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Original study

Association of a polymorphism in the 3' untranslated region of the *OLR1* gene with milk fat and protein in dairy cows

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Abstract

Oxidized low density lipoprotein receptor 1 (OLR1) is the major cell surface receptor for oxidized low density lipoprotein (Ox-LDL). The role of OLR1 in lipid metabolism and the existence of milk-related QTL in the vicinity of the *OLR1* gene have prompted the investigation of *OLR1* as a candidate gene influencing milk production traits. The present study explored the association of a single nucleotide polymorphism (SNP) in the 3' untranslated region of the *OLR1* gene ($OLR1_{g.8232C>A}$) with milk-related traits in 408 Iranian Holstein cows. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was performed for genotyping the animals. Animals with genotype CC had the highest and animals with genotype AA had the lowest fat percentage while genotype AC was intermediate ($P<0.05$). Cows carrying genotype CC showed more milk fat yield compared to the genotypes AC ($P<0.1$) and AA ($P<0.01$). Cows of genotypes CC and AC had a higher milk protein percentage than those of genotype AA ($P<0.01$). Regarding the association revealed, the SNP has the potential to be considered as a marker in marker-assisted selection.

Keywords: *OLR1* gene, polymorphism, marker-assisted selection, milk-related traits, dairy Holstein

Abbreviations: bp: base pairs, OLR1: oxidized low density lipoprotein receptor 1, Ox-LDL: oxidized low density lipoprotein, PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism, QTL: quantitative trait loci, SNP: single nucleotide polymorphism, UTR: untranslated region

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Introduction

Milk production traits in dairy cattle are controlled by a large number of quantitative trait loci (QTL) and environmental factors. Identification of genes underlying QTL can provide not only the most accurate markers for marker-assisted selection, but also provokes the investigation of critical biochemical pathways underlying effects of the genes on traits of interest. Additionally, selection programmes could start at early ages before traits of interest can be expressed phenotypically.

Oxidized low density lipoprotein receptor 1 (OLR1) is a type II membrane protein belonging to the C-type lectin family which is encoded by the *OLR1* gene (Chen *et al.* 2001). This receptor acts as the major cell surface receptor for oxidized low density lipoprotein (Ox-LDL) (Kataoka *et al.* 2000).

Based on combined data from different cattle maps, the gene has been estimated to be located in the interval of 106 to 108 cM of the bovine chromosome 5 and it consists of five exons which encode a 270-amino acid protein (Khatib *et al.* 2006). Several QTLs affecting milk production traits have been reported on the bovine chromosome 5 near the *OLR1* gene (Heyen *et al.* 1999, De Koning *et al.* 2001, Rodriguez-Zas *et al.* 2002, Olsen *et al.* 2002, Awad *et al.* 2010, Schopen *et al.* 2011).

Considering the role of OLR1 in lipid metabolism in degrading Ox-LDL and also QTL studies for milk production traits, *OLR1* has been regarded as a candidate gene affecting milk production traits in dairy cattle (Khatib *et al.* 2006). Using direct cDNA and genomic sequencing of *OLR1* gene in the US Holstein cattle population, Khatib *et al.* (2006) revealed two single nucleotide polymorphisms (SNPs) in exon 4, five SNPs in intron 4 and one in the 3' untranslated region (3'-UTR) of the gene. Their study also showed that allele C of SNP in the 3'-UTR had significant effects on fat yield and percentage. Komisarek & Dorynek (2009) and Schennink *et al.* (2009) revealed a significant effect of the *OLR1* gene on milk fat percentage in Polish and Dutch Holstein Friesian cattle, respectively. Wang *et al.* (2012) reported an association between the *OLR1* SNP and protein percentage in the Israeli Holstein population.

We did this experiment to determine the association of polymorphism in the 3'-UTR of the *OLR1* gene and milk-related traits in Iranian Holstein dairy cows. Applying different breeding goals and selection criteria in Iran compared to other countries over generations may have resulted in differences in the genetic background of Iranian Holstein cattle compared to other populations. Also, there are differences in environmental conditions like climate, feeding and management between Iran and other countries. These differences in genetic background and environmental conditions can lead to different results in association analysis.

Materials and methods

Animals and data collection

A total of 408 Iranian Holstein cows which had registered records of milk production traits were randomly selected from five different farms in Isfahan province of Iran. Feeding regimes were different for the five farms and all cows were milked three times a day. For 305-day milk yield, fat percentage and protein percentage phenotypic data, and for milk yield and fat yield breeding values were collected by the Vahdat Industrial Agriculturists & Dairymen

Cooperative of Isfahan. The data for our analysis were obtained through the Cooperative. First-parity data of milk production traits were used for statistical analysis. Blood samples were collected with vacuum venoject tubes containing EDTA and stored at –20 °C until DNA extraction.

SNP genotyping

Genomic DNA was extracted from the whole blood samples using the salting out method (Miller *et al.* 1988). Genotyping was carried out using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The PCR mixture (in a total volume of 20 µL) contained 50 ng genomic DNA, 10 pmol of each primer, 2 µL 10× PCR buffer, 2 mM MgCl2, 200 µmol dNTPs and 2.5 units of Taq polymerase. Sequences of the primers that were used in PCR were as reported previously by Khatib *et al.* (2006). The sequences of the forward and reverse primers were 5'- AAG GCG AAT CTA TTG AGA GC -3' and 5'- ACT TCT CTG AAG TCC TGC A -3', respectively. The 270 bp fragment of the bovine *OLR1* gene was amplified using PCR under the following conditions: initial denaturation at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 s, 57 °C annealing temperature for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. Digestion of PCR products of 270 bp was carried out with 5 U of PstI enzyme (Fermentas, St. Leon-Rot, Germany) in a reaction volume of 20 µL at 37 °C for 4 h. The digested products were run on a 2.5 % agarose gel and visualized with UV light. The A allele (uncut) was indicated by a band of 270 bp and the C allele (cut) resulted in two bands of 250 bp and 20 bp. The allele and genotype frequencies were estimated by direct counting.

Statistical analysis

Test for deviation from Hardy-Weinberg equilibrium was performed using the POPGENE software (Yeh *et al.* 2000). The association between genotypes and traits of interest was analysed through the general linear model (GLM) procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA) according to the following general model. Least squares means (LSMeans) of the genotypes were compared by the LSMeans test:

$$Y_{ijkl} = \mu + G_i + HYS_j + S_k + b1\,(X_{ijkl} - X) + b_2\,(W_{ijkl} - W) + e_{ijkl} \quad (1)$$

where Y_{ijkl} is the value for each milk-related trait, μ is the overall mean, G_i is the fixed effect of the ith genotype (AA, AC and CC), HYS_j is the fixed effect of herd (1, 2, 3, 4, and 5), year and season of parturition, S_k is the random effect of sire (1,…,155), b_1 is the linear regression coefficient of 305-day milk yield, X_{ijkl} is the 305-day milk yield, X is the mean 305-day milk yield, b_2 is the linear regression coefficient of open days, W_{ijkl} represents the open days, W is the mean of open days and e_{ijkl} is the random residual effect.

For analysis of the traits milk yield and fat yield, breeding values were used, so only the effect of genotype was included in the model. Also, for analysis of the trait 305-day milk yield, the covariate 305-day milk yield was excluded from the model.

Results

Allele and genotype frequencies

Both alleles A and C were observed in all five herds with overall frequencies of 0.517 and 0.483, respectively (Table 1). The frequencies for genotypes AA, AC and CC were 0.289, 0.456 and 0.255, respectively, and did not show any significant deviation from Hardy-Weinberg equilibrium proportions within and among all five herds ($P>0.05$; Table 1).

Table 1
Genotype and allele frequencies of the *OLR1* polymorphism in Holstein dairy cows

Herd	Number of animals	Allele		Genotype			Chi-square
		A	C	AA	AC	CC	
1	65	0.585	0.415	0.308	0.554	0.138	1.143^{ns}
2	96	0.417	0.583	0.197	0.437	0.366	1.064^{ns}
3	79	0.456	0.544	0.240	0.430	0.330	1.522^{ns}
4	83	0.500	0.500	0.277	0.446	0.277	1.088^{ns}
5	85	0.653	0.347	0.435	0.435	0.130	0.176^{ns}
Total	408	0.517	0.483	0.289	0.456	0.255	3.187^{ns}

ns: not significant at $P<0.05$

Association analysis

The results of the GLM analysis of association between the *OLR1* gene and our traits of interest are summarized in Table 2.

Table 2
Least squares means and standard error (LSMeans±SE) for milk production traits in Holstein dairy cows with different *OLR1* genotypes

Trait[1]	Genotype (Mean±SE)			Root mean square error
	AA	AC	CC	
305-day milk yield, kg	9322.0 ± 163^{a}	9581.0 ± 150^{a}	9492.0 ± 178^{a}	1246
Breeding value for milk yield, kg	400.8 ± 58.1^{a}	461.4 ± 46.2^{a}	565.5 ± 62.1^{a}	630.8
Breeding value for fat yield, kg	6.66 ± 2.01^{a}	11.44 ± 1.60^{a}	17.24 ± 2.15^{b}	21.82
Fat percentage	2.99 ± 0.05^{a}	3.17 ± 0.05^{b}	3.31 ± 0.05^{c}	0.41
Protein percentage	2.90 ± 0.02^{a}	2.98 ± 0.02^{b}	3.02 ± 0.02^{b}	0.17

Different superscript letters in each row indicate significant differences at $P<0.05$.

The SNP located in the 3'-UTR of the *OLR1* gene was associated with milk fat percentage. Genotype CC had the highest and AA the lowest fat percentage, while AC was intermediate ($P<0.01$). Similarly, there was an association between *OLR1* polymorphism and the breeding value for milk fat yield. Cows homozygous for the C allele had a significantly higher milk fat yield than those homozygous for the A allele ($P<0.01$) and tended to have higher milk fat yield than the AC group ($P<0.1$). The present study revealed an association between SNP in the 3'-UTR of the *OLR1* and milk protein percentage. Cows carrying genotypes CC and AC were found to have more milk protein percentage in comparison with the AA genotype ($P<0.01$). No significant association ($P>0.1$) was evident between the *OLR1* polymorphism and 305-day milk yield and breeding value for milk yield in the current study.

Discussion

The allele frequencies found in the present study were consistent with those of Khatib *et al.* (2006), Komisarek & Dorynek (2009) and Wang *et al.* (2012) who reported 0.46, 0.43 and 0.42 for allele A and 0.54, 0.57 and 0.58 for allele C in US, Polish and the Israeli Holstein cattle populations, respectively. However, they are not consistent with the frequencies reported by Schennink *et al.* (2009) with 0.29 and 0.71 for alleles A and C in an experiment with a Dutch Holstein population.

Our association of the C allele with higher milk fat percentage was consistent with those of Khatib *et al.* (2006), Komisarek & Dorynek (2009) and Schennink *et al.* (2009). Association of the SNP with milk fat yield shown in this study was in line with that of Khatib *et al.* (2006). In contrast, Komisarek & Dorynek (2009) and Schennink *et al.* (2009) did not find any association of this kind in their studies. The present study showed that the allele C of the SNP was associated with higher milk protein percentage. Khatib *et al.* (2006) and Komisarek & Dorynek (2009) did not report this association, but Wang *et al.* (2012) reported such an association in an Israeli Holstein population. The lack of an association between the *OLR1* polymorphism and 305-day milk yield and breeding value for milk yield was in accordance with results from Khatib *et al.* (2006) and Komisarek & Dorynek (2009).

The revealed association of the C allele with higher milk fat and protein may be attributed to more gene expression in the case of allele being C. This SNP might be in linkage disequilibrium with a functional polymorphic site in *OLR1* or another closely linked gene that influences the expression level of the *OLR1*. This hypothesis is supported by the finding that the expression of the *OLR1* was higher in CC cows compared to AA cows (Khatib *et al.* 2006).

Using murine 3T3-L1 adipocytes, Scazzocchio *et al.* (2009) showed that Ox-LDL can cause the impairment of insulin signalling likely because of insulin receptor substrate-1 degradation through enhanced Ser^{307}phosphorylation. On the other hand, there is increasing evidence that Ox-LDL induces beta cell dysfunction and reduction of insulin production (Maziere *et al.* 2004, Abderrahmani *et al.* 2007, Favre *et al.* 2011). Considering the role of insulin in stimulating protein synthesis in mammary gland (Mackle *et al.* 2000, Molento *et al.* 2002, Menzies *et al.* 2009, Appuhamy *et al.* 2011), it can be hypothesized that Ox-LDL may decrease protein synthesis by reducing insulin production in beta cells and its signalling in mammary cells. OLR1, as a major receptor for Ox-LDL, is able to remove Ox-LDL from circulating blood and as a result reduces its deleterious effect on insulin production and signalling. The increased milk protein percentage observed in cows carrying genotype CC may result from a higher *OLR1* gene expression and sufficient reduction of plasma Ox-LDL concentration.

The role of insulin in regulation of lipoprotein lipase activity and mRNA has been reported for the mammary gland of the lactating mouse (Jensen *et al.* 1994). Iverson *et al.* (1995) revealed a relationship between lipoprotein lipase activity and high milk fat during lactation in grey seals. There may be an association of this kind in bovine mammary gland also. Ringseis *et al.* (2007) reported the effect of oxidized fat on reducing milk triacylglycerol concentrations by inhibiting gene expression of lipoprotein lipase and fatty acid transporters in the mammary gland of rats. As an oxidized fat, Ox-LDL may induce a decrease in milk fat in the same way in bovine mammary gland. Association of the C allele with higher milk fat can be a consequence of more *OLR1* gene expression and removal of Ox-LDL from blood.

Possible reasons for obtaining different results in the present study and those of other researchers might include 1) the statistical models used to analyse the data: for 305-day milk yield, fat percentage and protein percentage, phenotypic values were available, while others used BV, DYD or adjusted values for their analysis. So, to adjust the data, we included the effects in our statistical model which were different from those included by others. These differences in statistical models can lead to different results, 2) different environment to which populations were exposed or genotype by environment interaction: effect or expression of genes varies depending on the environmental conditions like feeding, climate and management which may differ in various countries, 3) different genetic background of the animals and possible interactions between the *OLR1* polymorphism and background genes: in Iran, breeders put more emphasis on increasing milk yield rather than milk composition, but in other countries they put emphasis on both milk yield and composition. Different breeding goals and selection criteria will lead to differences in genetic background over generations.

In summary, the present study demonstrates a significant association of the 3'-UTR polymorphism in the *OLR1* gene with protein percentage and fat yield and percentage. The association results suggest that this SNP may be a potential genetic marker in selection programs for dairy cattle through marker-assisted selection. Further investigations are needed to confirm or refute the revealed results and find mechanisms underlying the association found in this study.

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