



Conserved *MC3R* gene in yaks and inferred phylogeny with other mammals

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Melanocortin 3 receptor gene (*MC3R*) is an intronless exon gene and is involved in regulation of feeding efficiency and fat storage (Chen *et al.* 2000). *Bos grunniens* is the most important grazing livestock species which have adapted to the alpine climates on the Qinghai-Tibetan Plateau and adjacent highlands for thousands of years and the number of yaks inhabiting these regions account for over 90% of the animals distributed all over of world (Cai *et al.* 2011).

The *MC3R* gene of *Bos grunniens* was amplified by using the homologous primers F (5' -TCCTTCAGCAACCAGA GCAGCAGC-32) and R (5' -AAGACGAAGACCCCGAG CAGGA TG-32), designed according to the *MC3R* gene sequence of *Bos taurus* (acc. no. FJ433881). All the *MC3R* gene sequences of *Bos grunniens* were deposited in GenBank with the acc. no. JN904083~JN904112. The corresponding sequences of other mammals were downloaded from GenBank, namely, *Bos taurus* (XM_002701108), *Ailuropoda melanoleuca* (XM_002921212), *Vulpes lagopus* (FJ468296), *Vulpes vulpes* (FJ005194), *Nyctereutes procyonoides procyonoides* (FJ468295), *Elephas maximus* (DQ648864), *Canis lupus familiaris* (NM_001135124), *Equus caballus* (XM_001489123), *Callithrix jacchus* (XM_002806779), *Oryctolagus cuniculus* (XM_002720876), *Rattus norvegicus* (NM_001025270), *Macaca mulatta* (XM_001091108), *Pan troglodytes* (XM_525362), *Nomascus leucogenys* (XM_003252957), *Pongo abelii* (XM_002830450), *Homo sapiens* (NM_019888), *Sus scrofa* (NM_001123137), *Rattus rattus* (X70667), *Mus musculus* (BC105668), *Syncerus caffer* (FJ483972), *Monodelphis domestica* (XM_001378078). Sequences were aligned using the ClustalW 1.82 on line.

Polymorphic sites of nucleotide were explored and analyzed in MEGA 4. The haplotype diversity (Hd) was analyzed by DnaSP 5.1. The NJ tree was reconstructed in MEGA, and the reliability of the tree topology was assessed by the bootstrap analyses carried out with 1000 replicates.

In this work, partial sequences of *MC3R* gene of *Bos grunniens* were cloned and only one nucleotide substitution was observed between 30 yaks, lacking of variations in *MC3R* gene might be attributed to the reasons that *MC3R* gene is a housekeeping gene and plays a very important function in regulation of feeding efficiency and fat storage. Interestingly, recent reports also showed the same situation in arctic fox (Skorczyk *et al.* 2011), porcine (Fan *et al.* 2008) and humans (Mencarelli *et al.* 2011). Based on these facts, we considered that the conservation of *MC3R* gene was common within the same species of mammals and the gene may have withstood the pressure of natural and artificial selections during the long period of evolution. On the other hand, we could not exclude the possibility that genetic drifts might have led to the very few naturally occurring mutations in coding sequences of *MC3R* gene, in that we have only sampled 30 individuals from the huge yak population.

Abundant variations of *MC3R* genes were exhibited between yaks and other mammals from different orders or families. Totally 257 variable sites were examined in 51 sequences from 22 different mammals, and 22 haplotypes were defined from the variations with a haplotype diversity of 0.6810. In addition, phylogenetic relationships of all mammals analyzed in this work were reasonably classified into the hierarchy, namely, Primates, Ursidae, Elephantidae, Suidae, Equidae, Didelphidae, Lagomorpha, Rodentia, Canidae and Bovidae (Fig. 1).

Only 10 substitution mutations were observed between yak and *Bos taurus*, and they converged into the family of Bovidae. Therefore, *MC3R* gene may have been subjected to substantial constraints during several million years of yak and cattle evolution. Moreover, highly conserved sequences of *MC3R* were also exhibited within other mammal's phylogenetic clades. Comparatively, the sequences of *MC3R* of primates also showed high conservation (98.29%) in this

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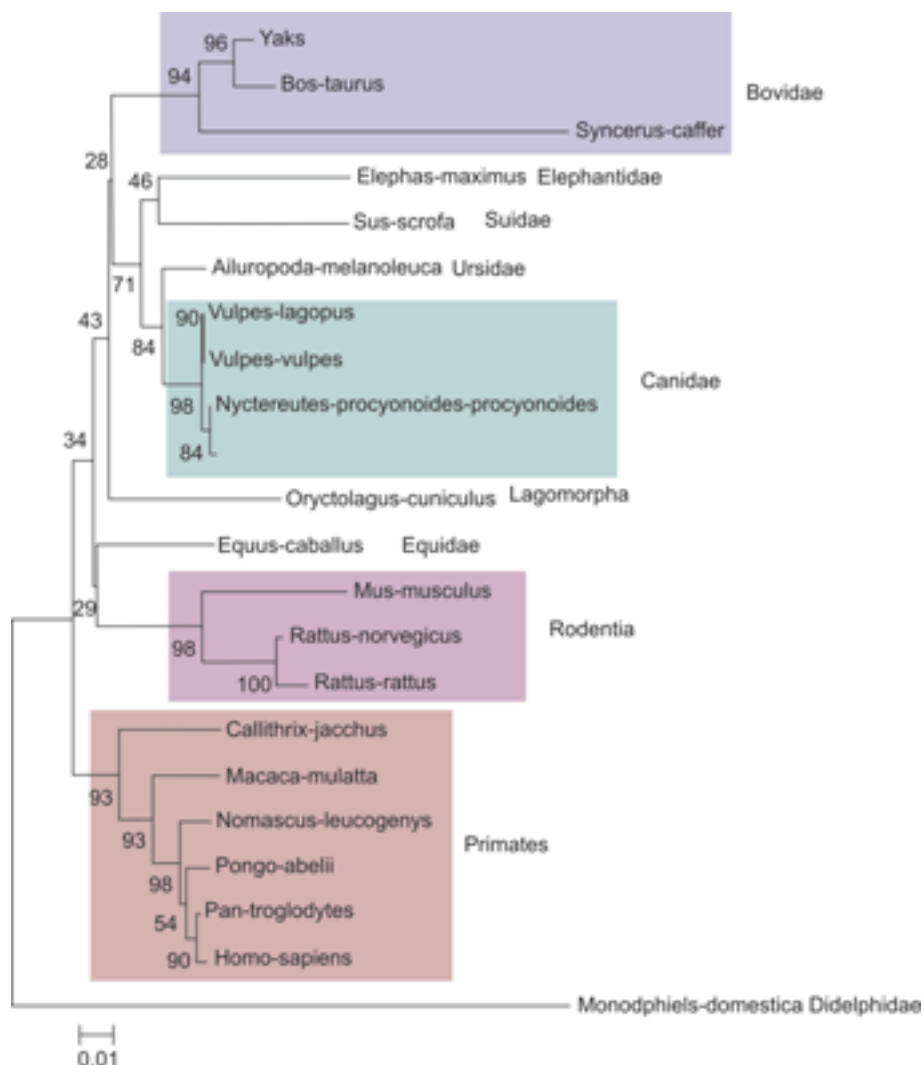


Fig. 1. Unrooted neighbour-joining tree constructed from *MC3R* genes of yak and other mammals. The numbers at the branches stand for bootstrap values for 1000 replications. Shaded rectangle-shaped areas indicated the phylogenetic lineages of different mammal orders or families.

work. This result could be explained by the view that *MC3R* gene has been subjected to high levels of continuous purifying selection during primate evolution, which resulted in most silent mutations either quickly fixed or removed from the population and non-synonymous mutations impacting *MC3R* function that have been subjected to very strong purifying selection (Hughes *et al.* 2009, Cai *et al.* 2011).

SUMMARY

High conservation observed between yaks showed that *MC3R* might be a housekeeping gene and also be a very important functional gene. The conservation of *MC3R* within the same order or family could be explained that *MC3R* gene may have been subjected to substantial constraints or strong purifying selection during several million years of mammal evolution. Whereas, *MC3R* gene showed abundant variations among different mammal phylogenetic clades.

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