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Application of the melanocortin 1 receptor (MC1R) gene for discrimination of Hanwoo from Holstein beef using real-time polymerase chain reaction (PCR)

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Abstract

This study was carried out to discriminate Hanwoo from the milking and hybrid cattle by detection of MC1R gene related to bovine hair color. One hundred sixty six samples were collected from the abattoir (n=106) and local market (n=60). The beef from abattoir were originated from Hanwoo (n=27), Holstein (n=29), Hybrid (n=45) and imported cattle (n=5), respectively. The beef from market consisted of Hanwoo (n=36), Holstein (n=7) and imported ones (n=17). Commercialized screening kit (Kogenebiotec, Korea) was used for MC1R gene analysis. As a result, Hanwoo was discriminated from Holstein. However, 9 of 45 hybrid and 11 of 22 imported beef samples were indistinguishable from Hanwoo. It could be explained by second generation of crossing of Hanwoo with Holstein or the cattle with silver or yellow hair. This results suggest that additional tests as well as MC1R gene detection be needed to confirm Hanwoo beef among cattle beef.

Key Words : MC1R gene, Real-time PCR, Coat color, Hanwoo beef

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Introduction

To distinguish the Hanwoo beef from the Holstein or imported beef, a variety of methods have been developed. Since 2000, assay for the gene of hair color has been focused to discriminate the species of cattle. Melanocortin 1 receptor (MC-1R), a hormone receptor stimulating the synthesis and proliferating of melanin, plays an important role in synthesis of two pigments including phaeomelanin and eumelanin in melanocyte. Accordingly,

the mutation of the MC1R gene causes various expression of the hair colors in mammalians[1,2].

Analysis on sequences of MC1R from Hanwoo and Holstein showed the difference of a base pair in Hanwoo. There is a single nucleotide polymorphism (SNP) missing the second guanine in a codon deciding the 104th amino acid. PCR-RAPD(random amplified polymorphic DNA) have been conducted to distinguish Hanwoo from Holstein and imported beef in Korea. With a complimentary measures, PCR-SSCP(single strand conformation polymorphism) and PCR-RFLP (restriction fragment length polymorphism) have improved their efficiencies. However, reproducibility of their techniques was poor and they took much time to make the gels for electrophoresis and dye them[1,3-5]. We used real-time PCR to differentiate Hanwoo from other meat and to increase reproducibility. Real-time PCR has been widely used for checking the amplified products with real-time and quantization of the product within a short time. As a result, we obtained more precious and reproducible results in this study.

Materials and Methods

Animals

Samples were 166 beef consisting of 106 from abattoir and 60 from local market. The meat from abattoir in Incheon were 27 Hanwoo, 29 Holstein, 17 hybrids and 5 imported cows (4 Red Angus and 1 Black Angus) and 28 frozen beef of hybrid species transferred from abattoir in Jeju island. Thirty-six Hanwoo, 7 Holstein and 17 imported beef from Aus-tralia were purchased from local market around the Incheon area (Table 1).

Table 1. Details of the beef samples used in this study

Source	Domestic beef				Imported beef	Total
	Hanwoo	Holstein	Hybrid[a)]	Angus		
Abattoir	27	29	45	5*		106
Market**	36	7	-		17***	60

[a)] Hybrid means interbreeding of Hanwoo with Holstein and other crossbred.
* imported from Australia (4 Red Angus and 1 Black Angus).
- Imported cow was recognized as domestic in case of 6 month breeding within our country.
** from Incheon Metropolitan city. *** Country of origin : Australia.

DNA extraction from muscle

DNA isolation was performed using Power-Prep™ DNA extraction kit (Kogenebiotech, Korea). Ten to twenty milligram of muscle were mixed with 440㎕ of nucleic lysis buffer and incubated 1 hour at 65℃. Chloroform were added to the mixture to remove protein and remnants, then the mixture was centrifuged for 10 minutes at 12,000rpm. Binding buffer and iso-propanol were added to supernatant and transfer the mixture to column to absorb the DNA. The DNA was washed three times with 75% ethanol

and eluted with sterile-water.

Primer, probe and standard DNA template

Commercialized Powercheck™ Screening kit (Kogenebiotech, Korea) was used for DNA amplification and gene type detection. Dye activity was calibrated to allelic discrimination during DNA amplification and 2 kind of MGB probe were used. One is FAM-dye labeled MGB probe that was expressed recognizing the missing base of Hanwoo MC1R gene and the other is VIC-dye labeled MGB probe that was expressed recognizing the Hanwoo MC1R gene.

Thermal cycling parameters

Real-time PCR amplification of MC1R was carried out in a 10㎕ reaction containing 2X Taqman® universal PCR mastermix 5㎕, primer-probe set 3㎕, and DNA template 2㎕. Genomic DNA was denatured for 2 min at 50℃ and 10 min at 95℃, and PCR was run for 40 cycles at 92℃ for 15 sec, 60℃ for 1 min. All quantification experiments were carried out on an ABI prism® 7000 Real-time PCR system (Applied Biosystems, USA).

Results

DNA amplification was checked up in every cycle using SDS v1.2 software. Cycle threshold was 23~32 cycle, so most samples could be detected after 32 cycles (Fig 1).

After amplifying the genes, the activities of FAM-dye of Hanwoo and VIC-dye of Holstein were shown in a graph. Hanwoo gene related to hair color was displayed with FAM dye (y axis of the graph, ◆). Holstein gene was indicated by VIC dye (x axis of the graph, ●) and hybrid type was located in the middle (▲) of the both (Fig 2).

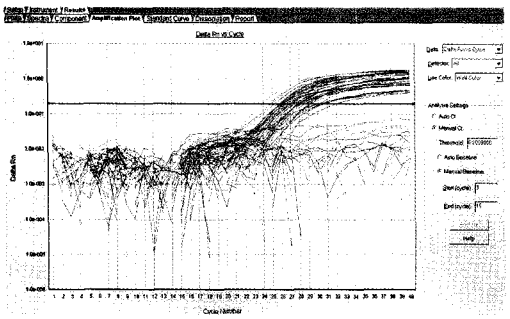

Fig 1. Amplification plot
(The portion of experiment)

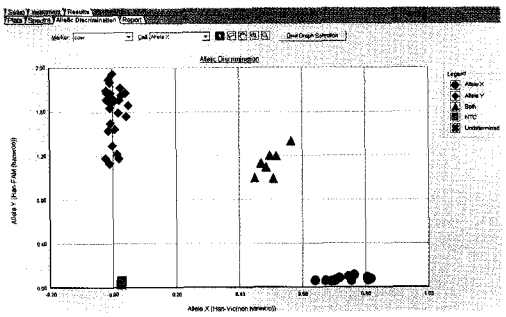

Fig 2. The graph of allelic discrimination

In slaughtered cattle, all of 27 Hanwoo beef were confirmed to Hanwoo type.

Among 29 meat samples from Holstein cattle, however, 27 were identified to Holstein type and 2 were from hybrid type. In marketed beef, all of 36 Hanwoo beef were found to be Hanwoo type, but 6 meats were identified as Holstein type and 1 was to be hybrid type among 7 samples from Holstein cattle. Therefore, Hanwoo meat was clearly distinguished from Holstein meat by using this method.

Among 45 beef from slaughtered hybrid cattle, 9(20%) were identified to Hanwoo type. Among 5 angus beef, 4 meats (80%) were found to Hanwoo type. In addition, 7(41.2%) of 17 cow meats imported from Australia were identified to Hanwoo type by this method. Details of the results according to types and hair colors of materials were shown in Table 2 and Table 3.

Table 2. Detection of the MC1R gene in beef samples from various slaughtered cattle

Beef from	Hair color	Gene type			Total
		Hanwoo	Holstein	Hybrid	
Hanwoo	yellow	27(100%)	-	-	27
Holstein	black/white	-	27	2	29
Hybrid	black	2	-	35	37
	yellow	3	-	-	3
	white spot in yellow	-	-	1	1
	Ivory	3	-	-	3
	silver	1	-	-	1
	subtotal	9(20%)	-	36	45
Angus	red	4	-	-	4
	black	-	-	1	1
	subtotal	4(80%)	-	1	5
Total		40	27	39	106

Table 3. Detection of the MC1R gene from the beef marketed in Incheon area

Beef from	Hair color	Gene type			Total
		Hanwoo(%)	Holstein	Hybrid	
Hanwoo (Domestic)	unknown	36 (100.0)	-	-	36
Holstein (Domestic)	unknown	-	6	1	7
Imported (Australia)	unknown	7 (41.2)	5	5	17
Total		43	11	6	60

Discussion

Cattle has their basic hair colors including black, red and white. There are many genes to regulate their colors[6]. Among them, MC1R is located on melanocyte and expresses hair color in cooperation with MSH (α-melanocyte stimulate hormone). By inhibition of binding MSH to MC1R, Hanwoo express yellowish brown hair color. Holstein express their color mixing with black and white by increa-

sing of the binding with MSH[4].

Using above principle, we tried to determine whether real time PCR with probe for MC1R was an useful method to distinguish Hanwoo beef from Holstein or crossbred breed beef. It was usually took within three hours to amplify genes and detect them in this study, and Hanwoo beef were distinguished perfectly (100.0%) from Holstein beef. This result was in accordance with those of Park et al[2]. However, differential ability of this method was not high because 9 (20.0%) of 45 hybrid beef were known from Hanwoo type. This result suggested that it is hard to distinguish specific breed among similar breeds by one difference in genes due to various hair color. Kim et al[3] tried to conform a breed among others such as Hanwoo, Holstein, Black Angus, Hereford and Muck cow (Hanwoo mixed with yellow to black) but that was hard. In additional study, Park et al[2] also showed the similar results with our outcome in their study.

As like Table 2 and Table 3, all 7 samples from hybrid with yellow, ivory and silver color hair were classified as Hanwoo beef and 7 (41.2%) among 17 cases of imported cow meat from Australia were also determined as Hanwoo type although their coat colors were not identified. Therefore, the hair colors of Australian cows were assumed to be similar with those of Hanwoo. Actually, hair colors of beef cows raised in Australia including Hereford, Angus, Brahman and others were various range from light yellow to red.

A few years ago, marketing beef were generally from Hanwoo and Holstein cattle. However, imported meat from Australia, New Zealand and the United States has increased in recent years so that the differentiation between these imported meat and Hanwoo beef was necessary. National Livestock Research Institute of Rural Development Administration announced a method which can distinguish Hanwoo beef from imported cow meat by developing 7 single nucleotide polymorphism (SNP) markers and 17 microsatellites (MS) markers. However, it was hard for general people to apply the method because the it was complex and did not include results on imported cow meat from the United States. Therefore, it did not used practically yet.

In addition, Korea Food and Drug Administration revised Food Hygiene Law and announced a draft of Method for Identification of Cow Meat Origin to differentiate Hanwoo beef from imported cow beef on December 2006, which was related to Required Labeling of Cow Meat Origin in Restaurants enacting from 2007. This method analyzed 70 differences in base sequences of genes between imported cow beef and Hanwoo beef and showed 100.0% accuracy in 1,000 imported cow beef and Hanwoo beef as a SNP genotyping method by using a new technique, Beadchip[7]. However, as it needs high specialty, it was not applied realistically. An easier method is expected to be confirmed and announced on the first half of 2008.

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