GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Brief Note

Variability in the cathelicidin 6 (*CATHL-6*) gene in Tianzhu white yak from Tibetan area in China

G.X. E, R.S. Na, Y.J. Zhao, L.P. Chen, X.Y. Qiu and Y.F. Huang

College of Animal Science and Technology,
Chongqing Key Laboratory of Forage & Herbivore,
Chongqing Engineering Research Centre for Herbivores Resource Protection and Utilization, Southwest University, Chongqing, China

Corresponding author: Y.F. Huang
E-mail: H67738337@swu.edu.cn

Genet. Mol. Res. 14 (2): 3129-3132 (2015)
Received September 29, 2014
Accepted January 30, 2015
Published April 10, 2015
DOI http://dx.doi.org/10.4238/2015.April.10.23

ABSTRACT. Cathelicidins are a major family of antimicrobial peptides (AMPs), an important component of innate immune system, playing a critical role in host defense and disease resistance in virtually all living species. Polymorphism and functional studies on cathelicidin of Tianzhu white yak contribute to understanding the specific innate immune mechanism in animals living at high altitudes in comparison to cattle and domesticated white yak. Thirty-six individuals of Tianzhu white yak, originating from the area of three ecotypes (Gansu in China), were investigated. The total length of the aligned Yak cathelicidin 6 (*CATHL-6*) sequences was 1923 bp, including six single nucleotide polymorphisms and one indel. Ten haplotypes were identified, and phylogenetic analyses resolved those 10 haplotypes in two clusters. The results indicate that the white yak originated from two domestication sites. In addition, lack of significant pairwise difference between sequences (Tajima's D = 0.92865, P > 0.10) in the

CATHL-6 region indicates absence of population size expansion in current white yak population.

Key words: Tianzhu white yak; Antimicrobial peptides; Cathelicidins (CATHL)

INTRODUCTION

The population of the Tianzhu white yak from Tibetan area of Gansu Province in China inhabits the upper slopes at 3000 m altitude, in the alpine steppe ecoregion. It constitutes a large and commercially important species of domestic animals from the genus *Bos* (Leslie Jr. and Schaller, 2009). Cathelicidins are a major family of antimicrobial peptides, an important component of innate immune system playing a critical role in host defense and disease resistance in virtually all living species (Hancock and Sahl, 2006; Takahashi et al., 2010). Polymorphisms and functional properties of cathelicidin in cattle have been the subject of many studies (Das et al., 2006; Gillenwaters et al., 2009). Identification of cathelicidin members will further our understanding of the specific innate immune mechanism in animals living at high altitude in comparison to cattle and domesticated white yak.

MATERIAL AND METHODS

Investigations were carried out in 36 individuals representing three ecotypes each represented by 12 individuals. The three ecotypes originate from three villages in the area of Gansu in China: Duoshixiangheigou village (37°18.0425' N 103°2.1588' E), Zhaxixiulongxiang village (37°11.7309' N 102°46.9758' E), and Xidatan village (37°16.6396' N 103°10.1656' E). Screening for polymorphisms was performed after direct sequencing of PCR products. Information about primers see Table 1, and applied PCR protocol as following: each 50-µL PCR contained 2 ng template DNA, 5 µL 10X PCR buffer, 1.25 U TransStart Taq DNA polymerase (TransGen Biotech, Inc., Beijing, China), 4 µL dNTP (2.5 µM, including 25 mM $MgCl_2$), 1.5 µL of each primer (10 µM), and added ddH_2O to a 50-µL final volume.

Table 1. PCR primer information of yak *CATHL-6* region.

Name	Sequences (5'-3')	Function
Yak-CATHL-6F	AGGCAAGTCCACCTCACATC	Amplifies complete gene region
Yak-CATHL-6R	CAGAATCCAAAAGCCTGAGC	Amplifies complete gene region
Yak-H-2R	GTCCAGCCCTGGGCTTGGTGCCG	Used for sequencing
Yak-H-3R	CCTATCACCCTAGCGGTTCCTGC	Used for sequencing

Primers were designed using Primer3 (http://frodo.wi.mit.edu/primer3/).

The PCR procedure consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 64.5°C for 1 min, and extension at 72°C for 2 min, and completed by an incubation period at 72°C for 15 min. Amplified DNA products were electrophoresed on 1-2% agarose gels and sequenced on an ABI3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA) following the manufacturer protocol.

Screening for haplotypes and the Tajima test were conducted by DnaSP5.10 (Librado and Rozas, 2009; http://www.ub.edu/dnasp/). Phylogenetic analyses based on neighbor-join-

ing algorithm were carried out to determine the evolutionary relationships of the haplotypes using the MEGA 5.0 software (Tamura et al., 2011).

RESULTS

The total length of the aligned Yak *CATHL-6* sequences was 1923 bp including six single nucleotide polymorphisms (C>T at 695 bp/1st intron, C>T at 723 bp/1st intron, G>A at 765 bp/1st intron, G>A at 943 bp/2nd intron) as well as two non-synonymous substitutions, C>T (p.Arg>Trp) at 142 bp/1st exon and A>G (p.Glu>Gly) at 1743 bp/4th exon, and one in-del (G>Δ at 457 bp/1st intron), using the GenBank sequence KM514704 as a reference. Ten haplotypes were identified using the seven sites given above in the CATHL-6 gene of 36 white yaks, and their frequencies supplemented with the data available in the GenBank database are given in Table 2. In addition, these 10 haplotypes formed two clusters on the phylogenetic tree (Figure 1).

Table 2. Structure and frequency of the *CATHL-6* genomic haplotype in white yaks.

Hap_No.	Site location (from the initial codon, ATG)							Frequency	GenBank accession No.
	142	457	695	723	765	943	1743		
H_1	C	Δ	C	T	G	A	A	42	KM514696
H_2	C	C	T	T	G	A	G	1	KM514697
H_3	T	G	T	T	A	G	A	13	KM514698
H_4	T	Δ	C	T	G	A	A	1	KM514699
H_5	C	Δ	C	T	G	G	A	1	KM514700
H_6	C	G	C	T	G	A	A	2	KM514701
H_7	C	G	C	T	G	G	A	7	KM514702
H_8	T	G	C	T	G	G	A	1	KM514703
H_9	T	G	C	T	A	G	A	1	KM514704
H_10	C	Δ	C	C	G	A	A	3	KM514705

Hap_No.: haplotype number; Δindicates deletion.

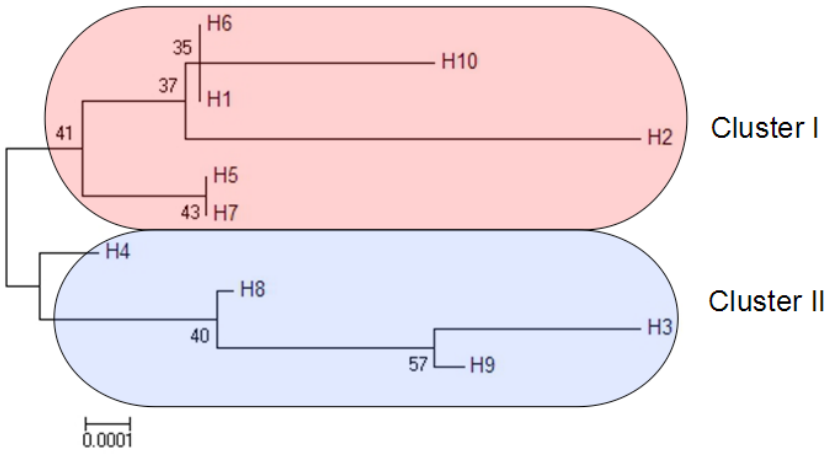

Figure 1. Neighbor-joining phylogenetic network of white yak *CATHL-6* haplotypes using MEGA6.0.

We did not detect any significant pairwise difference between the sequences (Tajima's D = 0.92865, P > 0.10) in the *CATHL-6* region, indicating the absence of population size expansion in the current white yak population.

DISCUSSION

The two clusters from the *CATHL-6* haplotype suggest that the white yak originated from two domestication sites.

ACKNOWLEDGMENTS

Research supported by the Fundamental Research Funds for the Central Universities (#SWU114023) and the 2013 Innovation Team Building Program in Chongqing Universities (#KJTD201334).

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