Relationship between Genetic Variants of Mitochondrial DNA and Growth Traits in Hanwoo Cattle

G. J. Jeon*, H. Y. Chung, J. G. Choi, M. S. Lee, C. W. Lee, J. J. Park, J. M. Ha, H. K. Lee[1] and H. H. Sung
Hanwoo Experiment Station, National Livestock Research Institute, Kangwon-do 232-952, Korea

ABSTRACT : Genetic variants of Hanwoo mtDNA in the region of cytochrome oxidase subunit I, II and III complex were detected using restriction enzymes. PCR primers were designed based on the bovine mtDNA sequence, and 6 primer sets (Mt4, Mt5, Mt6, Mt7, Mt8 and Mt9) were used. A total of 20 restriction enzymes were used, and 6 restriction enzymes, which were Hinf I, Pvu II, Rsa I, Eco RI, Bgl II, and Msp I, showed genetic polymorphisms. Significant associations between genetic variants and weight traits were observed at WT15 ($p<0.05$) and WT18 ($p<0.01$) with Pvu II for Mt9, Bgl II for Mt6 and Rsa I for Mt8 segments in the region of cytochrome oxidase subunit complex. Significant associations were also observed at Mt9-Pvu II and Mt6-Bgl II segments for WT9 ($p=0.01$), WT12 ($p=0.02$), respectively. These results suggest that genetic variants of mtDNA in the region of cytochrome oxidase subunit complex may be candidate segments for improvement of animal growth as weight traits. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 301-307)***

Key Words : mtDNA, Cattle, Polymorphism, Growth Traits

INTRODUCTION

Mitochondrial (mt) DNA, which is maternally inherited and non-recombining patterns in the nature of animals, is closed circular double helix DNA and the length of the sequence is approximately 16,500 bp (Brown, 1980). MtDNA encodes for 13 hydrophobic polypeptides, 22 tRNAs and 2 rRNAs, which are related to respiratory-chain and oxidative phosphorylation systems (Anderson et al., 1981). The respiratory chain is a series of 5 multi subunit enzyme complexes located on the inner mitochondrial membrane. Most of the mitochondrial protein-coding genes, particularly cytochrome subunits, as well as rRNAs, have been used in phylogenetic or population genetic studies.

Interesting findings were focused on the genetic or non-genetic differences on the cytochrome subunits in mtDNA because these subunits may be related to aging and muscle development process. Harman (1972) and Fleming (1985) were the first to postulate that mitochondria may play a role in the aging process. The aging hypothesis of mitochondria proposes that aging results from the accumulation of detrimental mitochondrial DNA mutations during life (Linnane et al., 1989). In human skeletal muscle, muscle fibers that lack cytochrome oxidase activity also appear and accumulate in an age related manner (Muller et al., 1990; Brieiley et al., 1996). Several reports have described a decrease in mitochondrial respiratory enzyme activities in human skeletal muscle (Trounce et al., 1989; Cooper et al., 1992; Boffoli et al., 1994; Boffoli et al., 1996) and liver (Yen et al., 1989) with aging. Therefore, genetic variants in the region of cytochrome oxidase subunits may explain different levels of cytochrome subunit activities, which may effect on early muscle development as well as aging. Marin et al. (1998) also reported that bovine cardiac mitochondrial function significantly increased in mitochondrial oxidative phosphorylation and mitochondrial gene expression during the early stages of growth and development. Mitochondrial respiratory function also decreased during aging (Marin et al., 1994). Therefore, genetic differences on the region of cytochrome oxidase subunit in mtDNA may explain some variation of growth and aging process.

To further understand the regulation of mitochondrial function and biogenesis from early to late muscle development and aging, point mutations in the cytochrome oxidase subunit region of mtDNA were aimed to analyze genetic effects on animal growth as weights.

* Corresponding Author: Gi Jun Jeon. Tel: +82-33-330-0613, Fax: +82-33-330-0660, E-mail: jeon7257@rda.go.kr
[1] Hankyong National University, Seokjongdong 67, Ansung-si, Kyonggi-do, 456-749, Korea.
Received May 5, 2004; Accepted November 13, 2004

MATERIALS AND METHODS

Animals

Two hundred thirty one Korean native steers, which were part of the 33rd progeny test in 2002 and 143 bulls, were used from Hanwoo Experiment Station of the National Livestock Research Institute (NLRI). The cattle were fed a postweaning corn and soybean meal diet, which was formulated to meet NRC (1984) requirements for growing beef cattle.

Sample preparation

Total DNA was prepared from EDTA-blood samples of all animals from Hanwoo Experiment Station of National Livestock Research Institute. The high salt procedure including proteinase K and SDS lysis steps was adapted for the DNA extraction. DNA was ethanol precipitated and resuspended in distilled water.

Table 1. Primer sequences for the regions of the cytochrome oxidase subunits (COX) I, II and II of the mitochondrial DNA

Primer	Sequence	Position	Region
Mt5F[1]	ATAGCCCCATTTCACTTC	4,587-4,604	COX I
Mt5R[2]	CGTTGTAGATTTGGTCGTC	5,834-5,852	
Mt6F	TTGGGCCGGTATAGTAGGAACAGC	5,781-5,758	COX II
Mt6R	TCGTCGAGGCATGCCAGATAGTC	6,981-7,003	
Mt7F	CGACGATACTCCGACTAC	6,998-7,015	COX II
Mt7R	TTTTATAATATTGACGCAGAT	8,039-8,059	
Mt8F	GTCCAGGCTTATATTACGGTCAA	7,936-7,958	COX III
Mt8R	TAGGCCAATTATTAGCAGGGTCAT	9,087-9,110	
Mt9F	CTAATCGGAGGAGCTACACTTG	8,806-8,827	COX III
Mt9R	ATTTATTATTCTTTTTCGGACTA	9,806-98,281	

[1] Forward primer in mitochondrial DNA cytochrome oxidase subunit region.
[2] Reverse primer in mitochondrial DNA cytochrome oxidase subunit region.

Design of primers

In Table 1, the primers for the cytochrome oxidase subunit I (COX I), II (COX II) and III (COX III) region in the mtDNA were designed based on the bovine mtDNA sequence (GenBank accession number, J01394). For the optimal size of the PCR fragments in the analysis procedures of single strand conformation polymorphisms (SSCP) or restriction fragment length polymorphism (RFLP), length of the PCR products were mediated to be expected around 1,000 bp to 1,200 bp.

Polymerase chain reaction

Polymerase chain reaction (PCR) was conducted with a final volume of 20 μl, including 2 μl of 10 × reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 10 μM dNTP, 10 pM of each primer, 50 ng of genomic DNA, and two units of Taq DNA polymerase. After denaturation for 2 min at 95°C, PCR cycles for the mt primers were adapted to 94°C for 45 sec for denaturation, 57°C for 1 min for annealing, and 72°C for 1.5 min for polymerization (MJ research P-200).

Restriction enzyme digestion

Six microliter aliquots from each PCR reaction mixture were used for restriction endonuclease digestion using 2 units of enzymes under the conditions suggested by the manufacturers. The enzyme used in this experiment were: Alu I, Hae III, Rsa I, Sca I, Msp I, Bgl I, Bgl II, Hinf I, Bam HI, Hind III, Pst I, Pvu II, Hae II, Xho I, Hph I, Kpn I, Apa I, Eco RI, Sma I and Taq I (Promega). These restriction enzymes showed sufficient activity to be used directly in the PCR mixture. Six microliters of the reaction solution for each digestion were loaded on 2% LE agarose gels. Restriction fragments were separated by electrophoresis at 4 V/cm for 1h. The gels were stained with ethidium bromide and photographed under UV light. All the Mt primer sets showed restriction enzyme sites, and therefore, SSCP analysis, which is a powerful tool to detect any single mutation site, was not performed.

Weight traits

Weights were recorded at birth (BW), 6 months of age (WT6), 9 (WT9), 12 (WT12), 15 (WT15) and 18 (WT18) during progeny testing period.

Statistical analysis

Least squares means and standard errors were determined for all measurements with a model including fixed effects of the cytochrome oxidase subunit genotypes, parity, and castration, and a covariate for age of animal. Analysis of variance was conducted using Statistical Analysis System (SAS) general linear models (GLM) procedures, and least squares means were compared using Fisher's least significant difference test (SAS, 1985) with a comparison error rate of 0.05. A total of eight mtDNA segments were genotyped, and all allele effect for the segments was analyzed separately.

The model was

$$Y = \mu + g_i + p_j + c_k + bX_{ijkl} + e_{ijkl}$$

where, Y_{ijkl} = dependent variables,
μ = overall mean,
g_i = fixed effect of the i^{th} allele genotype (A and B),
p_j = fixed effect of the j^{th} parity (1, 2, 3, 4 and 5),
c_k = fixed effect of the k^{th} castration (1 and 2),
bX_{ijkl} = covariate of dependent variable y on age
e_{ijkl} = residual error

RESULTS

Genetic variants

Genetic variants of mtDNA were revealed by restriction endonuclease analysis. A total of 20 restriction enzymes (Alu I, Hae III, Rsa I, Sca I, Msp I, Bgl I, Bgl II, Hinf I, Bam HI, Hind III, Pst I, Pvu II, Hae II, Xho I, Hph I, Kpn I, Apa I, EcoR I, Taq I and Sma I) were used. All the restriction enzymes had more than 2 restriction cleavage sites for the region of cytochrome oxidase subunit in

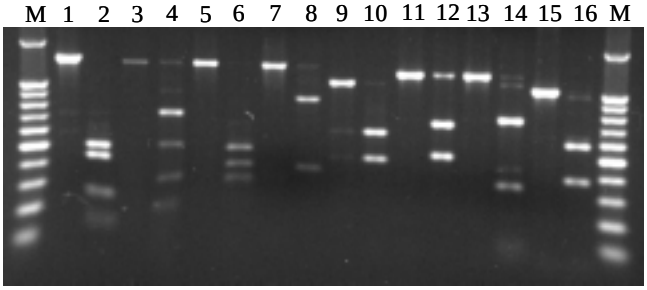

Figure 1. Restriction fragment length polymorphism patterns for Hanwoo mtDNA using 8 restriction enzymes (M: size marker, lane 1 and 2: mt5 digested by Hinf I, lane 3 and 4: mt6 digested by Rsa I, lane 5 and 6: mt6 digested by Msp I, lane 7 and 8: mt6 digested by Bgl II, lane 9 and 10: mt7 digested by EcoR I, lane 11 and 12: mt8 digested by Hinf I, lane 13 and 14: mt8 digested by Rsa I, lane 15 and 16: mt9 digested by Pvu II).

Table 2. Number of restriction fragments with each enzyme digestion of polymerase chain reaction amplicons for the region of cytochrome oxidase subunit in Hanwoo mtDNA

	Primer sets					Reaction
Enzyme	Mt5[1]	Mt6	Mt7	Mt8	Mt9	temperature
Pst I	-	-	-	2	-	37°C
Hae III	-	-	2	2	4	37°C
Rsa I	3	3	-	2	3	37°C
Alu I	3	2	-	2	2	37°C
Bgl II	-	2	-	-	-	37°C
Pvu II	-	-	-	-	2	37°C
Eco R I	-	-	2	-	-	37°C
Sca I	2	-	3	-	2	37°C
Msp I	2	-	-	-	-	37°C
Hinf I	5	3	4	2	2	37°C
Hph I	2	-	2	3	3	37°C
Taq I	-	2	2	-	3	65°C

[1] mitochondrial DNA priming region for cytochrome oxidase subunit I (Mt5), II (Mt6 and Mt7) and III (Mt8 and Mt9).

Hanwoo mtDNA (Figure 1). Eight out of 20 restriction enzymes (Bgl I, Bam HI, Hind III, Hae II, Xho I, Kpn I, Apa I and Sma I) were found no restriction cleavage sites in the region of cytochrome oxidase subunit of Hanwoo mtDNA. Restriction sites were simulated using mtDNA sequences from GenBank to compare the differences of sequences and restriction sites for our findings. The results were corresponding to the results from simulated restriction sites using the sequences from GenBank. Otherwise, the restriction enzymes (Hae III, Alu I, Sca I, Hinf I, Hph I and Taq I) had restriction cleavage sites on mtDNA without genetic variants. Genetic variants were detected by Rsa I, Msp I, Bgl II, EcoR I, Pst I, Pvu II restriction enzymes from Mt 5 through Mt 9 segments (Table 2).

Genotyping of mtDNA

For genotyping of Hanwoo mtDNA in each segment, two alleles were detected in all restriction cleavage experiments using 2% agarose gel electrophoresis, designated A or B, which were existence of restriction sites or not by base substitution. Allele frequencies were estimated for all segments (Table 3). Allele frequencies by Rsa I restriction endonuclease for Mt6 (COX II region) and Mt8 (COX III region) segments showed similar results.

Table 3. Allele frequencies of mtDNA restricted by Rsa I, Msp I, Bgl II, Eco RI, Hinf I, and Pvu II restriction enzymes in the region of cytochrome oxidase subunit in Hanwoo mtDNA

Segment	Allele	Frequency	Segment	Allele	Frequency
Mt5-Hinf I[1]	A	0.85	Mt7-EcoR I	A	0.15
	B	0.15		B	0.85
Mt6-Rsa I	A	0.72	Mt8-Rsa I	A	0.58
	B	0.28		B	0.42
Mt6-Msp I	A	0.84	Mt8-Hinf I	A	0.13
	B	0.16		B	0.87
Mt6-Bgl II	A	0.12	Mt9-Pvu II	A	0.45
	B	0.89		B	0.55

[1] Mitochondrial DNA segment of cytochrome oxidase subunit I restricted by restriction endonuclease.

Effects of genotypes

Statistical significant effects for the weight traits were detected by several restriction segments in the region of cytochrome oxidase subunits in Hanwoo mtDNA (Table 4). Mt9 segment in the region of COX III restricted by Pvu II enzyme showed genetic variation and significant differences among genotypes for WT9 ($p<0.05$), WT15 ($p<0.05$) and WT18 ($p<0.01$). Significant association between MT6-Bgl II segment and weight traits ($p<0.05$ at WT12, $p<0.05$ at WT15 and $p<0.01$ at WT18) in the region of COX I. Mt8 restricted by Rsa I ($p<0.01$) segments also explained some variations in WT15 and WT18 as shown in Table 5. No significant genotype effects were observed at Mt5 and 7 segments. Significant genetic effects for weight traits at the late age stages were detected, but there was no significant genotype effect detected at the early age stages.

DISCUSSION

The nucleotide sequence data for polymorphic sites in mtDNA have been widely used for the studies of molecular evolution, the genetic structure of populations and the estimation of genetic relationships between and within in species (Liu et al., 2004). Therefore, genetic studies for understanding of genetic functions related to particular phenotypic traits are necessary using mitochondrial DNA because of their fluent genetic variants, which may explain genetic diversity of animals. Therefore, a couple of protocols for finding genetic variants were applied using molecular genetics technologies. Restriction fragment length polymorphism (RFLP) is one of the easiest methods to detect genetic variants for unknown regions, and therefore, restriction enzymes were used to detect genetic

Table 4. Least squares for ANOVA for birth weight, weight on 6, 9, 12, 15 and 18 month by genotypes including parity and castration for the region of cytochrome oxidase subunit in Hanwoo mtDNA

Segment	MS											
	df	BW	df	WT6	df	WT9	df	WT12	df	WT15	df	WT18
Mt5-Rsa Ⅰ	1	0.009	1	2,157.779	1	3,477.868	1	4.9670	1	0.390	1	627.940
Error	287	6.827	263	920.682	245	1,066.055	227	1,474.201	156	1,416.735	128	2,015.606
Mt6-Rsa Ⅰ	1	7.272	1	27.066	1	64.73	1	2.272	1	164.777	1	2.802
Error	105	6.656	93	935.734	89	983.034	82	1,307.409	62	1,417.322	52	1,736.194
Mt6-Msp Ⅰ	1	0.508	1	97.165	1	5.769	1	758.466	1	364.257	1	130.759
Error	311	6.828	277	936.728	266	1,111.820	248	1,519.034	174	1,539.033	142	2,032.637
Mt6-Bgl Ⅱ	1	0.225	1	1856.421	1	1,588.191	1	7,702.716*	1	12,186.517*	1	33,632.569**
Error	319	6.793	284	924.267	272	1,103.710	253	1,471.499	178	1,446.730	146	1,798.156
Mt7-EcoR Ⅰ	1	12.902	1	73.696	1	323.975	1	0.484	1	147.935	1	0.001
Error	190	7.320	170	966.136	161	1,135.453	153	1,498.520	100	1,534.549	81	2,029.083
Mt8-Rsa Ⅰ	1	24.963	1	49.929	1	800.276	1	2,108.509	1	9,898.851**	1	24,451.807**
Error	114	8.215	107	915.730	100	1,107.608	92	1,614.076	69	1,369.545	59	1,594.118
Mt8-Pst Ⅰ	1	0.129	1	50.946	1	1.147	1	433.640	1	408.477	1	691.403
Error	234	6.406	214	909.827	205	1,113.035	189	1,406.267	131	1,425.798	111	2,037.058
Mt9-Pvu Ⅱ	1	18.198	1	3011.701	1	6,233.872**	1	7,335.916	1	7,762.767*	1	15,795.512**
Error	84	7.709	77	919.396	96	916.929	73	1,169.260	48	1,318.279	43	2,018.675

*,** Denotes that the genotype effect on corresponding trait was statistically significant ($p<0.05$, 0.01).

variants in Hanwoo mtDNA using a single digestion method. A double digestion method, however, was not conducted because actual restriction sites had to be proved by sequencing to compare exact base differences between our sequences and previous reported sequences. As the abundant polymorphic DNA, many genetic variants in mtDNA using restriction enzymes were reported for cattle by Laipis et al. (1982), Watanabe et al. (1989), Bhat et al. (1990) and Amano et al. (1994). The first mtDNA polymorphisms between and within a single lineage of Holstein cows were reported by Laipis et al. (1982). Our results for restriction patterns and enzymes were similar to previous studies. From our results, mtDNA polymorphisms were detected by restriction enzymes (Pst I, Pvu II, Rsa I, Eco RI, Bgl II and Msp I), showing genetic differences in the region of cytochrome oxidase subunit I, II and III in Hanwoo. Watanabe et al. (1985) have used 17 restriction enzymes to find genetic variants in mtDNA, and they observed genetic differences using Hind III, Taq I and Msp I restriction enzymes. Later, the same group reported more genetic variants in mtDNA using Bam HI, Bgl II, EcoR V, Hind III, Pst I and Sca I in Philippine cattle. Bhat et al. (1990) also reported genetic variants in mtDNA using Bam HI, Bgl II, Hind III, Hpa I, Pst I and Ava II in Holstein and Indian water buffalo. Chung et al. (1995) also found polymorphism using Pst I, Sca I and Hpa I in Holstein. Genetic variants in our findings, however, might not be the same restriction sites with many previous studies because previous reports did not make any location of the restriction cleavage sites on the mtDNA. The previous studies were focused on finding genetic variants at the whole mt genome or D-loop region, which contains many mutation sites, and also, concerned with construction of physical maps for mtDNA. Even though genetic polymorphisms using same restriction enzymes were found comparing with previous studies, the exact cleavages sites might not be the same. Therefore, because this experiment was aimed to find genetic variants in the specified regions, which were the cytochrome oxidase subunit I, II and III of the mitochondrial DNA in Hanwoo, comparisons for the detection of restriction sites were improper.

To compare restriction sites at the region of cytochrome oxidase in this study with the published sequence data from NCBI, cleavage sites were generated for mtDNA sequences based on Bos Taurus mtDNA sequences from GenBank accession number (J01394). Forty-one different types of restriction enzymes having 214 cleavage sites in the region of cytochrome oxidase were simulated. All the restriction cleavage sites detected in this experiment were belonged to the 214 of simulated cleavage sites, which were generated by computer programs except for Msp I restriction site. The mtDNA sequence reported in GenBank did not have Msp I restriction sites on the region of cytochrome oxidase complex I, II and III. It may be explained by hypotheses that either Hanwoo has different genetic composition comparing with Bos Taurus cattle or the Msp I restriction site is an SNP (single nucleotide polymorphism). Even though Hanwoo is in a phylogenetic line of Bos Taurus for the evolutional relationship, some part of genetic constitution may differ.

Mitochondria have been suggested as being responsible for genetic variation in cytogenetic effects on traits of

Table 5. Least squares means and standard errors for birth weight, weight on 6, 9, 12, 15 and 18 month by genotypes including parity and castration for the region of cytochrome oxidase subunit in Hanwoo mtDNA

Segment	Allele	BW (kg)	WT6 (kg)	WT9 (kg)	WT12 (kg)	WT15 (kg)	WT18 (kg)
		p=0.97	p=0.13	p=0.72	p=0.95	p=0.98	p=0.57
Mt5-Hinf I[1]	a	25.02±0.41	166.35±5.68	234.25±6.17	314.51±7.49	381.39±9.69	456.21±11.07
	b	25.52±0.62	163.30±7.68	230.09±8.50	314.95±9.10	393.01±12.41	477.80±12.93
		p=0.27	p=0.86	p=0.79	p=0.96	p=0.73	p=0.90
Mt6-Rsa I	a	24.93±0.60	164.82±7.24	237.99±7.60	310.78±9.05	389.30±21.56	474.50±52.25
	b	22.37±2.25	138.25±26.22	219.43±26.33	282.29±39.92	430.30±.81	477.80±12.84
		p=0.78	p=0.74	p=0.94	p=0.48	p=0.62	p=0.80
Mt6-Msp I	a	24.44±0.41	162.44±5.10	226.05±5.22	313.62±7.91	392.07±9.89	472.29±12.48
	b	26.73±1.39	166.82±16.55	238.18±15.65	310.28±30.14	418.56±48.70	540.79±50.67
		p=0.85	p=0.18	p=0.23	p=0.02	p=0.04	p=0.01
Mt6-Bgl II	a	24.22±0.64	160.83±8.06	222.00±8.33	328.28±20.54*	420.34±16.68*	466.23±24.40*
	b	24.93±0.47	167.77±6.15	236.04±5.97	291.39±14.25	381.71±10.73	473.69±13.70
		p=0.18	p=0.78	p=0.59	p=0.95	p=0.75	p=0.99
Mt7-EcoR I	a	24.92±1.39	164.55±11.44	231.15±16.47	312.37±22.85	373.52±43.46	441.01±31.13
	b	24.76±0.76	164.16±6.77	241.69±8.38	331.54±11.89	420.17±15.19	487.33±11.16
		p=0.08	p=0.81	p=0.39	p=0.25	p=0.01	p=0.01
Mt8-Rsa I	a	25.23±0.60	159.40±5.48	231.27±6.36	306.33±9.98	389.58±7.33*	452.16±17.06*
	b	28.39±3.49	135.27±29.47	221.20±31.37	259.64±47.38	329.76±28.84	432.47±45.85
		p=0.86	p=0.81	p=0.97	p=0.57	p=0.59	p=0.56
Mt8-Hinf I	a	24.90±0.62	159.97±8.48	230.96±8.411	301.01±19.46	381.23±19.26	479.77±22.66
	b	25.65±0.43	162.33±5.93	235.22±6.33	287.22±14.91	371.13±16.40	460.41±20.24
		p=0.12	p=0.07	p=0.01	p=0.09	p=0.02	p=0.01
Mt9-Pvu II	a	24.74±0.50	156.19±5.84	224.23±6.28*	300.60±13.49	376.66±11.37*	454.94±16.21**
	b	25.36±0.51	164.25±5.55	239.36±6.02	304.58±12.35	409.44±9.75	492.12±13.80

[1] Mitochondrial DNA segment of cytochrome oxidase subunit restricted by restriction endonuclease.
*,** Denotes that the genotype effect on corresponding trait was statistically significant ($p<0.05, 0.01$).

economic importance because they possess DNA and cytogenetic inheritance. Therefore, it is possible that genetic variants of the mtDNA may be a useful molecular marker for genetic improvement of beef cattle. Faust et al. (1989) suggested that cytogenetic effects of mtDNA could affect animal growth as well as reproduction. Therefore, finding genetic differences within breed should be done in mtDNA as well as genomic DNA. Especially, the region of cytochrome oxidase subunit may be a candidate segment for muscle development and aging because the mitochondria are the main source of energy in the cell. Aging hypothesis proposes that aging results from the accumulation of detrimental mitochondrial DNA mutations during life (Linnane et al., 1989). If the detrimental mitochondrial DNA mutations during life in the region of cytochrome oxidase subunit are inferred to animal production at certain stages of aging and growth, they would cause cellular and tissue dysfunction.

From the genotype effects on weight traits, if there were significant genotype effects on weight traits in a certain growing stage, we may expect that significances from early to late growing stages because genotype is not changed from young to old like proteins are turned over. However, no significant genotype effects were detected at early growing stages even though significant genotype effects were observed in the late of growing stages. If mtDNA polymorphism is highly related to the aging process of animals, then genetic variants can affect in the late of growing stages rather than early growing stages. Therefore, we may expect that significant genetic effects on growth traits may be observed in the late age stages. In this study, genotypic effects on weight were found in the late age stages from WT15 and WT18. However, it is still unclear that these results are from either hypothesis of mtDNA mutation correlated to animal aging and development process or statistical differences of different rate of gene expression for individuals in the late growing stages. Mannen et al. (1998) stated that growing stages from birth to 18 month in animal are varied, and therefore, higher individual variation would be expected in beef cattle.

MtDNA is more vulnerable to damage than nuclear DNA, and therefore, mutation rate comparing with nuclear DNA is 10 times higher. The reason is that mtDNA lacks protective histones, and has few and inefficient repair mechanisms as well as a high rate of turnover. Therefore, numerous mitochondrial DNA mutations (Lin et al., 2004), which are nucleotide deletions in general, have been demonstrated to appear and accumulate with age in a variety of animal tissues. Also, mitochondrial DNA shows increased damage with age. Muller et al. (1990) and

Brierley et al. (1996) reported that muscle fibers that lack cytochrome oxidase activity also appear and accumulate in an age related manner in human skeletal muscle. If mtDNA polymorphisms in the region of cytochrome oxidase are highly related to aging and growth process of animals, the genetic variants can be directly used in the breeding area as genetic markers because all the genetic facts for mtDNA are well known unlikely genomic DNA. However, we believe that the root of the growth and aging process are almost certainly multifactorial events. Even though our research purpose was aimed to detect genetic differences in mtDNA that may effect on growth and aging, major problems are still remained unclear.

The cytochrome oxidase subunit is too great to be explained from the very low levels of mitochondrial DNA mutations. In general, many studies reported that high frequency of mutation sites are around D-loop region. Elizabeth et al. (1997) insisted that mitochondrial DNA is extremely recessive and high levels of mutated mtDNA must be present before there is either a biochemical or clinical abnormality in cells. Our results may be reached to the same aspects with high levels of mutation in the region of cytochrome oxidase subunit. Consequently, with further investigation, our finding suggested that mtDNA mutation in the region of cytochrome oxidase subunit might be a factor to explain growth of animals at different developmental stages in Hanwoo population.

CONCLUSION

Genetic variants of the mtDNA for the cytochrome oxidase subunit I and III significantly influenced weight on late developmental stages, but genotypes from cytochrome oxidase subunit II did not explain significant variation in weight traits. As many previous studies suggested that mtDNA polymorphisms have been linked to differences in performance of certain traits in beef cattle, our findings indicate that mtDNA segments in the region of cytochrome oxidase subunit may be used as a candidate segment for weight traits for beef cattle. Consequently, results of the present study, and future genotypic data from these animals, based on variation in the mtDNA segments, will provide critical information of genetic improvement as a source of candidate genes. Even though some preliminary results for mtDNA effects on important traits, extensive researches were not taken because of shortage for the structured data set, which is materially related Hanwoo population in several generations. Therefore, to better understand the effects of mtDNA genotype on growing stages, it may be necessary further investigation with well unequivocally assigned Hanwoo population.

ACKNOWLEDGMENT

This study was supported in part by a grant from BioGreen 21 program, Rural Development Administration, Republic of Korea.

REFERENCES

Amano, T., Y. Miyakoshi, T. Tokada, T. Kikkawa and M. Suzuki. 1994. Genetic variants of ribosomal DNA and mitochondrial DNA between swamp and river buffaloes. Anim. Genet. 25:29-36.

Anderson, S., A. T. Bankier, B. G Barrell, M. H. De Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. Smith, R. Staden and I. G. Young. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457-465.

Bhat, P. P., B. P. Mishar and P. N. Bhat. 1990. Polymorphism of mitochondrial DNA in cattle and buffaloes. Biochem. Genet. 28:311-318.

Boffoli, D., S. C. Scacco, Vergari, R. Solarino, G. Santacroce and S. Papa. 1994. Decline with age of the respiratory chain activity in human skeletal muscle. Biochem. Biophys. Acta 1226:7382.

Boffoli, D., S. C. Scacco, R. Vergari, M. T. Persio, G. Solarino, R. Laforgia and S. Papa. 1996. Ageing is associated in females with a decline in the content and activity of the *b-c1* complex in skeletal muscle mitochondria. Biochem. Biophys. Acta. 1315:6672.

Brierley, E. J., M. A. Johnson, O. F. W. James and D. M. Turnbull. 1996. Effects of physical activity and age on mitochondrial function. Q J. Med. 89:251258.

Brown, W. M. 1980. Polymorphism in mitochondrial DNA of human as revealed by restriction endonuclease analysis. Proc. Natl. Acad. Sci. USA 77:3605-3609.

Chung, H. Y. and E. R. Chung. 1995. Polymorphism of mitochondrial DNA based on restriction endonuclease cleavage patterns in Holstein and Korean native cattle. Korean J. Dairy Sci. 17(2):102-112.

Cooper, J. M., V. M. Mann and A. H. V. Schapira. 1992. Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. J Neurol. Sci. 113:9198.

Elizabeth, J. B., A. J. Margaret, F. W. J. Oliver and M. T. Douglass. 1997. Mitochondrial involvement in the ageing process. Facts and controversies. Molecular and Cellular Biochemistry 174:325-328.

Faust, M. A., O. W. Robinson and B. T. McDaniel. 1989. The effects of cytoplasm on reproduction and production in Holsteins. J. Dairy Sci. 72:52.

Fleming, J. E., J. Miquel, S. F. Cottrell, L. S. Yengoyan and A. C. Economos. 1982. Is cell aging caused by respiration-dependent injury to the mitochondrial genome? Gerontology 28:4453.

Harman, D. 1972. The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20:145147, 1972

Laipis, P. J., C. J. Wilcox and W. W. Hauswirth. 1982. Nucleotide sequence variation in mitochondrial deoxyribonucleic acid from bovine liver. J. Dairy Sci. 65:1655-1662.

Linnane, A. W., S. Marzuki, T. Ozawa and M. Tanaka. 1989.

Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 1:42645.

Liu, Z. U., C. Z. Lei1, J. Luo, C. Ding, G. H. Chen, H. Chang, K. H. Wang, X. X. Liu, X. Y. Zhang, X. J. Xiao and S. L. Wu. 2004. Genetic variability of mtDNA sequences in Chinese native chicken breeds. Asian-Aust. J. Anim. Sci. 17:903-909.

Mannen, H., T. Kojima, K. Ojama, F. Mukai, T. Ishida and S. Tsuji. 1998. Effects of mitochondrial DNA variation on carcass traits of Japanese Black cattle. J. Anim. Sci. 76:36-41.

Marin-Garcia, J., R. Ananthakrishnan, N. Agrawal and M. J. Goldenthal. 1994. Mitochondrial gene expression during bovine cardiac growth and development. J. Mol. Cell Cardiol. 26:10291036.

Marin-Garcia, J., R. Ananthakrishnan, N. Agrawal and M. J. Goldenthal. 1997. Human mitochondrial function during cardiac growth and development. Molecular and Cellular Biochemistry 210:47-52.

Muller-Hocker, J. 1990. Cytochrome c oxidase deficient fibres in the limb muscle and diaphragm of man without muscular disease: an age-related alteration. J. Neurol Sci. 100:1421.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC. SAS (1985) SAS Inst. Inc., Cary, NC.

Trounce, I., E. Byrne and S. Marzuki. 1989. Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. Lancet 1:637639.

Watanabe, T., Y. Hayashi, R. Semba and N. Ogasawara. 1985. Bovine mitochondrial DNA in restriction endonuclease cleavage patterns and the location of the polymorphic sites. Biochem. Genet. 26:947-957.

Watanabe, T., T. S. Masangkay, S. Wakana, N. Saitou and T. Tomita. 1989. Mitochondrial DNA polymorphism in native Philippine cattle based on restriction endonuclease cleavage patterns. Biochem. Genet. 27:431-438.

Yen, T. C., Y. S. Chen, K. L. King, S. H. Yeh and Y. H. Wei. 1989. Liver mitochondrial respiratory functions decline with age. Biochem. Biophys. Res. Comm. 165:994-1003.