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LONG J. R.[1,2], TANG L. M.[1], QIU X. P.[3], ZENG F.T.[3], XIE X. H.[1], ZHANG Y. P.[2,4]

1 Sichuan Academy of Animal Sciences, Chengdu, Sichuan, 610066, China
2 Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China
3 Sichuan Agricultural University, Ya'an, Sichuan, 625014, China
4 Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, 650091, China

jrlongyou@yahoo.com

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[1] Sichuan Academy of Animal Sciences, Chengdu, Sichuan, 610066, China
[2] Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China
[3] Sichuan Agricultural University, Ya'an, Sichuan, 625014, China
[4] Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, 650091, China jrlongyou@yahoo.com

ABSTRACT

The major histocompatibility complex (*MHC*) genes, especially class II genes, are correlated with the ability of disease resistance and some economic traits in most vertebrates. In this study, we sequenced exon 2 of rabbit *MHC DQA* gene, *RLA-DQA*, in 20 animals raised in China. Sixty-one polymorphisms were observed and 18 haplotypes were defined. Aligning these haplotypes and seven sequences from GenBank, 70 nucleotide polymorphic sites were identified and 24 haplotypes were obtained. Among the 70 polymorphisms, there were 32 transitions, including 20 A/G and 12 C/T, and 32 transversions. At the remaining six polymorphic sites, at least three kinds of nucleotides were observed. On average, *RLA-DQA* exon 2 was composed of 28.1%A, 25.9%T, 23.2%G, and 22.8%C. Among the 24 haplotypes, 41 amino acid (AA) polymorphisms were observed. The rate of nonsynonymous substitution was higher than that of synonymous substitution ($P<0.05$), which means *RLA-DQA* may undergo positive selection. Overall, *RLA-DQA* has a very high degree of polymorphism not only at the nucleotide level, but also at the AA level. Further studies are needed to discover whether these polymorphisms can be used as genetic markers for association studies in the future.

Key words: rabbit, MHC, *DQA,* polymorphism.

INTRODUCTION

The major histocompatibility complex (*MHC*) genes, especially class II genes, are correlated with the ability of disease resistance and some economic traits in most vertebrates (MARGULIES 1997). For example, the *DR* gene of bovine *MHC* was correlated

with the occurrence of clinical mastitis caused by Staphylococcus species (SHARIF *et al*., 2000) and cattle production traits (SHARIF *et al*., 1999). Swine *MHC* was associated with immune responsiveness to a variety of microbes and metazoan parasites and production and reproduction performance (VAIMAN *et al*., 1998). A functional class II *MHC* molecule is formed by an α and a β chain peptide encoded by A and B genes, respectively (SENA *et al*., 2003). In both A and B genes, exon 2 is functionally important and encodes amino acids (AA) associated with the peptide-binding sites in the first domain of the class II molecule.

Rabbit major histocompatibility complex, *RLA*, is located at 12q1.1 (ROGEL-GAILLARD *et al*., 2001). The genetic composition of *RLA* closely parallels that of the human and most other mammals. *RLA-DQA* exhibited an unusually high degree of polymorphism, especially at exon 2 (MARCHE *et al*., 1989; HAN *et al*., 1994). *RLA-DQA* alleles were found to be associated with the regression of skin warts induced by the Shope cottontail rabbit papillomavirus, as well as malignant conversion of persistent warts (HAN *et al*., 1994). Therefore, this gene may be interesting not only for phylogenetic studies, but also for the association studies to search for diseases genes or quantitative trait loci (QTL) for economic traits.

Initially, *RLA* alleles were defined using mixed lymphocyte reaction and cellular transplantation experiments (TISSOT and COHEN 1972) and later by restriction fragment length polymorphisms, RFLP (MARCHE *et al*., 1989). In the present study, we directly sequenced exon 2 of the *RLA-DQA* gene to find polymorphisms and other characteristics of this gene.

MATERIAL AND METHODS

DNA samples

Whole blood samples of 20 domestic rabbits raised in China were collected. Details about the samples were described elsewhere (LONG *et al*., 2003). Total DNA was extracted by standard phenol/chloroform methods.

PCR and Sequencing

Primers specific for the second exon of *RLA-DQA* were designed. The forward primer was *DQA*F: 5'- TCATCAGCTGACCACGTTGG -3' and the reverse primer was *DQA*R: 5'-GCAGCAGTAGAGTTGGAG -3'. The expected size of the PCR product was 248 bp. PCR was performed using about 20 ng of DNA in a 25 μl reaction volume with 35 cycles (94℃ for 30 sec, 59℃ for 30 sec, and 72℃ for 30 sec). The products were purified with an

agorase gel extraction kit (Watson Biomedical Inc., Shanghai, China) and sequenced on an ABI 377 Sequencer using the Bigdye^{TM} Terminator Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA). Both strands were sequenced using the primers *DQA*F and *DQA*R. The *RLA-DQA* sequences obtained in this study have been submitted to GenBank (http://www. ncbi.nlm.nih.gov/) with accession nos. AY422757 to AY422774.

Statistics

Sequences were aligned by using the software of Editseq, Seqman and Megalign, which are implemented in the DNASTAR package (DNASTAR Inc.). All polymorphic sites of nucleotide and amino acid were identified by using the Mega 2.0 program (KUMAR *et al*., 2001). The one-tail Z-test implemented in Mega 2.0 was used to test positive selection.

RESULTS AND DISCUSSION

Nucleotide polymorphisms in *RLA-DQA* exon 2

After aligning the 20 sequences obtained in the present study, 61 nucleotide polymorphic sites were observed and 18 haplotypes were defined. Taken with the 18 haplotypes and the seven sequences from GenBank, 70 polymorphic sites were observed and 24 haplotypes were obtained (Table 1). Among the 70 polymorphisms, there were 32 transitions, including 20 A/G and 12 C/T, and 32 transversions including three G/T and three other kinds of transversions happening with similar frequencies. At the remaining six polymorphic sites, at least three kinds of nucleotides were obtained. After analyzing the 24 haplotypes, on average, *RLA-DQA* exon 2 was composed of 28.1%A, 25.9%T, 23.2%G, and 22.8%C.

In mammals, nucleotide substitution rate at the control region (D-loop) is about 5-10 times higher than the other regions in the mitochondrial DNA (mtDNA), whose substitution rate is about 6-17 times of the genomic genes (BROWN *et al*., 1979). A fragment of rabbit D-loop (~700 bp) was sequenced in 104 domestic rabbits raised in China (LONG *et al*., 2003). We only found 19 nucleotide polymorphic sites and eight haplotypes. Even when 24 haplotypes for both domestic and wild rabbits were extracted from GenBank to analyze, only 48 polymorphisms were observed. The nucleotide diversity π was only 0.003, about 1/10 of the value of π (0.031) according to the *DQA* gene. Therefore, it is easy to see that *RLA-DQA* is much higher polymorphic.

Table 1. DNA sequence variations of RLA-DQA exon 2

	11111	111111111	111111111	111112222	222222222	2222222
	211222	222333444	688900122	333334445	567778888	999990001	111122222	3344444
	479135234	679048069	167826508	012561450	170270289	236891270	135812345	8901235
AY422757	AAGTGTGCA	AACGAGCGC	AAGGCAAGC	TGYATCGAA	CGTCGACCA	?TATAAAGA	TAGTACGCT	CTGCAAC
AY422758	?????????	?????..AT	.GCA.R.AG	.TCGAAA.G	..C.....T	GGGCT.C.G	...G.....	....
AY422759	.....G...	....G....	.GCA...AG	.TCGAAAG.	....	GCG.....C	.C...GA..	.....T?
AY422760	??????...	....G....	.GCA...AG	.TCGAAAG.	....	GCG.....C	.C...GA..	....
AY422761	....	....	....	C.C.....	....	A........	....	....
AY422762	........G	....	.GCA...AG	.TCGAAAGG	....	GA.......	..C.....	......?
AY422763	C........	....R....	.RCA.R.AG	.TCGAAARG	....A...T	G?GCT.C.G	.????????	???????
AY422764	....	....	....	..C.....	....	A........	....	....
AY422765	.....G...	....G....	.GCA...AG	.TCGAAAG.	....	GCG..T..C	.C...GA..	......?
AY422766	.....?...	....	.GCA.R.AG	.TCGAAA.G	....A...T	GCGCT.C.G	...??????	???????
AY422767	.....?...	....	.GCA...AG	.TYGAAAGG	....	GC.......	..A.CG???	???????
AY422768	.TAA.....	....	..CA...?.	.?C??AARR	....	.?.......	..A...?..	???????
AY422769	??????...	....R....	.RCR.....	.?C?AA?..	....	GC....C.C	..C??????	???????
AY422770	????CA...	....	TGCA...AG	.TCGAAAGG	....	A........	..A.....	.....??
AY422771	??????...	....	.GCA...AG	.T.GAAAG?	....	GC.......	....	......?
AY422772	....	....	.GCA...AG	.T.GAAAG?	....	GC.......	..A.....	......?
AY422773	....	....	......G..	..C.....	G........	GC....C..	..C.....	....
AY422774	??????...	....R....	?GCA...AG	.TCGAAAG?	....	..?.....C	.C...??..	TGCA???
X71614	.....GCG.	....	.GCA...AG	.TCGAAAG.	....	GC.......	....	....TG.
X71613	....	....	.GCA...AG	.TTGAAAGG	....	GC.......	..A.....	....TG.
M15557	....	....	.GCA...AG	.TTGAAAGG	....	GC.......	..A.....	....TG.
X71615	.....GCG.	....G....	.GCA...AG	.TCGAAAG.	....	GCG.....C	.C...GA..	....T..
X71616	..A.....	....	....	..C.....	....	A........	....	....TG.
X71617	.....GCG.	...T...AT	.GCA.G.AG	.TCGAAA.G	....A...T	GGGCT.C.G	...G.....	....T..
AF212830	....	GGAC.AA..	.GCAT...G	.TCGAAA..	.A.GAGGT.	GC.....A.	AC.G...TA	....TGT

. denoting same as AY422757, ? denoting unsure, R denoting the heterozygote of A/G, Y denoting the heterozygote of C/T. AY422757 to AY422774 were obtained in this study; the others were extracted from GenBank.

AA polymorphisms in *RLA-DQA* exon 2,

In the 24 *RLA-DQA* haplotypes, there were 41 polymorphic sites among the total 82 amino acids. At the 13th, 29th, 33rd, 35th, 56th, 57th and 63rd AA positions, the nucleotide polymorphisms did no cause AA change. At the 5th and 81st AA positions, the DNA sequences were not complete, and it was unknown whether the polymorphisms at these positions resulted in AA change.

Generally, in the coding region of a protein, nucleotide substitution at the third codon is

usually synonymous and substitutions at the first and the second codon normally result in AA change. Since the AA changes will affect the normal function of the protein, they are easily purified by purification selection. However, sometimes the AA changes may benefit the function of the protein and the polymorphisms will be fixed in the population. From this study, nucleotide substitution in the *RLA-DQA* gene happened more often at the first and the second codon than the third codon. The rate of nonsynonymous substitution was significantly higher than that of synonymous substitution ($P<0.05$). Therefore, *RLA-DQA* may be under positive selection as the *HLA-DQA* gene (OLERUP *et al*., 1991) and other primate *DQA* genes (BERGSTROM and GYLLENSTEN 1995).

CONCLUSION

We sequenced exon 2 of the *RLA-DQA* gene in 20 domestic rabbits raised in China. The *RLA-DQA* gene had a very high polymorphism both in the DNA and AA sequences. Further studies are needed to determine whether these polymorphisms can be used as genetic markers for association studies in the future.

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