Journal of Animal and Veterinary Advances 10 (13): 1714-1718, 2011
ISSN: 1680-5593

Difference in PNLIP Allele Frequency Distribution Between High-Marbled and Low-Marbled Cattle

[1]Hideki Tanomura, [2]Youji Muramatsu, [1]Takuji Yamamoto, [3]Takeshi Ohta,
[4]Hiroyuki Kose and [1]Takahisa Yamada
[1]Laboratory of Animal Genetics, Graduate School of Science and Technology,
Niigata University, Nishi-Ku, 950-2181 Niigata, Japan
[2]Department of Nutritional Sciences for Well-Being, Faculty of Health Sciences for Welfare,
Kansai University of Welfare Sciences, Kashiwara, 582-0026 Osaka, Japan
[3]Central Pharmaceutical Research Institute, Japan Tobacco, Inc.,
Takatsuki, 569-1125 Osaka, Japan
[4]Department of Life Science, Division of Natural Sciences,
International Christian University, Mitaka, 181-8585 Tokyo, Japan

Abstract: Marbling characterized by the amount and distribution of intramuscular fat is one of the economically important traits of beef cattle. The *Pancreatic lipase* (Pnlip) gene, involved in the hydrolysis of dietary triglycerides to fatty acids has been previously shown to be regarded as possible candidate for the gene responsible for intramuscular fat content in the rat. It is located within the genomic region of a bovine marbling quantitative trait locus and thus was considered as a positional functional candidate for the marbling gene. In this study, researchers investigated the allele frequency distribution of the Single Nucleotide Polymorphisms (SNPs) in the PNLIP in high and low-marbled cattle. The frequencies of the rs41648171 C, rs41648172 T, rs41648173 T, rs41648174 C, rs41648176 C, rs41648178 T and rs42104801 T alleles were higher in Japanese Black sires with extremely high predicted breeding value for marbling than in the sires with extremely low one. Further as compared to the frequencies of the rs41648172 T, rs41648176 C and rs41648178 T alleles in Japanese Black cattle that has been subjected to a strong selection for high marbling, those in Holstein cattle that has not been selected for high marbling were lower. The findings suggest that the PNLIP SNPs are associated with marbling and may be useful for effective marker-assisted selection to increase the levels of marbling.

Key words: Allele frequency, high-marbled cattle, low-marbled cattle, marbling, PNLIP, single nucleotide polymorphism, Japan

INTRODUCTION

Marbling characterized by the amount and distribution of intramuscular fat in a cross section of musculus longissimus muscle is one of the economically important traits of beef cattle (JMGA, 1998). High levels of marbling improve the palatability and acceptability of beef by affecting the taste and tenderness of the meat (Busboom *et al*., 1993; Boylston *et al*., 1995; Matsuishi *et al*., 2001). Thus, there is great interest in gaining a better knowledge on the molecular architecture of marbling and in generating new opportunities for more effective marker-assisted selection.

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat has been established as a mutant that exhibits higher levels of intramuscular fat content in musculus longissimus muscle (Umezu *et al*., 2001). Researchers previously reported that a genomic region on chromosome 1 shows a linkage with intramuscular fat content with the OLETF allele acting on an increase in fat content by whole genome scanning (Tanomura *et al*., 2002). Further, there was demonstrated that Pnlip gene encoding *Pancreatic lipase* is possible candidate for the gene responsible for the intramuscular fat content using a congenic strain (Tanomura *et al*., 2003) trapping the OLETF allele of the genomic region linked to intramuscular fat content on the genetic background of normal rat strain in a monogenic context (Tanomura *et al*., 2011).

Pancreatic lipase encoded by PNLIP is known to be involved in the hydrolysis of dietary triglycerides to fatty acids which is essential for the intestinal absorption of

Corresponding Author: Takahisa Yamada, Laboratory of Animal Genetics, Graduate School of Science and Technology, Niigata University, Nishi-ku, 950-2181 Niigata, Japan

long-chain triglyceride fatty acids (Lowe, 2002). An increase in pancreatic lipase amount or activity is likely to result in excess energy income and then increase of intramuscular fat content leading to high levels of marbling. It has been reported that a marbling quantitative trait locus was mapped to genomic region containing PNLIP on bovine chromosome 26 using a half-sib family of Japanese Black beef cattle (Takasuga *et al*., 2007). Thus, the PNLIP was considered as a positional functional candidate for the gene responsible for marbling. Researchers herein analyzed the allele frequency distribution of the Single Nucleotide Polymorphisms (SNPs) in the bovine PNLIP in high-marbled and low-marbled cattle as the 1st trial to examine association of the PNLIP with marbling.

MATERIALS AND METHODS

Samples: There were used 34 Japanese Black unrelated sires (17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one) selected from 101 unrelated sires for SNP genotyping in this study. The sires were used either at present or previously at the Oita Prefectural Institute of Animal Industry (Oita, Japan). There was no strong bias for a specific father or a specific maternal grandfather of the sires and the sire panel likely represents a variety of the sire lines (OPIAI, 1999).

The predicted breeding values were obtained from the Oita recording system for beef cattle previously reported by Sasaki *et al*. (2006a). The accuracy of the predicted breeding values in the 101 sires was 0.935±0.008, ranging from 0.770-0.990. Furthermore, there were used 34 Holstein cows for SNP genotyping in this study. There was no strong bias for a specific father or a specific maternal grandfather of the cows and the cow panel likely represents a random sample of Holstein dairy cattle population. Semen, blood or adipose tissue were collected from these animals for SNP genotyping. These materials were sampled by the Oita Prefectural Institute of Animal Industry (Oita, Japan). DNA was prepared from the materials according to standard protocols. This study conformed to the guidelines for animal experimentation of the Graduate School of Science and Technology, Niigata University (Niigata, Japan).

SNP genotyping: Researchers genotyped 13 SNPs, rs41648166, rs41648167, rs41648171, rs41648172, rs41648173, rs41648174, rs41648165, rs41648176, rs41648178, rs41648179, rs41648180, rs42104800 and rs42104801 in dbSNP which are located in the intron 5, intron 5, intron 6, intron 6, intron 6, intron 6, intron 7, exon 7, intron 7, intron 10, intron 10, intron 12 and intron 12 regions of the PNLIP, respectively. The SNPs were genotyped using PCR-restriction Fragment Length Polymorphism (RFLP) method. PCR primers and restriction enzymes used for PCR-RFLP were shown in Table 1. PCR amplifications were performed using 25 ng of the prepared DNA as template in a final volume of 25 μL containing 0.5 μM of each primer, 0.2 mM of each dNTP, 0.625 U of Ex Taq polymerase (Takara, Shiga, Japan) and 1X Ex Taq buffer (Takara). The PCR conditions were as follows: 94°C for 3 min, 35 cycles of 94°C for 50 sec, 66°C for 50 sec and 72°C for 50 sec followed by a further 5 min extension at 72°C. An aliquot of PCR amplified product was digested at 37°C for 1 h with restriction enzyme *HpyCH4IV* for the rs41648166 and rs41648172 SNPs, *BsaXI* for the rs41648167 SNP, *NlaIII* for the rs41648171 SNP, *HphI* for the rs41648173 SNP, *MboI* for the rs41648174 SNP, *BamHI* for the rs 41648175 SNP, *MslI* for the rs41648176 SNP, *BccI* for rs41648179 SNP, *MboII* for rs41648180 SNP, *AluI* for the rs42104801 SNP and at 65°C for 1 h with restriction enzyme *TaqI* for the rs41648178 SNP andelectrophoresed on a 3.0% agarose gel. Agarose gels were stained with ethidium bromide and photographed under an ultraviolet light.

Table 1: PCR primers and restriction enzymes used for PCR-RFLP

Entrez SNP accession no.	Locations in Pnlip	Primer sequences Forward	Reverse	Restriction enzyme
rs41648166	Intron 5	GCCTTTGACTGTGTGGATCA	GATTCCAGCTTGTGCTTCCT	*HpyCH4*IV
rs41648167	Intron 5	GCCTTTGACTGTGTGGATCA	GATTCCAGCTTGTGCTTCCT	*Bsa*XI
rs41648171	Intron 6	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*Nla*III
rs41648172	Intron 6	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*HpyCH4*IV
rs41648173	Intron 6	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*Hph*I
rs41648174	Intron 6	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*Mbo*I
rs41648175	Intron 7	TACCACCTGCACTTGCACTC	GAAATCTAGGTGGCCCACAA	*Bam*HI
rs41648176	Exon 7	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*Msl*I
rs41648178	Intron 7	CAGTGCTATCTCCCGGAGTC	GAAATCTAGGTGGCCCACAA	*Taq*I
rs41648179	Intron 10	AGGATTCTTGCCTGGGAAAT	CCAAATTGGAAAGGCGATAA	*Bcc*I
rs41648180	Intron 10	AGGATTCTTGCCTGGGAAAT	CCAAATTGGAAAGGCGATAA	*Mbo*II
rs42104800	Intron 12	TCTGCAATGGTTCTCCTCTG	CACACAGTGAAAGCGAAAGC	*Bst*NI
rs42104801	Intron 12	AAGATTGGAGTGTGCGTGAA	CTGTCTGTTTCAGTGGGAAGG	*Alu*I

Statistical analysis: The allele frequency distributions of the PNLIP SNPs were compared between 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one or between 34 Japanese Black beef cattle and 34 Holstein dairy cattle by Chi-square (χ^2) test. Statistical analysis was performed by the FREQ procedure of SAS program (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Using PCR-RFLP method, researchers genotyped 34 Japanese Black unrelated sires consisting of 17 sires with extremely high predicted breeding value for marbling and 17 sires with extremely low one for the 13 SNPs. For the rs41648171 SNP, the GG homozygotes, the CC homozygotes and the CG heterozygotes resulted in three bands (91, 163 and 292 bp), four bands (42, 91, 121 and 292 bp) and five bands (42, 91, 121, 163 and 292 bp), respectively. For the rs41648172 SNP, the TT homozygotes, the CC homozygotes and the CT heterozygotes resulted in one band (546 bp), two bands (131 and 415 bp) and three bands (131, 415 and 546 bp), respectively. For the rs41648173 SNP, the CC homozygotes, the TT homozygotes and the CT heterozygotes resulted in two bands (113 and 433 bp), three bands (113, 176 and 257 bp) and four bands (113, 176, 257 and 433 bp), respectively. For the rs41648174 SNP, the AA homozygotes, the CC homozygotes and the AC heterozygotes resulted in four bands (48, 53, 139 and 306 bp), five bands (42, 48, 53, 139 and 264 bp) and six bands (42, 48, 53, 139, 264 and 306 bp), respectively. For the rs41648176 SNP, the CC homozygotes, the TT homozygotes and the CT heterozygotes resulted in three bands (53, 223 and 270 bp), four bands (53, 116, 154 and 223 bp) and five bands (53, 116, 154, 223 and 270 bp), respectively. For the rs41648178 SNP, the TT homozygotes, the CC homozygotes and the CT heterozygotes resulted in one band (546 bp), two bands (74 and 472 bp) and three bands (74, 472 and 546 bp), respectively. For the rs42104801 SNP, the TT homozygotes, the CC homozygotes and the CT heterozygotes resulted in two bands (64 and 193 bp), three bands (54, 64 and 139 bp) and four bands (54, 64, 139 and 193 bp), respectively. Genotyping, the 34 sires for the six SNPs, rs41648166, rs41648167, rs41648175, rs41648179, rs41648180 and rs42104800 showed no detection of the A, C, C, T, G and T alleles, respectively, suggesting that the SNPs seem to fix at the G, G, T, C, A and G alleles and not to segregate in Japanese Black beef cattle.

Statistically significant difference in the allele frequency distribution between 17 sires with extremely high breeding value and 17 sires with extremely low one

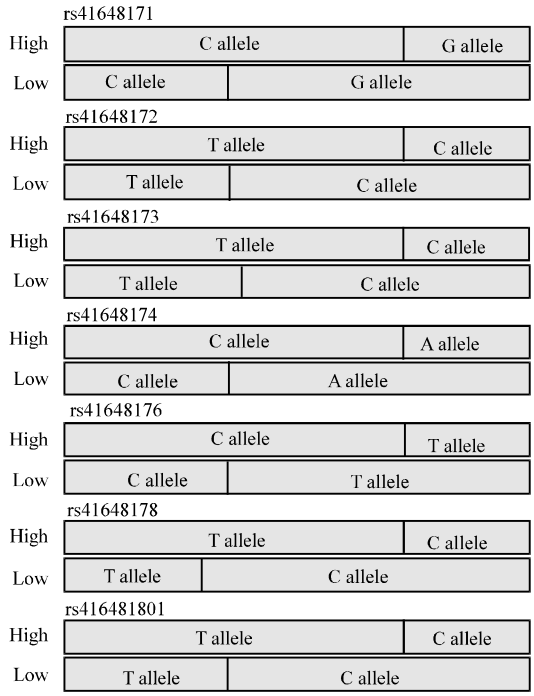

Fig. 1: The allele frequency distribution between 17 sires with extremely high breeding value (High) and 17 sires with extremely low one (Low) for the rs41648171, rs41648172, rs41648173, rs41648174, rs41648176, rs41648178 and rs42104801 SNPs

was detected for all of the seven polymorphic SNPs, rs41648171 ($p = 0.0154$), rs41648172 ($p = 0.0154$), rs41648173 ($p = 0.0154$), rs41648174 ($p = 0.0154$), rs41648176 ($p = 0.0154$), rs41648178 ($p = 0.0066$) and rs42104801 ($p = 0.0154$) (Fig. 1). The frequencies of the C, T, T, C, C, T and T alleles at the rs41648171, rs41648172, rs41648173, rs41648174, rs41648176, rs41648178, rs42104801 SNPs, respectively were higher in animals with the high breeding value than with the low one andthe G, C, C, A, T, C and C allele frequencies in animals with the low one than with the high one (Fig. 1).

There has been a strong selection for high marbling in Japanese Black breed but not in other breeds such as Holstein, over the past 50 years (Sasaki *et al*., 2006b; Sumio, 2007). Thus, the rs41648172, rs41648176 and rs41648178 SNPs were genotyped by PCR-RFLP method in 34 Holstein cows and statistical comparisons for the allele frequency distribution between 34 Japanese Black cattle and 34 Holstein cattle were performed. Statistically significant differences were detected between Japanese Black cattle and Holstein cattle for all of the 3 SNPs (Fig. 2). The frequencies of the rs41648172 T, rs41648176 C and rs41648178 T alleles which were higher in animals with the high breeding value than with the low one were

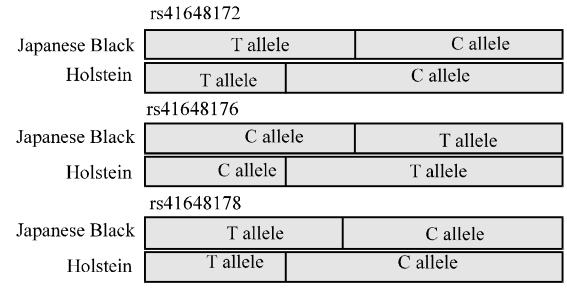

Fig. 2: The allele frequency distribution between 34 Japanese Black cattle (Japanese Black) and 34 Holstein cattle (Holstein) for the rs41648172, rs41648176 and rs41648178 SNPs

0.50, 0.50 and 0.47, respectively in Japanese Black cattle that has been subjected to a strong selection for high marbling (Fig. 2). As expected as compared to these frequencies in Japanese Black cattle, those in Holstein cattle that has not been selected for high marbling were lower for all the 3 SNPs (Fig. 2). There should note that all the p-values reported in this study were nominal with no correction for multiple testing. This finding leads to the hypothesis that the pressure of the strong selection for high marbling in Japanese Black cattle has increased the frequencies of the rs41648172 T, rs41648176 C and rs 41648178 T alleles assuming that the three alleles have experienced a selective sweep.

Thus, there were showed the higher frequencies of the rs41648171 C, rs41648172 T, rs41648173 T, rs41648174 C, rs41648176 C, rs41648178 T and rs42104801 T alleles in high-marbled cattle as compared to low-marbled cattle. This study, together with the mapping of a marbling quantitative trait locus within genomic region containing PNLIP on bovine chromosome 26 (Takasuga *et al*., 2007), suggests that the PNLIP SNPs are associated with marbling andmay be useful for effective marker-assisted selection to increase the levels of marbling. In previous study, there is supposed that the PNLIP is responsible for the intramuscular fat content in the rat (Tanomura *et al*., 2011). There can hypothesize that the PNLIP SNPs have a direct impact on bovine marbling. In addition although, it may be true that the SNPs themselves are functional and directly affect marbling, a more likely event is that the SNPs are in linkage disequilibrium with an unidentified true causal mutation.

CONCLUSION

In this study, there is showed that the frequencies of the C, T, T, C, C, T and T alleles at the PNLIP SNPs, rs41648171, rs41648172, rs41648173, rs41648174, rs41648176, rs41648178 and rs42104801, respectively are higher in high-marbled cattle than in low-marbled cattle. This study suggests that the PNLIP SNPs are associated with marbling, providing an useful information for the establishment of effective marker-assisted selection to increase the levels of marbling.

ACKNOWLEDGEMENTS

This research was supported by a Grant-in-Aid for Scientific Research (B) (no. 14360166) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the research funds of Japanese Livestock Technology Association.

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