The correlation analysis of polymorphisms of Prion-Related Doppel (PRND) with prion (PRNP) alleles in Gansu Alpine Merino sheep

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ABSTRACT. The interaction between the ovine prion protein gene (PRNP) and Doppel (PRND) gene polymorphisms is essential for understanding the role of prion proteins in scrapie. In this study, the blood genomic DNA samples of 111 Gansu Alpine Merino sheep were used to define the PRNP alleles and the haplotypes of PRND by PCR-SSCP (single-strand conformation polymorphism) and PCR. The results shown that the frequency of PRNP genotype ARQ/ARQ and ARR/ARR has a certain advantage, especially the ARQ/ARQ (46%). In addition, PRND 26 haplotypes and 29 polymorphisms sites were firstly defined, and the dominant polymorphic sites are at codons 28, 51 and 158. The frequency of the haplotype GTC of PRND is 98%, the ARQ/ARQ has great advantage

in the PRNP genotype. These results may be helpful in future studies of the relationship between PRND with PRNP in prion diseases.

KEY WORDS: Polymorphism; PRND; PRNP alleles; Correlation

INTRODUCTION

Prion (PrP) and Doppel belong to the prion protein family. The conversion of the normal cellular prion protein (PrPC) to abnormally folded isoform (PrPSc) is the main molecular mechanism of prion diseases including the scrapie in sheep and the transmissible spongiform encephalopathies in cow (Dandoydron and Benboudjema et al., 2000). PrPC plays important roles in many physiological functions, such as apoptosis, nucleic acid metabolism, lymphocyte signal transduction and anti-oxidant effect (Río and Gavín, 2016). In addition, PrP has a context-dependent neuro-protective effect (Steele and Zhou et al., 2009, Weise and Sandau et al., 2006). Prion-like protein Doppel gene (PRND) is 16 ~52 kb and locates in the downstream of prion protein gene (PRNP) (Mesquita and Batista et al., 2010). The main function of PRND is to regulate the male sterility (Ferreira and Garcia-Herreros et al., 2016). PRND also plays regulatory roles in central nervous system and blood vessel formation, especially in blood brain barrier (Peoc'H and Guérin et al., 2000).

Although, Doppel and PrP have a similar biochemical and structural characteristics, Doppel has opposite effects of PrP in the body (Qin and Zhao et al., 2006；Sakaguchi, 2008；Li and Dong et al., 2009；Sakudo and Onodera, 2011). The protein structures of PrPC and Doppel are highly conserved in animals (Wopfner and Weidenhöfer et al., 1999). The PRNP are highly expressed in central nervous system and peripheral organs of sheep (Han and Liu et al., 2006；Wang and Wu et al., 2011). However，PRND is highly expressed in testis (Wang and Wu et al., 2011；Mo and Moore et al., 2001). Meanwhile, the expression of PRND and PRNP are influenced by genotype of PRNP in sheep (Wang and Wu et al., 2011).

The susceptibility of scrapie was affected by the polymorphism of PRNP alleles, 136, 154 and 171 (González and Martin et al., 2002). The scrapie-susceptibility alleles of ARQ in sheep population accounted for a large proportion in China (Zhao and Wu et al., 2012). Although the polymorphism of PRND codon 174 loci associated with human sporadic Creutzfeld-Jakob (CJD) (Croes and Alizadeh et al., 2004), PRND genotype and the correlation analysis of polymorphisms of PRND with PRNP is still unclear. In the present study, the PRNP allele genotypes and the haplotypes of PRND were analyzed by PCR-SSCP and PCR. We evaluated the correlation polymorphisms of PRND with PRNP alleles.

MATERIAL AND METHODS

Animals and samples

The blood samples of Gansu Alpine Merino sheep（n=111）were collected from the Emperor Sheep Farm located in Suna county, Gansu province of China and treated with acid citrate dextrose. The genomic DNA was extracted following the instruction of Blood Genome DNA Extraction Kits (cat No.MD1001, ABigen, China).

Determination of PRNP gene with PCR-SSCP

The PRNP alleles were analyzed by PCR-SSCP, which were performed according to the literature (Zhao and Wu et al., 2012).

Polymorphism of PRND gene with PCR

The PCR primers of PRND gene were designed based on the sequence from NCBI (number：DQ408533.) 5′-TCTGTTGCAGATTCCGACAC-3 (forward), 5′-GGGTTTGGGGTTAGAGGAGA-3′ (reverse). The PCR reaction mixture (50 μL volume) contained 1.0 μL (50 pmol/L) of genomic DNA, 2.0 μL (10 pmol/L) of each primer set, 25.0 μL of 2xEasyTaq Super mix (cat No AS111-01, TransGen Biotech, China) and 20 μL of ultrapure Millipore water and was carried out by Bio-Rad PCR (BIO-RAD, Hercules, CA, USA).

The PCR amplification conditions were; initial denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 30s, 55 °C for 45s and 72 °C for 1 min. Final extension step was 72 °C for 10 min. The products of PCR reactions were electrophoresed on an agarose gel (1.5%), Purified PCR products were cloned into pMD-18T (cat No.6011, TaKaRa, Japan) following standard protocols. The clones were sequenced by Suzhou GENEWIZ，Inc biological Co., Ltd.

Amino acid alignment analysis was conducted by the codon usage table using the DNAMAN program (Version 5.2.2, production) and DNASP (Version 5.10.01, production) based on the obtained DNA sequences.

RESULTS

The Polymorphism of PRNP codons 136, 154 and 171

The major genotypes and the frequencies of PRNP from all samples were: ARH/ARH, 1.8% (2/111); ARH/ARQ, 1.8% (2/111); ARQ/ARH, 2.7% (3/111); ARR/ARQ, 27% (30/111); ARR/ARR, 20.7% (23/111); ARQ/ARQ, 46% (51/111). Sheep with ARQ/ARQ, ARR/ARQ, ARQ/ARH, ARH/ARQ, ARH/ARH, genotypes accounted for 79%, ARQ/ARQ genotype with absolute advantage accounted for 46% (Table 1).

Table1 Genotype percentage of PRNP allele at codons 136, 154 and 171 in sheep

Number	2	2	3	30	23	51	111
Percentage (%)	1.8	1.8	2.7	27	20.7	46	100

The haplotype of PRND

Total of 29 polymorphic sites were defined based on the 111 PRND PCR sequences. According to these polymorphic sites, 26 PRND haplotypes were defined, the most important haplotypes are GTC, DTC, DAR, the relevant site of these three haplotypes are 28, 51,158 respectively (FIGURE 1). In addition, the frequency of polymorphic site 18 (GTCI18) is higher (5.4%, 6/111) than the frequency of other polymorphic sites (Table 3). The GTC frequency is 95.5%, followed by DTC, DAR (Table 2).

Table 2 haplotypes percentage of PRND at codons 28, 51 and 158 in sheep

Genotype	GTC	DTC	DAR	Total
Number	106	3	2	111
Percentage (%)	95.5	2.7	1.8	100

Table 3 Other site percentage of PRND in sheep (%)

Haplotype	N	%	Haplotype	N	%	Haplotype	N	%
$DTCK_{75}$	1	0.9	$GTCI_{13}$	1	0.9	$GTCD_{90}$	1	0.9
$DTCD_{11}$	1	0.9	$GTCI_{18}$	6	5.4	$GTCL_{30}$	1	0.9
$DTCC_{119}$	1	0.9	$GTCL_{138}$	1	0.9	$GTCL_{136}$	1	0.9
$DTCI_{166}$	1	0.9	$GTCI_{24}$	1	0.9	$GTCR_{25}$	1	0.9
$DTCI_{19}$	1	0.9	$GTCI_{92}$	1	0.9	$GTCS_{16}$	1	0.9
$DARA_{24}$	1	0.9	$GTCR_{12}$	1	0.9	$GTCG_{72}$	1	0.9
$DARI_{99}$	1	0.9	$GTCE_{26}$	1	0.9	$GTCS_{14}$	1	0.9
$GTCI_{19}$	1	0.9	$GTCD_{48}$	1	0.9	$GTCP_{11}$	1	0.9
$GTCY_{69}$	1	0.9	$GTCK_{113}$	1	0.9			

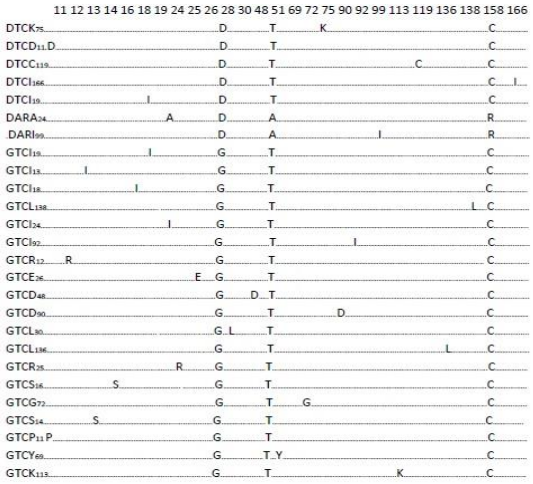

Figure 1 Define the haplotype at codons 28, 51 and 158 in sheep of PRND

The correlation analysis of polymorphisms of PRND and PRNP alleles

The results shown that the distribution of PRND haplotype were under the control of the PRNP genotype. GTC of PRND haplotype were occupied most of the majority of the PRNP genotype but did not appeared in the PRNP genotype ARH/ARH. DTC of PRND haplotype only appeared in the PRNP genotype ARR/ARR (8.7%, 2/23) and ARQ/ARQ (2%, 1/51), meanwhile DAR only appeared in the PRNP genotype ARH/ARH (100%, 2/2). In addition to, the frequencies of GTCI92 (33.3%, 1/3), GTCG72 (33.3%, 1/3) were higher than the frequencies of other polymorphic loci in genotype ARQ/ARH of PRNP (TABLE 4).

DISCUSSION

It has been known that sheep scrapie was associated with PRNP polymorphisms at codons 136, 154 and 171 (Hunter and Moore et al., 1997; Andréoletti and Morel et al., 2006; Goldmann and Houston et al., 2006). Genotypes of ARR and AHQ were associated with the scrapie resistance, ARQ, ARH and VRQ were associated with scrapie susceptibility (Mesquita and Batista et al., 2010).

As a homologue of PrPC, the Doppel protein is like PrPC and is also a membrane glycoprotein with a GPI anchor and is formed by the precursor protein undergoing a series of modifications (RC, M. and L. IY, et al., 1999). Although structurally similar, but Doppel is not as widely distributed as PrPC and functions more differently. The N-terminal domain of PrPC antagonizes Doppel neurotoxicity thus showing antagonism. (Qin and Zhao et al., 2006). In addition, the expression of PRND genes and protein levels has been identified in a variety of cancers, it is suggesting that PRND may be a tumor marker or a potential new target for therapy (Comincini, S. and A. Facoetti, et al., 2004). PRNP genotypic polymorphism studies have demonstrated a significant correlation between the 136, 154 and 171 codons and susceptibility / resistance to scrapie in sheep.

However, the sheep PRND genotype polymorphism has not been reported, the relationship between PRNP polymorphism and PRND polymorphism is still unclear. In this study, PCR and PCR-SSCP were applied to determine the ovine PRNP allele and PRND haplotype genotypes. Scrapie-susceptibility genotypes ARQ/ARQ, ARQ/ARH, ARH/ARQ and scrapie-resistant genotypes ARR/ARR, ARQ/ARQ genotype of PRNP account the major genotypes of PRNP, these results are in line with our previous studies (Zhao and Wu et al., 2012). Polymorphic site 129 of PRNP and polymorphic site 174 of PRND are highly associated with the sporadic CJD disease and Alzheimer's disease in humans (Pan and Baldwin et al., 1993; Jeong and Kim et al., 2014; Mesquita and Garcia et al., 2016). Although Mesquita et al suggested that there is an association between the PRND polymorphism and scrapie susceptibility5, the relationship between PRND genotype and the polymorphisms of PRND with PRNP in sheep is unclear.

We are firstly defined PRND 26 haplotypes and 29 polymorphisms sites, which the dominant polymorphic sites are codons 28, 51 and 158. PRND haplotype majority is GTC. We also found that there is no significant difference between the PRND haplotype GTC and DTC with scrapie-susceptibility genotype ARQ/ARQ as well as scrapie-resistant genotypes of ARR/ARR. We speculate that the haplotype GTC of PRND plays an important role in balancing scrapie susceptibility and resistance. Taken together, our study provided the relationship between PRNP allels with PRND polymorphism for further studies of prion diseases.

Table 4 haplotypes of PRND associated with PRNP Genotype

Genotype / Haplotype	ARH/ARH N=2		ARH/ARQ N=2		ARQ/ARH N=3		ARR/ARQ N=30		ARR/ARR N=23		ARQ/ARQ N=51	
	N	%	N	%	N	%	N	%	N	%	N	%
GTC	0	0	2	100	3	100	30	100	21	91.3	50	98
$GTCY_{69}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCI_{19}$	0	0	0	0	0	0	4	13.3	1	4.3	1	2
$GTCI_{13}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCI_{18}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCL_{138}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCI_{24}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCI_{92}$	0	0	0	0	1	33.3	0	0	0	0	0	0
$GTCR_{12}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCE_{26}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCD_{48}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCK_{113}$	0	0	0	0	1	33.3	0	0	0	0	0	0
$GTCD_{90}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCL_{30}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCL_{136}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCR_{25}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCS_{16}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$GTCG_{72}$	0	0	0	0	1	33.3	0	0	0	0	0	0
$GTCS_{14}$	0	0	0	0	0	0	0	0	0	0	1	2
$GTCP_{11}$	0	0	0	0	0	0	0	0	1	4.3	0	0
DTC	0	0	0	0	0	0	0	0	2	8.7	1	2
$DTCK_{75}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$DTCD_{11}$	0	0	0	0	0	0	0	0	0	0	1	2
$DTCC_{119}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$DTCI_{166}$	0	0	0	0	0	0	1	3.3	0	0	0	0
DAR	2	100	0	0	0	0	0	0	0	0	0	0
$DARA_{24}$	0	0	0	0	0	0	1	3.3	0	0	0	0
$DARI_{99}$	0	0	0	0	0	0	0	0	0	0	1	2

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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