.56)	94(0.44)	118(0.56)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		TGTGC/TGTGC	TGTGC/-----	-----/-----	TGTGC	-----
E2(-14)-E2(-13)	Case	47(0.44)	0(0)	59(0.56)	94(0.44)	118(0.56)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		A/A	A/T	T/T	A	T
E2(-1)	Case	1(0.01)	1(0.01)	104(0.98)	3(0.01)	209(0.99)
	Control	0(0)	0(0)	92(1)	0(0)	184(1)
		GG	GA	A/A	G	A

463	Case	103(0.97)	3(0.03)	0(0)	209(0.99)	3(0.01)
	Control	90(0.98)	2(0.02)	0(0)	182(0.99)	2(0.01)
		A/A	A/G	G/G	A	G
478	Case	45(0.42)	0(0)	61(0.58)	90(0.42)	122(0.58)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		A/A	A/G	G/G	A	G
489	Case	10(0.09)	5(0.05)	91(0.86)	25(0.12)	187(0.88)
	Control	10(0.11)	5(0.05)	77(0.84)	25(0.14)	159(0.86)
		C/C	C/T	T/T	C	T
494	Case	2(0.02)	4(0.04)	100(0.94)	8(0.04)	204(0.96)
	Control	1(0.01)	11(0.12)	80(0.87)	13(0.07)	171(0.93)
		G/G	G/T	T/T	G	T
496	Case	45(0.42)	1(0.01)	60(0.57)	91(0.43)	121(0.57)
	Control	54(0.59)	0(0)	38(0.41)	108(0.59)	76(0.41)
		A/A	A/G	G/G	A	G
520	Case	103(0.57)	0(0)	3(0.03)	206(0.97)	6(0.03)
	Control	91(0.99)	0(0)	1(0.01)	182(0.99)	2(0.01)
		C/C	C/T	T/T	C	T
531	Case	45(0.42)	0(0)	61(0.58)	90(0.42)	122(0.58)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		C/C	C/G	G/G	C	G
537	Case	91(0.86)	6(0.06)	9(0.08)	188(0.89)	24(0.11)
	Control	78(0.85)	13(0.14)	1(0.01)	169(0.92)	15(0.08)
		C/C	C/T	T/T	C	T
563	Case	99(0.93)	2(0.02)	5(0.05)	200(0.94)	12(0.06)
	Control	87(0.95)	2(0.02)	3(0.03)	176(0.96)	8(0.04)
		A/A	A/G	G/G	A	G
581	Case	35(0.33)	4(0.04)	67(0.63)	74(0.35)	138(0.65)
	Control	21(0.23)	5(0.05)	66(0.72)	47(0.26)	137(0.74)
		C/C	C/T	T/T	C	T
586	Case	50(0.47)	1(0.01)	55(0.52)	101(0.48)	111(0.52)
	Control	56(0.61)	2(0.02)	34(0.37)	114(0.62)	70(0.38)
		C/C	C/T	T/T	C	T
611	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53(0.58)	78(0.42)	106(0.58)
		C/C	C/T	T/T	C	T
653	Case	75(0.71)	13(0.12)	18(0.17)	163(0.77)	49(0.23)
	Control	70(0.76)	19(0.21)	3(0.03)	159(0.86)	25(0.14)
		A/A	A/C	C/C	A	C
669	Case	34(0.32)	5(0.05)	67(0.63)	73(0.34)	139(0.66)
	Control	21(0.23)	5(0.05)	66(0.72)	47(0.26)	137(0.74)
		A/A	A/G	G/G	A	G

672	Case	102(0.96)	0(0)	4(0.04)	204(0.96)	8(0.04)
	Control	88(0.96)	2(0.02)	2(0.02)	178(0.97)	6(0.03)
		C/C	C/T	T/T	C	T
695	Case	50(0.47)	1(0.01)	55(0.52)	101(0.48)	111(0.52)
	Control	56(0.61)	2(0.02)	34(0.37)	114(0.62)	70(0.38)
		G/G	G/T	T/T	G	T
821	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53(0.58)	78(0.42)	106(0.58)
		A/A	A/C	C/C	A	C
831	Case	1(0.01)	2(0.02)	103(0.97)	4(0.02)	208(0.98)
	Control	0(0)	0(0)	92(1)	0(0)	184(1)
		A/A	A/G	G/G	A	G
832	Case	40(0.38)	3(0.03)	63(0.59)	83(0.39)	129(0.61)
	Control	26(0.28)	3(0.03)	63(0.68)	55(0.3)	129(0.7)
		A/A	A/T	G/G	A	G
836	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53 (0.58)	78(0.42)	106(0.58)
		A/A	A/G	G/G	A	G
851	Case	85(0.8)	4(0.04)	17(0.16)	174(0.82)	38(0.18)
	Control	79(0.86)	3(0.03)	10(0.11)	161(0.88)	23(0.12)
		AAG/AAG	AAG/---	---/---	AAG	---
E2(+6)- E2(+8)	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53(0.58)	78(0.42)	106(0.58)
		C/C	C/T	T/T	C	T
E2(+11)	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53(0.58)	78(0.42)	106(0.58)
		G/G	G/-	-/-	G	-
E2(+14)-E2(+15)	Case	45(0.42)	0(0)	61(0.58)	90(0.42)	122(0.58)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		A/A	A/-	-/-	A	-
E2(+22)-E2(+23)	Case	53(0.5)	0(0)	53(0.61)	106(0.5)	106(0.5)
	Control	53(0.5)	0(0)	36(0.39)	112(0.61)	72(0.39)
		C/C	C/-	-/-	C	-
E2(+23)-E2(+24)	Case	61(0.58)	0(0)	45(0.42)	122(0.58)	90(0.42)
	Control	39(0.42)	0(0)	53(0.58)	78(0.42)	106(0.58)
		A/A	A/G	G/G	A	G
E2(+24)	Case	63(0.59)	1(0.01)	42(0.4)	127(0.6)	85(0.4)
	Control	63(0.68)	3(0.03)	26(0.28)	129(0.7)	55(0.3)
		C/C	C/T	T/T	C	T
E2(+26)	Case	45(0.42)	0(0)	61(0.58)	90(0.42)	122(0.58)
	Control	53(0.58)	0(0)	39(0.42)	106(0.58)	78(0.42)
		C/C	C/T	T/T	C	T

E2(+37)	Case	61(0.58)	2(0.02)	43(0.41)	124(0.58)	88(0.42)
	Control	39(0.42)	1(0.01)	52(0.57)	79(0.43)	105(0.57)
		A/A	A/G	G/G	A	G
E2(+38)	Case	6(0.06)	0(0)	100(0.94)	12(0.06)	200(0.94)
	Control	2(0.02)	1(0.01)	89(0.97)	5(0.03)	179(0.97)

NA: Deletion Form

Relationship between SNP-site and Tuberculosis susceptibility

The correlation between each SNP site and tuberculosis susceptibility was analyzed, which was based on the online software SNP stats (http://bioinfo.iconcologia.net/snpstats), and the association between a single site and tuberculosis susceptibility was calculated by using the logistic regression analysis method (0.01<P<0.05, showing that the site has a significant correlation with tuberculosis susceptibility / resistance; P< 0.01, indicating that the site has an extremely significant correlation with tuberculosis susceptibility/ssresistance; P>0.05, indicating that this site has no significant correlation with tuberculosis susceptibility / resistance); the best genetic method to locate the was based on the principle of minimum AIC value (Codominant: Codominance; Dominant: Dominant; Recessive: Hidden; Over dominant: Hyper-dominant; Log-additive: Additivity). The association between each site of BoLA-DRB3.2 gene and tuberculosis susceptibility was calculated by using the logistic regression analysis method. The best genetic method to locate the was based on the principle of minimum AIC value; there are three sites of BoLA-DRB3.2 gene of cows ---E2(-54), 537, 653, in Yunnan Province, have an extremely significant correlation with tuberculosis susceptibility / resistance (P<0.01); the best genetic model for the site E2 (-54) is Dominant, which has a genotype G/A-A / A that increases the risk of tuberculosis (OR >1); The site 537 is codominant, and genotype G/C is tuberculous protective type (OR<1), and G/G is tuberculosis susceptibility (OR>1); the site 653 is recessive, and genotype T/T is tuberculosis susceptibility (OR>1).Multiple sites are significantly related to tuberculosis susceptibility (0.01<P<0.05), including E2 (-57), E2 (-40), 478, 494, 496, 531, 586, 611, 695, 821, 836, E2 (+6)-E2 (+8), E2 (+11), E2 (+14)-E2 (+15), E2 (+23), E2 (+26), E2 (+37). The best genetic model for these sites--- E2 (-57), 586,695, E2 (+37), is recessive. The E2 (-57)genotype T/T, 586 genotype T/T, and 695 genotype T/T belong to the type of tuberculous protection (OR<1). E2(+37) genotype T/T is a tuberculosis susceptibility type(OR>1); The site 494 is an over dominant gene, and the site genotype T/C is a tuberculosis susceptibility type(OR>1); 496 is a dominant inheritance, and the site genotype T/G-T/T is tuberculosis protection type(OR<1); Site 478 genotype A/A, 531 genotype C/C, 611 genotype T/T, 821 genotype T/T, 836 genotype G/G, E2(+11) genotype T/T, E2(+14)-E2(+15) genotypes G/G and E2(+26) genotype C/C is tuberculosis susceptibility type(OR>1); The loss of the bases E2(+6)-E2(+8) and E2(+23) can lead to an increased risk of tuberculosis(OR>1). The details of the correlation between the SNP-sites and tuberculosis susceptibility are shown in Table 4.

Table 4: SNP association with TB (n=198, crude analysis) in DRB3 gene

Sites	Model	Genotype	TB	control	OR (95% CI)	P-value	AIC
E2(-57)	Recessive	C/C-C/T	73 (68.9%)	76 (82.6%)	1.00	0.024	272.4
		T/T	33 (31.1%)	16 (17.4%)	0.47 (0.24-0.92)		
E2(-55)	Dominant	A/A	77 (72.6%)	76 (82.6%)	1.00	0.093	274.7
		A/G-G/G		16 (17.4%)	0.56 (0.28-1.11)		
E2(-54)	Dominant	G/G	90 (84.9%)	61 (66.3%)	1.00	0.0021	268
		G/A-A/A	16 (15.1%)	31 (33.7%)	2.86 (1.44-5.67)		
E2(-40)	Overdominant	A/A-T/T	102 (96.2%)	82 (89.1%)	1.00	0.05	273.7
		A/T	4 (3.8%)	10 (10.9%)	3.11 (0.94-10.28)		
E2(-21)	Overdominant	T/T-A/A	100 (94.3%)	88 (95.7%)	1.00	0.67	277.3
		T/A	6 (5.7%)	4 (4.3%)	0.76 (0.21-2.77)		
E2(-16)-E2(-15)	---	T/T	47 (44.3%)	53 (57.6%)	1.00	0.062	274
		G/G	59 (55.7%)	39 (42.4%)	0.59 (0.33-1.03)		
E2(-14)-E2(-13)	---	TGTGC/TGTGC	47 (44.3%)	53 (57.6%)	1.00	0.062	274
		NA	59 (55.7%)	39 (42.4%)	0.59 (0.33-1.03)		
E2(-1)	Dominant	T/T	104 (98.1%)	92 (100%)	1.00	0.11	275
		A/T-A/A	2 (1.9%)	0 (0%)	0.00 (0.00-NA)		

463	---	G/G	103 (97.2%)	90 (97.8%)	1.00	0.77	277.4
		G/A	3 (2.8%)	2 (2.2%)	0.76 (0.12-4.67)		
478	---	G/G	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		A/A	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
489	Dominant	G/G	91 (85.8%)	77 (83.7%)	1.00	0.67	277.3
		G/A-A/A	15 (14.2%)	15 (16.3%)	1.18 (0.54-2.57)		
494	Overdominant	T/T-C/C	102 (96.2%)	81 (88%)	1.00	0.028	272.7
		T/C	4 (3.8%)	11 (12%)	3.46 (1.06-11.28)		
496	Dominant	G/G	45 (42.5%)	54 (58.7%)	1.00	0.022	272.3
		T/G-T/T	61 (57.5%)	38 (41.3%)	0.52 (0.29-0.91)		
520	---	A/A	103 (97.2%)	91 (98.9%)	1.00	0.37	276.7
		G/G	3 (2.8%)	1 (1.1%)	0.38 (0.04-3.69)		
531	---	T/T	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		C/C	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
537	Codominant	C/C	91 (85.8%)	78 (84.8%)	1.00	0.0067	269.5
		G/C	6 (5.7%)	13 (14.1%)	2.53 (0.92-6.96)		
		G/G	9 (8.5%)	1 (1.1%)	0.13 (0.02-1.05)		
563	Recessive	C/C-C/T	101 (95.3%)	89 (96.7%)	1.00	0.6	277.2
		T/T	5 (4.7%)	3 (3.3%)	0.68 (0.16-2.93)		
581	Recessive	G/G-G/A	71 (67%)	71 (77.2%)	1.00	0.11	274.9
		A/A	35 (33%)	21 (22.8%)	0.60 (0.32-1.13)		
586	Recessive	C/C-C/T	51 (48.1%)	58 (63%)	1.00	0.035	273
		T/T	55 (51.9%)	34 (37%)	0.54 (0.31-0.96)		
611	---	C/C	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		T/T	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
653	Recessive	C/C-T/C	88 (83%)	89 (96.7%)	1.00	0.001	266.6
		T/T	18 (17%)	3 (3.3%)	0.16 (0.05-0.58)		
669	Recessive	C/C-A/C	72 (67.9%)	71 (77.2%)	1.00	0.15	275.4
		A/A	34 (32.1%)	21 (22.8%)	0.63 (0.33-1.18)		
672	Over dominant	A/A-G/G	106 (100%)	90 (97.8%)	1.00	0.079	274.4
		A/G	0 (0%)	2 (2.2%)	NA (0.00-NA)		
695	Recessive	C/C-C/T	51 (48.1%)	58 (63%)	1.00	0.035	273
		T/T	55 (51.9%)	34 (37%)	0.54 (0.31-0.96)		
821	---	G/G	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		T/T	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
831	Dominant	C/C	103 (97.2%)	92 (100%)	1.00	0.052	273.7
		A/C-A/A	3 (2.8%)	0 (0%)	0.00 (0.00-NA)		
832	Log-additive	---	---	---	0.81 (0.60-1.09)	0.16	275.6
836	---	A/A	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		G/G	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
851	Log-additive	---	---	---	0.80 (0.53-1.20)	0.27	276.3

E2(+6)-E2(+8)	---	AAG/AAG	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		NA	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
E2(+11)	---	C/C	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		T/T	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
E2(+14)-E2(+15)	---	G/G	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)	0.033	273
		NA	61 (57.5%)	39 (42.4%)	1.00		
E2(+22)-E2(+23)	---	A/A	53 (50%)	56 (60.9%)	1.00	0.12	275.1
		NA	53 (50%)	36 (39.1%)	0.64 (0.37-1.13)		
E2(+23)	---	C/C	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		NA	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
E2(+24)	Recessive	A/A-G/A	64 (60.4%)	66 (71.7%)	1.00	0.092	274.7
		G/G	42 (39.6%)	26 (28.3%)	0.60 (0.33-1.09)		
E2(+26)	---	T/T	61 (57.5%)	39 (42.4%)	1.00	0.033	273
		C/C	45 (42.5%)	53 (57.6%)	1.84 (1.05-3.24)		
E2(+37)	Recessive	C/C-C/T	63 (59.4%)	40 (43.5%)	1.00	0.025	272.5
		T/T	43 (40.6%)	52 (56.5%)	1.90 (1.08-3.35)		
E2(+38)	Recessive	G/G-A/G	100 (94.3%)	90 (97.8%)	1.00	0.2	275.9
		A/A	6 (5.7%)	2 (2.2%)	0.37 (0.07-1.88)		

NA: Deletion form

Analysis of haplotype and tuberculosis susceptibility

Based on the analysis of online software SNP Stats, a group of haplotypes is obtained. The haplotypes with frequencies greater than 0.01 are named DRB 3.2-1 to DRB 3.2-19, ranging from high frequency to low frequency. As what can be seen from Table 5, DRB 3.2-2, DRB 3.2-4, DRB 3.2-5 are significantly related to tuberculosis susceptibility (0.01 <P<0.05), and the frequencies of these three haplotypes in the positive group are higher than in the negative group of tuberculosis, which are the haplotype of tuberculosis susceptibility to (OR>1); DRB3.2-13 is very significantly related to tuberculosis susceptibility (P<0.01), frequencies of which in the positive group are higher than in the negative group of tuberculosis, belonging to the haplotype of tuberculosis protection, with the exceeding of OR value over the normal value (OR<<1); The haplotype DRB3.2-15, DRB3.2-18, and DRB3.2-19 are unique to the tuberculosis group, and the haplotype DRB3.2-17 is unique to the negative tuberculosis group, indicating that the haplotype DRB3.2-15, DRB3.2-18, and DRB3.2-19 may be the haplotype of tuberculosis susceptibility, and haplotype DRB3.2-17 may be the tysspe of tuberculosis protection.

Table 5: Association analysis of DRB3 haplotypes with TB

Sequence Number	Haplotype	case	control	Freq	OR (95% CI)	P-value
DRB3.2-1	CAAATTTGTGCTGAGTGACCCGCTCCACTCGGAAAGTGACACTG	0.1038	0.2337	0.1641	1	---
DRB3.2-2	CAGATTTGTGCTGGGTTATCCATCCAATGCAAAAAGCGACGTCG	0.1509	0.1196	0.1364	1.88(1.04 – 3.44)	0.037
DRB3.2-3	CAGAATTGTGCTGGATTATCCATCCAATGCAAAAAGCGACGTCG	0.099	0.0924	0.0959	1.64 (0.86- 3.13)	0.14
DRB3.2-4	CAGATTTGTGCTGAGTGACCCGCTTCACTCGGAAAGTGACACTG	0.0991	0.0694	0.0873	2.94 (1.30 -6.67)	0.01
DRB3.2-5	CAGATTTGTGCTGAGTGACGCGCTTCACTCGGAAAGTGACACTG	0.0943	0.061	0.0794	3.03(1.26 – 7.14)	0.013

DRB3.2-6	CAGATTTGTGCTGAGTGACCCGCTCCACTCGGAAAGTGACACTG	0.033	0.1153	0.0693	0.40 (0.14 – 1.15)	0.089
DRB3.2-7	TGGATTTGTGCTGGGTTATCCGTCCCATGCGAGAAGCGACATCG	0.066	0.0652	0.0656	1.61(0.26 – 3.23)	0.19
DRB3.2-8	CAGTTTTGTGCTGAGCGACCCGCTCCACTCGGAAAGTGACACTG	0.0283	0.0652	0.0455	0.78(0.26 – 2.33)	0.65
DRB3.2-9	TGGATTTGTGCTGGGTTATCCATCCAATGCAAAAAGCGACGTCG	0.066	0.0109	0.0404	0.00 (-Inf - Inf)	1
DRB3.2-10	TGGATTTGTGCTGGGTTATCTGCCCCACGCAAAAAGCGACGTCA	0.0425	0.0217	0.0328	2.22 (0.86 – 5.88)	0.1
DRB3.2-11	TGGATTTGTGCTGGGTTATCCGTCCCGTGCGAGAAGCGACATCG	0.0189	0.0217	0.0202	0.67 (0.23 - 1.95)	0.46
DRB3.2-12	TAGATTTGTGCTGGGTTGTCCGTCCCATGCGAGAAGCGACATCG	0.0189	0.0109	0.0152	2.13 (1.45 – 7.69)	0.25
DRB3.2-13	TGGAATTGTGCTGGATTATCCATCCAATGCAAAAAGCGACGTCG	0.0048	0.0217	0.0127	3.07e20 (8.55e19 – 1.1e21)	<0.0001
DRB3.2-14	TAGATTTGTGCTGGGTTATCCGTCCCGTGCGAGAAGCGACATCG	0.0142	0.0109	0.0126	1.79 (0.13 – 8.33)	0.48
DRB3.2-15	CAGATTTGTGCTGGGTTATCCGTCCCATGCGAGAAGCGACATCG	0.0189	NA	0.0102	0.00 (-Inf - Inf)	1
DRB3.2-16	CAGATTTGTGCTAAGTGACCCGCTCCACTCGGAAAGTGACACTG	0.0094	0.0109	0.0101	0.44 (0.05 - 3.81)	0.46
DRB3.2-17	TAGATTTGTGCTGGGTTATCTGCCCCACGCAAAAAGCGACGTCG	NA	0.0217	0.0101	2.06e23 (2.05e22 – 2.71e24)	<0.0001
DRB3.2-18	TGGATTTGTGCTGAGTGACCCGCTTCACTCGGAAAGTGACACTG	0.0189	NA	0.0101	0.00 (-Inf - Inf)	1
DRB3.2-19	TGGATTTGTGCTGAGTGACGCGCTTCACTCGGAAAGTGACACTG	0.0189	NA	0.0101	0.00 (-Inf - Inf)	1

NA indicates that this genotype does not exist in this category.

Discussion

The epidemic of cattle tuberculosis is not only greatly harmful to the industry of cattle breeding, causing huge economic losses, but also tremendously influential to human public health security and human tuberculosis control. The BoLA-DRB3 gene is a kind of bovine Leukocyte antigen gene, and the encoded MHC-II molecule plays a very important role in the immune system. Researches have indicated that the DRB3 gene has a close relationship with the resistance and susceptibility of livestock [13].It was also found that the alleles of BoLA-DRB3 are related to diseases, such as dairy tcowmasitis, persistent-lymphocytosis, pheoderma, and bovine para-tuberculosis. Many scientific studies have shown that human HLA-DR genes are associated with human tuberculosis. The research made by Rojas found that tuberculosis patients in north-eastern Mexico are closely related to HLA-DR1l(5) and DR16(2) and the haplotypes of DR11(5)-DQ7(3), DR14(6)-DQS(1) and DR16(2)-DQ7(3) has a higher frequency in the case group; the alleles of HLA-DRl7(3) and DQ8(3) and the haplotypes of DR17(3)-DQ2 and DR4-DQ8(3) have a higher frequency in the control group [14]. Hyun et al. found that DRB1*0803 is closely related to patients of Korean tuberculosis resistance [15]. Dubaniewicz et al. found that tuberculosis in northern Poland was related to DRB1*14 and DRB1*16 [16]. Pospelova proved that the allele of HLA-DRB1*13,*14 is closely related to tuberculosis in Tuvalu [17].

The thesis focuses on studying on the correlation between dairy cows and dairy-tuberculosis for the first time in Yunnan, which analyzed the gene sequence of BoLA-DRB3.2 of tuberculosis group and tuberculosis negative control group with case-control design principle, the high polymorphism of which was found; The study found that there are 38 SNPs in the BoLA-DRB3.2 gene in dairy cows in Yunnan Province, of which 19 are new sites. Meanwhile, it was found that these sites---E2(-54),537, and 653 were extremely related to tuberculosis susceptibility(P<0.01); those sites--- E2(-57), E2(-40), 478, 494, 496, 531, 586, 611, 695, 821, 836, E2(+6)-E2(+8), E2(+11), E2(+14)-E2(+15), E2(+23), E2(+26), E2(+37) were significantly related to tuberculosis susceptibility(0.01<P<0.05).19 haplotypes with frequencies greater than 0.01 were obtained through analysis, of which DRB3.2-2, DRB3.2-4, and DRB3.2-5 were significantly related to tuberculosis susceptibility (0.01<P<0.05), and the frequencies of these three haplotypes were higher in the positive group than in the negative group; DRB3.2-13 is tremendously significantly related to tuberculosis susceptibility (P<0.01), and the frequency of the haplotype is higher in the tuberculosis positive group than in the negative group; The haplotype DRB3.2-15, DRB3.2-18, and DRB3.2-19 are unique to the tuberculosis group, and the haplotype DRB 3.2-17 is unique to the tuberculosis negative group.

Research shows that, due to the differences in the frequency and location of genes for each site, which caused different genetic effects, expressions of the tuberculosis susceptibility are also different. Through the identification and analysis of the functional SNP/haplotype in the BoLA-DRB3.2 gene, the functional site and haplotype of the genes related to tuberculosis susceptibility were revealed, and the results would be applied to actual production, which is of great significance to eliminate tuberculosis susceptible cattle, breed the tuberculosis resistant cattle and prevent the cattle tuberculosis, laying a scientific foundation for the complete elimination of cattle tuberculosis.

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