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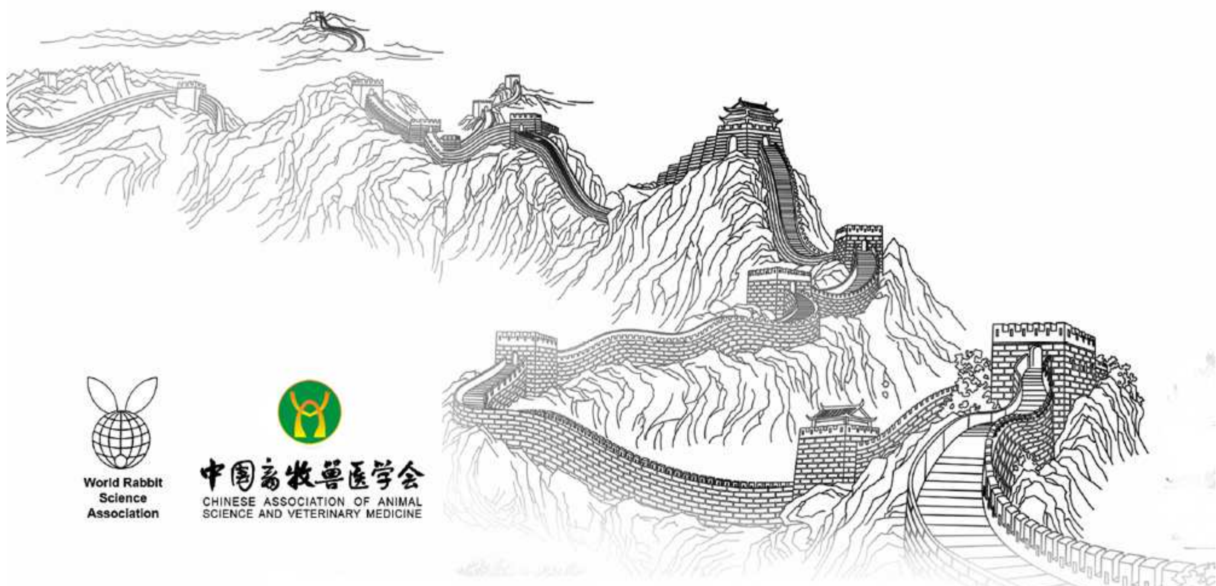

SINGLE NUCLEOTIDE POLYMORPHISMS IN THE RABBIT TOLL-LIKE RECEPTOR 1 (TLR1), TLR4 AND TLR5 GENES

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ABSTRACT

Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns, which are derived from pathogens and participate in activation of the immune responses of the host. Therefore, we think that TLRs are significant candidate genes for rabbit diseases resistance research. In this study, we detected genetic polymorphisms of TLRs in 12 meat rabbits from 4 breeds. The sequence analysis indicated that 18 SNPs were identified, 10 of which were non-synonymous SNPs. Four nonsynonymous SNPs (G563A, G628T, A653C and A654C) were located in the LRR domains of the TLR4 predicted protein. It is possible that these replacements, which change the amino acid characteristics, will change their extracellular pathogen recognition capabilities. The identification of these polymorphisms reported here increases the resource of genetic markers useful for mapping and association analyses with disease resistance. Future researches are needed to survey potential associations.

Key words: Toll-like receptors, Single nucleotide polymorphism, Innate immunity, Rabbit.

INTRODUCTION

Toll-like receptors (TLR) play important roles in recognizing "pathogen-associated molecular patterns (PAMPs)" of pathogens (such as viruses, bacteria and fungi). TLR genes variance might influence the function of combining receptor with ligand and signal transduction capacity after combining with ligand, having an important influence on the pathogen recognition and disease resistance of the organism. There have been manyreports on research of correlation between polymorphisms of TLR genes and disease, for example TLR4 might play an important role in the response of host to the intramammary infection in cattle (Werling et al., 2004). Researches on human diseases show that there is a significant correlation between TLR4 polymorphism and disease of digestive and respiratory systems (Lien et al., 2002). The mutation Arg392stop in the human TLR5 gene is associated with a decreased risk of Crohn's disease (Gewirtz et al., 2006). At present, few studies investigate the genetic polymorphisms in the rabbit TLR genes. In the present study, we sequenced 12 rabbits from 4 breeds to discover genetic polymorphisms in the TLR genes.

MATERIALS AND METHODS

DNA Samples And Extraction.

Some DNA extracted from muscle of a New Zealand White buck was used for primer optimization, and the identification of amplicon sequence was done via BLAST. Additional rabbit DNA samples of 12 unrelated rabbits from 4 breeds (New Zealand White, Californian, Felmish Giant and Fu Jian Yellow) were extracted from whole-venous blood and readily stored at -20°C. Three were Californian, 3 were Fu Jian Yellow, 3 were New Zealand White, 3 were Flemish Giant. Genomic DNA was extracted using a universal genomic DNA extraction kit (TaKaRa, Dalian, China) according to the manufacturer’s instructions.

PCR And Sequencing.

The coding sequences (CDs) of the TLR genes (NM_001082732, HQ874605 and XM_002709270) were used to design the PCR primers. Fourteen PCR primer pairs (Table 1) that produced overlapping fragments within CDS of the TLR genes were designed using the Web interface for Primer3 (http://frodo.wi.mit.edu/primer3). All PCR amplicons were visualized via agarose electrophoresis and

subsequently purified using the QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's recommendations. The PCR amplicons purified were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA).

Table 1: PCR primers for amplification

Gene	Fragment nos.	Forward primer (5′-3′)	Reverse primer (5′-3′)	Fragment size (bp)
TLR1	1	CTCACCCTTCAACCCCATCC	GGCAGAGCATCAAACGCATT	660
	2	CTGCGCTTCAGACAGCAGAT	GGCCAAACCAACTGGAGGAT	637
	3	CTGCGTGCTACATGACAACG	AAGCACCTTGACCTTGGGAG	685
	4	ACAACAGTTGGATGCTAGCCA	CTCAGATACCAGGGCACGTC	633
	5	ACCCAGAAGGCTACAAGGGA	CTCCTTCGGCCATTCCAAGT	611
	6	TCCATCTTTGTCTTGTCTCCCA	AGAAACAGGCGAATGCTCCA	502
TLR4	1	TGTGGCCCATAATCTTATCCA	CACCTTTGTTGGCAGTGAAA	626
	2	CAACCAAGAAATTTTTCCTAAAGA	GACTCCAGTCTGGGGAGTGA	612
	3	ATGCTTTCCAGGACAACAGG	TTCTCACCCAGTCCTCATCC	621
	4	TGTGCATCACACCTCCAAAT	GGAAGCCCCTCAGGAGTATT	722
TLR5	1	TCCCTTCACCCCTCCTTTGA	AGGTGAGCTCAGGTAGTCCA	610
	2	TGGACTACCTGAGCTCACCT	AAGACGCTCAGTGAGGCAAA	651
	3	TGTGTCCAAGAACCAGCTCC	CTGCTCACCACACAGACGAT	618
	4	GGACAGGCAGTACAGTGACC	GCGCATTCTGCAGTCGATAC	717

Detection Of Polymorphisms.

Sequence quality was evaluated by Sequencher4.7 (Gene Codes, Ann Arbor, MI, USA). Then, the low quality data at the ends of the sequence were trimmed by Sequencher4.7. The SNPs were automatically detected using Sequencer4.7 and each of them was verified by manual identification of the chromatograms. All heterozygous nucleotides were annotated with the appropriate IUPAC-IUB code.

RESULTS AND DISCUSSION

We detected 5 SNPs in TLR1, of these 4 were nonsynonymous SNPs and 1 was a synonymous SNPs. All were transitions (A ↔ G; C ↔ T). The genomic positions of all SNPs, SNP genotypes and amino acid positions are also provided in Table 2. Polar changes of substitutions in amino acids were detected at bases 136 and 163 in TLR1. It is possible that these replacements in amino acids in TLR1, which change the amino acid characteristics, will change their extracellular pathogen recognition capabilities. We detected 6 SNPs in TLR4, of these, 5 were nonsynonymous SNPs, 1 was a synonymous SNPs. Of the 6 SNPs, 3 were transitions (A ↔ G; C ↔ T) and 3 were transversions. One nonsynonymous SNP that changed the charges on the amino acids was identified at base 757 in TLR4. Moreover, polar changes of substitutions in amino acids were detected at bases 628, 653 and 654 in TLR4. We detected 7 SNPs in TLR5, of these 1 was a nonsynonymous SNP and 6 were synonymous SNPs. All of the 7 SNPs were transitions (A ↔ G; C ↔ T). The rate of occurrence (bp/polymorphism) of nonsynonymous SNPs and synonymous SNPs in the coding sequence were 2877 and 480, respectively.

TLRs play critical roles in identification of invading PAMPs in host, with recent study providing further evidence for this conclusion in domestic animals (Bochud et al., 2007). We detected 18 SNPs in rabbit TLR1, TLR4 and TLR5, we anticipated that these SNPs attribute resistance or sensitivity to infection with particular pathogens. Polymorphisms in the sequences encoding the regions might have allowed TLRs to adapt to various kind of PAMPs derived from the rapid mutations of microbes. It is generally believed that Asp299Gly and Thr399Ile of TLR4 associate with the inflammatory reaction of human (Barber *et al.*, 2004). The TLR4 allele polymorphism Asp299Gly is related with the susceptibility of Gram-negative bacteria and sepsis, the carriers of this mutation are more easily infected by Gram-negative bacteria, suffer from sepsis and the death rate is extremely high (Lorenz et al., 2002). TLR1 and TLR2 can form a kind of heterologous dimers and identify mycobacteria triglyceride lipoprotein and enhance the recognition for the specificity of PAMPs. Therefore, TLR1 is considered to be a candidate gene for susceptibility to breast

inflammation. TLR5 can form homologous dimers or form heterologous dimers with TLR4 and it can recognize the flagellum, activate certain signal transduction pathways, induce synthesis of cytokines, activate the body's natural immune response. Studies on human disease have shown that C1174T (rs5744168) single nucleotide polymorphism of TLR5 gene is associated with the susceptibility of lupus erythematosus (Graham et al., 2001; Hawn et al., 2005).

Table 2: Distribution of single nucleotide polymorphisms (SNP) among 12 individuals from 4 rabbit breeds.

Gene	Alleles[a]	Genomic Position[b]	Amino acid Position[c]	Amino Acid[d]	Character	SNP genotype[e]	Domain[f]
TLR1	**G**/A	65	22	R/K	-	R	None
	G/A	136	46	D/N	Anionic /Polar	R	None
	G/A	163	55	D/N	Anionic /Polar	R	None
	G/A	195	65	Q/Q	-	R	None
	G/A	965	322	S/N	-	R	low complexity
TLR4	**G**/A	563	188	S/N	-	R	LRR
	G/T	628	210	A/S	Nonpolar/Polar	K	LRR
	C/A	653	218	P/Q	Nonpolar/Polar	M	LRR
	C/A	654	218	P/Q	Nonpolar/Polar	M	LRR
	G/A	757	253	E/K	Anionic/Cationic	R	None
	G/A	1095	365	R/R	-	R	None
TLR5	**G**/A	573	191	R/R	-	R	LRR
	G/A	894	298	G/G	-	R	None
	G/A	978	326	K/K	-	R	LRR
	C/T	1842	614	D/D	-	Y	LRRCT
	A/G	1953	651	T/T	-	R	transmembrane region
	C/T	2320	774	H/Y	Cationic /Nonpolar	Y	TIR
	G/A	2358	786	A/A	-	R	TIR

[a] The reference sequence allele depicted in bold, underlined text. [b] Genomic position based on the first base of the start codon. [c] Amino acid position based on the start codon .[d] Amino acid(s) encoded by the alleles, with predicted amino acid replacements. [e] Heterozygous SNP genotypes are depicted using the IUPAC codes for heterozygosity. [f]TLR protein domain architectures were predicted by the SMART.

CONCLUSIONS

Our work aimed to identify polymorphisms of rabbit TLR genes so as to provide candidate polymorphisms for association analyses and signal transduction research. Finally, 18 putative SNPs were detected. The polymorphisms reported here increases the resource of the genetic markers useful for mapping and association analyses with disease resistance.

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