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A Research on Association between *SCD1* and *OLR1* Genes and Milk Production Traits in Iranian Holstein Dairy Cattle

Research Article

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ABSTRACT

The present study was carried out to investigate the association of C/T single nucleotide polymorphism (SNP) in exon 5 of stearoyl-CoA desaturase 1 (*SCD1*) gene and A/C SNP in the 3' untranslated region of oxidized low density lipoprotein receptor 1 (*OLR1*) gene with milk production traits in Iranian Holstein dairy Cattle. The blood samples of 153 (for *OLR1*) and 308 (for *SCD1*) dairy cattle from three different farms were used for genotyping. A 146 bp fragment of 3'UTR of *OLR1* gene and a 400 bp fragment of exon 5 of *SCD1* gene were amplified by standard PCR. Single nucleotide polymorphism of *OLR1* and *SCD1* gene was determined by polymerase chain reaction single-restriction fragment length polymorphism (PCR-RFLP) technique. The association between genotypes of *OLR1* and *SCD1* genes with milk production traits was studied by general linear models (GLM) procedure of SAS package and Duncan test was used for comparing means of traits. The frequency of AA, AV and VV genotype of *SCD1* gene were 0.60, 0.32 and 0.08 respectively. The frequency of alleles A and V were 0.76 and 0.24. The genotype frequencies of AA, AC and CC in *OLR1* gene were 0.22, 0.50 and 0.28 respectively. The frequency of allele A and C was 0.47 and 0.53. Thus, this population was in Hardy-Weinberg equilibrium for *OLR1* but not for *SCD1*. The means of fat percentage for *SCD1* genotypes were 3.43% and 3.33 for VV and AA respectively ($P<0.05$). The means of *OLR1* genotypes were 8273 kg (CC), 8344 kg (AC) and 7178 kg (AA) for milk yield; 276.3 kg (CC), 277.6 kg (AC) and 239.7 kg (AA) for fat yield and for protein yield were 286.7 kg (CC), 290.5 kg (AC) and 253 kg (AA) ($P<0.05$). The results revealed these two SNP are appropriate for marker assisted selection.

KEY WORDS gene, Holstein, *OLR1*, *SCD1*.

INTRODUCTION

Quantitative traits are controlled by large numbers of genes and also influenced by environmental factors. Recent researches with farm animals have quantified the effect of candidate genes on economically important traits. Identification of genes with large effects on milk production traits would be useful for genetic improvement programs in dairy cattle (Wang *et al.* 2016; Cecchinato *et al.* 2015; Hosseinpour *et al.* 2013). Stearoyl-CoA desaturase (SCD) is an enzyme that plays an important role in the biosynthesis of fatty acids. This enzyme belongs to a large family of enzymes that are involved in the synthesis of saturated fatty acids and they are found in both animals and plants. Stearoyl-CoA desaturase (SCD) is the enzyme responsible for conversion of saturated fatty acids into 9-monounsaturated fatty acids in mammalian adiposities (Campbell *et al.* 2001; Taniguchi *et al.* 2004). Two SCD isoforms are known in cattle, *SCD5* is expressed in brain and is located on chromosome 6 and *SCD1* is located on

chromosome 26 and expressed in adipose and mammary tissue (Schennink *et al.* 2008). The cytosine to thymine substitution causes alanine change to valine amino acids on protein (A293V). Several researches have shown significant effects of genotypes SCD1A293V in exon 5 on the composition of fatty acids in milk and milk production traits (Moioli *et al.* 2007; Kgwatalala *et al.* 2009; Clark *et al.* 2010).

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) (Mehta and Li, 2002). The genomic sequence of bovine *OLR1*, released by Baylor College of Medicine, contains five exons and located on chromosome 5. The length of this gene is 11373 base pairs (GenBank accession no. NW_215807). The bovine *OLR1* gene encodes 270 AA that has a 72% identity to the human protein (Sawamura *et al.* 1997).

Many studies have been conducted on QTL in bovine chromosome 5 near *OLR1* affecting milk production traits between 1999 and 2004 (Heyen *et al.* 1999; Olsen *et al.* 2002; Ashwell *et al.* 2004; De Koning *et al.* 2001; Viitala *et al.* 2003; Rodriguez-Zas *et al.* 2002). 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 (3'UTR) (Khatib *et al.* 2006). Some researchers reported that allele C of SNP in the 3'UTR had significant effects on fat yield and fat percentage. Khatib *et al.* (2006) reported significant effects of A/C SNP in the 3′-untranslated region of OLR1 on milk fat yield and fat percentage in a granddaughter-design 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) reported a significant association between *OLR1* and milk fat percentage in Dutch Holstein-Friesian cattle.

Because of the role of *OLR1* in lipid metabolism and degradation of Ox-LDL, the *OLR1* gene has been regarded as a candidate gene affecting milk production traits in dairy cattle (Khatib *et al.* 2006). Thus, the objective of this research was to study the association between genes *OLR1* and *SCD1* and milk production traits in Iranian Holstein dairy cattle.

MATERIALS AND METHODS

Samples and phenotypic data

Blood samples of 153 (for *OLR1*) and 308 (for *SCD1*) of Iranian Holstein dairy cattle were randomly selected for genotyping from three different farms in the Khorasan Razavi province of Iran. Records of 136 (*OLR1*) and 274 (*SCD1*) cows were used for the association study. The Studied traits were 305-day milk, fat and protein yield and fat and protein percentage.

Genotyping of SNP

Approximately 5 mL of blood were collected from the jugular vein of each animal in EDTA tubes. The aliquots of whole blood were stored at -20 ˚C. The genomic DNA from blood samples was extracted using the GuSCN-Silica Gel method and standard protocol with commercial kit of DIAtom DNA Prep (Biokom). The quality of DNA was examined by agarose gel electrophoresis. Genotyping was carried out using the PCR-RFLP technique. The 146 bp fragment of *OLR1* gene from 3'UTR (GenBank accession no. NC_007303.4; 107080852; 107092157 described in Khatib *et al.* 2006) was amplified by standard PCR by Biometra thermo cycler (Germany). The sequences of the primers were (F 5′-TCCCTAACTTGTTCCAAGTCCT-3′) and (R 5′-CTCTACAATGCCTAGAAGAAAGC-3'), respectively (Komisarek and Dorynek, 2009). The total volume of reaction was 25 µL that contained one unit (0.2 µL) of *Taq* polymerase, 200 µ*M* (0.5 µL) of dNTP, 2 m*M* $MgCl_2$, 10 p*M* (3 µL) primer mix and 2.5 µL standard buffer in 13.8 µL dH_2O. Fifty nano grams (5 µL) DNA were added to the reaction mix. The thermal cycling conditions for the PCR were as follows: initial denaturation at 94 ˚C for 5 min, cyclic denaturation at 94 ˚C for 30 s, cyclic annealing of primers at 62 ˚C for 30 s, cyclic elongation at 72 ˚C for 45 s (for 30 cycles) and final elongation at 72 ˚C for 5 min (Komisarek and Dorynek, 2009). The PCR products were separated by electrophoresis on 2% agarose gel and visualized on gel documentation system (UVP, 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 37 ˚C. Polyacrylamide gel electrophoresis methods were used to identify genotypes. The 400bp fragment of *SCD1* gene that included exon 5 was amplified with standard PCR based on GenBank accession no. AY241932. The primers were as follow: F (5′-CCC ATT CGC TCT TGT TCT GT-3′) and R (5′-CCC ATT CGC TCT TGT TCT GT-3′).

The total volume of PCR reaction was 25 µL similar to *OLR1* fragment. The thermal program of PCR reaction was initial denaturation at 94 ˚C for 3 min, cyclic denaturation at 94 ˚C for 45 s, cyclic annealing of primers at 54 ˚C for 30 s , cyclic elongation at 72 ˚C for 90 s (for 34 cycles) and final elongation at 72 ˚C for 10 min (Kgwatalala *et al.* 2009).

The PCR products were separated by electrophoresis on 2% agarose gel and visualized on UVP gel documentation system. The PCR product was digested with restriction enzyme *NcoI* (Fermentas). The 3 µL of PCR product was mixed with 2 µL 10X buffer, 4.5 µL dH_2O and 2 units of *NcoI* and digested over 5 hours at 37 ˚C . The digested fragment was loaded in 2% agarose gel and visualized on UVP gel documentation system.

Statistical analysis

The Hardy-Weinberg equilibrium for allele and genotype frequencies was analyzed with Chi-square test using POPGENE software (Yeh *et al.* 1999). The association between genotypes and traits was assessed with the (GLM) procedure of SAS (2004) according to the following general linear model:

$$Y_{ijnl} = \mu + G_i + HYS_j + L_k + S_l + e_{ijkl}$$

Where:

Y_{ijkl}: value for each milk-related trait.

µ: overall mean.

G_i: fixed effect of the i^{th} genotype (3 genotypes for each gene: AA, AV and VV for SCD1 or AA, AC and CC for *OLR1*).

HYS_j: fixed effect of herd (1, 2, 3), year and season of parturition.

L_k: k^{th} lactation (1 and 2).

S_l: random effect of sire (1,…,128).

e_{ijkl}: residual effects.

Due to use the records of traits, the fixed effects and the sire effect (as random genetic effect) were considered in the model. Genotype means were compared with the Duncan test ($P<0.05$).

RESULTS AND DISCUSSION

Gene and genotypic frequencies

Genotype and allele frequencies are shown in Table 1. The *SCD1* genotypic frequencies for AA, AV and VV were 0.60, 0.32 and 0.08, respectively. The frequencies of alleles A and V of *SCD1* were 0.76 and 0.24. The population was not in Hardy Weinberg equilibrium for gene *SCD1*. Because of population was under selection and probably other dispersive factor such as population size was affected.

The higher frequency of the A allele is in agreement with results reported by other studies. Schennink *et al.* (2008) reported the frequency of allele A and V as 0.73 and 0.27. Kgwatalala *et al.* (2009) genotyped 525 Canadian Jersey cows for the *SCD1* gene and reported genotypic frequencies as 0.686, 0.244 and 0.07 for the AA, AV and VV genotypes, respectively and frequencies of alleles A and V of 0.808 and 0.192. Genotype frequencies were in Hardy-Weinberg equilibrium. Cows of 3 breeds of northern Italy, Jersey, Valdostana and Piedmontese were genotyped at exon 5 of the *SCD* gene; the frequency of A allele was 0.94 for Jersey, 0.65 for Valdostana and 0.42 for Piedmontese (Moioli *et al.* 2007). Clark *et al.* (2010) determined the polymorphism of 143 and 215 cows in two studies. The distribution of genotypes among 143 dairy cows was 72 AA, 60 AV, and 11 VV animals. Therefore, allele frequencies were 0.71 (A) and 0.29 (V). In study 2, the distribution of genotypes for 215 dairy cows was 111 (AA), 85 (AV) and 19 (VV) animals. Allele frequencies were 0.71 and 0.29 for the A and V alleles. Frequencies of *SCD* genotypes in the sample of Italian Friesian cows were 0.27, 0.6 and 0.13 for AA, AV and VV genotypes, respectively (Mele *et al.* 2007). The higher frequency of the A allele (0.57) compared to allele V (0.43) agreed with the result of this study.

The genotypic frequencies of gene *OLR1* (AA, AC and CC) were 0.22, 0.5, and 0.28 respectively. Frequencies of A and C alleles were 0.47 and 0.53. The Chi-square test revealed that the population was in Hardy-Weinberg equilibrium, the two alleles frequencies were near to 0.5 so the population was in equilibrium and probably the selection and other factors were not effective. Khatib *et al.* (2006) and Komisarek and Dorynek (2009) reported the same frequency of 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) estimated the frequency of allele C and A to be 0.483 and 0.517 in Holstein dairy cattle from 5 farms in the Esfahan province of Iran. Schennink *et al.* (2009) reported frequencies of 0.29 and 0.71 for alleles A and C in a Dutch Holstein population.

Association analysis

In general, the used regression model was significant for all studied traits with *OLR1* or *SCD1* genotypes included in model. The coefficient of determination (R^2) was higher than 0.8 and 0.9 for most of traits that showed the fixed and random effects in general linear model justify the variation in traits. Trait means, standard errors, and coefficients of variation are also shown in Table 2.

Table 1 Genotype and allele frequencies

Gene	Genotype			Allele		Index		HWE
SCD1	AA	AV	VV	A	V	Shanon	Nei	χ^2
	0.60	0.32	0.08	0.76	0.24	0.55	0.36	20.37**
OLR1	AA	AC	CC	A	C	-	-	-
	0.22	0.50	0.28	0.47	0.53	0.69	0.50	0.17^{ns}

SCD1: stearoyl-CoA desaturase 1 and *OLR1*: oxidized low density lipoprotein receptor 1.
HWE: Hardy-Weinberg equilibrium.
** (P<0.01).
NS: non significant.

Table 2 Analysis of general linear model for studied traits including *SCD1* or *OLR1* genotypes

Model	Parameter	MY (kg)	FY (kg)	PY (kg)	FP %	PP %
With *SCD1* genotype	Pr > F	0.0002	0.0001	0.0001	0.0019	0.0001
	R^2	0.77	0.82	0.80	0.74	0.87
	(Mean±SE)	8115±1364	272±42.8	285±53.6	3.36±0.231	3.5±0.254
	CV%	16.8	15.7	18.8	6.9	7.3
With *OLR1* genotype	Pr > F	0.0175	0.0008	0.0018	0.0219	0.0001
	R^2	0.88	0.92	0.91	0.88	0.94
	(Mean±SE)	8077±1302	269±39.3	281±48.6	3.33±0.208	3.47±0.243
	CV %	16	14.6	17.2	6.25	7.02

SCD1: stearoyl-CoA desaturase 1 and *OLR1*: oxidized low density lipoprotein receptor 1.
MY: milk yield; FY: fat yield; PY: protein yield; FP: fat percentage and PP: protein percentage.
SE: standard error.
CV: coefficient of variation.

The coefficient of variation range for 305-d milk, fat and protein yield were between 14.7 (for fat yield) to 18.8 (for protein yield), this value for fat and protein percentage were lower and the range was between 6.25 (for fat percentage) and 7.3 (for protein percentage). The results of comparing traits means with different *SCD1* and *OLR1* genotype by Duncan multiple rang test are shown in Table 3.

SCD1

The *SCD1* genotypes showed significant effects on fat percentage but no effect on the other traits. The means of fat percentage for VV and AA genotype were 3.43% and 3.33% (P<0.05), this result was similar to Komisark and Dorynek (2009), that showed a positive effect of VV genotype on fat percentage. We found 305-d milk and protein yield means of cows with genotype AA to be higher than those of cows with the VV genotype. Kgwatalala *et al.* (2009) reported that allele A was positively associated with increased 305-d milk and protein yields. Effects of *SCD* genotype were not observed for milk yield or composition (Clark *et al.* 2010). The *SCD1* genotype did not significantly affect fat or protein percentage and fat, protein, or milk yield (Schennink *et al.* 2008).

OLR1

The comparison of traits mean for *OLR1* genotypes are shown in Table 3. The milk yield mean of cows with genotype AA (7178 kg) was significantly higher (P<0.05) than those of cows with genotypes AC (8344 kg) and CC (8273 kg).

The means of fat and protein yield for cows with the AC and CC genotypes were higher than the corresponding values for cows with the AA genotype (P<0.05). The means for fat and protein percentage in AA cows were somewhat higher than those for AC and CC cows, but differences were not significant (P>0.05). 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) reported the positive effect of allele C on fat and protein percentage and fat yield (P<0.05). Soltani-Ghombavani *et al.* (2013) revealed a significant effect of *OLR1* gene on fat and protein percentage and breeding value of fat yield in Iranian Holstein cattle, they found the value of milk yield for CC and AC genotype more than AA genotype but there was no significant association. 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).

Komisarek and Dorynek (2009) reported a significant association between C allele and fat yield and fat percentage. The significant effect of allele C on fat yield in this study was similar to those reported by Khatib *et al.* (2006); Komisarek and Dorynek (2009) and Soltani-Ghombavan *et al.* (2013). The association of CC genotype with milk yield shown in this study disagreed with the findings of Khatib *et al.* (2006) and Komisarek and Dorynek (2009).

Table 3 Compare genotypes mean for milk production traits with different genotypes of *SCD1* or *OLR1* gene

Gene	Genotype	(MY (kg)±SE)	(FY (kg))±SE)	(PY (kg)±SE)	(FP %±SE)	(PP %±SE)
	AA (no 172)	8130±134	271±4.8	285.8±5.8	3.33^{b}±0.02	3.49±0.03
SCD1	AV (no 72)	8113±202	276±7.2	285.6±8.7	3.40^{ab}±0.03	3.5±0.05
	VV (no 30)	8036±285	274 ±10.5	283.7±10.6	3.43^{a}±0.06	3.53±0.04
P-value	-	0.31	0.41	0.50	0.048	0.68
	AA (no 29)	7178^{b}±267	239^{b}±9.4	253^{b}±12	3.35±0.052	3.52±0.09
OLR1	AC (no 73)	8344^{a}±209	277^{a}±7.5	290^{a}±9.2	3.33±0.032	3.46±0.06
	CC (no 34)	8273^{a}±291	276^{a}±8.3	286^{a}±11.7	3.34±0.045	3.46±0.06
P-value	-	0.049	0.038	0.039	0.41	0.71

SCD1: stearoyl-CoA desaturase 1 and *OLR1*: oxidized low density lipoprotein receptor 1.
MY: milk yield; FY: fat yield; PY: protein yield; FP: fat percentage and PP: protein percentage.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

Conversely, Schennink *et al*. (2009) also found no association between *OLR1* gene and fat percentage in Dutch Holstein Friesian cows agreeing on results here. Similarly, no association was observed between polymorphism in *OLR1* and either milk production traits or reproduction traits in a Czech Fleckvieh population (Rychtarova *et al.* 2014).

CONCLUSION

The frequency of allele A of *SCD1* was 0.76 in the sample of Iranian Holstein dairy cattle studied here, and the association of genotype with fat percentage was significant. The population was in linkage disequilibrium for *SCD1*, perhaps due to selection for fat percentage in this population. Although the frequency of the C allele of gene *OLR1* was somewhat higher than that of the A allele, the population was in linkage equilibrium. However, the C allele had significant effects on milk, fat and protein yields, it could be concluded the favorable allele for *SCD1* and *OLR1* gene are A and C allele, thus these alleles could be used in a marker-assisted selection program to increase favorable alleles frequencies in the population and also these SNPs are proper for having on commercial SNP panels.

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