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Genetic variability of the coding region for the prion protein gene (*PRNP*) in gayal (*Bos frontalis*)

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Abstract The gayal (*Bos frontalis*) is a rare semi-wild bovid species in which bovine spongiform encephalopathy (BSE) has not been reported. Polymorphisms of the prion protein gene (*PRNP*) have been correlated significantly with resistance to BSE. In this study, the coding region of *PRNP* was cloned and characterized in samples from 125 gayal. A total of ten single nucleotide polymorphisms (SNPs), including six silent mutations (C60T, G75A, A108T, G126A, C357T and C678T) and four mis-sense mutations (C8A, G145A, G461A and C756G), corresponding to amino acids T3K, G49S9, N154S and I252M were identified, revealing high genetic diversity. Three novel SNPs including C60T, G145A and C756G, which have not been reported previously in bovid species, were retrieved. There also was one insertion–deletion (187Del24) at the N-terminal octapeptide repeat region. Alignment of nucleotide and amino acid sequences showed a high degree of similarity with other bovid species. Using phylogenetic analyses it was revealed that gayal has a close genetic relationship with Zebu cattle. In short, preliminary information is provided about genotypes of the *PRNP* in gayal. This could assist with the study of the pathogenesis of transmissible spongiform encephalopathies and cross species transmission as well as a molecular breeding project for gayal in China.

Keywords Gayal (*Bos frontalis*) · Prion protein gene (*PRNP*) · Polymorphisms

Introduction

Bovine spongiform encephalopathy (BSE) is one of the prion diseases, which also include scrapie in sheep, goats and mice, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in moose, deer and elk, feline spongiform encephalopathy (FSE) in cats, puma and cheetah, and also Creutzfeldt–Jacob disease (CJD), Gerstmann–Straussler–Sheinker (GSS) syndrome, fatal familial insomnia (FFI) and historical kuru in humans [1, 2]. A characteristic of BSE is the accumulation of a modified conformational neurotoxic isoform ($PRNP^{Sc}$) switching from the normal prion protein ($PRNP^{C}$) in the bovine brain and spinal cord, forming deposits of β-amyloid resistant to proteolytic enzymes and resulting in the formation of specific histopathological lesions [3–5]. Normal prion protein is encoded by the prion protein gene (*PRNP*) which is a single gene with considerable genomic differences in human and domestic animal populations [6].

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The gayal or mithun (*Bos frontalis*) is a rare semi-wild bovid species distributed throughout Bangladesh, Bhutan, China, India, Malaysia and Myanmar [7, 8]. In China, gayal is found predominantly in the narrow valleys of the Dulong and Nujiang Rivers and adjacent mountainous areas of Yunnan Province where they are described as 'Dulong Cattle' [9, 10]. Gayal has a chromosome complement of $2n = 58$, which differs from those of cattle (*Bos taurus*, $2n = 60$) and gaur (*Bos gaurus*, $2n = 56$) [9, 11, 12]. Gayal browse tree leaves and graze grasses, bamboo leaves, reeds and other local plant species and reveals a very wide range of adaptive activities under the harsh conditions which range from cold to tropical belts [13, 14]. Due to the remoteness of their habitats and other ecological and sociopolitical factors, gayal remain one of the least studied ungulates [15] and has been catalogued in the red list of threatened species of the International Union for Conservation of Nature and Natural Resources (IUCN)—see www.redlist.org.

To date, the coding region of *PRNP* has been cloned and characterized extensively in European (*B. taurus*) [16–19] and Zebu (*Bos indicus*) cattle and their hybrids (*B. indicus* × *B. taurus*) [20], yak (*Bos grunniens*) [17], buffalo (*Bubalus bubalis*) [17], bison (*Bison bison*) and some wild ruminants [21–23]. However, for the "unique" gayal, the polymorphisms of *PRNP* have been investigated seldom. Although no natural case of BSE has been reported in gayal, it is considered to be very important to know more about the sequences of polymorphisms of *PRNP* in gayal. This could assist with the study of the pathogenesis of transmissible spongiform encephalopathies, cross species transmission and also a molecular breeding project for gayal in China. Therefore, in this report, we provide a detailed survey the *PRNP* coding region polymorphisms of gayal and have compared those with the sequences of *PRNP* from a range of bovid species.

Materials and methods

Sample collection

From 2005 to 2009, 125 blood or tissues samples were collected from apparently healthy gayal (*B. frontalis*) at the National Jiumudang Stud Gayal Farm (located at N25°47′02.6″, E099°05′56.5″ at an altitude of 2260 m above sea level), Dulong Town, Gongshan County, Yunnan Province, China. The appearance and mitochondrial DNA analyses of the animals were criteria used to exclude individuals showing evidence of interspecific hybridization with local Yunnan cattle (*B. taurus*) [24 and unpublished data]. All samples were snap frozen in liquid nitrogen then stored at −80°C pending analyses.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from blood or tissues using standard proteinase K digestion followed by extraction with the phenol–chloroform method [25].

The *PRNP* sequence was amplified using previously described primers *PRNP*-CDS-F (5′-CTAGGGTCCCCAC AAGAACAAG-3′) and *PRNP*-CDS-R (5′-ACGGGGCT GCAGGTAGATA-3′) [26]. The PCR was carried out in a reaction volume of 25 μl, containing 2.0 μl DNA (approximately 50 ng μl^{-1}), 2.0 μl 250 μmol/l dNTPs, 2.5 μl buffer ($MgCl_2$ in the buffer provided by the manufacture already), 2.0 μl 10 μmol/l forward primer, 2.0 μl 10 μmol/l reverse primer and 0.2 μl 10× Taq DNA polymerase (5 U/μl, Beijing TransGen Biotechnology Co., Ltd, China). Thermal cycling parameters were as follow: 5 min at 95°C, 35 cycles of amplification (45 s at 94°C, 45 s at 54°C, 60 s at 72°C), and finally 7 min at 72°C. The purified 125 PCR products were sequenced bidirectionally using an ABI3730 DNA Analyzer (Applied Biosystems Inc.) at the Sango Biotechnology Company (Shanghai, China).

Bioinformatic analyses

Genotype and allele frequencies were checked by counting directly, then the Hardy–Weinberg (H–W) equilibrium was assessed by application of the χ^2 test. The haplotype frequencies were analyzed from the genotypic data by using the program package Haploview 4.0 [27]. Population genetic diversity indices, in terms of gene heterozygosity (He), gene homozygosity (Ho), effective allele numbers (Ne) and polymorphism information content (PIC) also were calculated using the previously described method by Nei and Li [28]. Nucleotide sequence alignment and construction of a phylogenetic tree were achieved by using the DNAStar program (Version 5.2.2) and MEGA software (Version 4.0). Protein motif prediction was made by using the PROSITE motif search tool http://www.expasy.org/prosite and http://www.cbs.dtu.dk/services/NetNGlyc/.

Results

Identification and distribution of the *PRNP* polymorphisms in the open reading frame (ORF)

The PCR amplicons covering the coding region of *PRNP* exon 3 were amplified successfully from 125 genomic DNAs and sequenced in both strands. The lengths of PCR amplicons were 1281 bp or 1257 bp. The sequences have been deposited in the GenBank database under accession numbers HQ262495 and HQ262496.

Ten single nucleotide polymorphisms (SNPs; C8A, C60T, G75A, A108T, G126A, G145A, C357T, G461A, C678T and C756G, numbered relative to the ORF) and one Indel polymorphism (187Del24) were found. Six SNPs (C60T, G75A, A108T, G126A, C357T and C678T) were silent mutations corresponding to encoding amino acids at D20, K25, T36, P42, N119 and I226, respectively. The remaining four SNPs (C8A, G145A, G461A and C756G) were mis-sense mutations and resulted in a T–K conversion at codon 3, a G–S at codon 49, an S–N at condon 154 and a T–M at codon 252, respectively. The deletion 187Del24 was identified as a deletion of the second octapeptide repeat (R2) at nucleotides 187–210 (Fig. 1).

Genotype, allele and haplotype frequencies of the *PRNP*

The genotype and gene frequencies of 11 polymorphic sites are summarized in Fig. 2. All sites, except G461A, were consistent with the Hardy–Weinberg equilibrium. Haplotypes covering the 11 loci were identified (Fig. 3). The majority (71.2%, $n = 89$) of the gayal had the I/I genotype (6:6) in the *PRNP* N-terminal octapeptide repeat region, 26.4% ($n = 33$) carried the I/D genotype (6:5) and 2.4% ($n = 3$) of the animals expressed the D/D genotype (5:5).

Gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (Ne) and polymorphism

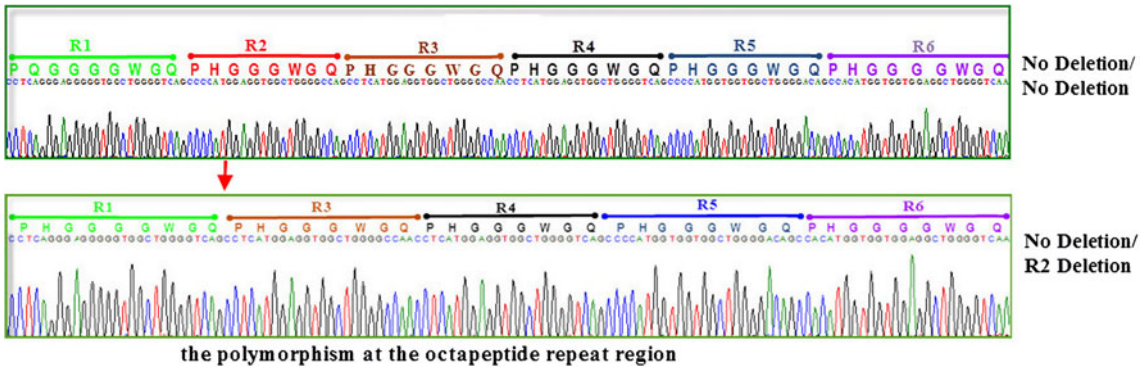

Fig. 1 Polymorphism of the octarepeat region of gayal *PRNP*

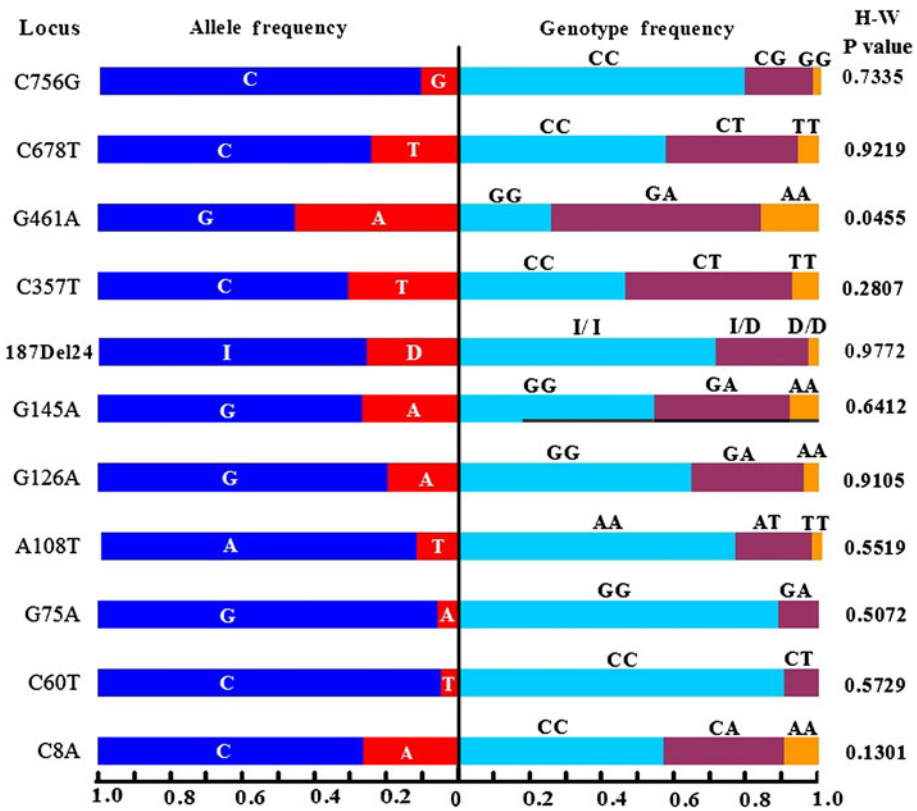

Fig. 2 The genotype and gene frequencies of 11 polymorphic sites of the *PRNP* in gayal. The numbers at the right are the probability values for the test of the Hardy–Weinberg (H–W) equilibrium

Haplotypes	C8A	C60T	G75A	A108T	G126A	G155A	187Del24	C357T	G461A	C678T	C756G	Number	Percentage
ht1	C	C	G	A	G	G	I	C	A	C	C	40	0.336
ht2	*	*	*	*	*	A	*	T	G	*	*	21	0.173
ht3	*	*	*	*	*	A	D	T	G	*	*	7	0.059
ht4	A	*	*	*	A	*	*	*	G	T	*	6	0.045
ht5	*	*	*	*	*	*	*	T	G	*	*	5	0.036
ht6	A	*	*	T	A	*	*	*	G	T	*	4	0.037
ht7	*	*	*	*	*	*	*	*	G	*	*	3	0.026
ht8	A	*	A	*	*	*	*	*	*	*	*	3	0.025
ht9	A	*	*	*	A	*	D	*	G	T	G	3	0.022
ht10	*	*	*	*	*	*	D	*	*	*	*	2	0.013
ht11	*	T	*	*	*	*	*	*	*	*	*	2	0.012
											Total	96	0.784

Fig. 3 Haplotype frequencies covering 11 polymorphic sites of the *PRNP* in gayal. *Same symbol as in the first line of each column

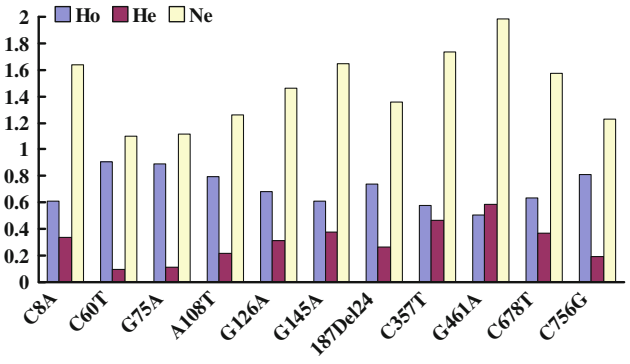

Fig. 4 Genetic diversity parameters at 11 polymorphic sites of the *PRNP* in gayal. *Ho* gene homozygosity, *He* gene heterozygosity, *Ne* effective allele numbers

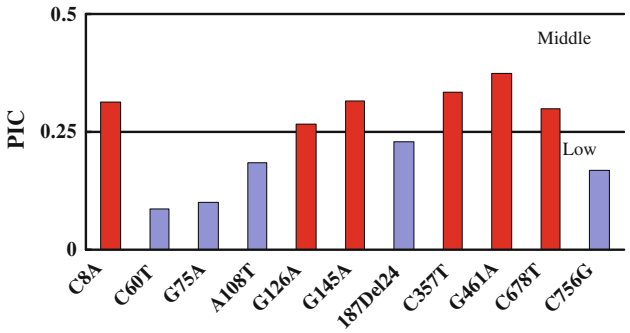

Fig. 5 Polymorphism information content (PIC) of 11 polymorphic sites of the *PRNP* in gayal

information content (PIC) of all loci are shown in Figs. 4 and 5. According to the classification of PIC (low polymorphism for a PIC value <0.25, median polymorphism for a PIC value >0.25 and <0.5, and high polymorphism for a PIC value >0.5) [29], six of eleven loci from the *PRNP* had a median polymorphic level.

Sequence characteristics of the *PRNR* and proteins

Compared with bovine *PRNP* (GenBank accession number AJ298878), the entire coding region of the *PRNP* from gayal was obtained, which had a 795 bp or 771 bp ORF encoding a protein of 264 or 256 amino acid residues with a predicted molecular mass of 28614 Da (pI 9.40) or 27837 Da (pI 9.40).

The nucleotide sequence alignments of 20 mammalian *PRNP* analyses are shown in Fig. 6. The *PRNP* sequence for gayal shared high identity with the prion protein gene sequences of Zebu (AY720681, 99.4%) and European cattle (EF139165, 99.2%), gaur (AY720697, 99.4%), bison (AY769958, 99.2%), Banteng cattle (AY720693, 98.9%), yak (AY367635, 99.4%), buffalo (AY768533, 98.6%), cape buffalo (AY720686, 97.6%), red deer (AY748455, 97.1%), sika deer (EF057409, 97.4%), fallow deer (EF165089, 97.0%), sheep (DQ149388, 97.9%), goats (EF140716, 97.7%) and cats (AF003087, 97.4%). There also was 80–90% homology with the *PRNP* sequences for mink (S46825), dogs (EF139170), arctic foxes (EU365392), humans (AF076976) and mice (M18070). Moreover, amino acid alignments for the 20 species revealed higher homology than the nucleotide level (data not shown).

Based on bioinformatic analyses, the signal peptide (SP) was identified from amino acids 1–24 at the N-terminus. An octapeptide repeat region was identified between amino acids 54–103 comprising two nonapeptides (PQ/HGGGGWGQ) surrounding a tandem repeat of three or four copies of an octapeptide (PHGGGWGQ). Three α-helices corresponded to residues 157–163, 184–204, and 211–237, respectively. Two β-sheets were located at residues 136–139 and 178–181. Two N-linked glycosylation sites were located at amino acids 192–194 and 208–210 which represented NIT and NFT, respectively. The putative topology of the gayal PRNP harbored one hydrophobic domain from amino acids 122–145 near the C-terminus. A glycosylphosphatidylinositol (GPI) anchor site between amino acids 241–264 was found. Two cysteine residues (amino acids 190 and 225) involved in the formation of a disulfide bridge were also found (Fig. 7).

The phylogenetic tree analyses revealed that the gayal *PRNP* had a very close genetic relationship with the *PRNP* gene of Zebu cattle (Fig. 8).

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		99.4	99.2	99.4	99.2	98.9	99.4	98.6	97.6	97.1	97.4	97.0	97.9	97.7	86.2	97.4	87.3	86.7	86.9	82.0	1	Gayal
2	0.6		99.4	99.2	99.4	99.2	99.2	98.5	97.7	97.0	97.3	96.9	97.8	97.5	86.3	97.3	87.3	86.8	86.4	82.0	2	Zebu cattle
3	0.8	0.6		99.9	100.0	99.5	99.6	98.6	97.9	97.1	97.4	97.0	97.9	97.7	86.4	97.4	87.3	86.7	87.0	81.8	3	European cattle
4	0.6	0.8	0.1		99.9	99.4	99.7	98.7	98.0	97.3	97.5	97.1	98.1	97.8	86.6	97.5	87.5	86.8	87.1	82.0	4	Gaur
5	0.8	0.6	0.0	0.1		99.5	99.6	98.6	97.9	97.1	97.4	97.0	97.9	97.7	86.4	97.4	87.3	86.7	87.0	81.8	5	Bison
6	1.1	0.8	0.5	0.6	0.5		99.1	98.1	97.6	96.6	96.9	96.5	97.4	97.1	86.2	96.9	87.1	86.6	86.2	81.4	6	Banteng cattle
7	0.6	0.8	0.4	0.3	0.4	0.9		98.7	97.7	97.3	97.5	97.1	98.1	97.8	86.3	97.5	87.2	86.6	87.1	81.8	7	Yak
8	1.4	1.5	1.4	1.3	1.4	1.9	1.3		98.1	96.9	97.0	96.6	97.5	97.3	86.0	97.0	87.0	86.3	86.4	81.4	8	Buffalo
9	2.4	2.3	2.2	2.0	2.2	2.4	2.3	1.9		96.2	96.8	96.4	97.0	96.8	86.2	96.8	87.2	86.8	86.4	81.0	9	Cape buffalo
10	2.9	3.1	2.9	2.8	2.9	3.5	2.8	3.2	4.1		99.5	99.1	97.4	97.4	86.3	98.3	87.3	86.6	86.5	82.1	10	Red deer
11	2.6	2.8	2.6	2.5	2.6	3.2	2.5	3.0	3.7	0.5		99.4	97.4	97.4	86.8	98.6	87.8	87.2	86.5	81.8	11	Sika deer
12	3.1	3.2	3.1	2.9	3.1	3.6	2.9	3.5	4.2	0.9	0.7		97.3	97.3	86.6	98.3	87.4	86.8	86.5	81.6	12	fallow deer
13	2.1	2.2	2.1	2.0	2.1	2.6	2.0	2.5	3.5	2.6	2.6	2.8		99.5	86.8	98.4	87.7	87.0	86.7	81.7	13	Sheep
14	2.4	2.5	2.4	2.2	2.4	2.9	2.2	2.8	3.7	2.6	2.6	2.8	0.5		87.1	98.4	87.9	87.3	86.7	81.6	14	Goat
15	15.0	14.8	14.7	14.5	14.7	15.0	14.8	15.2	15.3	14.8	14.2	14.5	14.2	13.9		87.2	91.4	91.0	84.4	82.0	15	Mink
16	2.6	2.8	2.6	2.5	2.6	3.2	2.5	3.0	3.7	1.7	1.4	1.7	1.6	1.6	13.7		88.2	87.7	86.9	81.7	16	Cat
17	13.4	13.2	13.4	13.2	13.4	13.6	13.6	13.9	13.7	13.5	12.9	13.4	13.2	12.9	8.7	12.4		99.0	86.4	81.8	17	Dog
18	14.2	14.0	14.2	14.0	14.2	14.4	14.4	14.7	14.2	14.3	13.7	14.2	14.0	13.7	9.1	13.1	1.0		85.8	81.4	18	Arctic fox
19	13.4	13.4	13.2	13.0	13.2	13.5	13.0	13.5	14.0	13.5	13.5	13.5	13.5	13.5	15.5	13.2	14.2	14.9		85.7	19	Human
20	20.0	20.0	20.2	20.0	20.2	20.7	20.2	20.5	20.3	19.1	19.4	19.8	19.8	20.2	18.4	19.6	19.8	20.3	16.3		20	Mouse
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		

Fig. 6 Nucleotide sequences alignments of 20 mammalian prion protein genes. 1. Gayal (*Bos frontalis*, HQ262495), 2. Zebu cattle (*Bos indicus*, AY720681), 3. European cattle (*Bos taurus*, EF139165), 4. Gaur (*Bos gaurus*, AY720697%), 5. Bison (*Bison bison*, AY769958), 6. Banteng cattle (*Bos jsvanicus*, AY720693), 7. Yak (*Bos grunniens*, AY367635), 8. Buffalo (*Bubalus bubalus*, AY768533), 9. Cape buffalo (*Syncerus caffer*, AY720686), 10. Red deer (*Cervus elaphus*, AY748455), 11. Sika deer (*Cervus nippon*, EF057409), 12. Fallow deer (*Dama dama*, EF165089), 13. Sheep (*Ovis aries*, DQ149388), 14. Goat (*Capra hircus*, EF140716), 15. Mink (*Mustelan vison*, S46825), 16. Cat (*Felis catus*, AF003087), 17. Dog (*Canis familiaris*, EF139170), 18. Arctic fox (*Vulpes lagopus*, EU365392), 19. Human (*Homo sapiens*, AF076976), 20. Mouse (*Mus musculus*, M18070)

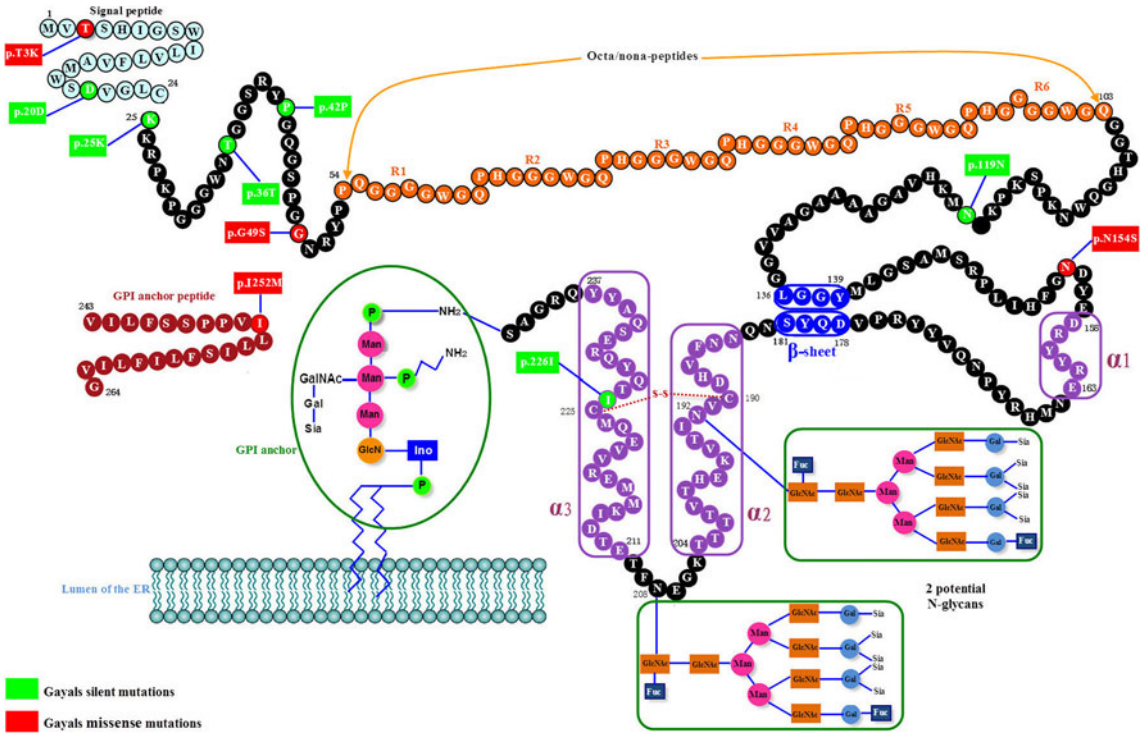

Fig. 7 Two dimensional structure of the deduced amino acid sequence of prion protein of gayal with the identified amino acid changes indicated. The numbering of signal and glycosylphosphatidylinositol (GPI) anchor peptides is indicated according to the precursor sequence

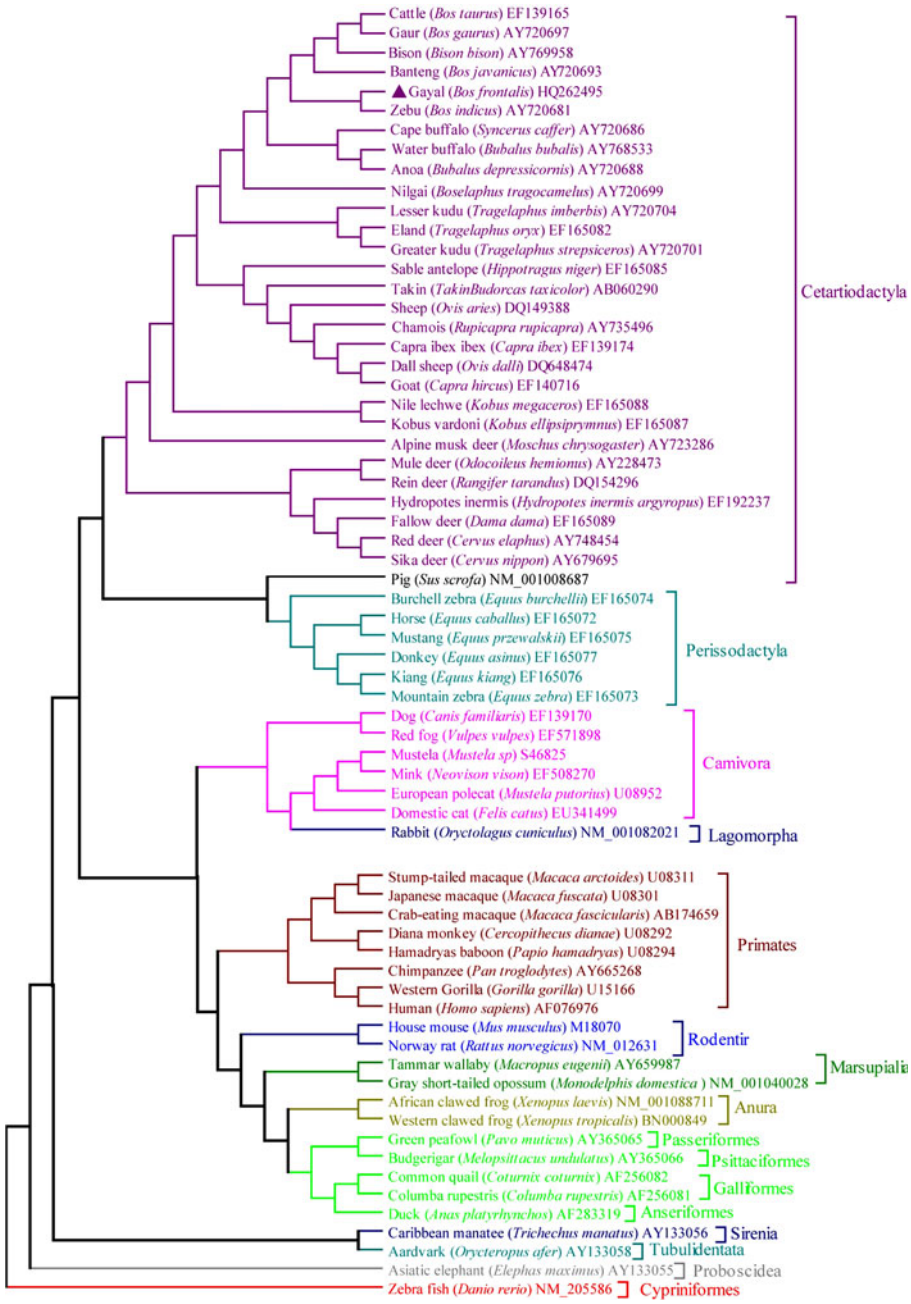

Fig. 8 Phylogenetic tree for 66 animals prion protein genes

Discussion

In the present study, the gayal ORF of *PRNP* was isolated for the first time. Novel polymorphism analyses expanded the collection of known *PRNP* from animals within the *Bos* genus. Collectively, 10 SNPs including 6 silent and 4 mis-sense mutations were identified. Comparing with known SNPs of *PRNP* from European cattle, yak and swamp buffalo (*Bubalus bubalis*) in China [17, 19, 30, 31], as well as European and Zebu cattle and their hybrids, Banteng cattle (*Bos javanicus*), American bison, American buffalo (*Syncerus caffer nanus*), forest buffalo (*Syncerus caffer nanus*), lowland anoa (*Bubalus depressicornis*), Asian water buffalo (*Bubalus bubalis*), nilgai (*Boselaphus tragocamelus*) in countries other than China [16, 18, 21, 32, 33], three novel SNPs in the coding region including C60T (D20), G145A (G49S) and C756G (T252M), which have not been reported previously in bovid species, were identified.

Eight classical type structural characters, consisting of a SP, a tandem repeat, three α-helices, two β-sheets, a hydrophobic region, two glycosylation sites, a GPI anchor site and two cysteine residues possibly involved in the formation of a disulfide bridge, which were reported as

quite conserved elements in mammals [34], were also identified in the present study. However, two non-silent mutations (one T3K and another I252M) were identified in the SP and GPI anchor regions, respectively (Fig. 7).

The SP encodes the determinants for topogenesis and plays a role in targeting proteins to the endoplasmic reticulum [35]. Increased generation of ^{Ctm}PRNP, which is a topologic form of PRNP formed at the endoplasmic reticulum, is related to the development of transmissible spongiform encephalopathies (TSE) in humans and transgenic mice [36, 37]. Furthermore, the SP was divided into three significant fragments including the n-region (amino acids 1–9), h-region (amino acids 10–16) and c-region (amino acids 17–24). The n-region is a polar and charged domain. Mutation of the n-region often results in the introduction of charged residues and alternations in the topogenesis of PRNP and the introduction of basic residues that decrease the level of ^{Ctm}PRNP [35].

In the current study, the K3T mutation (reside 3, comprising the positively charged and basic amino acid lysine) within the n-region of SP was converted to the uncharged and aromatic amino acid threonine. Therefore, more attention should be paid to the topogenesis of PRNPs and their expression level in future research. It is noteworthy that this mutation has been reported in the native Chinese southern yellow cattle (*B. taurus*) [19] but not in the native Chinese northern yellow cattle [17, 30, 31]. In the present report, we also identified this site in gayal from the south west of China. This observation is consistent with a previous report that gayal may have derived genetic material from Chinese southern yellow cattle [24].

The second non-silent mutation G49S was located within the N-terminal region and included amino acids 1–91, comprising the signal peptide (amino acids 1–24) and the tandem repeat region (amino acids 54–103). These play an important role in TSE pathogenesis through possible combination of the N-terminal region with copper ions [38]. Within this area, one linkage is between the octarepeat and the structural domain at His^{107} and His^{122} and another is between W^{60} and G^{93} in bovine PRNP [39]. However, G49S occurs outside of these regions. Therefore, this mutation G49S may not be related to susceptibility to BSE. Significantly, this site has been identified only in gayal and never in other bovid animals [17, 19–21].

The third non-silent mutation S154 N (analogous to mouse residue 142) is located at the tail of first α-helix [40]. This site was retrieved previously in swamp buffalo and also European and Zebu cattle and their hybrid offspring [17, 19–21]. In transgenic mice carrying chimeric murine–ovine $PRNP^c$, mushp-$PRNP^c$N142S is established after converting asparagine to serine at residue 142. The change leads into a large reduction in Me7 prion-induced conversion, but not the BSE-induced conversion [41]. Serine and asparagine are located on an uncharged polar side chain and serine is considerably smaller than asparagine. Moreover, serine lacks the terminal carboxyamide group which in asparagine enables the formation of hydrogen bonds and improves the intrinsic stability of the molecule [19, 41]. It is possible that this mutation in gayal could lead to the synthesis of a highly stable of prion protein, unlike mushp-$PRNP^c$N142S of transgenic mice, in which a negative result was obtained in a cell-free conversion experiment.

The fourth non-silent mutation, T252M, occurred in the GPI anchor site region which enables the protein prion coded for to associate with sphingolipid- and cholesterol-rich membranes [42] leading to binding and replication [43]. This association is critical for the conformational conversion of normal $PRNP^c$ to pathogenic $PRNP^{Sc}$ [44, 45]. Only one SNP within the GPI anchor site has been reported in 751 (G or A, V251M) in forest buffalo [21]. In the present study, SNP C756G was predicted to alter the amino acid at 252 from isoleucine to methionine which had not been reported previously. These two amino acids are non-polar but, the functional significance of a T252M variant is unknown.

Turning to the octapeptide repeat, in previous studies it has been found that most healthy cattle from modern breeds have similar distributions of the *PRNP* octarepeat 6:6 and 6:5 genotypes. This has been found in studies from Japan [46, 47], Korea [48–51], Vietnam, Laos, Myanmar, Mongolia and Bangladesh [52, 53], China [19], USA [54], Croatia [55], Czechoslovakia [56] and Poland [57]. In four comparative studies from Germany [58], Japan [59], Czechoslovakia [26] and Scotland [60] no differences between the frequencies of these *PRNP* genotypes in healthy and BSE-affected cattle were found. The number of octarepeats in cattle has been found to vary from 4 to 7 [61]. An increased number of octapeptide repetitions in bovine PRNP may be associated with increased host susceptibility to BSE agents [62, 63]. The observed allele frequencies (6, 5 and 4) of octapeptide repeat units were 0.77, 0.14 and 0.09 from 11 gayal samples which were collected in Myanmar where is close to our sampling site near Dulong Town, Gongshan County, Yunnan Province, China [52]. In the current study, based on 125 tested samples, it was found that gayal has a small number of octapeptide repeats, lacking the four, seven or larger number of repeats reported by others for cattle [61]. Furthermore, the genotype and gene frequencies of the *PRNP* do not differ from those reported above. Therefore, the tandem repeat with R2 del polymorphism in gayal appears not to affect resistance or susceptibility of gayal to BSE.

Recently, a novel E211K mutation (analogous to human reside E200K) was reported to have a relationship

with a case of H-type BSE [64–66]. However, this SNP has never been found in gayal or in so-called cattle (including Chinese yellow cattle), swamp buffalo and yak [17, 19, 30, 31].

In conclusion, the present genetic analyses extend knowledge of the DNA polymorphism of the *PRNP* in gayal. It appears that there is scope for exploring the relationship between the diversity of the coding region for *PRNP* and resistance to BSE. Further studies should be focused on the Indel polymorphisms in the promoter and intron of this gene.

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