www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Polymorphism of oxidized low density lipoprotein receptor1 (OLR1) gene in the Iranian Holstein dairy cattle

Mojtaba Hossienpour Mashhadi[1*], Saeed Sobhanirad[1], Seyed Eeman Nooraee[1] and Reza Bahari Kashani[1]

Department of Animal Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran

Abstract

The present study was carried out to determine the polymorphism in the 3' untranslated region of OLR1 gene in Iranian Holstein dairy Cattle. Oxidized low density lipoprotein receptor1 (LOX1) also known as oxidized low density lipoprotein receptor1 (OLR1) is a type II trans-membrane receptor which belongs to the C type lectin family. Polymorphism as well as significant associations was reported between single nucleotide polymorphism (SNP) in 3'untranslated region of OLR1 gene with milk production traits so OLR1 gene was chosen as a candidate gene. The blood samples of 153 dairy cattle from three different farms were used for extracting genomic DNA. A 146 bp fragment of 3'-untranslated region of OLR1 gene was amplified by standard polymerase chain reaction (PCR) procedure. Polymorphism of OLR1 gene was determined by PCR-RFLP technique. The number of animals with AA, AC and CC genotype were 32, 79 and 42 individuals respectively. The frequency of allele A and C was 0.4673 and 0.5327 respectively. The results of Chi square test showed that the population was under Hardy Weinberg equilibrium. The frequency of allele A and C is near 0.5 suggesting the effects of allele C on milk fat which may be increased in population.

Keywords: Dairy cattle; polymorphism, OLR1, Holstein

To cite this article: Mashhadi MH, S Sobhanirad, SE Nooraee and RB Kashani, 2014. Polymorphism of Oxidized Low density lipoprotein Receptor1 (OLR1) Gene in the Iranian Holstein dairy cattle. Res. Opin. Anim. Vet. Sci., 4(5): 237-240.

Introduction

Quantitative traits are controlled by genes and environmental factors. The recent research in farm animals shows the effect of candidate genes on economically important traits of dairy cattle. For programming in dairy cattle breeding, detection of major genes associated with dairy cattle performance could be useful for improvement of milk production traits (Boichard et al., 2003; Ogorevc et al., 2009).

The major protein oxidized low density lipoprotein receptor1 (OLR1) was initially identified in bovine aortic endothelial cells. This protein binds, internalizes, and degrades oxidized low-density lipoprotein (Sawamura et al., 1997). The oxidized form of the low-density lipoprotein (oxLDL) is involved in endothelial cell injury, dysfunction and activation, all of which are implicated in the development of atherosclerosis (Mehta and Li, 1998).

The oxLDL and its lipid constituents have numerous damaging effects on secretory activities of the endothelium, including induction of apoptosis (Imanishi et al., 2002).

The OLR1 gene encodes a vascular endothelial cell-surface receptor that binds and degrades the oxidized forms of low-density lipoproteins (oxLDL) (Metha and Li., 2002). The genomic sequence of bovine OLR1, released by Baylor College of Medicine Texas, US, contains five exons. The length of this gene is 11373 base pair GenBank accession no. NW_215807.

OLR1 gene is located on chromosome 5 and has five exons. The bovine OLR1 gene encodes 270 amino acids (AA) that has 72% identity to the human homologous protein (Sawamura et al., 1997). From 1999 to 2004, many researchers studied the effects of quantitative trait loci (QTL), which is located on bovine chromosome 5 near OLR1, on milk production traits (Heyen et al., 1999; De Koning et al., 2001; Olsen et al.,

[*]**Corresponding author:** M. Hossienpour Mashhadi, Department of Animal Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran

2002; Rodriguez-Zas et al., 2002; Viitala et al., 2003; Ashwell et al., 2004).

Direct cDNA and genomic sequencing of OLR1 revealed 2 single nucleotide polymorphisms (SNP) in exon 4, 5 SNP in intron 4, and 1 in the 3' untranslated region (UTR) (Khatib et al., 2006).

Many researchers reported that allele C of SNP in the 3'-UTR had significant effects on milk fat yield and percentage. Khatib et al. (2006) indicated significant effect of A/C SNP in the 3′-untranslated region (UTR) region of OLR1 with milk fat yield and fat percentage in a Holstein bull population. Association between OLR1 haplotypes and milk production traits was further confirmed in a daughter design study of Holstein cows and in an Italian Brown Swiss population (Khatib et al., 2007). Schennink et al. (2009) also reported a significant association between *OLR1* and milk fat percentage in Dutch Holstein-Friesian cattle. Soltani-Ghombavan et al. (2013) revealed a significant effect of OLR1 gene on milk fat yield and fat percentage in Iranian Holstein cattle. Therefore, based on the role of OLR1 in lipid metabolism and degrading ox-LDL, OLR1 has been regarded as a candidate gene affecting milk production traits in dairy cattle (Khatib et al., 2006).

The objective of this study was to determine polymorphisms in the 3'untranslated region of OLR1 gene in Iranian Holstein dairy cattle.

Materials and Methods

A total of 153 Iranian Holstein dairy cattle were randomly selected from three different herds in Khorasan Razavi province of Iran. Approximately, 5 ml of blood was collected from each animal from the jugular vein, into BD-vacutainer®K2EDTA tubes (BD Diagnostics, Franklin Lakes, NJ). The aliquots of whole blood were stored at -20°C.

The genomic DNA from blood samples were extracted based on GuSCN-Silica Gel method and standard protocol from the commercial kit of DIAtom DNA Prep (Biokom, Russia). The quality and quantity of DNA were examined by way of agarose gel electrophoresis by spectrophotometry (Bio Aquarius, Cecil, UK). 5µl of DNA was diluted with 100µl of dH_2O.

Genotyping was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The 146 bp fragment of OLR1 gene was amplified with standard PCR by Biometra thermo cycler (Göttingen, Germany). The sequences of the forward and reverse primers were (F 5′-TCCCTAACTT GTTCCAAGTCCT-3′) and (R 5′-CTCTACAATGCCT AGAAGAAAGC-3'), respectively (Komisarek and Dorynek, 2009). The total volume of reaction was 25µl that contained one unit (0.2µl) of Taq polymerase, 200µM (0.5µl) of dNTP, 2mM MgCl2, 10pM (3µl) primer mix and 2.5µl standard buffer in 13.8µl dH_2O. Fifty nanograms (5µl) DNA were added to the reaction mix.

The thermal cycling conditions for the PCR were as follows: initial denaturation at 94ºC for 5 min, cyclic denaturation at 94ºC for 30 s, cyclic annealing of primers at 62ºC for 30 s, cyclic elongation at 72ºC for 45 s (for 30 cycles) and final elongation at 72ºC for 5 min (Komisarek and Dorynek, 2009).

The PCR products were separated by electrophoresis in 2% agarose gel and visualized on gel documentation system (UVP, California, USA). The PCR product was digested with *PstI* restriction enzyme (Fermentas). The 5 µl of PCR product was mixed with 2 µl 10X buffer, 5 µl dH_2O and 2 units of *PstI* enzyme and digested over 5 hours at 37ºC. Polyacrylamide gel electrophoresis methods were used to determine the genotypes. The A allele (uncut) was indicated by a band at 146 bp and the C allele (cut) resulted in two bands at 116 bp and 30 bp.

The Hardy Weinberg equilibrium for allele and genotype frequencies was analyzed with Chi square test using Popgene software (Yeh et al., 1999).

Results and Discussion

The fragment of 146 bp of OLR1 gene was amplified by PCR (Fig. 1).

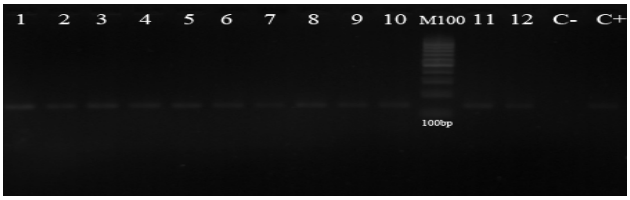

Fig 1: ORL-1 PCR products. Molecular marker (M100). C-: negative control, C+:positive control

Results of digestion by *PstI* restriction enzyme revealed three band patterns in 17% polyacrylamide gel. Band pattern of AA genotype was observed when the fragment of 146 bp was not digested. This is due to the absence of restriction site for *PstI*, and thus only one band with a length of 146 bp can be observed on the gel. The CC genotype was observed when the amplified fragment was digested on both strands. Therefore, two bands of 116 bp and 30 bp length were present on the gel. The heterozygote genotype of AC was also clearly observed with three bands that related to fragments with length of 146 bp, 130 bp and 16bp. This indicated that in one strand of DNA the restriction site of *PstI* is present and the alternate strand lacks this site. All restriction patterns are presented in Fig. 2.

Number of cows with AA, AC and CC genotype in present study was 32, 79 and 42 respectively. The genotypic frequencies of AA, AC and CC were 0.218, 0.498, and 0.284 respectively. Frequencies of A and C alleles were 0.4673 and 0.5327. The Chi square test results (at one degree of freedom and one per cent level)

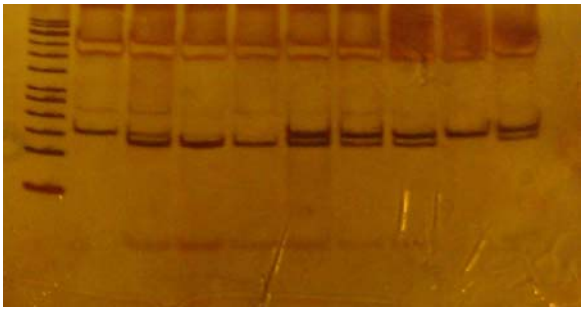

Fig 2: RFLP-PCR results with molecular marker (M50)

Table 1: Chi square test of data

Genotype	Observed	Expected	$\frac{(O-E)^2}{E}$
AA	32	33.2885	0.0499
AC	79	76.4230	0.0869
CC	42	43.2885	0.0384
Total	153	153	0.1752

Table 2: Summary of heterozygosity statistics for OLR1 locus

Locus	Sample size	Obs-Hom	Obs-Het	Exp-Hom	Exp-Het	Ave-Het
OLR1	306	0.4837	0.5163	0.5005	0.4995	0.4979

revealed that the population was in Hardy-Weinberg equilibrium (Table 1).

Observed and expected homozygosity and heterozygosity of OLR1 gene in this study are shown in Table 2.

The frequency of allele C in present study was 0.5327 that is a little more than frequency of allele A (0.4673). Khatib et al. (2006) and Komisarek and Dorynek (2009) reported the same frequency as 0.46 and 0.43 for allele A and 0.54 and 0.57 for allele C in the US, Polish and Holstein cattle populations respectively. Soltani-Ghombavan et al. (2013) studied in five Holstein dairy cattle farm in Esfahan province of Iran, the frequency of allele C and A was 0.483 and 0.517 respectively. Schennink et al. (2009) reported 0.29 and 0.71 frequencies for alleles A and C with experiment on Dutch Holstein population respectively.

Although other SNP were identified in OLR1, only the 3′-UTR SNP was found to be associated with milk traits in the Holstein population (Khatib et al., 2006). Interestingly, quantitative real-time PCR analysis revealed that the expression level of OLR1 was higher in individuals bearing the CC genotype compared with the AA genotype of the 3′-UTR SNP, suggesting that C is the nucleotide causing increased expression of OLR1 or is in strong linkage disequilibrium with the causative SNP (Khatib et al., 2006). The mutation located in the 3'UTR region of OLR1 (C223A) was found to be associated with milk fat percentage. The EBV for this trait was significantly lower in AA bulls than in AC and CC individuals (Komisarek and Dorynek, 2009). Soltani-Ghombavan et al. (2013) reported that the SNP located in the 3'-UTR of OLR1 gene was associated with milk fat percentage. Genotype CC had the highest and AA the lowest fat percentage, while AC was intermediate.

The results of present study showed that frequency of favourable allele (C) was intermediate. Regarding the effects of allele C on milk fat percentage, this allele could be used as a marker in marker-assisted selection with other candidate genes in cattle to increase the frequency of allele C in population for the improvement of milk fat.

References

Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., Van Tassell, C.P., Da, Y., VanRaden, P.M., Ron, M., Weller, J.I. and Lewin, H.A. 2004. Detection of quantitative trait loci affecting milk production, health, and reproductive traits in Holstein cattle. *Journal of Dairy Science*, 87: 468-475.

Boichard, D., Grohs, C., Bourgeois, F., Cerqueira, F., Faugeras, R., Neau, A., Rupp, R., Amigues, Y., Boscher, M.Y. and Levézil, H. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. *Genetic Selection Evolution*, 35: 77-101.

De Koning, D.J., Schulmant, N.F., Elo, K., Moisio, S., Kinos, R., Vilkki, J. and Maki-Tanila, A. 2001. Mapping of multiple quantitative trait loci by simple regression in half-sib designs. *Journal of Animal Science*, 79: 616-622.

Heyen, D.W., Weller, J.I., Ron, M., Band, M., Beever, J.E., Feldmesser, E., Da, Y., Wiggans, G.R., VanRaden, P.M. and Lewin, H.A. 1999. A genome scans for QTL influencing milk production and health traits in dairy cattle. *Physiological Genomics*, 1: 165-175.

Imanishi, T., Hano, T., Sawamura, T., Takarada, S. and Nishio, I. 2002. Oxidized low density lipoprotein potentiation of Fas-induced apoptosis through lectin-like oxidized-low density lipoprotein receptor-1 in human umbilical vascular endothelial cells. *Circulation Journal*, 66: 1060-1064.

Khatib, H., Leonard, S.D., Schutzkus, V., Luo, W. and Chang, Y.M. 2006. Association of the OLR1 gene with milk composition in Holstein dairy cattle. *Journal of Dairy Science*, 89: 1753-1760.

Khatib, H., Rosa, G.J., Weigel, K., Schiavini, F., Santus, E. and Bagnato, A. 2007. Additional support for an association between OLR1 and milk fat traits in cattle. *Animal Genetics*, 38: 308-310.

Komisarek, J. and Dorynek, Z. 2009. Effect of ABCG2, PPARGC1A, OLR1 and SCD1 gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein-Friesian bulls. *Journal of Applied Genetics*, 50: 125-132.

Mehta, J.L. and Li, D.Y. 1998. Identification and autoregulation of receptor for OX-LDL in cultured human coronary artery endothelial cells. *Biochemical and Biophysical Research Communications*, 248: 511-514.

Metha, J.L. and Li, D.Y. 2002. Identification, regulation and function of a novel lectin-like oxidized low-density lipoprotein receptor. *Journal of the American College of Cardiology*, 39: 1429-1435.

Ogorevc, J., Kunej T., Razpet, A. and Dovc, P. 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. *Animal Genetics*, 40: 832-851.

Olsen, H.G., Gomez-Raya, L., Vage, D.I., Olsaker, I., Klungland, H., Svendsen, M., Adnoy, T., Sabry, A., Klemetsdal, G., Schulman, N., Kramer, W., Thaller, G., Ronningen, K. and Lien, S. 2002. A genome scan for quantitative trait loci affecting milk production in Norwegian dairy cattle. *Journal of Dairy Science*, 85: 3124-3130.

Rodriguez-Zas, S.L., Southey, B.R., Heyen, D.W. and Lewin, H.A. 2002. Interval and composite interval mapping of somatic cell score, yield, and components of milk in dairy cattle. *Journal of Dairy Science*, 85: 3081-3091.

Sawamura, T., Kume, N., Aoyama, T., Moriwaki, H., Hoshikawa, H., Aiba, Y., Tanaka, T., Miwa, S., Katsura, Y., Kita, T. and Masaki, T. 1997. An endothelial receptor for oxidized low-density lipoprotein. *Nature*, 386: 73-77.

Schennink, A., Bovenhuis, H., Le´on-Kloosterziel, K.M., van Arendonk J.A.M. and Visker, M.H.P.W. 2009. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. *Animal Genetics*, 40: 909-916.

Soltani-Ghombavan, M., Ansari-Mahyari, S., Ghorbani, G.R. and Edriss, M.A. 2013. Association of a polymorphism in 3' untranslated region of OLR1 gene with milk fat and protein in dairy cows. *Archiv Tierzucht*, 56: 32-40

Viitala, S.M., Schulman, N.F., de Koning, D.J., Elo, K., Kinos, R., Virta, A., Virta, J., Maki-Tanila, A. and Vilkki, J.H. 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. *Journal of Dairy Science*, 86: 1828-1836.

Yeh, F.C., Yang, R.C. and Boyle, T. 1999. Popgene. Microsoft Windows-Based Freeware for Population Genetic Analysis. Version 1.31. *University of Alberta, Canada.*