Journal of Animal and Veterinary Advances 11 (7): 1000-1006, 2012
ISSN: 1680-5593

Bioinformatics Analysis of Melanocortin-1 Receptor Gene for Silver Fox and Other Species

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Abstract: The *MC1R* gene plays an important role in pigment synthesis in many species including human being. In the study, the coding sequence of the silver fox *MC1R* gene was obtained by the method of PCR direct sequencing and aligning, the length was 954 bp. A total of 119 *MC1R* gene sequences with the complete Coding regions (CDS) belonging to 23 species were analyzed and the differentiation within and among the species was also studied. The results showed that most of the species have the stop codon TGA. Only *Gallus gallus* and *Cereopsis novaehollandiae* of Phasianidae and *Danio rerio*, respectively used TAG and TAA as the stop codon. The length of the *MC1R* gene with the complete CDS varies greatly, from 924-972 bp but most of the species had 954 bp. Observed genetic diversity was higher among species than within species and *Canis lupus familiaris* had more polymorphisms than any other species. Differentiation of the *MC1R* gene was obvious among species and the reconstructed phylogenetic tree was basically consistent with the taxonomy in the National Center for Biotechnology Information.

Key words: *MC1R* gene, CDS, silver fox, variation, species, China

INTRODUCTION

Melanocortin-1 Receptor (MC1R) plays a major role in pigmentation in many species. The pigmentation of domestic animal including human beings is controlled by eumelanin and pheomelanin in melanocytes. Eumelanin synthesis is stimulated in melanocytes by the binding of a-Melanocyte Stimulating Hormone (a-MSH) to MC1R, resulting in black/brown coat color whereas pheomelanin synthesis is stimulated by the binding of ASIP to MC1R, resulting in yellow coat color. *MC1R* gene only contains one exon in the coding region. In recent years, some reports have focused on Single Nucleotide Polymorphism (SNP) identification of the *MC1R* gene and more attention has been focused on its relationship with coat, hair or skin colour in many species (for fox, Vage *et al*., 1997, 2005; for dog, Newton *et al*., 2000; Dreger and Schmutz, 2010; for cattle, Mohanty *et al*., 2008; Li *et al*., 2008; for cat, Peterschmitt *et al*., 2009; for rabbit, Fontanesi *et al*., 2010; for human beings, Peng *et al*., 2001). Previous investigations of the *MC1R* gene focused on its variation in single species and only on part of the Coding region (CDS) and little attention was paid to multiple species especially to its evolution and differentiation on the complete CDS within and among species. The silver fox, a variant of the red fox (*Vulpes vulpes*) is a close relative of the dog (*Canis familiaris*) (Kukekova *et al*., 2004). Complete coding sequences of red fox and artic fox have issued in GenBank database while that of silver fox have not been reported so far.

In this study, the coding region sequence of the silver fox *MC1R* gene was obtained by direct sequencing and aligning. The total number of 119 *MC1R* gene sequences with the complete CDS from 23 species were studied to investigate its evolution and differentiation within and among species.

MATERIALS AND METHODS

DNA extraction, primer design and PCR amplification: Genomic DNA from spleen samples of a silver fox was isolated according to the standard phenol: Chloroform Extraction method. The 1057 bp fragment of *MC1R* gene was amplified with a pair of primers designed by primer 3 (http:/frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) based on red fox MC1R sequence (X90844). The forward and reverse primers were 5'-AGCCAGGGGTAAGTGG CCGC-3' and 5'-AAGACCC TG CCTGGCTCCTGCT-3'),

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Table 1: *MC1R* gene sequences of 23 species

Species	Common name	N	Length (bp)	GenBank accession No.	Stop codon
Vulpes vulpes	Red fox and Siver fox	2	954	X90844, from silver fox (this study)	TGA
Vulpes lagopus	Arctic fox	2		AJ786717, AJ786718	TGA
Canis lupus familiaris	Dog	10	954	NM_001014282, GU233655, GU233656, GU220378, GU220379, AF064455, JF501537, JF501538, JF501539, JF501540	TGA
Nyctereutes procyonoides	Raccoon dog	1	954	HM852533	TGA
Felis catus	Domestic cat	9	954	NM_001009324, FM877776, FM180571, AY237395, JF501541, JF501542, JF501543, JF501544, JF501545	TGA
Panthera onca	Jaguar	2	954	AY237396, AY237397	TGA
Lama pacos	Alpaca	5	954	FJ517582, FJ502229, FJ502230, EU220010, EU135880	TGA
Equus caballus	Horse	2	954	NM_001114534, AF288357	TGA
Homo sapiens	Human	19	954	AB598376, AB598377, AB598378, AB598379, AB598380, AB598381, NM_002386, EU151499, AY363619, AY363620, AY363621, AY363622, AY363623, AY363624, AY363625, AY363626, AY363627, AY225228, AF529884	TGA
Pan paniscus	Chimpanzee	7	954	AB598371, AB598372, AB598373, AB598374, AB598375, AB296236, AB296237	TGA
Hylobates lar	Common gibbon	2	954	AB296235, AY205089	TGA
Papio anubis	Olive baboon	2	954	NM_001164589, AY205104	TGA
Oryctolagus cuniculus	Rabbit	5	924, 948, 954	FN658679, FN658678, FN658677, FN658676, FN658675	TGA
Gallus gallus	Chicken	5	945	HQ699517, NM_001031462, AY220305, AY220304, AY220303	TAG
Cereopsis novaehollandiae	Cape Barren goose	1	945	FJ170063	TAG
Danio rerio	Zebrafish	4	972	NM_180970, AY161847, BC162848, BC162836	TAA
Mus musculus	House mouse	7	948	NM_008559, AB306322, BC119294, BC119296, AB177607, AB177608, AB177609	TGA
Rattus tanezumi	Oriental house rat	6	954	AB576595, AB576596, AB576597, AB576599, AB576600, AB576601	TGA
Camelus bactrianus	Bactrian camel	1	954	AB495001	TGA
Sus scrofa	Wild boar	5	963	DQ191188, GQ900668, GQ900669, GQ900670, GQ900671	TGA
Sus scrofa	Pig	11	963	GQ240943, FJ665469, FJ665471, FJ665473, FJ665474, FJ665475, FJ665479, EU443657, EU443644, AY916522, AY916519	TGA
Bos taurus	Cattle	4	954	NM_174108, GU982927, AF445641, AF445642	TGA
Capra hircus	Goat	1	954	FM212940	TGA
Ovis aries	Sheep	6	954	AM884255, AM884255, FN600553, FN600554, FN600555, FN600556	TGA

respectively and they were synthesized by Shanghai Sangon Biological Engineering and Technology services Co. Ltd. (Shanghai, China). The amplified fragment spanned bases from 10-1066 including part of 5' UTR, 3' UTR and the complete CDS.

PCR amplification was carried out in a PTC-100TM PCR instrument (MJ Research, Inc., Massachusetts, USA) with a total reaction volume of 30 μL. The reaction volume containing 4 μL (75 ng μL^{-1}) of silver fox genomic DNA, 3 μL of 10×PCR standard reaction buffer, 2.4 μL deoxynucleoside triphosphates (2.5 pmol L^{-1} of each deoxynucleotide), 1.2 μL (10 pmol L^{-1}) of each forward and reverse primer, 0.3 μL (5 U μL^{-1}) of Taq DNA Polymerase (TaKaRa Biotechnology Co. Ltd. Dalian, China) and 17.9 μL of distilled water. After predenaturation for 3 min at 94°C, the PCR profile consisted of a denaturation step at 94°C for 45 sec, an annealing step at 64°C for 45 sec and an elongation step at 72°C for 1 min for a total of 34 cycles followed by a final extension of 10 min at 72°C. PCR products were detected on 1.5% agarose gel including 0.5 μg mL^{-1} of ethidium bromide, photographed under UV light and sequenced by Shanghai Sangon Biological Engineering Technology, Biological and Technology and Service Co., Ltd. (Shanghai, China).

Sequence analysis and database search of *MC1R* gene: Sequences of the silver fox *MC1R* gene was examined and edited using the BioEdit Version 7.0.5.2 (Hall, 1999) and DNAMAN software. Searches for the other sequence similarity were performed with the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST). A total of 118 sequences with the complete CDS of the *MC1R* gene belonging to 23 species were searched from GenBank (Table 1). All the sequences were aligned using the Clustal W program implemented in BioEdit Version 7.0.5.2. The DnaSP Version 4.0 software (Rozas and Rozas, 1999) was used to analyze the Haplotype diversity (Hd), the average number of nucleotide differences (Tajima, 1983), the nucleotide diversity (π), synonymous nucleotide diversity (π_s), nonsynonymous nucleotide diversity (π_a), the polymorphic site (S), the Singleton variable sites (SP) and the Parsimony Informative sites (PIP) for each species and the average number of nucleotide substitutions per site between species (D_{xy}) (Lynch and Crease, 1990). The phylogenetic tree among species based on the D_{xy} was constructed by MEGA 4.0.2 Software using Unweighted Pair Group Method with Arithmetic mean (UPGMA) method, Neighbor Joining (NJ) method and Minimum Evolution (ME) method.

RESULTS AND DISCUSSION

PCR amplification, sequencing and alignment of silver fox *MC1R* gene: The 1057 bp fragment of silver fox *MC1R* gene was obtained by amplifying and sequencing (Fig. 1). The alignment results of the sequence with those of red fox (X90844), artic fox (allele wild-type, AJ786717) and artic fox (allele blue fox, AJ786718) revealed that the silver fox *MC1R* gene had a coding region of 954 bp which had 99.66% identity and the same length with red fox (X90844) and the artic fox (AJ786717, AJ786718) (Fig. 2). It is noteworthy that the silver fox was one of Canidae family.

Variation of stop codon: Three kinds of stop codon mutation were found in the *MC1R* gene within and among different species. Most species use TGA as stop codon for the *MC1R* gene with only the *Danio rerio* variation (NM_180970, AY161847, BC162848, BC162836) and the *Gallus gallus* variation (HQ699517, NM_001031462, AY220305, AY220304, AY220303) and *Cereopsis novaehollandiae* variation (FJ170063) of Phasianidae using TAA and TAG, repectively. There might be stop codon usage bias among species and for different genes (Ghosh *et al*., 2000). Kang *et al*. (2008) also found stop codon variation of the *LF* gene within and among different families and indicated that most species had the stop codon TGA and only *Gallus gallus* and *Cereopsis novaehollandiae* of Phasianidae and Danio rerio, respectively used TAG and TAA as the stop codon. Usually, the stop codon TAA has the highest stopping efficiency in translation because both the tripeptides of the release factor 1 and the release factor 2 can recognize the third stop codon UAA leading to translation stop (Robert, 2001).

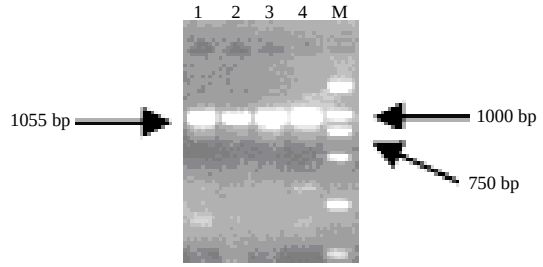

Fig. 1: PCR products (1057bp) of silver fox *MC1R* gene (1-4: PCR products; M: DL2000)

Sequence length variation among species: It was shown that the length of the *MC1R* gene with the complete CDS varies greatly among species, ranging from 924-972 bp (Table 1). Most of the analyzed species have a coding region of 954 bp for the *MC1R* gene but there is also length variation within and among species. *Danio rerio* had the longest CDS (972 bp) and conserved. The shortest CDS (924 bp) is one variant type of *Oryctolagus cuniculus* (FN658678) due to an 30 bp deletion of AGGCG GGCGCCTTGGCCGGCCGGGCCGCAG, from 305-334, a stretch that exists in all other sequences. Seven

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Silver fox (this paper)          1 bp   ATGTCTGGGCAGGGCCCCCAGAGAAGGCTGCTGGGCTCTCCCAATGCCACCTCCCCAACC  60 bp
Red fox (X90844)                        ATGTCTGGGCAGGGCCCCCAGAGAAGGCTGCTGGGCTCTCCCAATGCCACCTCCCCAACC
Arctic fox (allele wild-type, AJ786717)ATGTCTGGGCAGGGCCCCCAGAGAAGGCTGCTGGGCTCTCTCAATGCCACCTCCCCAACC
Arctic fox (allele blue fox, AJ786718) ATGTCTGGGCAGTGCCCCCAGAGAAGGCTGCTGGGCTCTCTCAATGCCACCTCCCCAACC
                                       *********** ************************** ********************

Silver fox (this paper)          121 bp CCCAACGGGCTGTTCCTCAGCCTGGGGCTGGTGAGCGTTGTGGAAAATGTGCTGGTGGTG 180 bp
Red fox (X90844)                        CCCAACGGGCTGTTCCTCAGCCTGGGGCTGGTGAGCGTTGTGGAAAATGTGCTGGTGGTG
Arctic fox (allele wild-type, AJ786717)CCCGACGGGCTGTTCCTCAGCCTGGGGCTGGTGAGCGTTGTGGAAAATGTGCTGGTGGTG
Arctic fox (allele blue fox, AJ786718) CCCGACGGGCTGTTCCTCAGCCTGGGGCTGGTGAGCGTTGTGGAAAATGTGCTGGTGGTG
                                       *** ******************************************************

Silver fox (this paper)          241 bp GCTGTGTCCGACCTGCTGGTGAGCGTGAGCAATGTGCTGGAGACGGCCGTCATGCTGCTG 300 bp
Red fox (X90844)                        GCTGTGTCCGACCTGCTGGTGAGCGTGACGAATGTGCTGGAGACGGCCGTCATGCTGCTG
Arctic fox (allele wild-type, AJ786717)GCTGTGTCCGACCTGCTGGTGAGCGTGACGAATGTGCTGGAGACGGCCGTCATGCTGCTG
Arctic fox (allele blue fox, AJ786718) GCTGTGTCCGACCTGCTGGTGAGCGTGACGAATGTGCTGGAGACGGCCGTCATGCTGCTG
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Silver fox (this paper)          361 bp GACGTGCTCATCTGTGGTTCCATGGTATCCAGCCTCTGCTTCCTGGGCGCCATCGCCGTG 420 bp
Red fox (X90844)                        GACGTGCTCATCTGTGGTTCCATGGTATCCAGCCTCTGCTTCCTGGGCGCCATCGCCGTG
Arctic fox (allele wild-type, AJ786717)GACGTGCTCATCTGTGGTTCCATGGTATCCAGCCTCTGCTTCCTGGGCGCCATTGCCGTG
Arctic fox (allele blue fox, AJ786718) GACGTGCTCATCTGTGGTTCCATGGTATCCAGCCTCTGCTTCCTGGGCGCCATTGCCGTG
                                       ************************************************** ******

Silver fox (this paper)          661 bp ATTGCCCGGCTCCGTAAGCGGCAGCACTCCGTCCACCAGGGCTTTGGCCTCAAGGGCGCT 720 bp
Red fox (X90844)                        ATTGCCCGGCTCCGTAAGCGGCAGCACTCCGTCCACCAGGGCTTTGGCCTCAAGGGCGCT
Arctic fox (allele wild-type, AJ786717)ATTGCCCGGCTCCGTAAGAGGCAGCACTCCGTCCACCAGGGCTTTGGCCTCAAGGGCGCT
Arctic fox (allele blue fox, AJ786718) ATTGCCCGGCTCCGTAAGAGGCAGCACTCTGTCCACCAGGGCTTTGGCCTCAAGGGCGCT
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Fig. 2: Alignment of *MC1R* gene coding region among silver fox, red fox (X90844), arctic fox (allele wild-type, AJ786717) and arctic fox (allele blue fox, AJ786718); variable sites were showed in panes

Table 2: Genetic diversity of the *MC1R* gene in 19 species

		Diversity parameter[b]								
Species[a]	Common name	h	H_d	K	π	π_s	π_a	S	SP	PIP
Vulpes vulpes	Red fox and Siver fox	2	1.000	2.000	0.0022	0.0020	0.0021	2	2	0
Vulpes lagopus	Arctic fox	2	1.000	2.000	0.0022	0.0040	0.0029	3	3	0
Canis lupus familiaris	Dog	4	0.644	8.067	0.0091	0.0137	0.0103	23	3	20
Felis catus	Domestic cat	4	0.833	1.278	0.0014	0.0030	0.0007	3	0	3
Panthera onca	Jaguar	2	1.000	3.000	0.0034	0.0000	0.0045	3	3	0
Lama pacos	Alpaca	4	0.900	3.000	0.0034	0.0016	0.0043	8	7	1
Equus caballus	Horse	1	0.000	0.000	0.0000	0.0000	0.0000	0	0	0
Homo sapiens	Human	15	0.942	1.778	0.0020	0.0033	0.0015	17	16	1
Pan paniscus	Chimpanzee	2	0.571	1.143	0.0013	0.0022	0.0025	4	0	4
Hylobates lar	Common gibbon	1	0.000	0.000	0.0000	0.0000	0.0000	0	0	0
Papio anubis	Olive baboon	1	0.000	0.000	0.0000	0.0000	0.0000	0	0	0
Oryctolagus cuniculus	Rabbit	3	0.7000	0.800	0.0009	0.0016	0.0006	2	2	0
Gallus gallus	Chicken	5	1.0000	4.2000	0.0047	0.0099	0.0026	8	3	5
Danio rerio	Zebrafish	2	0.667	0.667	0.0008	0.0022	0.0009	2	1	1
Mus musculus	House mouse	3	0.667	1.429	0.0016	0.0024	0.0012	3	1	2
Rattus tanezumi	Oriental house rat	2	0.533	0.533	0.0006	0.0022	0.0000	2	0	2
Sus scrofa	Wild boar Pig	8	0.883	1.967	0.0022	0.0020	0.0021	5	1	4
Bos taurus	Cattle	2	0.5000	1.500	0.0017	0.0020	0.0015	3	3	0
Ovis aries	Sheep	6	1.0000	3.467	0.0039	0.0026	0.0041	8	4	4

[a]*Nyctereutes procyonoides*, *Cereopsis novaehollandiae*, *Camelus bactrianus* and *Capra hircus* have no effective data and are not shown in Table 2; [b]h: Number of haplotypes; Hd: Haplotype diversity; K: Average number of nucleotide differences; π: Nucleotide diversity; π_s: Synonymous nucleotide diversity; π_a: Nonsynonymous nucleotide diversity; S: Number of polymorphic Sites; SP: Singleton variable sites and PIP: Parsimony Informative sites

sequences of *Mus musculus* (NM_008559, BC119294, BC119296, AB306322, AB177609, AB177608, AB177607) and one sequence of *Oryctolagus cuniculus* (FN658677) were all 948 bp but *Mus musculus* due to a 6 bp deletion from 52-57 bp, *Oryctolagus cuniculus* from 281-286 bp. All sequences of *Gallus gallus* and one sequence of *Cereopsis novaehollandiae* were 945 bp which had a deletion of 3 bp (from 669-671) besides a 6 bp deletion (from 52-57 bp).

Only *Sus scrofa* including five sequences of wild boar (DQ191188, GQ900668, GQ900669, GQ900670, GQ900671) and eleven sequences of pig (GQ240943, FJ665469, FJ665471, FJ665473, FJ665474, FJ665475, FJ665479,EU443657,EU443644,AY916522,AY916519)had an insertion of AACCAGACG encoding Asn-Gln-Thr from 94-102 bp and other species do not have this. No variations were also found in this insertion region. The length variation of the *MC1R* gene among species might result from evolution and differentiation.

At present, most reports have focused on the MC1R coat colour variation related to the amino acid mutation. Vage *et al*. (2005) discovered two mutations causing amino acid substitutions in the complete coding region of the *MC1R* gene by sequencing for arctic fox. In position 5, the glycin residue is substituted by a cysteine in the blue fox while a phenylalanine is replaced by a cysteine at position 280. These findings suggest that the MC1R/agouti regulatory system is involved in the seasonal changes of coat color found in arctic fox. Peterschmitt *et al*. (2009) reported that amber colour is caused by a single MC1R allele called e (c. 250G>A→p. Asp84Asn) in the Norwegian Forest cat. Dreger and Schmutz (2010) discovered a new mutation called E^G in the MC1R sequence of several Saluki. The mutation was a g.233G>T substitution resulting in an amino acid change from a glycine to valine at residue 78 (p.Gly78Val) (GenBank GU220379).

Polymorphism and genetic diversity within and among species: The alignment of 115 sequences within the region of 989 bp and containing gaps was carried out using BioEdit. The results of DnaSP analysis showed that the selected region (1-989) of the 115 sequences from different species have 891 sites excluding sites with gaps (98). There were 326 invariable sites and 565 variable sites that include 11 singleton variable sites and 554 parsimony informative sites. The nucleotide diversity (π = 0.1565) and the average number of nucleotide differences (K = 139.48) for all sequences were higher than the highest values in *Canis lupus familiaris* (π = 0.0091, K = 8.067). Distinct differentiation of the species could be concluded based on the high genetic diversity of the *MC1R* gene. The polymorphic information and haplotype diversity of the *MC1R* gene for each species were shown in Table 2. *Canis lupus familiaris* had the largest number of total mutations (23), parsimony informative sites (20), average number of nucleotide differences (8.067), nucleotide diversity (0.0091), synonymous nucleotide

Table 3: Average nucleotide substitutions per site (D_{xy})

Species	*V. vulpes*	*V. lagopus*	*C. lupus familiaris*	*F. catus*	*P. onca*	*L. pacos*	*E. caballus*	*H. sapiens*	*P. paniscus*	*H. lar*	*P. anubis*	*O. cuniculus*	*G. gallus*	*D. rerio*	*M. musculus*	*R. tanezumi*	*S. scrofa*	*B. taurus*
V. lagopus	0.007																	
C. lupus familiaris	0.012	0.012																
F. catus	0.087	0.089	0.087															
P. onca	0.088	0.090	0.088	0.014														
L. pacos	0.139	0.139	0.139	0.107	0.101													
E. caballus	0.140	0.138	0.139	0.116	0.116	0.123												
H. sapiens	0.155	0.155	0.154	0.138	0.135	0.141	0.144											
P. paniscus	0.153	0.153	0.153	0.134	0.132	0.136	0.141	0.019										
H. lar	0.146	0.146	0.145	0.133	0.132	0.146	0.145	0.046	0.043									
P. anubis	0.143	0.140	0.142	0.133	0.131	0.139	0.137	0.056	0.055	0.058								
O. cuniculus	0.169	0.171	0.168	0.142	0.140	0.154	0.147	0.141	0.140	0.147	0.143							
G. gallus	0.282	0.286	0.284	0.258	0.256	0.260	0.275	0.263	0.261	0.263	0.268	0.261						
D. rerio	0.376	0.373	0.377	0.382	0.376	0.383	0.382	0.386	0.381	0.381	0.386	0.388	0.362					
M.musculus	0.202	0.200	0.200	0.179	0.180	0.171	0.164	0.193	0.191	0.189	0.180	0.180	0.295	0.395				
R. tanezumi	0.190	0.190	0.189	0.168	0.166	0.159	0.165	0.181	0.182	0.174	0.174	0.173	0.287	0.392	0.080			
S. scrofa	0.146	0.146	0.146	0.115	0.111	0.102	0.127	0.129	0.126	0.130	0.132	0.141	0.252	0.387	0.188	0.173		
B. taurus	0.162	0.161	0.163	0.145	0.143	0.130	0.156	0.151	0.151	0.158	0.149	0.171	0.261	0.388	0.197	0.184	0.130	
O. aries	0.158	0.158	0.158	0.133	0.129	0.117	0.152	0.142	0.141	0.147	0.143	0.166	0.256	0.391	0.194	0.181	0.121	0.045

diversity (0.0137) and nonsynonymous nucleotide diversity (0.0103) which showed that *Canis lupus familiaris* had the highest genetic diversity. Usually, the more abundant the genetic diversity of species is the more useful for the artificial selection. The higher genetic diversity of the *MC1R* gene in *Canis lupus familiaris* might be related to their extensive adaptability and survival for a polyembryonic animal. The haplotype diversity of *MC1R* gene for each species was shown in Table 2. *Vulpes vulpes*, *Vulpes lagopus*, *Panthera onca*, *Gallus gallus* and *Ovis aries* had the largest haplotype diversity (1.000) indicating abundant genetic diversity in those species. No mutation was detected in *Equus caballus*, *Hylobates lar* and *Papio anubis* according to the comparison within corresponding species which might be due to the limited range of the samples.

DNA divergence and phylogenetic analysis: The average number of nucleotide substitutions per site (D_{xy}) of the *MC1R* gene between species was shown in Table 3. D_{xy} is the index of DNA divergence between or among the sequences. The larger the D_{xy} is the larger the genetic distance is. Based on D_{xy}, a phylogenetic tree was constructed for all the species using UPGMA, NJ and ME method, respectively and obtained the same cluster results (Fig. 3). The divergence time among different species was also labeled on the scale bar calculated from the average nonsynonymous nucleotide rate (0.85×10^{-9} $year^{-1}$, Li and Graur, 1991). The phylogenetic tree of all species was basically consistent with the taxonomy of NCBI. *Vulpes vulpes*, *Vulpes lagopus* and *Canis lupus familiaris*, *Felis catus* and *Panthera onca*,

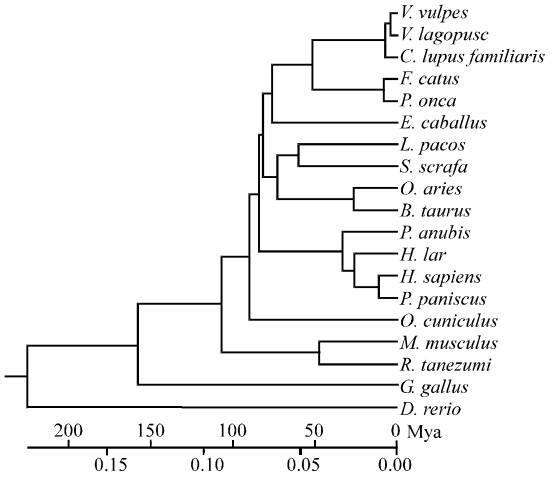

Fig. 3: Phylogenetic tree of the *MC1R* gene among 19 species. Mya = Million years ago

Lama pacos and *Sus scrofa*, *Ovis aries* and *Bos taurus*, *Homo sapiens*, *Pan paniscus*, *Hylobates lar* and *Papio anubis*, *Mus musculus* and *Rattus tanezumi* fall into one small clade, respectively. The smallest D_{xy} (0.007) and divergence time (about 5 Mya) showed the closest relationship between *Vulpes vulpes* and *Vulpes lagopus* (belong to Canidae) which basically consistent with that of Zhong *et al.* (2010). Zhong *et al.* (2010) analyzed 12 concatenated heavy-strand protein-coding genes and discovered that arctic fox was the sister group of red fox and they both belong to the red fox-like clade in family Canidae. *Felis catus* and *Panthera onca* (belong to Felidae) had smaller D_{xy} which was 0.014. The D_{xy} (0.045) and divergence time (about 26 Mya) between *Ovis aries* and *Bos taurus* (belong to Bovidae) is basically

consistent with that of Nikaido *et al.* (2001) and Kang *et al.* (2008). Nikaido *et al.* (2001) pointed out that the divergence time between *Bos taurus* and *Ovis aries* was 30.9 Mya based on an analysis of mtDNA. Kang *et al.* (2008) reported the divergence time among *Ovis aries*, *Capra hircus* and *Bos taurus* ranged approximately from 10-35 Mya. The average D_{xy} (0.040) of the branch containing *Homo sapiens*, *Pan paniscus*, *Hylobates lar* and *Papio anubis* (all belong to Hominidae) was relatively larger, *Homo sapiens* and *Pan paniscus* clustered first and then *Hylobates lar* and *Papio anubis* was added successively. The divergence time of the *MC1R* gene between *Homo sapiens* and *Pan paniscus* was about 10 Mya (Fig. 3). The result basically accords with that of Nie *et al.* (2008), Kang *et al.* (2008), Yang and Yoder (2003) and Wildman *et al.* (2003). Nie *et al.* (2008) discovered that the closest relationship existing between human and Chimpanzee by constructing the un-rooted phylogenetic tree of *MC1R* gene for 10 animal species.

Kang *et al.* (2008) estimated the divergence time of *Homo sapiens* and *Pan troglodytes* (about 6 Mya) by analysing bioinformation of *Lactoferrin* gene for several species. Yang and Yoder (2003) analyzed two mitochondrial protein-coding genes, cytochrome oxidase II and cytochrome b to estimate the divergence times of human and chimpanzee (7.1 Mya). Wildman *et al.* (2003) discovered that the coding DNA of nuclear divergence separated from the most recent common ancestor of human and chimpanzee about 5 or 6 million years ago. The close relationship between *Lama pacos* (belong to Camelidae) and *Sus scrofa* was also in accordance with the results of Yang *et al.* (2004), Tang *et al.* (2006) and Kang *et al.* (2008). Both Yang *et al.* (2004) and Tang *et al.* (2006) showed that the comparability of cDNA sequences was highest between the pig and the camel by alignment of the full-length sequences of gene caBD21 cDNA of camel, pig, cattle and sheep.

As is known, camel is also one of the Camelidae. Kang *et al.* (2008) pointed out that *Camelus dromedarius* and *Sus scrofa* had the close relationship based on an analysis of *LF* gene. The divergence time between *Lama pacos* and *Sus scrofa* is about 62 Mya. Delsuc *et al.* (2004) compared nuclear genomic data of *Lama* and *Sus scrofa* to show the reconstruction of phylogeny and evolutionary time (62 Mya).

The divergence time was 68.2 Mya based on analysis of mitochondrial genomes of *Lama* and *Sus scrofa* (Nikaido *et al.*, 2001). *Mus musculus* and *Rattus norvegicus* had the higher D_{xy} (0.080) and the larger divergence time (about 48 Mya) which is consistent with the result of Wang *et al.* (2007). The largest D_{xy} (0.395) and divergence time (about 250 Mya) displayed the earliest differentiation between *Danio rerio* and *Mus musculus*.

CONCLUSION

In this study, the coding sequence of the silver fox *MC1R* gene was obtained and the length was 954 bp. Most species use TGA as stop codon for the *MC1R* gene. The length of the *MC1R* gene with the complete CDS varies greatly from 924-972 bp and most of the species had 954 bp. Observed genetic diversity was higher among species than within species and *Canis lupus familiaris* had more polymorphisms than any other species. The reconstructed phylogenetic among species tree was basically consistent with the taxonomy in the National Center for Biotechnology Information.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (No. 31040048) and Special Fund for Agro-scientific Research in the Public Interest (200903014-07).

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