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# Analysis of the Prion Protein Gene (PRNP) in Bottle-nosed Dolphin

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Prion diseases, as a group of fatal neurodegenerative diseases, have deeply affected human and a variety of other mammals. Science being clarified as the causative agent of prion diseases, prion protein (PrP) has attracted lots of scientists to research on (Prusiner and Prions, 1998). The prion protein has two types of conformations which are encoded by the same genes. The infectious agent of prion disease is the abnormal form PrP, namely $PrP^{SC}$, which is rich in β-sheet, $PrP^{SC}$ has a resistant character to proteinase K compared with the normal form PrP, $PrP^{C}$. The Bovine Spongiform Encephalopathy (BSE) in cattle, the scrapie in sheep, and the variant Creutzfeldt-Jakob disease (vCJD) in human are all fatal neurodegenerative disease caused by $PrP^{SC}$. It is believed that vCJD is derived from cattle infected with BSE.

Bottlenose dolphin (*Tursiops truncatus*), which belong to Class Mammalia, order Cetacea, suborder Odontoceti, Delphinidae, is an ocean mammal. Marino (2004) and Herman *et al.* (2002) showed that the bottlenose dolphin possess Encephalization Quotient (EQ) ranging from 4 to 5, which is tantalizingly close to human level and higher than any other animals. It is interesting that the bottle-nosed dolphin has special capacity of short-term and long-term memory for visual, auditory and multimodal information and abstract concepts. The dolphins are capable of understanding semantics and syntax, and even can understand symbolic references to objects that are absent. All of these evidences may suggest that the dolphin brain is still very much like a mystery and well worth carrying out deep exploration (Herman *et al.*, 2001).

There are no reported Transmissible Spongiform Encephalopathy (TSE) cases in dolphins in the world yet. The only related report about prion protein gene (PRNP) of bottle-nosed dolphin was described by Wopfner *et al.* (1999). The gene sequence is 425 bp long. Just like other marine animals, dolphins have already interested scientists on many others aspects, it would be worthy to research on and take appropriate precautions in order to protect them from the prion disease. Through the study on the PRNP which originated from different animal species, it has been believed that the prion diseases transmission was affected by the species barrier phenomena. The polymorphism within the Open Reading Frame (ORF) of the single-copy gene of prion protein has been demonstrated to be associated with susceptibility to scrapie in sheep and variant Creutzfeldt-Jacob disease in humans. Therefore the comparative analysis of variations in the ORF sequence of PRNP will provide more details to clarify the pathogenesis of TSE.

EDTA blood sample was collected from 30 bottle-nosed dolphins (ten males and twenty females with 3-6 years old) from two ocean parks (Beijing and Shanhai Col ocean parks). Genomic DNA was isolated from the blood samples using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The PRNP was amplified by Polymerase Chain Reaction (PCR) as described by Meng and Zhao (2005). The PCR reactions contained 200 ng genomic

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DNA, 10 pmol of each primer, 250 μmol of dNTP and 5 Unit/μL Takara Taq™ DNA polymerase (Takara, Japan). Sterilized distilled water was used to make a final volume of 50 μL. The PCR was performed under conditions as follows: denaturation at 94°C for 4 min, 35 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 45 sec, followed with a final elongation step at 72°C for 10 min. The PCR product was ligated into a pGEM-T-easy vector (Takara, Japan) and the resulted recombinant was used to transform competent *E. coli.* (DH5α, Promega, USA) following standard protocols. After amplification, positive clones were collected to re-extract plasmid using Qiagen Plasmids Mini Kit (Qiagen, UK). The positive clones were confirmed by restriction enzyme *E.coli* digestion followed by gel electrophoresis showing a 3 kb band which is the vector and a 774 bp band which is the target PRNP. The target gene band was purified using Wizard® PCR preps DNA purification System (Promega, USA) and was sequenced in both orientations using an ABI 377 system machine (Applied Biosystems, USA). The sequence was analysed using the Genetyx 7.0 software. The sequences were submitted to the GenBank (accession number AY964056, DQ130069, DQ130070).

Through analysis the 30 sequences cloned in the experiment, three single nucleotide polymorphisms (T534C, T543C, C552G) were found in the ORF of the dolphin PRNP gene. The changes of nucleotide between the C and G lead to the amino acid difference between Asparagine and lysine. It has been confirmed that the asparagine 184 is very conservative in the prion protein which has been reported. The substitution of lysine should be studied deeply. (Wopfner *et al*., 1999; Rheede *et al*., 2003).

According to the reports, there are many dolphins will run aground and suicide together with no apparent reasons. No reasonable explanation has been given. It has been demonstrated that the polymorphisms of the codons 131, 154 and 171 affect the susceptibility in scrapie disease, and also showed that the A136-R154-Q171 (ARQ) type has the moderate susceptibility. (Goldmann *et al*., 1996). This phenomenon has not been discovered in other animal species except in some Creutzfeldt-Jakob disease patients. It was found out that the PRNP of dolphin is ARQ style, future investigation will be carried out to illustrate the relationship between PRNP types and TSE susceptibility.

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