

Identification of a SNP in Hanwoo MC4R Gene with its Effect on Economic Traits

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Abstract. Mutation of the Melanocortin-4 receptor(MC4R) gene are associated with the appetite, obesity and growth in pig, mice, chicken and human. But the function of cattle MC4R gene still published very few. The objective of this study was to identify SNP in Hanwoo MC4R gene to detection of Novel Mutations about the correlation of SNP affecting on economic traits. In this study, 200 Hanwoo was screened for the polymorphisms of the MC4R gene using PCR-Restriction Fragment Length Polymorphism(PCR-RFLP) and DNA sequencing methods. Genetic polymorphism was screened by DNA sequencing detected a nucleotide in exon 1 SNP of MC4R gene. The result found that all are missense mutations that exists in exon. Among MC4R gene mutation have a five mutation as new creates discovery. SNP analysis of the amino acid mutations found that G709A and C1069G SNP was identified as missense mutation,C927T, C1343A, C1786T was identified as the silent mutation in exon1. In case of G709A was varied Valine(GTG) to Methionine(ATG) and C1069G was varied Leucine(CTG) to Valine(GTG). Holstein in the MC4R gene G709A polymorphism loci for the analysis of the correlation between economic traits; backfat thickness, carcass weight and dressing percentage were detected as significant association but no significant in any loci in case of Hanwoo. The relationship between Haplotype and economic traits was analyzed. However non significant correlation with economic traits have been found.

Keywords: MC4R gene, SNP, Sequencing, Economic traits, Korean cattle (Hanwoo)

1. Introduction

Recently various of molecular biological techniques have been developed and used in various fields. In livestock ,the economic traits have been concerned and improved by using these techniques such as the major genes that affect on the accelerating characteristics. Livestock breeding improvement in molecular level is becoming increasingly possible rapidly. By the SNP(Single Nucleotide Polymorphism) technique appear differently between the traits is increasing interested in genetic variation. In case of beef cattle industry DNA markers are available commercially. Thus, in Korean cattle(Hanwoo) the sufficient value is so strong at industrial level that means it should be very useful if we can develop the DNA markers that fit to economic transformation of Korean cattle(Hanwoo).

Even through livestock genetic analysis including cattle have been done and going on human genes, also found at the microarray field which generated in several genes related to meat quality and meat characteristics has been concerned. G-protein coupled receptor(GPCRs) have function effecting to the central nervous system receptor as same as protein in the energy metabolism and food intake that effecting to weight control and their functions involved in the MC4R(melanocortin 4 receptor) in the development of obesity and related drugs has been concern. In pig MC4R gene mutation D298N substitution of aspartate to asparagines mutation has been reported. The wild boar and Large White pigs Mating was accepted in Poland and in other breeds of MC4R genotypes, growth traits ADG, feed conversion, backfat thickness have been reported with non significant association. In this study, by the using of molecular breeding technology in

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term of MC4R mutations to explore economic traits of the livestock by using the correlation and comparative analysis of the transgenic cattle for the future as important data for analysis and improvement have been done.

2. Materials and Methods

2.1. Testing materials

SNP to explore other 24 friven sire were used and carcass determine to the SNP genotype of 124 Holstein and 200 Korean cattle(Hanwoo) tissue samples were used as materials.

2.2. Genomic DNA extraction and PCR amplification

DNA samples were extracted from tissue by QuickGene DNA tissue kits(FUJIFILM). Genomic DNA extracted using a ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA) by measuring the concentration and purity were used in the experiment.

The MC4R region of genomic DNA was amplified using PCR with the following primers(Accession No. NC_007325)(Table 1). The Polymerase Chain Reaction was conducted in 20ul volumes, each containing 100ng of genomic DNA, 10 pmole of each primer, 10x PCR buffer 2.5ul(10mM Tris-HCl, 50mM KCl, 1.5mM MgCl₂, pH 8.3), DdNTP 2.5ul(2.5mM), Taq polymerase 0.1ul(10 unit/ul)(Genetbio, Korea). The condition of PCR was a first denaturation step of 5 min at 94°C followed by 35 cycles, each consisting of 30 sec at 94°C, 30 sec at each temperature, 30 sec at 72°C and then, a final step of 5 min at 72°C using PTC 200 peltier thermal cycler(MJ Research, USA).

Table 1. MC4R gene primer information for PCR analysis

Primer Name	sequence of forward and reverse (5`-3`)	Annealing Temperature(°C)
MC4R_1	tgagagcatgcgcacataga agtagcctttgccaggac	58.3
MC4R_2	gatgctgaccagagccacac ttggaaacgctcaccaacat	58.4
MC4R_3	gctctttgtctctcccagg gccagcatggtgaagaacac	60.4
MC4R_4	gtcgggcgtcttgttcac gcttgtgttagcatcgcgt	58.1
MC4R_5	tgcaattccatcattgaccc agcaagcaaagtgcacatcc	56.6
MC4R_6	tccacatcacaggttataggcac tcccaaattgctgtgagaa	58.0
MC4R_7	ttctcattcattcttctgtgc gtaagtcactgcatgtggaaaa	57.1

2.3. Sequencing

PCR reaction were performed to explore the SNP in MC4R gene. After purification steps we get pure PCR products. The presence of PCR products produced by the SNP and by DNA sequencing attached fluorescent dye(Big dye Terminator v3.1 Cycle Sequencing Kits, Applied Biosystems, USA) were prepared.

After the final purification step by ABI 3130 Genetic Analyzer(Applied Biosystems, USA), each of the nucleotide sequences were determined. Sequences were determined by using seqMAN II program (DNA STAR Inc.)for each SNP.

2.4. PCR-RFLP

The following PCR products of the G709A region were digested with *Fau I* restriction enzyme(CCCGC[^]) and separated on 2% gel. The uncut fragment represents the valine variant, whereas the *Fau I* RFLP fragments of 460bp, 280bp, 180bp represent the methionine variant. And the other PCR products of the C1069G region were digested with *HpyCH4IV* restriction enzyme(AC[^]GT) and separated on a 2% gel. The uncut fragment represents the leucine variant, whereas the *HpyCH4IV* RFLP fragments of 493bp, 318bp, 175bp represent the valine variant.

2.5. Statistical analysis

SAS linear covariant models least squares due to genotypes of Korean cattle(Hanwoo) for the MC4R gene was analyzed association with economic traits.

$$Y_{ijk} = u + T_i + G_j + e_{ijk}$$

Where Y_{ijk} = a phenotypic record; u = overall mean; T_i = effect of considered as a covariate; G_j = effect of RFLP genotypes; and e_{ijk} = random residual.

3. Result and Discussion

Genetic polymorphism was screened by DNA sequencing detected a nucleotide in exon 1 SNP of MC4R gene. MC4R gene mutation are five mutation(G709A, C927T, C1069G, C1343A, C1786T) that creates a new discover. SNP analysis of the amino acid mutations found in a result of the G709A and C1069G SNP was identified as missense mutation,C927T, C1343A, C1786T was identified as the silent mutation in exon1. In the case of G709A was varied Valine(GTG) to Methionine(ATG) and C1069G was varied Leucine(CTG) to Valine(GTG). Missense mutation identified in the polymorphism of PCR-RFLP technique have been done and found the genotype and economic traits correlation . MC4R gene G709A locus is cut by the restriction enzyme *Fau I* 180bp, 280bp, 460bp size polymorphism has been observed, C1069G locus with the restriction enzyme cut *HpyCH4IV* the 175bp, 318bp, 493bp size polymorphism was observed.

Table 2. Least squares means and standard errors for economic traits of G709A genotype in MC4R gene in Holstein

Genotype	BF	LMA	CW	DP	MS
AA	16.31±1.34 ^a	83.57±2.56	448.26±14.91 ^a	58.09±0.98 ^b	4.93±1.12
GA	19.67±0.57 ^b	86.98±1.09	486.57±6.34 ^b	55.51±0.41 ^a	6.96±0.44
P	<u>0.0228</u>	0.2244	<u>0.0196</u>	<u>0.0172</u>	0.0975

BF : Backfat Thickness, LMA : Longissimus Muscle Area,
 CW : Carcass Weight, DP : Dressing Percentage, MS : Marbling Score
^{a, b} : Different superscripts within row are significantly differ (p<0.05)

Holstein in the MC4R gene G709A polymorphism loci for the analysis of the correlation between economic traits; backfat thickness, carcass weight and dressing percentage were detected as significant association(Table 2).

Table 3. Least squares means and standard errors for economic traits of C1069G genotype in MC4R gene in Holstein

Genotype	BF	LMA	CW	DP	MS
GC	19.68±0.78	84.53±1.45	480.91±8.72	55.32±0.57	6.03±0.60
GG	18.69±0.73	88.15±1.36	480.51±8.18	56.42±0.53	7.26±0.56
P	0.3558	0.0726	0.9735	0.1621	0.1401

BF : Backfat Thickness, LMA : Longissimus Muscle Area,
 CW : Carcass Weight, DP : Dressing Percentage, MS : Marbling Score

C1069G in the MC4R gene locus of Holstein for the genotype analysis of the correlation between economic traits in the GC genotype of backfat thickness were 19.68 ± 0.78 , GG genotype of longissimus muscle area were 88.15 ± 1.36 . Marbling score in the GG genotype was 7.26 ± 0.56 . However, the genotype of the C1069G locus is non significant association with economic traits (Table 3).

G709A locus in Korean cattle (Hanwoo), GA genotype was found. so economic traits correlation did not analyse.

Korean cattle (Hanwoo) in the MC4R gene and genotype of the C1069G locus have correlated with economic traits for the CC genotype results in backfat thickness and dressing percentage was the highest, CG genotype showed the highest of marbling score and longissimus muscle area. But for economic traits was non significant statistically (Table 4).

Table 4. Least squares means and standard errors for economic traits of C1069G genotype in MC4R gene in Korean cattle (Hanwoo)

Genotype	BF	LMA	CW	MS
CC	15.25 ± 1.18	89.03 ± 2.12	435.85 ± 10.02	5.50 ± 0.42
CG	13.51 ± 0.53	90.02 ± 0.96	425.87 ± 4.56	5.57 ± 0.19
GG	13.35 ± 1.02	89.59 ± 1.84	440.59 ± 8.72	5.29 ± 0.37

BF : Backfat Thickness, LMA : Longissimus Muscle Area, CW : Carcass Weight, MS : Marbling Score

In this study MC4R gene analyzed the Haplotype for G709A and C1069G. Haplotype analysis result showed the 4 types that A-C, A-G, G-C, G-G. G-G type (0.42335) was the highest type and A-C type, A-G type analyzed 0.23383 and 0.34278. So G-C type (0.00004) appeared the least association with economic traits were excluded analysis.

Table 5. Least squares means and standard errors for economic traits of Haplotype in MC4R gene

Haplotype	BF	LMA	CW	DP	MS
A-C	19.68 ± 0.77	84.53 ± 1.46	480.91 ± 8.67	55.32 ± 0.56	6.03 ± 0.60
A-G	18.16 ± 0.64	87.12 ± 1.21	473.30 ± 7.16	56.79 ± 0.46	6.80 ± 0.50
G-G	19.67 ± 0.57	86.98 ± 1.09	486.57 ± 6.44	55.51 ± 0.42	6.96 ± 0.44

BF : Backfat Thickness, LMA : Longissimus Muscle Area, CW : Carcass Weight, DP : Dressing Percentage, MS : Marbling Score

The relationship between Haplotype and economic traits was analyzed. We found that, A-G type (87.12 ± 1.21) was the highest in longissimus muscle area and G-G type was the highest in dressing percentage (486.57 ± 6.44) and marbling score (6.96 ± 0.44) (Table 5). However non significant correlation with economic traits have been found.

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5. References

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