

## Cloning and analysis of *PRNP* gene of *Vulpes corsac* in Qinghai plateau, China

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### ABSTRACT

*PRNP* gene encodes PrP protein, which is conservative among different species and associates with the susceptibility of prion disease. In this report, we cloned and sequenced the full-length *PRNP* gene of *Vulpes corsac* in Qinghai plateau, China. The amino acid sequence of *Vulpes corsac* PrP showed 100% homology with those of the other three species of foxes. The taxa relationship of *Vulpes corsac* PrP with other species of animals, including human, canine, bovine, cervus, capra, ovis, camelus, felis, *Mustela*, mouse and hamster were also analysed.

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## Introduction

Prion diseases, or transmissible spongiform encephalopathies (TSE), are a group of transmissible, fatal neurodegenerative diseases affecting a wide variety of mammals including humans [1,2]. The conversion of the normal and non-infectious cellular form of the host prion protein (PrP<sup>C</sup>) into the abnormal and pathogenic form (PrP<sup>Sc</sup>) is critical for prion disease [3]. Prion protein (PrP) is encoded by a host gene, namely *PRNP*, usually contains one open reading frame (ORF) [4]. Despite that the sequence of *PRNP* is fairly conservative among different species of animals, the full extent of *PRNP* allele is closely associated with the susceptibility of the infections of different prion strains both naturally and experimentally [5–8].




*Vulpes corsac* belongs to fox species, which mainly inhabits steppe, desert and semi-desert areas and distributes in Central Asia, ranging into Mongolia and northern China [9]. This kind of animal has three subspecies including *Vulpes corsac corsac*, *Vulpes corsac kalmykorum* and *Vulpes corsac turkmenicus* [10]. In this report, we have described the full-length of *PRNP* gene of *Vulpes corsac* that was captured in Qinghai plateau, China.

## The sequence of *Vulpes corsac* PrP

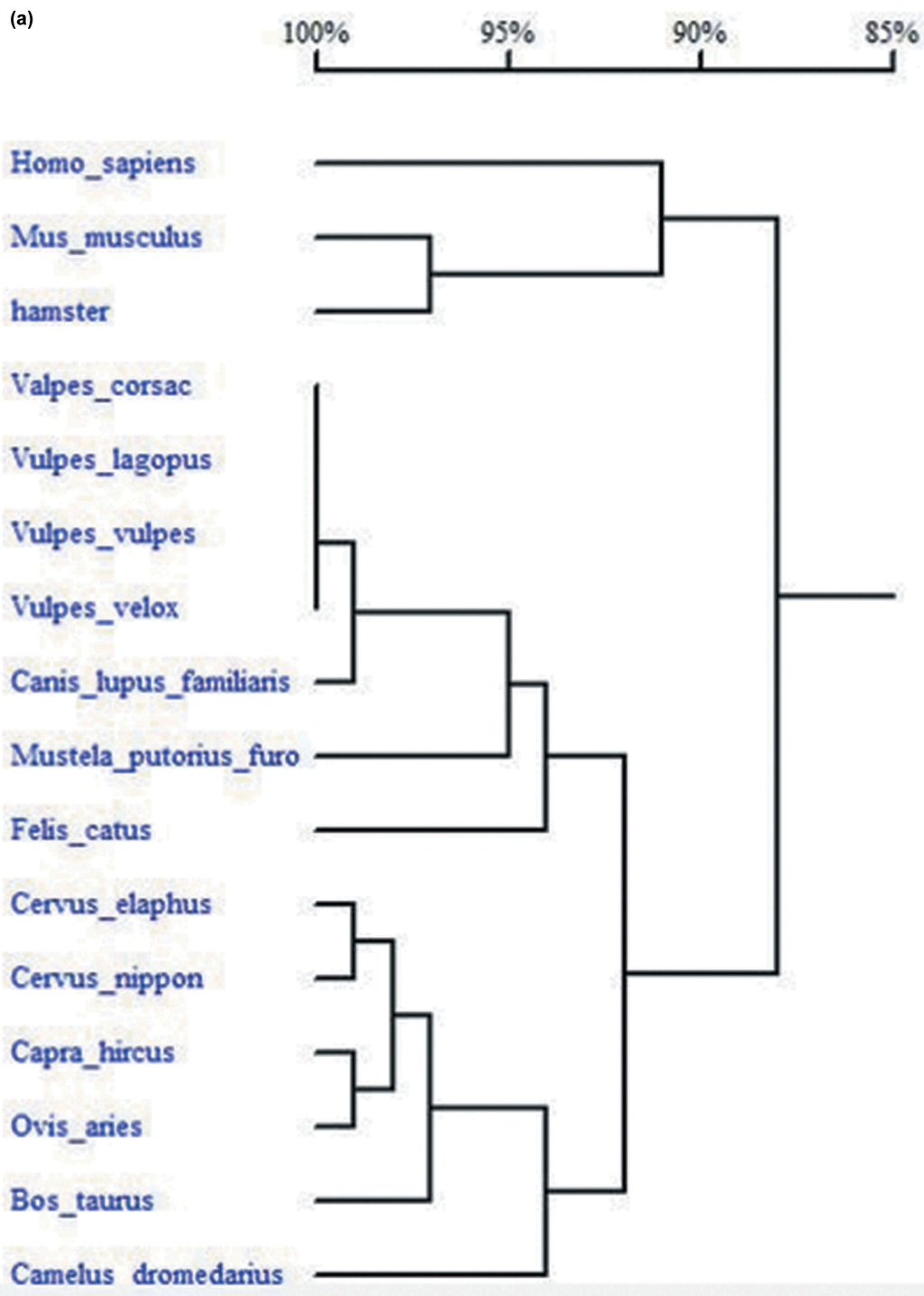
The genomic DNA from the liver tissue of a natural death Corsac fox collected in Qinghai province was extracted using QIAamp DNA Mini Kit. The *PRNP*

sequence was amplified by PCR technique with the designed primers (upstream primer: 5'-ATGGTGA AAAGCCACATAG-3'; downstream primer: 5'-TCA TCCCACTATCAAGAGA-3) based on the *PRNP* sequences of *Vulpes vulpes* (EF571898), *Vulpes velox* (EU341513) and *Vulpes lagopus* (EU365392) in NCBI website. The reaction conditions were 94°C for 1 min, 52°C for 30 s, 72°C for 40 s, totally 35 cycles. After purification, the PCR product was inserted into a clone vector pMD19 and sequenced with the primer designed according to the sequence of the cloning vector.

According to the sequencing results, the *PRNP* sequence of the tested *Vulpes corsac* was 774 bp long, which may encode 257 amino acids (submitted to NCBI, MN381732). Compared with the published data of foxes, including red fox, Swift fox and Arctic fox, the amino acid sequence of *Vulpes corsac* PrP was 100% homology. The homology of *Vulpes corsac* PrP with human and other species of animals was also illustrated in the phylogeny tree (Figure 1(a)). It revealed an extremely close homology with canine PrP (NP\_001013441), while apparently remote relationship with the PrP of human (NP\_898902). Meanwhile, the PrP sequences of *Vulpes corsac*, as well as other foxes and dog, showed also remote relationship with that of the animal species with naturally occurred prion diseases, such as cattle (ABE02802), ovis (NP\_001009481, NP\_001301176), cervus (QAU19537, AAT72295), cat (ACA50727), ferret (XP\_012901521) and camel (AUM59985), as well as the

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(b)

Homology matrix of 16 sequences

Homo_sapiens	100%
Valpes_corsac	86.7% 100%
Vulpes_lagopus	86.7% 100.0% 100%
Vulpes_vulpes	86.7% 100.0% 100.0% 100%
Vulpes_velox	86.7% 100.0% 100.0% 100.0% 100%
Canis_lupus_familiaris	87.1% 99.2% 99.2% 99.2% 99.2% 100%
Cervus_elaphus	90.7% 91.9% 91.9% 91.9% 91.9% 92.3% 100%
Cervus_nippon	90.7% 92.7% 92.7% 92.7% 92.7% 93.1% 99.2% 100%
Capra_hircus	90.3% 94.0% 94.0% 94.0% 94.0% 94.4% 97.2% 97.2% 100%
Ovis_aries	91.1% 94.0% 94.0% 94.0% 94.0% 94.4% 98.0% 98.0% 99.2% 100%
Camelus_dromedarius	89.1% 89.9% 89.9% 89.9% 89.9% 89.5% 93.5% 93.5% 93.1% 94.0% 100%
Bos_taurus	91.5% 92.3% 92.3% 92.3% 92.3% 91.9% 97.2% 97.2% 96.8% 97.6% 93.5% 100%
Felis_catus	86.3% 94.4% 94.4% 94.4% 94.4% 94.0% 91.1% 91.1% 91.5% 91.5% 87.9% 90.7% 100%
Mustela_putorius_furo	87.1% 95.6% 95.6% 95.6% 95.6% 95.2% 91.9% 92.7% 94.0% 94.0% 90.7% 92.3% 93.1% 100%
Mus_musculus	90.7% 87.1% 87.1% 87.1% 87.1% 87.1% 89.5% 89.5% 89.1% 89.9% 88.3% 89.5% 85.5% 87.1% 100%
hamster	91.1% 87.1% 87.1% 87.1% 87.1% 87.1% 89.5% 89.5% 88.3% 89.1% 87.9% 89.1% 85.5% 86.7% 96.8% 100%

Figure 1. Homology analysis of amino acid sequences of PrP proteins of human and various species of animals. (a). Phylogenetic tree. (b). Homology matrix.

animals with experimental prion diseases, such as mouse (NP\_001265185) and hamster (AAA37013).

The homology matrix of human, *Vulpes corsac* and other species of mammalia is illustrated in Figure 1(b). Beside of the canine PrP showing 99.2% homology, PrP of *Vulpes corsac* revealed 95.6% homology with that of *Mustela putorius*, 94.4% with feline, 94.0% with ovis, 92.7% with *Cervus nippon*, 92.3% with *Bos taurus*, 91.9% with *Cervus elaphus*, 89.9% with camel, 87.1% with mouse and hamster, and 86.7 with human.

The exact differences of PrP sequences among the different species are shown in Figure 2. Similar to that of the fox, canine PrP consisted of 257 amino acids. Only two amino acids were different between fox and dog,

which located at the position of aa 101 from Gly (fox) to Ser (dog) and at the position of aa 163 from Asp (fox) to Glu (dog), indicating a high homology of PrP within the canine family. Human PrP consists of 253 amino acids. There were 36 amino acid differences in PrP peptides between the *Vulpes corsac* and human. The regions with more discrepancies in amino acid between fox and human PrPs were N-terminal signal sequence (39.2%) and C-terminal GPI anchor (22.7%). Amino acid variations were also identified within the regions of  $\alpha 3$  (22.7%) and  $\alpha 2$  (9.1%), but not in the regions of  $\alpha 1$ ,  $\beta 1$  and  $\beta 2$ . Further comparisons of the sequence of *Vulpes corsac* PrP with those of bovine and cervus PrPs also revealed the similar variation patterns. Besides of

Homo_sapiens	..MNLGCMVLVPMVATNSDILGLCKKRPKPGG.WNTGG.SRYPQGGSPGGRIP	50
Valpes_corsac	mvkshi-g-i-l-----v-----g-----	53
Vulpes_lagopus	mvkshi-g-i-l-----v-----g-----	53
Vulpes_velox	mvkshi-g-i-l-----v-----g-----	53
Vulpes_vulpes	mvkshi-g-i-l-----v-----g-----	53
Canis_lupus_familiaris	mvkshi-g-i-l-----v-----g-----	53
Cervus_elaphus	mvkshi-s-i-----m-v-----g-----	53
Cervus_nippon	mvkshi-s-i-----m-v-----g-----	53
Capra_hircus	mvkshi-s-i-----m-v-----g-----	53
Ovis_aries	mvkshi-s-i-----m-v-----g-----	53
Bos_taurus	mvkshi-s-i-----m-v-----g-----	53
Camelus_dromedarius	mvkshs-s-i-----v-v-----g-----	53
Felis_catus	mvkghi-g-i-----v-----g-----	53
hamster	..-sy-l-a-----t-v-----g-----	50
Mus_musculus	..-y-l-a-----tm-t-v-----g-----	50
Mustela_putorius_furo	mvkshi-s-l-----i-f-----g-----	53
Homo_sapiens	EQGGGGWQPHGGG.WSQPHGGG.WSQPHGGG.....WSQPHGGG.WSQGG	93
Valpes_corsac	.....g-----	97
Vulpes_lagopus	.....g-----	97
Vulpes_velox	.....g-----	97
Vulpes_vulpes	.....g-----	97
Canis_lupus_familiaris	.....g-----	97
Cervus_elaphus	.....g-----	97
Cervus_nippon	.....g-----	97
Capra_hircus	.....g-----	97
Ovis_aries	.....g-----	97
Bos_taurus	.....g-----	105
Camelus_dromedarius	.....g-----	96
Felis_catus	.....g-----	100
hamster	.....g-----	93
Mus_musculus	.....g-----	92
Mustela_putorius_furo	.....g-----	97
Homo_sapiens	GTHSQWNKPSKPKITNMKHMAGAAAGAVVGLGGTMTLSAMSRLPIHFGS	147
Valpes_corsac	-s-g-g-n-v-----l-n-----	151
Vulpes_lagopus	-s-g-g-n-v-----l-n-----	151
Vulpes_velox	-s-g-g-n-v-----l-n-----	151
Vulpes_vulpes	-s-g-g-n-v-----l-n-----	151
Canis_lupus_familiaris	-s-g-g-n-v-----l-n-----	151
Cervus_elaphus	.....l-n-----	150
Cervus_nippon	.....l-n-----	150
Capra_hircus	.....s-----l-n-----	150
Ovis_aries	.....s-----l-n-----	150
Bos_taurus	.....s-----l-n-----	158
Camelus_dromedarius	-a-g-----s-v-----l-n-----	150
Felis_catus	-g-g-----l-n-----	154
hamster	-n-----v-----ml-n-w-----	147
Mus_musculus	-n-----l-v-----m-n-w-----	146
Mustela_putorius_furo	-s-g-g-----i-v-----l-n-----	151
Homo_sapiens	.....HRIFNPVLIIRFMDEISN.....GENPT	201
Valpes_corsac	.....y-d-v-q-r-v-----	205
Vulpes_lagopus	.....y-d-v-q-r-v-----	205
Vulpes_velox	.....y-d-v-q-r-v-----	205
Vulpes_vulpes	.....y-d-v-q-r-v-----	205
Canis_lupus_familiaris	.....y-e-v-q-r-v-----	205
Cervus_elaphus	.....y-----v-q-n-t-v-----	204
Cervus_nippon	.....y-----v-q-n-t-v-----	204
Capra_hircus	.....y-----v-q-r-v-----	204
Ovis_aries	.....y-----v-q-r-v-----	204
Bos_taurus	.....y-----v-q-r-v-----	212
Camelus_dromedarius	.....y-----k-v-q-s-v-----	204
Felis_catus	.....y-----v-q-r-v-----	208
hamster	.....n-----v-q-n-----	201
Mus_musculus	.....y-----v-q-r-v-----	200
Mustela_putorius_furo	.....y-----k-v-q-r-v-----	205
Homo_sapiens	.....SQAITQ.RGSS.....	253
Valpes_corsac	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257
Vulpes_lagopus	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257
Vulpes_velox	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257
Vulpes_vulpes	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257
Canis_lupus_familiaris	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257
Cervus_elaphus	-i-----q-e-rga-v.i-----	256
Cervus_nippon	-i-----q-e-rga-v.i-----	256
Capra_hircus	-i-i-----q-----rga-v.i-p-----	256
Ovis_aries	-i-i-----q-----rga-v.i-----	256
Bos_taurus	-i-----q-----rga-v.i-----	264
Camelus_dromedarius	.....q-y-s-grga.....i-----	255
Felis_catus	-m-i-----v-qk-e-rga-a.i-p-----l-l-l-g-----	260
hamster	.....v-qk-dg-r-a-----	254
Mus_musculus	.....v-qk-dg-r-st-----	254
Mustela_putorius_furo	-m-i-----v-qk-e-rga-a.i-p-----l-l-----	257

Figure 2. Comparison of the variations of amino acids of PrP proteins of human and various animals. The various functional and secondary structural regions within PrP sequences are indicated with colours. The amino acid numbers are shown on the right.

differences in the regions of the signal peptide and GPI anchor, variations were also found in the regions of  $\alpha 3$  (18.1%) and  $\alpha 2$  (9.1%), but not in those of  $\alpha 1$ ,  $\beta 1$  and  $\beta 2$ .

## Discussion

In this report, we have for the first time described the *PRNP* sequence of *Vulpes corsac* in Qing-Tibet plateau. *Vulpes corsac* belongs to canine family, fox subfamily, fox genus. Generally, the animals of canine species are not sensitive to prion infection. So far, there is no naturally occurred canine TSE reported [11,12], including the animals of *Vulpes*. Among the factors that may contribute to the susceptibility or resistance to the prion strains, the host PrP sequence is believed as the most essential one. The high identity of *PRNP* sequence between *Vulpes corsac* and dog indicates that *Vulpes corsac* may also be insensitive to prion infection.

*Vulpes corsac* distributes widely in the different geographies of Central Asia and the western region of China, usually taking rodents and birds as the main source of food, such as yellow mouse, striped hamster and Brandt's vole. Meanwhile, many other carnivores may prey *Vulpes corsac*, such as caracal, jackal, etc. Qinghai-Tibet plateau is an important pastoral area in China. There are thousands of livestock, e.g., yaks, sheeps and goats, as well as wild herbivore, e.g., Tibetan antelope, kiang, wild yak, mongolian gazelle, etc. As a middle link of food cycle in a special geography, understating the sequence of *Vulpes corsac* PrP may help for evaluation its potential in the circulation of prions, e.g., scrapie, in a special region.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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