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Haplogroup Classification of Korean Cattle Breeds Based on Sequence Variations of mtDNA Control Region

Jae-Hwan Kim, Seong-Su Lee, Seung Chang Kim, Seong-Bok Choi, Su-Hyun Kim[1], Chang Woo Lee[2], Kyoung-Sub Jung[3], Eun Sung Kim[4], Young-Sun Choi[5], Sung-Bok Kim[6], Woo Hyun Kim[7], and Chang-Yeon Cho*
Animal Genetic Resources Research Center, NIAS, RDA, Namwon 55717, Korea

ABSTRACT: Many studies have reported the frequency and distribution of haplogroups among various cattle breeds for verification of their origins and genetic diversity. In this study, 318 complete sequences of the mtDNA control region from four Korean cattle breeds were used for haplogroup classification. 71 polymorphic sites and 66 haplotypes were found in these sequences. Consistent with the genetic patterns in previous reports, four haplogroups (T1, T2, T3, and T4) were identified in Korean cattle breeds. In addition, T1a, T3a, and T3b sub-haplogroups were classified. In the phylogenetic tree, each haplogroup formed an independent cluster. The frequencies of T3, T4, T1 (containing T1a), and T2 were 66%, 16%, 10%, and 8%, respectively. Especially, the T1 haplogroup contained only one haplotype and a sample. All four haplogroups were found in Chikso, Jeju black and Hanwoo. However, only the T3 and T4 haplogroups appeared in Heugu, and most Chikso populations showed a partial of four haplogroups. These results will be useful for stable conservation and efficient management of Korean cattle breeds. (**Key Words:** Haplogroup, mtDNA, Control Region, Korean Cattle Breed, Phylogenetic Tree)

INTRODUCTION

Cattle were introduced from North China to the Korean peninsula and Japan around A.D. 200 (Kim and Lee, 2000). Three cattle heads with brown, brindle or black coat colors, are estimated to be the origin of the Korean cattle breeds (Na, 2008). At present, four cattle breeds, Hanwoo, Chikso, Heugu, and Jeju black, exist in Korea, and are classified based on their different coat colors and geographical distribution (Suh et al., 2014). These breeds have been documented in the Domestic Animal Diversity Information System (DAD-IS) of the United Nations Food and Agriculture Organization (FAO; http://dad.fao.org/). Because the three Korean native cattle breeds, except for Hanwoo, were recently recognized as possessing valuable genetic resources in Korea, molecular studies were performed on them using genetic markers for characteristic evaluation.

Various molecular genetic markers have been used to determine genetic diversity, molecular evolution and genetic relationship within and among cattle breeds (Horsburgh et al., 2013; Ludwig et al., 2013; San et al., 2014; Tu et al., 2014; Xin et al., 2014; Hristov et al., 2015). Of these, mitochondrial DNA (mtDNA) contains a maternal genetic pattern, and is a very useful marker for evaluating the origin and gene flow of modern cattle breeds. Especially,

* Corresponding Author: Chang-Yeon Cho. Tel: +82-63-620-3520, Fax: +82-63-620-3590, E-mail: bloodtype@korea.kr
[1] Gyeonggi Livestock and Veterinary Service, Suwon 16381, Korea.
[2] Gangwon Provincial Livestock Research Center, Hoengseong 25266, Korea.
[3] Chungbuk Institute of Livestock and Veterinary Research, Cheongwon 28153, Korea.
[4] Jeonbuk Institute of Livestock and Veterinary Research, Gimje 54324, Korea.
[5] Jeonnam Agricultural Research and Extension Services, Gangjin 59213, Korea.
[6] Chungnam Institute of Livestock Experiment Research, Cheongyang 33350, Korea.
[7] Gyeongbuk Livestock Research Institute, Yeongju 36052, Korea.
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this marker has been used for the haplogroup classification of modern and extinct cattle (Gou et al., 2010; Dadi et al., 2012; Gravlund et al., 2012). At present, 6 haplogroups (T, T1, T2, T3, T4, and T5) have been reported among Europe, Africa, and Near East, and Asia cattle populations (Troy et al., 2001; Mannen et al., 2004; Achilli et al., 2008). The T3 haplogroup is distributed mainly in Europe and Asia, whereas T1 is found in Africa, T and T2 in the Near East, and T4 in the Northeast.

Korean cattle have been classified into the T2, T3, and T4 haplogroups, but mainly into the T3 haplogroup (Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). However, these studies did not classify the haplogroup at the breed level for Korean cattle. Therefore, the aims of the present study were to analyze the mtDNA control region sequence and to classify the mtDNA haplogroup of each Korean cattle breed.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 288 animals (182 Chikso, 20 Heugu, and 86 Jeju black) were used in this study. Chikso samples were collected from 6 Management Institutes, Gyeonggi Livestock and Veterinary Service (GG), Gangwon Provincial Livestock Research Institute (GW), Chungbuk Institute of Livestock and Veterinary Research (CB), Jeonbuk Institute of Livestock and Veterinary Research (JB), Chungnam Institute of Livestock Experiment Research (CN), and Jeonnam Agricultural Research and Extension Services (JN). Heugu and Jeju black samples were collected from CB and the Jeju Special Self-Governing Province Livestock Promotion (JJ), respectively. All samples were collected from each institute for detailed identification of haplogroups among the populations. The sequences for the Hanwoo breed were obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/), distributed in 6 regions througthout Korea (Jeju, Jungeup, Cheongwon, Incheon, Youngju, and Yeoju) (AY337521-26, AY337529-41, AY337543-46). Genomic DNA was extracted from blood using the DNeasy Blood Kit (Qiagen, Hilden, Germany). The concentration of genomic DNA was measured using a model ND 1000 spectrometer (Thermo Scientific, Waltham, MA, USA).

DNA amplification and sequencing

Two primer sets were designed using the sequences for tRNA-Pro, the control region, and tRNA-Phe, according to the mtDNA genome sequences (GenBank accession no. V00654). The sequences of the two primer sets were as follows:

Set 1: 5′- ACCCCCAAAGCTGAAGTTCT -3′ and 5′- AGATGAGATGGCCCTGAAGA-3′;

Set 2: 5′- GGGTCGCTATCCAATGAATT -3′ and 5′- GCATTTTCAGTGCCTTGCTT -3′.

Polymerase chain reaction (PCR) was carried out using 2.5 μL of 10× reaction buffer, 0.2 mM of dNTP, 1.5 mM $MgCl_2$, 1.5 units of *Taq* DNA polymerase (Takara, Tokyo, Japan), 10 mM of each primer, and 10 ng of genomic DNA in a final volume of 25 μL. The PCR amplification was performed using a PTC-200 (MJ Research, Waltham, MA, USA) under the following conditions: 35 cycles of 30 s at 94°C, 30 s at 63°C, and 60 s at 72°C. After purification of the PCR products using the QIAEX II Gel Extraction Kit (Qiagen, Germany), nucleotide sequencing was carried out by direct-sequencing with an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). The complete sequences of the complete control region were determined based on the complete sequences of the *Bos taurus* mtDNA genome sequence (Genbank accession no. V00654) as reported by Anderson et al. (1982). The determined sequences were deposited in the GenBank database (GenBank accession no. KR857524-74).

Data analysis

The sequences of the control region from four Korean cattle breeds were aligned in the CLUSTAL W program (Thompson et al., 1994) in the BioEdit software (Hall, 1999). Sites representing gaps in any of the aligned sequences were excluded from subsequent analyses. Identification of haplotypes was carried out using DNA sequence polymorphism version 5.1 (Librado and Rozas, 2009). Haplogroup classification was performed based on several previous reports (Troy et al., 2001; Achilli et al., 2008; Mannen et al., 2004). Genetic distances between haplotypes were estimated using the substitution model of Tamura and Nei (1993). The neighbor-joining (NJ) tree (Saitou and Nei, 1987) among haplotype sequences was reconstructed using the MEGA 5.05 package (Tamura et al., 2011).

RESULTS AND DISCUSSSION

In this study, 288 complete sequences of the mtDNA control region were determined. In additions, 30 Hanwoo sequences were obtained from the GenBank database. The entire length of the control region ranged from 908 to 912 bp, because two insertion/deletion mutation sites were found at nucleotide positions 216 to 221 and 352 to 363 in the complete control region sequences (GenBank accession no. V00654). These regions contained two poly-C tracts, and the mutations were excluded from subsequent analyses. 71 polymorphic sites were detected and 66 haplotypes were classified (Figure 1). Of these, 7 (H1, H4, H6-8, H10, H39) were major haplotypes that contained more than 10 sequences (Table 1). In addition, breed- and population-specific sequences were found.

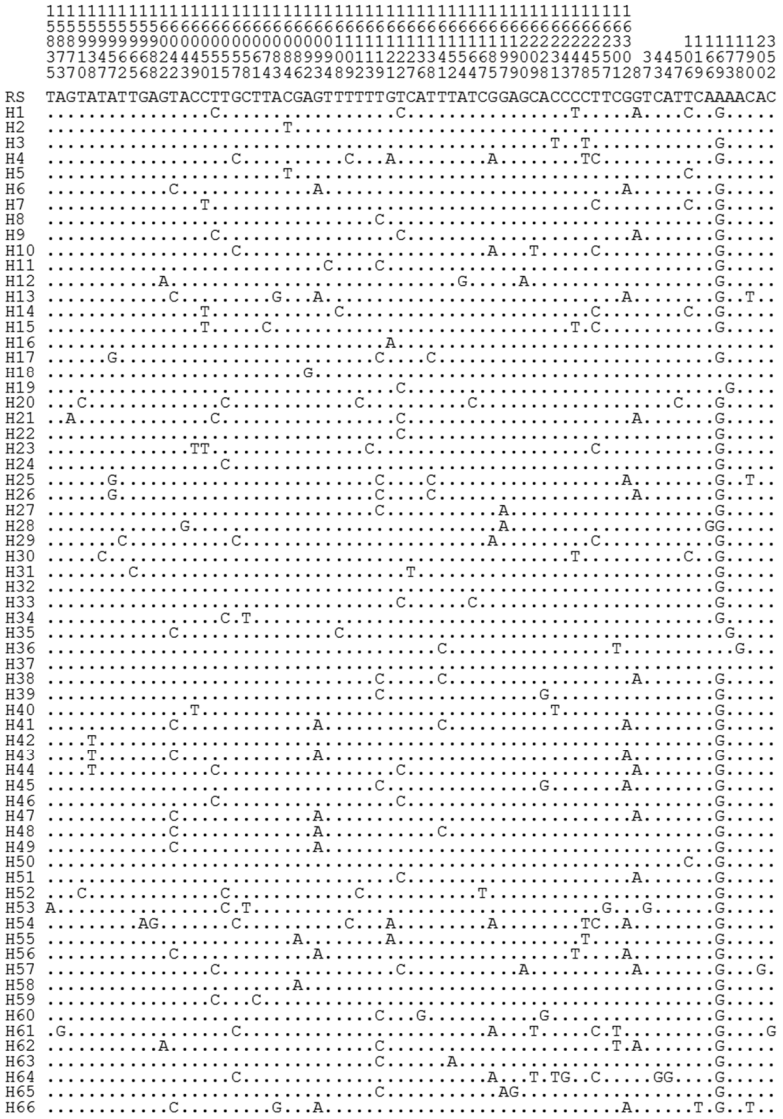

Figure 1. Sequence variations of 66 haplotypes in the mtDNA control region of four Korean cattle breeds. Mutations are scored relative to RS (GenBank accession no. V00654) of Anderson et al. (1982). Dots identify the RS sequence.

Table 1. i) Distribution of 66 haplotypes in four Korean cattle breeds based on sequence variations of the mtDNA control region

Haplotypes	Breeds										Total	Accession no.
	Chikso							Heugu	Jeju Black	Hanwoo		
	GW	CB	JB	GG	CN	JN	Sub-total					
H1	23					1	35				35	KR857525
H2	8						8				8	KR857543
H3	4						4				4	KR857529
H4	11						11				11	KR857544
H5	3						3				3	KR857535
H6	2	9				7	18	2	16	3	39	KR857553
H7		9					9		7	2	18	KR857574
H8		4			3		7	14		2	23	KR857524
H9		3					3		4		7	KR857552
H10		6				1	7		3	1	11	KR857547
H11		3					3				3	KR857530
H12		2					2				2	KR857555
H13		3					3				3	KR857540
H14		1	3	1			5				5	KR857532
H15			7				7				7	KR857527

Table 1. ii) Distribution of 66 haplotypes in four Korean cattle breeds based on sequence variations of the mtDNA control region (Continued)

Haplotypes	Breeds										Total	Accession no.
	Chikso							Heugu	Jeju Black	Hanwoo		
	GW	CB	JB	GG	CN	JN	Sub-total					
H16			2				2				2	KR857534
H17			7		1		8				8	KR857570
H18			3				3				3	KR857541
H19			4			1	5				5	KR857536
H20			1				1				1	KR857565
H21			7	1			8				8	KR857564
H22			3				3		5	1	9	KR857572
H23			1				1				1	KR857526
H24			1				1				1	KR857537
H25			1				1				1	KR857557
H26			1				1				1	KR857561
H27			2	2			4				4	KR857531
H28				1			1				1	KR857538
H29				1			1				1	KR857568
H30				1			1				1	KR857546
H31				5			5				5	KR857528
H32					1	4	5		3		8	KR857563
H33						2	2				2	KR857542
H34						1	1			2	3	KR857550
H35						1	1				1	KR857533
H36					2		2				2	KR857539
H37								4			4	KR857554
H38									8		8	KR857545
H39									18	1	19	KR857573
H40									9		9	KR857569
H41									3		3	KR857560
H42									1		1	KR857548
H43									1		1	KR857562
H44									1		1	KR857567
H45									1		1	KR857556
H46									1		1	KR857551
H47									1		1	KR857559
H48									1		1	KR857558
H49									1		1	KR857549
H50									1		1	KR857566
H51									1		1	KR857571
H52										1	1	AY337546
H53										1	1	AY337545
H54										1	1	AY337543
H55										1	1	AY337540
H56										1	1	AY337539
H57										1	1	AY337537
H58										4	4	AY337535
H59										1	1	AY337532
H60										1	1	AY337530
H61										1	1	AY337526
H62										1	1	AY337525
H63										1	1	AY337524
H64										1	1	AY337523
H65										1	1	AY337522
H66										1	1	AY337521
Total	51	43	51	12	7	18	182	20	86	30	318	

GW, Gangwon Provincial Livestock Research Center; CB, Chungbuk Institute of Livestock and Veterinary Research; JB, Jeonbuk Institute of Livestock and Veterinary Research; GG, Gyeonggi Livestock and Veterinary Service; CN, Chungnam Institute of Livestock and Veterinary Research; JN, Jeonnam Agricultural Research and Extension Services.

Consistent with the genetic pattern reported by Mannen et al. (2004) and Jia et al. (2010), four haplogroups (T1, T2, T3, and T4) were identified in Korean cattle breeds (Figure 2). T and T5 haplogroups have been defined by a transition at positions 16255 and 163 in reference sequences (RS, V00654), respectively (Troy et al., 2001; Achilli et al., 2008; Jia et al., 2010). However, these haplogroups were absent in this study. The T4 haplogroup was differentiated from T3 by a transition at position 16042, 16093, and 16302. Moreover, the T2 haplogroup was differentiatedd from T by a transition at position 16057 and a transversion at position 16185. T1, the African cattle specific haplogroup, was identified by a transition at position 16050 and 16113. In addition, T1a, a sub-haplogroup reported by Jia et al. (2010) and Achilli et al. (2008), differed in only one position, 16050.

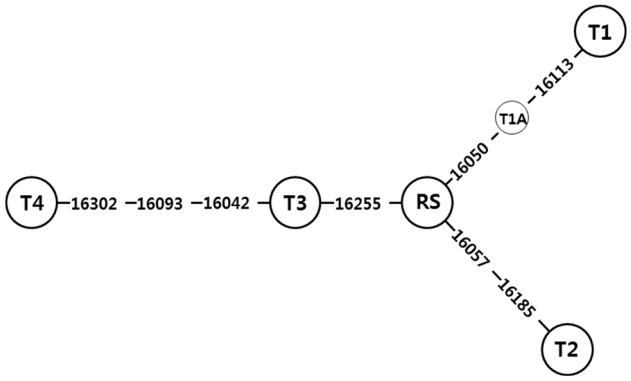

Figure 2. Genetic structure of haplogroups found in Korean cattle breeds. The numbers between haplogroups are variation sites that correspond to RS (GenBank accession no. V00654). T1a, a sub-haplogroup of T1, is shown as smaller circle than the haplogroups.

The phylogenetic tree was constructed using 66 haplotype sequences (Figure 3). This phylogenetic pattern was compared with the classified haplogroups. Each haplogroup formed an independent cluster. The T3 haplogroup was classified into T3a and T3b sub-haplogroups by previous reports (Achilli et al., 2008; Jia et al., 2010). In this study, T3a and T3b were differentiated by a mutation pattern at position 169. Partial haplotypes contained in the T3b sub-haplogroups showed distinct mutation patterns in three positions (16122, 16055, and 16119). The T4 haplogroup was separate from the 3a sub-haplogroup, and this phylogenetical pattern agreed with previous reports (Achilli et al., 2008; Jia et al., 2010). The T1 haplogroup and the T1a sub-haplogroup were located together in an independent group.

Several studies have reported the frequency and

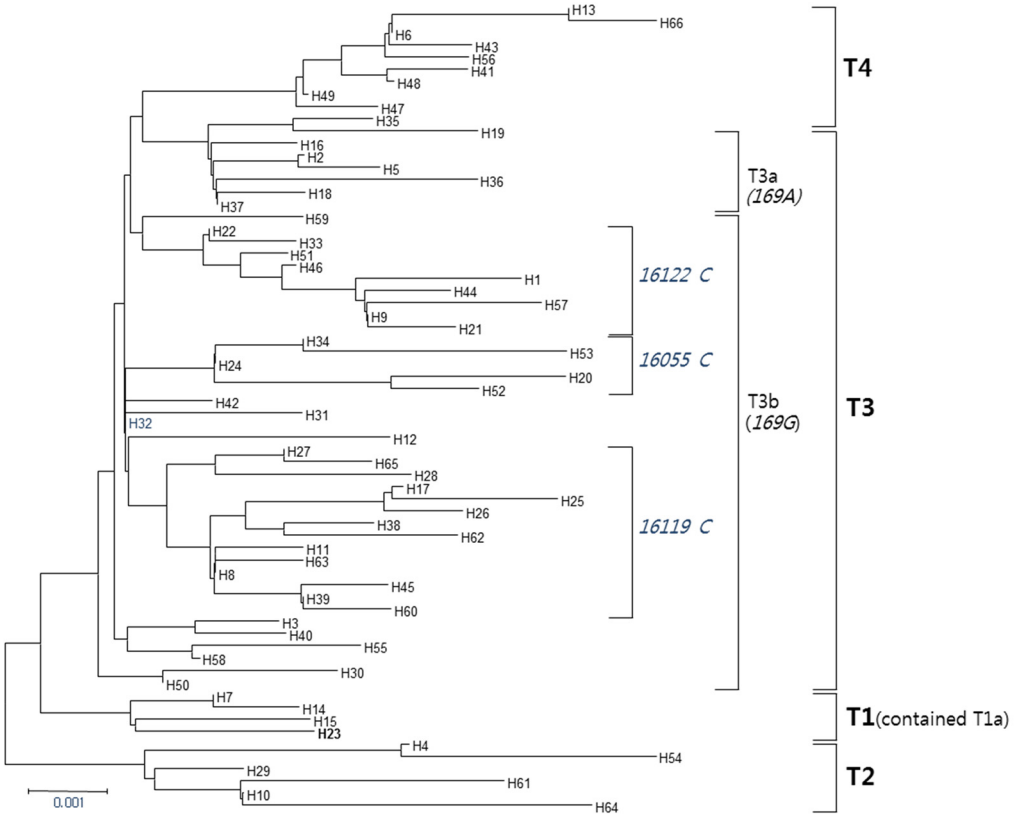

Figure 3. Neighbor-joining tree of 66 haplotypes in the mtDNA control region of four Korean cattle breeds. These haplotype were classified into four main lineages: T1 (containing T1a), T2, T3, and T4. T3a and T3b are sub-haplogroups of the T3 haplogroup according to published results (Jia et al., 2010). The italic numbers and letters in the brackets are variation sites and substituted nucleotides relative to RS (GenBank accession no. V00654), respectively.

Table 2. Frequencies of haplogroups found in Korean cattle breeds/populations

Breeds (populations)		Sample sizes	Haplogroups				
			T1	T1a	T2	T3	T4
Chikso*	Sub-total	182	1(0.01)	22(0.12)	19(0.10)	119(0.65)	22(0.12)
	GW	51			11	38	2
	CB	43		10	6	15	12
	JB	51	1	10		40	
	GG	12		1	1	10	
	CN	7				7	
	JN	18			1	9	8
Heugu		20				18(0.90)	2(0.10)
Jeju Black		86		7(0.08)	3(0.03)	53(0.62)	23(0.27)
Hanwoo		30		2(0.07)	4(0.13)	19(0.63)	5(0.17)
Total		318	1(>0.01)	30(0.10)	26(0.08)	209(0.66)	52(0.16)

GW, Gangwon Provincial Livestock Research Center; CB, Chungbuk Institute of Livestock and Veterinary Research; JB, Jeonbuk Institute of Livestock and Veterinary Research; GG, Gyeonggi Livestock and Veterinary Service; CN, Chungnam Institute of Livestock and Veterinary Research; JN, Jeonnam Agricultural Research and Extension Services.

distribution of haplogroups among various cattle breeds in East Asia (Mannen et al., 2004; Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). T3 was confirmed as the major haplogroup in Chinese cattle. On the other hand, the T4 haplogroup predominates (about 65%) in Japanese cattle with approximately two fold higher frequency than T3. In Korean cattle, the frequency of the T3 haplogroup ranged from 69% to 83% and was the highest among the haplogroups. In this study, the haplotype distribution was confirmed among four Korean native cattle breeds (Table 2). The frequencies of T3, T4, T1 (containing T1a), and T2 were 66%, 16%, 10%, and 8%, respectively. The frequency of the T3 haplogroup was 66%, which was lower than that of several previous reports (Mannen et al., 2004; Lai et al., 2006; Sasazaki et al., 2006; Jia et al., 2010). This difference may be explained by the number of samples and the haplogroups evaluated. Previous reports used a small number of samples (30 to 108). In addition, the T1 haplogroup and the T1a sub-haplogroup were found in only reports by Jia et al. (2010). Because we used over three times the number of samples, we estimate that our results more accurately reflect the haplogroup frequency in Korean cattle than previous reports.

T1 haplogroup contained only one haplotype and one sample (Table 2). Lei et al. (2006) investigated 231 animals from 20 Chinese native cattle breeds/populations. They reported that the T1 haplogroup was identified in only one animal from the Yanbian and Zaosheng breeds. Kim et al. (2013a, b) reported the phylogenetic relationship between Korean cattle breeds and these the two Chinese breeds. These results suggest that Korean cattle breeds and two Chinese breeds have similar maternal genetic patterns.

All four haplogroups were found in Chikso, Jeju black, and Hanwoo. However, only the T3 and T4 haplogroups appeared in Heugu. This breed is raised only one Management Institute, and the population size is small. Hanwoo was different from the other breeds, with a higher frequency of the T2 haplogroup than the T1 haplogroup. In the Chikso breed, only the CB population contained all haplogroups. However, most Chikso populations showed a partial of four haplogroups. To maintain the genetic diversity of Chikso in the event of various environmental changes, exchange and use of genetic materials among populations is necessary.

In conclusion, we detected sequence variations in the mtDNA control region in Korean cattle breeds, and performed haplogroup distribution at the breed level. These results will be useful for stable conservation and efficient management of Korean cattle breeds. Besides, genetic characteristics based on mtDNA analysis might serve as a source for identification of the maternal origins of cattle, containing Korean cattle breeds.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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