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Association of bovine *PPARGC1A* and *OPN* genes with milk production and composition in Holstein cattle

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ABSTRACT: Several studies have reported quantitative trait loci on chromosome 6 affecting milk production and composition traits in dairy cattle. Osteopontin (*OPN*) and peroxisome proliferator activated receptor-γ coactivator-1α (*PPARGC1A*) genes have been located on this chromosome and identified as positional candidates for milk traits. We investigated the associations of single nucleotide polymorphism (SNP) T>C at position 1892 and SNP A>C at position 3359 in *PPARGC1A* gene as well as SNP C>T at position 8514 in *OPN* gene with milk production and composition. Hence, 398 Iranian Holstein cows were genotyped through polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The Least Squares methods were used to examine the effects of genotypes on milk traits. The frequencies of the *C* allele at position 1892, *A* allele at position 3359, and *T* allele at position 8514 were 0.56, 0.64, and 0.53, respectively. In this study, c.1892T>C genotypes indicated significant associations with milk fat content adjusted for two milkings per day (FAT_{P2X}; %), estimated breeding value for milk (EBV_M; kg), milk protein yield adjusted for mature body weight (PRO_{ME}; kg), milk protein yield adjusted for 305 days (PRO_{305}; kg) ($P < 0.05$), and estimated breeding value for milk fat content (EBV_{FP}; %) ($P < 0.01$). There were significant associations between c.3359A>C genotypes and FAT_{P2X}, EBV_{FP} ($P < 0.01$). Moreover, significant associations were shown between c.8514C>T genotypes in *OPN* gene and FAT_{P2X} ($P < 0.05$), and PRO_{PER305} ($P < 0.01$). Thus, these SNPs would provide an excellent opportunity for marker assisted selection programs in dairy cattle.

Keywords: cattle; *OPN*; PCR-RFLP; *PPARGC1A*; SNPs

INTRODUCTION

An essential field of research in livestock species is detection of genes that influence important production traits. In dairy cattle, most researches have detected quantitative trait loci (QTL) on autosomal chromosomes affecting one or more of the five milk traits (i.e. milk yield, fat yield and percentage, and protein yield and percentage). Many studies have reported that the highest number of these QTL have been located on chromosome 6 (Khatkar et al. 2004). Osteopontin (*OPN*) and peroxisome proliferator activated receptor-γ coactivator-1α (*PPARGC1A* termed *PGC-1α*) are located in the middle of chromosome 6 of bovine about 6Mb apart, which is approximately 12 cM (Khatib et al. 2007). *PPARGC1A* is as activator of various nuclear hormone receptors and mediator in expression of genes involved in oxidative metabolism, adipogenesis, and gluconeogenesis. In humans and mice, the role of *PPARGC1A* in liver, fat, and muscle tissue is contributing in glucose and fat metabolism and energy balance (Liang and Ward 2006). The *PPARGC1A* gene is located on chromosome 5 in mice and chromosome 4 in humans. Also, this gene encodes a protein that contains 797 or 798 amino acids in mouse and human, respectively (Liang and Ward 2006). Structure of

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PPARGC1A gene is made from 13 exons composed of 6261 bp. Also, it is expressed at various levels in many tissues (Weikard et al. 2005).

OPN is a phosphoprotein secreted into body fluids firstly identified as a bone matrix protein. The recent researches reported that *OPN* could act as a cytokine (Eta-1) when produced by activated T cells (Denhardt et al. 2001). *OPN* gene has been located on chromosomes 4 and 5 in human and mouse, respectively (Nemir et al. 2000). *OPN* phosphorprotein is composed of about 300 amino acids (278 in cow, 280 in buffalo, 297 in mouse, 314 in human) (Liang and Ward 2006). It is reported that the *OPN* gene expression is higher in mammary gland epithelial cells and monocytes and macrophages in milk. It is recognized the range of *OPN* concentration is 3–10 µg/ml in human (Senger et al. 1989) and 8 mg/l in raw milk of cows (Bayless et al. 1997). The different roles of human *OPN* are: cell adhesion and survival, tissue remodelling, regulation of inflammation, fetal growth and development, and in initiating and maintaining pregnancy (Denhardt et al. 2001). The *OPN* gene is located in a region of bovine chromosome 6 that comprises QTL affecting milk production traits (Ron et al. 2001; Rodriguez-Zas et al. 2002; Ashwell et al. 2004).

Weikard et al. (2005) showed that SNP T>C of *PPARGC1A* gene had a significant effect on milk fat yield in the German Holstein population. Khatib et al. (2007) reported significant associations between SNP A>C of *PPARGC1A* gene and milk yield, milk protein percentage, and SCS in the North American Holstein population. Leonard et al. (2005) reported significant associations between SNP A>C of *OPN* gene and milk protein percentage and milk fat percentage in the North American Holstein population. Ron et al. (2001) detected QTL affecting protein percentage to a confidence interval of 4 cM in the region of *OPN*. A QTL affecting milk production traits was mapped to an interval of 420 kb between the genes *ABCG2* and *LAP3* on bovine chromosome 6. This fine region harbours only 6 genes, including *OPN* (Olsen et al. 2005). Also, a quantitative trait nucleotide has been situated upstream of *OPN* promoter region that has significant effects on percentage of milk protein and fat (Schnabel et al. 2005). Thus, in this study, the association of bovine *OPN* and *PPARGC1A* variants with milk production and composition were investigated in Iranian Holstein cows.

MATERIAL AND METHODS

A total of 398 Holstein-Friesian cows from 10 dairy herds were selected from Tehran and Esfahan provinces of Iran. They were fed standard diet and were milked twice a day using a pipeline machine. Rainfall (mm) and minimum daily temperature (°C) values are higher in Tehran than in Esfahan. The first lactation records of cows born within 2003–2006 were used. All phenotypic data ($MILK_{305}$ (milk yield adjusted for 305 days; kg), $MILK_{2X}$ (milk yield adjusted for two-milkings per day; kg), $MILK_{ME}$ (milk yield adjusted for mature body weight; kg), PRO_{305} (milk protein yield adjusted for 305 days; kg), PRO_{ME} (milk protein yield adjusted for mature body weight; kg), PRO_{PER305} (milk protein content adjusted for 305 days; %), FAT_{2X} (milk fat yield adjusted for two-milkings per day; kg), FAT_{P2X} (milk fat content adjusted for two milkings per day; %)) and estimated breeding values (EBV_M (estimated breeding value for milk; kg), EBV_F (estimated breeding value for milk fat yield; kg), EBV_{FP} (estimated breeding value for milk fat content; %)) used in this study were obtained from the Animal Breeding Center of Iran. DNA isolation was performed using salting out method. Analysis of genotypes was performed using the PCR-RFLP method. Two polymorphisms were studied in the *PPARGC1A* gene. These sites were found in intron 9 (T/C at position 1892) and in the 3′ untranslated region (A/C at position 3359) (GenBank Accession No. AY321517). The primers used to amplify two fragments of the *PPARGC1A* gene were designed by Weikard et al. (2005). One SNP was researched in intron 4 (C>T at position 8514) of the *OPN* gene (GenBank Accession No. NW_255516). The primers used to amplify a fragment of the *OPN* gene were designed by Leonard et al. (2005). The amplification of genomic DNA was performed in 20 µl of reaction volume, which included 100 ng of genomic DNA, 0.6 pm of each primer, 0.2mM of each dNTP, 2 µl of 10× PCR buffer, 2mM of $MgCl_2$, and 1.5 units of Taq DNA polymerase. The temperature cycles for amplification of DNA were as follows: the initial denaturation at 94°C for 5 min; 35 cycles were included: denaturation at 94°C for 50 s, touchdown annealing for *PPARGC1A* gene from 60 to 55°C (–0.5°C/cycle) for 50 s and for *OPN* gene from 60 to 53°C (–0.7°C/cycle) for 40 s, extension at 72°C for 50 s; and a final extension at 72°C for 7 min.

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The PCR products of *PPARGC1A* gene (195 and 357 bp fragments) were incubated with restriction enzymes *HaeIII* and *NheI* respectively at 37°C for 3 h. PCR products of *OPN* gene (290 bp fragment) were digested with the restriction enzyme *BsrI* (*BseNI*) at 65°C for 5 h. The digestion products were electrophoresed on a 2% agarose gel.

The Pop Gene software (Version 1.31, 1999) was used to estimate the Hardy-Weinberg test, gene and genotype frequency, observed and expected heterozygosity. The standard error of allele frequency was calculated by the following formula (Falconer and Mackay 1996):

$$\sqrt{p(1-p)/2n}$$

where:
n = sample size
p = frequency of the *C* allele

The haplotypes frequencies at a pair of loci were used to calculate linkage disequilibrium (r^2 statistics) between the studied SNPs (Hill and Robertson 1968). The effects of *PPARGC1A* and *OPN* genotypes on milk production and composition traits were analyzed using the Least Squares method of the GLM procedure of SAS (Statistical Analysis System, Version 8.0, 1999). Tukey's test was used to compare the means. The used model was as follows:

$$Y_{ijkmn} = \mu + G_i + S_j + M_k + N_m + e_{ijkmn}$$

where:
Y_{ijkmn} = observed trait
μ = overall mean
G_i = fixed effect of genotypes (3 levels)
S_j = fixed effect of herd (10 levels)
M_k = fixed effect of year (3 levels)
N_m = fixed effect of calving seasons (4 levels)
e_{ijkmn} = residual effect to each observation

In addition, the SNPs effects of *PPARGC1A* and *OPN* genes on estimated breeding values were analyzed using a statistical model including the genotypes effects. To calculate additive effects, the genotypes were ranked as the number of *C* alleles (0, 1, or 2) at the c.1892T>C and c.8514C>T loci and the number of *A* alleles (0, 1, or 2) at the c.3359A>C locus. To test for dominance, an additional regression covariate was added with value of 0 for homozygous and 1 for heterozygous animals. *PPARGC1A* and *OPN* genes variance was estimated using the $(1 - MS_{fullmodel}/MS_{reducedmodel})$ equation (Knott et al. 1996), where $MS_{fullmodel}$ and $MS_{reducedmodel}$ – residual mean squares of the model with the *PPARGC1A* and *OPN* effect fitted and the model without the *PPARGC1A* and *OPN* effect, respectively.

RESULTS AND DISCUSSION

The *PPARGC1A* and *OPN* genes were chosen for this research. The first reason was QTL studies that indicated an association of *PPARGC1A* and *OPN* regions on milk production and composition traits in dairy cattle (Khatkar et al. 2004; Olsen et al. 2005; Weikard et al. 2005; Khatib et al. 2007). The second reason was the involvement of these genes in regulating important physiological processes – *PPARGC1A* plays a fundamental role in the regulation of cellular energy metabolism such as adaptive thermogenesis, mitochondrial biogenesis, adipogenesis, gluconeogenesis, and glucose/fatty acid metabolism (Liang and Ward 2006). Also, the existence of OPN protein in milk and its high expression in mammary gland epithelial cells are the evidences for effects of different *OPN* genotypes on milk production and composition in Holstein dairy cattle population (Leonard et al. 2005). In the present study, three digestion pat-

Table 1. Summary of population genetic information for c.1892T>C, c.3359A>C, and c.8514C>T positions

Loci	Genotypic frequency			Allelic frequency		SEM	Heterozygosity		χ^2
							observed	expected	
c.1892T>C	*TT*	*TC*	*CC*	*T*	C				
	0.12	0.65	0.23	0.44	0.56	0.01	0.65	0.49	8.51**
c.3359A>C	*AA*	*AC*	*CC*	*A*	*C*				
	0.38	0.52	0.10	0.64	0.36	0.01	0.52	0.46	6.01*
c.8514C>T	*CC*	*CT*	*TT*	*C*	*T*				
	0.19	0.57	0.24	0.47	0.53	0.01	0.57	0.49	7.50**

*P = < 0.05, **P = < 0.01

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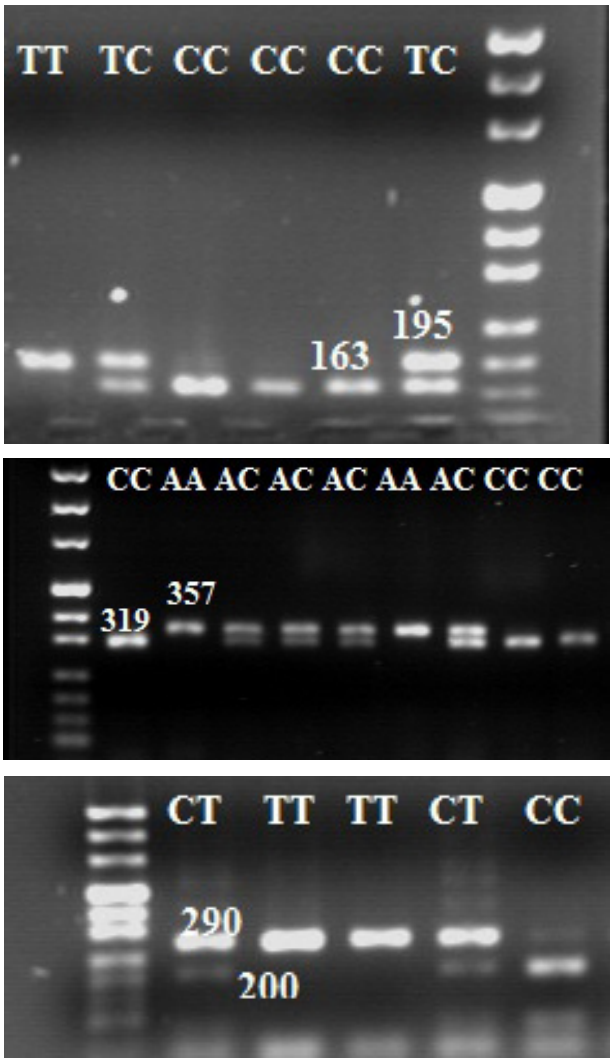

Figure 1. (**A**) Enzymatic digestion for the 195 bp fragment of *PPARGC1A* gene: *TT* (195 bp), *CC* (163 and 32 bp), *TC* (195, 163, and 32 bp); (**B**) enzymatic digestion for the 357 bp fragment of *PPARGC1A* gene: *AA* (357 bp), *CC* (319 and 38 bp), *AC* (357, 319, and 38 bp); (**C**) enzymatic digestion for the 290 bp fragment of *OPN* gene: *TT* (290 bp), *CC* (200 and 90 bp), *CT* (290, 200, and 90 bp). The 32, 38, and 90 bp fragments are not shown

terns were obtained for each of the three studied SNPs in Holstein cows (Figure 1).

In the examined group of 398 cows, the genotypes 46 *TT*, 259 *TC*, and 93 *CC* (c.1892T>C); 151 *AA*, 207 *AC*, and 40 *CC* (c.3359A>C); and 75 *CC*, 226 *CT*, and 97 *TT* (c.8514C>T) were identified. The allelic and genotypic frequencies, standard error of frequencies, observed and expected heterozygosity, and the value of chi-square test are shown in Table 1.

The highest and least frequencies of genotypes in c.1892T>C SNP were for *TC* and *TT*, respectively, consistent with the results obtained by Khatib et al. (2007). The highest allele frequency was reported for *C* allele in this study and other researches (White et al., 2007; Komisarek and Dorynek 2009; Schennink et al. 2009). The frequencies were reported in c.3359A>C position of *PPARGC1A* gene as follows: the highest frequency was found for the *AC* genotype and the lowest frequency for the *CC* genotype. These results are similar to those of Weikard et al. (2005) and Khatib et al. (2007), who reported the highest frequency for the *AC* genotype, as well as of Kowalewska-Luczak et al. (2010) who obtained the least genotypic frequency for the *CC*. It is interesting that the obtained allelic frequencies in our study are the same as those reported by Komisarek and Dorynek 2009 (*A* – 0.64 and *C* – 0.36). Also, we observed the highest and least genotypic frequencies at c.8514C>T position of *OPN* gene for the *CT* and *CC*, respectively. The obtained results are consistent with the previous studies (Khatib et al. 2007; Pareek et al. 2008). The differences in genotypic frequencies might be regarded as the reason of variation of the studied breeds or discrepancy in sample size. The chi-square test indicated the genotypes in all loci deviated from the Hardy Weinberg equilibrium ($P < 0.05$). These values indicate that the population has been under selection for milk production and composition traits for years.

Table 2 shows the effect of the c.1892T>C genotypes of the *PPARGC1A* gene on milk production and composition traits. The results showed that the *TT* genotype is associated with higher FAT_{P2X} ($P < 0.05$) and more EBV_{FP} ($P < 0.01$) if compared to other genotypes. The *CC* genotype had higher EBV_{M} compared to *TC* and *CC* cows ($P < 0.05$). Finally, the *TC* genotype group yielded more PRO_{305} and PRO_{ME} in comparison to the *TT* groups ($P < 0.05$). Significant differences were found for c.3359A>C genotypes of the *PPARGC1A* gene with FAT_{P2X} and EBV_{FP} (Table 2). Cows of the *AA* genotype showed more FAT_{P2X} compared to *AC* cows ($P < 0.01$). Also, cows of *AA* genotypes had higher EBV_{FP} compared to other genotypes ($P < 0.01$).

Khatib et al. (2007) reported that the *A* allele in c.3359A>C position of *PPARGC1A* gene was associated with increasing of protein percentage and decreasing of milk yield, whereas they did not find any association with c.1892T>C and milk production traits. Weikard et al. (2005) showed that cows with the *TT* genotypes of c.1892T>C position yielded higher milk fat than the other

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Table 2. Effect of c.1892T>C, c.3359A>C, and c.8514C>T positions genotypes of *PPARGC1A* and *OPN* genes on milk traits in Iranian Holstein cows (Least Squares Means ± standard errors)

Traits	c.1892T>C Genotypes			*P*-value
	TT (*n* = 46)	*TC* (*n* = 259)	*CC* (*n* = 93)	
$MILK_{305}$	6902 ± 612	7801 ± 479	7318 ± 508	0.125
$MILK_{2X}$	6876 ± 285	7349 ± 223	7291 ± 237	0.312
$MILK_{ME}$	7988 ± 327	8525 ± 256	8490 ± 271	0.259
FAT_{2X}	219.5 ± 10.6	234.5 ± 8.3	224.7 ± 8.7	0.388
FAT_{P2X}	3.29 ± 0.10^{a}	3.21 ± 0.08^{b}	3.10 ± 0.09^{c}	0.021
EBV_{M}	–16.79 ± 89.73^{c}	12.60 ± 58.84^{b}	50.08 ± 72.52^{a}	0.024
EBV_{F}	1.36 ± 3.07	3.69 ± 2.01	3.40 ± 2.48	0.892
EBV_{FP}	0.09 ± 0.02^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.001
PRO_{305}	207.2 ± 9.2^{b}	223.2 ± 7.0^{a}	218.6 ± 7.4^{ab}	0.038
PRO_{PER305}	3.09 ± 0.05	3.06 ± 0.04	3.04 ± 0.04	0.515
PRO_{ME}	240.6 ± 10.5^{b}	258.5 ± 8.0^{a}	253.7 ± 8.5^{ab}	0.040
	c.3359A>C genotypes			
	AA (*n* = 151)	*AC* (*n* = 207)	*CC* (*n* = 40)	
$MILK_{305}$	7297 ± 507	7656 ± 457	7495 ± 643	0.148
$MILK_{2X}$	7216 ± 264	7326 ± 222	7258 ± 300	0.782
$MILK_{ME}$	8379 ± 271	8511 ± 254	8464 ± 344	0.792
FAT_{2X}	232.2 ± 8.7	227.6 ± 8.2	233.7 ± 11.1	0.636
FAT_{P2X}	3.27 ± 0.08^{a}	3.14 ± 0.07^{b}	3.13 ± 0.10^{ab}	0.009
EBV_{M}	–44.12 ± 66.05	8.73 ± 60.08	94.10 ± 96.11	0.229
EBV_{F}	3.03 ± 2.24	3.09 ± 2.04	4.87 ± 3.27	0.870
EBV_{FP}	0.05 ± 0.01^{a}	0.02 ± 0.01^{b}	0.01 ± 0.02^{c}	0.004
PRO_{305}	218.5 ± 7.4	221.7 ± 6.8	229.7 ± 9.9	0.382
PRO_{PER305}	3.07 ± 0.03	3.07 ± 0.03	3.06 ± 0.05	0.189
PRO_{ME}	253.4 ± 8.5	257.1 ± 7.8	266.3 ± 11.4	0.366
	c.8514C>T genotypes			
	CC (*n* = 75)	*CT* (*n* = 226)	*TT* (*n* = 97)	
$MILK_{305}$	7588 ± 540	7428 ± 497	7606 ± 508	0.755
$MILK_{2X}$	7338 ± 252	7332 ± 232	7216 ± 237	0.497
$MILK_{ME}$	8506 ± 289	8514 ± 266	8399 ± 272	0.614
FAT_{2X}	222.0 ± 9.4	232.4 ± 8.4	230.2 ± 8.8	0.152
FAT_{P2X}	3.07 ± 0.09^{c}	3.24 ± 0.08^{a}	3.20 ± 0.08^{b}	0.025
EBV_{M}	77.72 ± 53.60	61.96 ± 28.43	69.67 ± 55.92	0.181
EBV_{F}	4.78 ± 2.63	3.49 ± 2.10	1.91 ± 2.36	0.291
EBV_{FP}	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.089
PRO_{305}	220.4 ± 7.9	224.2 ± 7.1	217.8 ± 7.6	0.512
PRO_{PER305}	3.05 ± 0.04^{b}	3.08 ± 0.03^{a}	3.06 ± 0.03^{b}	0.004
PRO_{ME}	255.3 ± 9.0	259.9 ± 8.1	252.8 ± 8.6	0.501

$MILK_{305}$ = milk yield adjusted for 305 days (kg), $MILK_{2X}$ = milk yield adjusted for two milkings per day (kg), $MILK_{ME}$ = milk yield adjusted for mature body weight (kg), FAT_{2X} = milk fat yield adjusted for two milkings per day (kg), FAT_{P2X} = milk fat content adjusted for two milkings per day (%), EBV_{M} = estimated breeding value for milk (kg), EBV_{F} = estimated breeding value for milk fat yield (kg), EBV_{FP} = estimated breeding value for milk fat content (%), PRO_{305} = milk protein yield adjusted for 305 days (kg), PRO_{PER305} = milk protein content adjusted for 305 days (%), PRO_{ME} = milk protein yield adjusted for mature body weight (kg)

$^{a–c}$in the same row significantly different at $P < 0.01$ and $P < 0.05$

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Table 3. Estimates (± SE) of additive and dominance effects associated with the single nucleotide polymorphism (SNP) at c.1892T>C, c.3359A>C, and c.8514C>T positions in Iranian Holstein cows

Traits	c.1892T>C SNP		c.3359A>C SNP		c.8514C>T SNP	
	additive effect	dominance effect	additive effect	dominance effect	additive effect	dominance effect
$MILK_{305}$	207.9 ± 254.8	690.9 ± 254.8*	–99.09 ± 25.18	260.2 ± 327.0	–8.98 ± 2.45	–219.0 ± 286.3
$MILK_{2X}$	207.6 ± 118.9	265.8 ± 140.5	–20.79 ± 12.25	88.79 ± 52.25	60.51 ± 10.10	34.11 ± 23.33
$MILK_{ME}$	251.2 ± 136.1	285.6 ± 160.9	–42.62 ± 13.65	89.40 ± 75.65	52.61 ± 11.57	36.98 ± 15.31
FAT_{2X}	2.62 ± 4.50	12.42 ± 5.41*	–4.53 ± 0.73	–5.35 ± 5.92	–4.09 ± 3.82	6.31 ± 5.19
FAT_{P2X}	–0.09 ± 0.04**	0.01 ± 0.05	0.06 ± 0.04*	–0.06 ± 0.05	–0.06 ± 0.03*	0.06 ± 0.05
EBV_{M}	109.0 ± 44.9**	71.53 ± 54.38	–69.10 ± 45.19	–16.23 ± 5.65	24.10 ± 24.19	–61.04 ± 50.67
EBV_{F}	1.02 ± 1.53	1.31 ± 1.86	–0.91 ± 1.53	–0.86 ± 1.96	1.44 ± 1.36	–0.01 ± 1.72
EBV_{FP}	–0.04 ± 0.01*	–0.01 ± 0.01	0.02 ± 0.01*	–0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01*
PRO_{305}	5.66 ± 4.13*	10.32 ± 4.13	–5.63 ± 4.13	–2.40 ± 5.40	1.30 ± 3.35	5.06 ± 4.54
PRO_{PER305}	–0.02 ± 0.02	–0.02 ± 0.01	0.01 ± 0.02	0.01 ± 0.02	–0.01 ± 0.01	0.02 ± 0.01**
PRO_{ME}	6.52 ± 4.73*	11.39 ± 5.46	–6.43 ± 4.72	–2.82 ± 6.17	1.24 ± 3.83	5.78 ± 5.19

$MILK_{305}$ = milk yield adjusted for 305 days (kg), $MILK_{2X}$ = milk yield adjusted for two milkings per day (kg), $MILK_{ME}$ = milk yield adjusted for mature body weight (kg), FAT_{2X} = milk fat yield adjusted for two milkings per day (kg), FAT_{P2X} = milk fat content adjusted for two milkings per day (%), EBV_{M} = estimated breeding value for milk (kg), EBV_{F} = estimated breeding value for milk fat yield (kg), EBV_{FP} = estimated breeding value for milk fat content (%), PRO_{305} = milk protein yield adjusted for 305 days (kg), PRO_{PER305} = milk protein content adjusted for 305 days (%), PRO_{ME} = milk protein yield adjusted for mature body weight (kg)
*$P < 0.05$, **$P < 0.01$

individuals, without significant effect on milk yield and milk fat percentage traits. These authors did not observe significant differences in the analyzed milk traits of cows with different c.3359A>C genotypes. No significant difference between c.1892T>C and c.3359A>C genotypes and milk yield, fat, and protein percentage traits was reported by Kowalewska-Luczak et al. (2010) in Jersey cows. Schennink et al. (2009) found an association between c.1892T>C and milk fat composition in Dutch Holstein-Friesian cattle.

Significant relationships were found between the c.8514C>T genotypes and FAT_{P2X} and PRO_{PER305} traits (Table 2). Cows with the *CT* genotype had higher FAT_{P2X} ($P < 0.05$) and more PRO_{PER305} ($P < 0.01$) than those carrying other genotypes. No associations were observed between the studied SNPs genotypes and the other traits ($P > 0.1$). The results of our study are consistent with those of other researches that have shown the significant association of c.8514C>T in the *OPN* gene with milk protein and fat percentage (Ron et al. 2001; Leonard et al. 2005; Schnabel et al. 2005; Khatib et al., 2007). Khatib et al. (2007) showed the significant additive effects for milk protein percentage, fat percentage, and fat yield traits at c.8514C>T position.

Additive and dominance effects of alleles are shown in Table 3. We observed an increase in FAT_{P2X} and EBV_{FP} associated with the *T* allele in the c.1892T>C SNP while animals with *TC* genotype had more PRO_{305} and PRO_{ME} in milk. Instead, Alim et al. (2012) indicated that this allele is associated with an increase in protein yield and protein concentration. Moreover, the *A* allele in c.3359A>C SNP was associated with an increase in FAT_{P2X} and EBV_{FP} traits. These results are consistent with those in the previous study by Khatib et al. (2007) that showed an association between allele *A* and milk production traits. The *T* allele at c.8514C>T SNP in *OPN* gene increases FAT_{P2X} whereas *CT* genotype is related to higher PRO_{PER305} as compared to *CC* and *TT* genotypes. In contrast, Leonard et al. (2005) reported that the *C* allele increases milk protein percentage and fat percentage.

The *PPARGC1A* and *OPN* variances for significant traits are shown in Table 4. We obtained the highest variance of the *PPARGC1A* gene for EBV_{FP} (4.32). This value suggests that *PPARGC1A* plays an essential role in lipid metabolism and was introduced as a candidate gene for milk fat. Also, the highest variance of the *OPN* gene was obtained for PRO_{PER305} (6.34). This result proves

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Table 4. *PPARGC1A* and *OPN* variances for significant milk traits in Iranian Holstein cows

Loci	Traits	Variance (%)
c.1892T>C	FAT_{P2X}	2.20
	EBV_{FP}	4.32
	EBV_{M}	1.03
	PRO_{305}	1.07
	PRO_{ME}	1.54
c.3359A>C	FAT_{P2X}	1.35
	EBV_{FP}	1.32
c.8514C>T	FAT_{P2X}	2.72
	PRO_{PER305}	6.34

FAT_{P2X} = milk fat content adjusted for two milkings per day (%), EBV_{M} = estimated breeding value for milk (kg), EBV_{FP} = estimated breeding value for milk fat content (%), PRO_{305} = milk protein yield adjusted for 305 days (kg), PRO_{PER305} = milk protein content adjusted for 305 days (%), PRO_{ME} = milk protein yield adjusted for mature body weight (kg)

that *OPN* is a functional candidate for the QTL affecting protein percentage (Schnabel et al. 2005).

The r^2 statistics was estimated to indicate if the effects of the SNPs in the *PPARGC1A* and *OPN* genes were partially explaining the same variation. The r^2 measure for the T>C SNP and T>C SNP in *PPARGC1A* gene was calculated 0.85. The r^2 measure between the two SNPs in *PPARGC1A* gene and C>T SNP in *OPN* gene were below 0.001. The figure of 0.85 means that the genotype of the T>C SNP explains 85% of the variation in the T>C SNP. So the SNPs are in a quite high linkage disequilibrium (LD) but not complete. On the other hand, the low r^2 values indicate that the effect of one SNP explains variation in the other SNPs just to a limited extent. Considering that the LD value between the c.1892T>C and the c.3359A>C loci was 0.85, the high LD can be the reason of significant associations between the genotypes and milk traits. Moreover, the significant associations between the c.1892T>C, c.3359A>C, and c.8514C>T SNPs and milk traits may be due to LD with a yet-undetected functional polymorphism closely to *PPARGC1A* and *OPN* genes.

The investigated SNPs of the current study were located in the intronic and untranslated regions. The introns consist of genes for small nuclear RNA, which is important to the translation of messenger RNA. Also, they can be essential in alternative splicing process, which can produce multiple types of messenger RNA from a single gene (Chorev and Carmel 2012). These functions of introns confirm that a variation in the noncoding region of these genes can influence glucose, fat, and energy metabolism during the high-lactation state.

The differences in the studied breed, sample size, and the applied statistical model are considered as the main reasons of the contradictory results. In addition, these inconsistent results might be due to gene fusion and paternal effects. On the other hand, factors like animal's age and health (particularly the mammary gland) would influence milk production and composition traits. The inconsistency of our findings with other researches necessitate more investigation in larger numbers of cows of different breeds to reveal molecular mechanisms causing the QTL effects.

CONCLUSION

Milk and its products are regarded as the most important nutritional resource, meeting the energy requirements and offering high quality protein and various vitamins and minerals. Earlier, most genetic improving programs of agriculturally important livestock population have been carried out through complete phenotypic and pedigree information. However, applying molecular genetic information in breeding stock may lead to a better understanding of quantitative traits. Hence, the present study investigated the association of different *PPARGC1A* and *OPN* genotypes with milk production and composition traits in Iranian Holstein cows. Generally, detection and estimation of associations of identified genes and genetic markers with economic traits are the basis of a successful application of marker-assisted selection (MAS) in breeding programs. The MAS strategies can be used for pre-selection of young bulls prior to progeny test. Finally, MAS can potentially raise annual genetic gain through increasing the accuracy of evaluation, increasing the selection intensity, and decreasing the generation interval.

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