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

Xue-Hua Yang\textsuperscript{a}, Kang Xiao\textsuperscript{a}, Yuezhang Wu\textsuperscript{a}, Liping Gao\textsuperscript{a}, Dongdong Chen\textsuperscript{a}, Xiao-Ping Dong\textsuperscript{a,b,c}, and Qi Shi\textsuperscript{a}

\textsuperscript{a}State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases (Zhejiang University), National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, People’s Republic of China; \textsuperscript{b}Center for Global Public Health, Chinese Center for Disease Control and Prevention, Beijing, China; \textsuperscript{c}Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan, People’s Republic of China

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.

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\textsuperscript{C}) into the abnormal and pathogenic form (PrP\textsuperscript{Sc}) 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 turcmenicus [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 TCCCACATCAAGAGA-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 (ACAS0727), ferret (XP_012901521) and camel (AUM59985), as well as the...
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

![Figure 2](image-url)

**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 α3 (18.1%) and α2 (9.1%), but not in those of α1, β1 and β2.

Discussion

In this report, we have for the first time described the PRNP sequence of Vulpes corsac in Qing-Tobent 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.

Funding

This work was supported by Research Project [2018 ZX10711001, 2017YFC1200500], Chinese National Natural Science Foundation Grants [81572048, 81630062] and SKLID Development Grant [2019SKLID501, 2019SKLID603, 2019SKLID307].

References

[1] Colby DW, Prusiner SB. Prions[J]. Cold Spring Harb Perspect Biol. 2011;3(1):13363–13383.
[2] Prusiner SB. Nobel lecture: prions[J]. Proc Natl Acad Sci U S A. 1998;95(23):13363–13383.
[3] Westergard L, Christensen HM, Harris DA. The cellular prion protein (PrP(C)): its physiological function and role in disease[J]. Biochim Biophys Acta. 2007;1772(6):629–644.
[4] Prusiner SB. Molecular biology of prion diseases[J]. Science. 1991;252(5012):1515–1522.
[5] Hadlow WJ, Race RE, Kennedy RC Experimental infection of sheep and goats with transmissible mink encephalopathy virus.[J]. Can J Vet Res Revue canadienne de recherche vétérinaire. 1987;51(1):135.
[6] Hanson RP, Eckroade RJ, Marsh RF, et al. Susceptibility of mink to sheep scrapie.[J]. Science. 1971;172(3985):859–861.
[7] Robinson MM, Hadlow WJ, Huff TP, et al. Experimental infection of mink with bovine spongiform encephalopathy.[J]. J Gen Virol. 1994;75(Pt 9):2151.
[8] Robinson MM, Hadlow WJ, Knowles DP, et al. Experimental infection of cattle with the agents of transmissible mink encephalopathy and scrapie[J]. J Comp Pathol. 1995;113(3):241–251.
[9] Murdoch JD 2014. Vulpes corsac. The IUCN red list of threatened species 2014: e.T23051A59049446. [EB/OL]. [cited 2019 Jul 7]. Available from: https://www.iucnredlist.org/en
[10] Search|BioLib.cz[EB/OL]. [cited 2019 Jul 7]; Available from: https://www.biolib.cz/en/formsearch?action=execute&searcharea=1&string=Vulpes+corsac
[11] Sanchez-Garcia J, Fernandez-Funez P. D159 and S167 are protective residues in the prion protein from dog and horse, two prion-resistant animals[J]. Neurobiol Dis. 2018;119:1–12.
[12] Won SY, Kim YC, Kim K, et al. The first report of polymorphisms and genetic features of the prion-like protein gene (PRND) in a prion disease-resistant animal, dog[J]. Int J Mol Sci. 2019;20(6):1404.