# Effect of polymorphisms in the *ABCG2*, *LEPR* and *SCD1* genes on milk production traits in Holstein cows

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*(Received 5 November 2014; Accepted 13 May 2016; First published online 24 June 2016)*



## Abstract

This study was performed to investigate the association between polymorphisms in the *ABCG2* (ATP-binding cassette sub-family G member 2), *LEPR* (leptin receptor) and *SCD1* (stearoyl-coenzyme A desaturase 1) genes and milk production traits in Holstein dairy cows in Iran. The analysis was conducted on 816 lactations from 408 Iranian Holstein cows. Genotyping was carried out using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. The results of the association study revealed that cows with genotype AC of the *ABCG2*-Y581S single nucleotide polymorphism (SNP) had lower breeding value for milk yield compared with the AA genotype, but showed significantly higher fat and protein percentages. Cows that were homozygous for allele T at the *LEPR*-T945M locus had higher breeding value for fat yield than those that were homozygous for the C allele. Regarding the *SCD1*-A293V SNP, cows with genotype AV produced a higher 305-day milk yield in comparison with cows with the VV genotype. Furthermore, cows that were homozygous for allele V showed a significantly higher protein percentage compared with AA and AV genotypes. The results of this study suggest that these SNPs have the potential to be used in programmes based on genomic selection in Iranian dairy herds.

**Keywords:** Genetic polymorphism, Iranian Holstein, milk fat, milk protein, SNP
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## Introduction

The application of marker-assisted selection has the potential to enhance genetic improvement in livestock by direct selection of genes or genomic regions that affect economic traits through genomic selection (Dekkers & Hospital, 2002). Individual genes, markers and quantitative trait loci (QTL) can be used to select desirable animals at an early age, based on their genotypes (Banos *et al*., 2008). Many genes have been chosen as candidates for economically important traits in dairy cattle based on their physiological role in traits of interest or on their being located in genome regions containing previously identified QTLs for these traits. Knowledge of the association between polymorphisms in these candidate genes and traits of economic importance is essential for their effective use in marker-assisted selection. Among candidates for milk production traits, the *ABCG2* (ATP-binding cassette sub-family G member 2), *LEPR* (leptin receptor) and *SCD1* (stearoyl-coenzyme A desaturase 1) genes seem promising.

*ABCG2* is a member of the ATP-binding cassette (ABC) superfamily, which transports various xenobiotics and cytostatic drugs across the plasma membrane (Litman *et al*., 2000). It has been demonstrated that the *ABCG2* is responsible for the active secretion of clinically important substrates into mouse milk, and that mice that are homozygous for an *ABCG2* knock-out mutation lack this function (Jonker *et al*., 2005). The expression of this gene in the mammary gland is increased significantly during lactation, compared with the dry period (Farke *et al*., 2008). The bovine *ABCG2* gene is located in the region of chromosome 6, harbouring a QTL for milk production traits (Olsen *et al*., 2005). Several genes, including *ABCG2,* have been proposed as candidates in the QTL region. Analysing the sequence variation of these genes SNP (A to C) in exon 14 of the *ABCG2*, causing the substitution of tyrosine to serine at position 581 (*ABCG2*-Y581S), has been demonstrated to be the only polymorphism that corresponded to the segregation status of the QTL based on the allele substitution effect (Cohen-Zinder *et al*., 2005). Using physical mapping and combined linkage disequilibrium mapping, the QTL region between the *ABCG2* and *LAP3* genes has been fine-mapped and it has been found that the *ABCG2*-Y581S is the only marker in perfect linkage

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v46i2.11

disequilibrium with the QTL (Olsen *et al*., 2007). The *ABCG2*-Y581S has been shown to be associated with milk production traits in Polish Holstein-Friesians (Komisarek & Dorynek, 2009).

Leptin is an important hormone that is involved in feed intake, energy partitioning and metabolism, and consequently has an effect on energy balance and milk production traits (Liefers *et al*., 2002; Silva *et al*., 2002). Since this hormone exerts physiological effects through receptors located in most bovine tissues (Silva *et al*., 2002), the leptin receptor can be regarded as a candidate that influences milk production traits. The *LEPR* gene is located on bovine chromosome 3 (Pfister-Genskow *et al*., 1997) and in the proximity of a QTL for milk fat yield (Rodriguez-Zas *et al*., 2002). This gene produces various leptin receptor isoforms through alternative splicing. The isoforms have identical extracellular domains, and differences between them are due to changes in the length of the intracellular domains. The long form of the receptor (LEPR-b) has the complete cytoplasmic domain and is responsible for most physiological effects of leptin (Tartaglia L.A., 1997). A non-synonymous SNP in exon 20 of the *LEPR* gene, causing the substitution of threonine with methionine in the intracellular domain of the LEPR at residue 945 (*LEPR*-T945M), has been shown to affect bovine leptin concentration during pregnancy (Liefers *et al*., 2004). Furthermore, this SNP has been reported to be associated with milk fat and protein contents (Komisarek & Dorynek 2006; Suchocki *et al*., 2010).

The *SCD1* gene, located on bovine chromosome 26 (Chung *et al*., 2000), encodes a key enzyme that introduces a double bond between carbons 9 and 10 of medium to long-chain saturated fatty acids (Corl *et al*., 2001). This enzyme is also involved in some aspects of metabolism, including lipogenesis and lipid oxidation (Flowers & Ntambi, 2008). The enzyme is abundantly expressed in the mammary gland of lactating ruminants and plays a major role in the synthesis of milk fat (Bionaz & Loor, 2008). An SNP in exon 5 of the bovine *SCD1*, which causes the replacement of valine with alanine at residue 293 (*SCD1*-A293V), has been reported to be associated with milk fatty acid composition in various cattle populations (Mele *et al*., 2007; Schennink *et al*., 2008; Kgwatalala *et al*., 2009). However, literature that reports a significant effect of this polymorphism on milk production traits is limited, though an effect of this SNP on milk and protein yield has been described in Italian Holsteins (Macciotta *et al*., 2008) and Canadian Jerseys (Kgwatalala *et al*., 2009). The objective of this study was therefore to examine the effect of polymorphisms in the *ABCG2*, *LEPR* and *SCD1* genes on milk production traits in Iranian Holstein dairy cattle to provide useful markers for these traits in genetic selection programmes.

## Materials and Methods

A total of 816 lactations from 408 Iranian Holstein cows (with a major genetic influence from USA and Canada) by 155 bulls (1 to 24 progeny per bull) were analysed in this study. The cows were randomly selected from five dairy herds in the Isfahan Province of Iran. Accurate phenotypic data for 305-day milk yield, fat and protein percentage, somatic cell count (SCC), and breeding value data (from national evaluations) for fat and milk yield were obtained from the Vahdat Industrial Agriculturists & Dairymen Cooperative of Isfahan Province in the centre of Iran, which hosts the biggest milk production herds across the country. Intensive production systems using open-shed and free-stall barns are the predominant housing systems. Most of the farms use feed rations that are relatively high in concentrates, with alfalfa and corn silage as the major roughage. Cows are milked three times a day. On average, Iranian Holstein cows produce 9000 kg milk, 300 kg fat and 270 kg protein in a 305-day lactation. SCC was transformed to somatic cell score (SCS) for each record ($SCS = \log_2 [SCC/100\ 000] + 3$) to achieve normality and homogeneity of variances. Blood samples for DNA extraction were collected using vacuum venoject tubes containing EDTA and stored at −20 ºC until DNA extraction.

Genomic DNA was extracted from blood samples by the salting-out method (Miller *et al*., 1988). Genotypes were determined using the PCR-RFLP technique. PCR was used to amplify the DNA fragments containing the polymorphisms of interest. The PCR reactions were carried out in a total volume of 20 µL, which contained 50 ng genomic DNA, 10 pmoL each primer, 2 µL 10X PCR buffer, 2 mM MgCl2, 200 µM dNTPs and 2.5 units Taq DNA polymerase (Fermentas, Germany). Sequences of the primers used in the PCR for the fragments containing *LEPR*-T945M, *ABCG2*-Y581S and *SCD1*-A293V were as presented in Table 1 (Komisarek & Dorynek, 2006; Kgwatalala *et al*., 2009; Komisarek & Dorynek, 2009).

Thermal cycling conditions were as follows: initial denaturation at 94 ºC for 2 min, 35 cycles of denaturation at 94 ºC for 30 s, annealing temperature (Table 1) for 1 min, extension at 72 ºC for 1 min, and a final extension at 72 ºC for 10 min.

The PCR products were digested with 5 U (Table 1) restriction enzymes (Fermentas, Germany), in 20 µL of reaction volume for 8 h at 37 and 60 ºC for the fragments containing the *ABCG2*-Y581S and *LEPR*-T945M, respectively, then subjected to electrophoretic separation in 2.5% ethidium bromide-stained agarose gel. The A allele of the *ABCG2*-Y581S polymorphism was characterized by a single 292-bp fragment, while the C allele was identified by the presence of two fragments of 268 and 24 bp. For the *LEPR*-T945M locus, the T allele was determined by a fragment of 400 bp and the C allele resulted in two fragments of 375 and

25 bp. Furthermore, allele A of the *SCD1*-A293V was characterized by a fragment of 200 bp, while the V allele was determined by a 400-bp fragment. Tests of Hardy-Weinberg equilibrium for each locus were conducted separately using the software POPGENE (Yeh *et al*., 2000).

**Table 1** Primers and PCR-RFLP conditions used for the analysed polymorphisms

| SNP | Primers (5'-3') | Annealing temp (ºC) | Restriction enzyme | Digestion product size (bp) |
|---|---|---|---|---|
| *ABCG2*-Y581S | F-AACAGCCTCAGCTCCAGAGAGATAT<br>R-CGGTGACAGATAAGGAGAACATACT | 57 | *PstI* | A : 292<br>C : 268, 24 |
| *LEPR*-T945M | F-GCAACTACAGATGCTCTACTTTTGT<br>R-CAGGGAAATTTCCCTCAAGTTTCAA | 60 | *Taq* | T : 400<br>C : 375, 25 |
| *SCD1*-A293V | F-CCCATTCGCTCTTGTTCTGT<br>R-CGTGGTCTTGCTGTCGACT | 59 | *NcoI* | V : 400<br>A : 200 |

SNP: single nucleotide polymorphism.

For the association studies, the traits of interest were analysed using the least squares method of the general linear model (GLM) procedure of SAS software [SAS, Version 8] according to the following model:

$$Y_{ijklmno} = \mu + A_i + L_j + S_k + HYS_l + L_m + S_n + b_1 (X_{ijklmno} - \bar{X}) + b_2 (W_{ijklmno} - \bar{W}) + e_{ijklmno}$$

where:

$Y_{ijklmno}$ = value for each trait 305-day milk yield, fat and protein percentage, somatic cell score, and breeding value for fat and milk yield; $\mu$ = overall mean;

$A_i$ = fixed effect of genotype *ABCG2*; $L_j$ = fixed effect of genotype *LEPR*;

$S_k$ = fixed effect of genotype *SCD1*; $HYS_l$ = combined effect of herd, year and season of parturition;

$L_m$ = fixed effect of lactation number; $S_n$ = random effect of sire;

$b_1$ = regression coefficient of milk yield; $X_{ijklmno}$ = milk yield; $\bar{X}$ = mean of milk yield;

$b_2$ = regression coefficient of open days; $W_{ijklmno}$ = open days; $\bar{W}$ = mean of open days;

$e_{ijklmno}$ = random residual effect.

For the analysis of 305-day milk yield, milk yield as a covariate was excluded from the model. Also, wherever breeding value was used instead of phenotypic value for a trait, effects other than genotype were omitted from the model. The average allele substitution effect of each SNP was estimated by regressing the number of copies of each allele against the phenotypic or breeding value for each trait (Sherman *et al*., 2008).

## Results and Discussion

All known SNP alleles of the *ABCG2*-Y581S (A and C), *LEPR*-T945M (T and C) and *SCD1*-A293V (A and V) polymorphisms were observed in this study (Table 2).

As a result of a very low frequency of the C allele of the *ABCG2*-Y581S locus, the CC genotype of this locus was not detected within the population. A much higher frequency of the A variant of the *ABCG2*-Y581S compared with the C variant in the current study (97% vs. 3%) is in agreement with previous reports. A high frequency of the A allele was reported in Israeli Holstein-Friesians (Cohen-Zinder *et al*., 2005). The same tendency was reported in 32 *Bos taurus* and three *Bos indicus* breeds (Ron *et al*., 2006). The A variant was found to be fixed in Indian cattle and buffalo breeds (Tantia *et al*., 2006). A higher frequency for the A allele of this SNP than the C allele was reported in Norwegian Red cattle (Olsen *et al*., 2002). Furthermore, a higher frequency of the A variant of *ABCG2*-Y581S compared with the C variant was evident in Polish Holstein-Friesian bulls (Komisarek & Dorynek, 2009). The higher frequency of the C allele of *LEPR*-T945M, compared with the T allele (65% vs. 35%), in the study population of Iranian Holstein cows confirms earlier studies on Holstein-Friesian cows (Liefers *et al*., 2004), Jersey cows (Komisarek & Dorynek, 2006), Polish dairy population (Szyda & Komisarek, 2007), UK Holstein cows (Banos *et al*., 2008), Polish Holstein-Friesian cattle (Komisarek, 2010), Jersey and Polish Holstein-Friesian cows (Suchocki *et al*., 2010) and Swedish Red and Swedish Holstein cows (Glantz *et al*., 2011; Glantz *et al*., 2012). Nevertheless, except for Jersey cows (Suchocki *et al*., 2010), the frequency for the C allele obtained in the current study was lower than those reported by these authors. Similar to previous reports (Asadollahpour Nanaei *et al*., 2013), the frequencies for the A and V alleles of the *SCD1*-A293V polymorphism in the current study were 0.58 and 0.42,

respectively. Consistent with results from the present study, a higher frequency of the A allele in comparison with the V allele has been reported in Japanese Black cattle (Taniguchi *et al*., 2004), Italian Holstein (Mele *et al*., 2007), Valdostana breed (Moioli *et al*., 2007) and Canadian Jersey (Kgwatalala *et al*., 2009). On the contrary, in the Piedmontese breed (Moioli *et al*., 2007) and Italian Brown cattle (Conte *et al*., 2010) the V allele had a higher frequency compared with the A allele. The result of the test for Hardy-Weinberg equilibrium for the loci *ABCG2*-Y581S and *LEPR*-T945M did not show significant deviation from Hardy-Weinberg equilibrium (Table 2). However, for the *SCD1*-A293V, the population of the current study was not in Hardy-Weinberg equilibrium ($P$ <0.01), which indicates non-random mating with respect to this locus in Iranian Holstein cows.

The results of analysis of association between the SNPs and traits of interest and allele substitution effects are presented in Tables 3 and 4, respectively.

**Table 2** Allele and genotype frequencies of the *ABCG2*-Y581S and *LEPR*-T945M polymorphisms in Iranian Holstein cows

| SNP | Allele | Frequency | Genotype | Frequency | Chi-square |
|---|---|---|---|---|---|
| | A | 0.97 | AA | 0.94 (25) | |
| *ABCG2*-Y581S | C | 0.03 | AC | 0.06 | 0.40 |
| | | | CC | - | |
| | T | 0.35 | TT | 0.11 | |
| *LEPR*-T945M | C | 0.65 | TC | 0.49 | 1.64 |
| | | | CC | 0.40 | |
| *SCD1*-A293V | A | 0.58 | | | 46.08** |
| | V | 0.42 | | | |

** Significant at $P$ <0.01; SNP: single nucleotide polymorphism.

**Table 3** Results of association analysis between the investigated single nucleotide polymorphisms (SNPs) and milk production traits in Iranian Holstein cows (least squares means ± SE).

| Genotype | | Trait | | | | | |
|---|---|---|---|---|---|---|---|
| | | 305-d MY | BV for MY | BV for FY | FP | PP | SCS |
| *ABCG2*-Y581S | AA (n = 766) | 10114.26 ± 138.42 | 530.17 ± 33.84$^{B}$ | 12.66 ± 1.19 | 3.20 ± 0.05$^{A}$ | 3.03 ± 0.02$^{A}$ | 1.94 ± 0.08 |
| | AC (n = 50) | 9618.99 ± 321.19 | 247.12 ± 94.39$^{A}$ | 12.26 ± 3.31 | 3.49 ± 0.11$^{B}$ | 3.10 ± 0.04$^{B}$ | 1.98 ± 0.17 |
| *LEPR*-T945M | TT (n = 86) | 9831.07 ± 275.03 | 453.99 ± 82.29 | 16.16 ± 2.88$^{A}$ | 3.34 ± 0.09 | 3.06 ± .03 | 1.83 ± 0.16 |
| | TC (n = 384) | 9837.13 ± 201.24 | 383.94 ± 56.73 | 11.86 ± 1.99$^{AB}$ | 3.36 ± 0.07 | 3.07 ± 0.02 | 2.06 ± 0.11 |
| | CC (n = 320) | 9931.66 ± 208.46 | 328.01 ± 56.07 | 9.35 ± 1.96$^{B}$ | 3.33 ± 0.07 | 3.07 ± 0.02 | 1.99 ± 0.11 |
| *SCD1*-A293V | AA (n = 212) | 9607.13 ± 204.74$^{A}$ | 341.59 ± 62.30 | 11.02 ± 2.18 | 3.31 ± 0.07 | 3.03 ± 0.02$^{A}$ | 1.97 ± 0.12 |
| | AV (n = 530) | 10086.36 ± 190.86$^{B}$ | 388.74 ± 53.14 | 13.73 ± 1.86 | 3.29 ± 0.06 | 3.00 ± 0.02$^{A}$ | 2.07 ± 0.10 |
| | VV (n = 74) | 9906.37 ± 311.22$^{AB}$ | 435.61 ± 85.77 | 12.63 ± 3.00 | 3.44 ± 0.11 | 3.17 ± 0.04$^{B}$ | 1.84 ± 0.18 |

305-d MY: 305-day milk yield; BV: breeding value; MY: milk yield; FY: fat yield; FP: fat percentage; PP: protein percentage; SCS: somatic cell score.
$^{A,B}$ Means within a row with different superscripts differ significantly at $P$ <0.05.

The polymorphism in the *ABCG2* gene was found to be significantly associated with breeding value for milk yield, and fat and protein percentages. The substitution of the A allele for the C allele increased the percentages of fat and protein by 0.29% and 0.07%, respectively, and decreased milk yield by 278.09 kg,

**Table 4** Estimates of the allele substitution effects of the investigated single nucleotide polymorphisms (SNPs) for milk production traits in Iranian Holstein cows

| Trait | Allele substitution effects | *P*-value |
|---|---|---|
| | *ABCG2*-Y581S (C vs. A) | |
| 305-d MY | −511.23 ± 295.52 | 0.084 |
| BV for MY | −278.09 ± 93.27** | 0.003 |
| BV for FY | −0.18 ± 3.26 | 0.957 |
| FP | 0.29 ± 0.10** | 0.003 |
| PP | 0.07 ± 0.03* | 0.027 |
| SCS | 0.18 ± 0.21 | 0.395 |
| | *LEPR*-T945M (T vs. C) | |
| 305-d MY | −68.90 ± 99.65 | 0.490 |
| BV for MY | 67.01 ± 34.66* | 0.054 |
| BV for FY | 3.80 ± 1.20** | 0.002 |
| FP | 0.03 ± 0.03 | 0.332 |
| PP | 0.01 ± 0.01 | 0.600 |
| SCS | −0.05 ± 0.10 | 0.588 |
| | *SCD1*-A293V (V vs. A) | |
| 305-d MY | 290.52 ± 123.11* | 0.019 |
| BV for MY | 47.51 ± 39.19 | 0.226 |
| BV for FY | 1.53 ± 1.37 | 0.266 |
| FP | 0.03 ± 0.04 | 0.540 |
| PP | 0.02 ± 0.01 | 0.083 |
| SCS | −0.04 ± 0.07 | 0.598 |

* Significant at *P* <0.05; ** Significant at *P* <0.01.
305-d MY: 305-day milk yield; BV: breeding value; MY: milk yield; FY: fat yield; FP: fat percentage; PP: protein percentage; SCS = somatic cell score.

(Table 4). Cows with the AC genotype had a significantly lower breeding value for milk yield than those with the AA genotype (*P* <0.01). The AC genotype had higher fat (*P* <0.01) and protein (*P* <0.05) percentages than those of the AA genotype. In line with these results, the effect of this SNP on these traits has been reported in other studies, though opposite effects were observed for the alleles C and A in the current research compared with these studies (Cohen-Zinder *et al*., 2005; Olsen *et al*., 2007). These studies found a negative effect of the A allele on fat and protein percentages and a positive effect on milk yield in Israeli Holstein-Friesians and Norwegian Red cattle, respectively. Interestingly, the allele substitution effect of 278.1 kg milk yield obtained in the current study was consistent with those reported by Cohen-Zinder *et al*. (2005). Nevertheless, the allele substitution effect for fat percentage (0.29%) was approximately half of that estimated by Cohen-Zinder *et al.* (2005). Moreover, for protein percentage, the allele substitution effect in this study (0.07%) was two times those reported by Cohen-Zinder *et al.* (2005). Also, an association of the A variant with higher fat and protein percentages, but not with milk yield, was reported in Polish Holstein-Friesian bulls (Komisarek & Dorynek, 2009). Contrary to the researchers' findings, Cohen-Zinder *et al*. (2005) and Komisarek & Dorynek (2009) have found an association between the A allele and breeding value for fat yield. No significant association was evident between the genotypes of the *ABCG2*-Y581S and 305-day milk yield, breeding value for fat yield and SCS in the current study (*P* >0.05). The differences between these results in the associations and values for the allele substitution effects may be attributed to a lack of CC individuals and a very low number of AC animals among the populations.

The *LEPR*-T945M polymorphism in the present study showed only a significant association with breeding value for fat yield. Cows carrying the genotype TT were found to be superior in this trait than those carrying the CC genotype (*P* <0.05). As shown in Table 4, replacing the C allele with the T allele increased

fat yield by 3.80 kg. The results of other studies (Komisarek & Dorynek, 2006; Suchocki *et al*., 2010; Glantz *et al*., 2012) revealed no relationship between this SNP and fat yield. This association implies that the SNP may be in linkage disequilibrium with a gene engaged in milk fat synthesis or may be the possible role of *LEPR* in milk fat synthesis which can be considered in marker-assisted selection for improving this trait. The association of this polymorphism with fat yield in the current study may be attributed to the physiological actions of the hormone, leptin, through its receptor. The *LEPR*-T945M was revealed to be correlated with the plasma leptin concentration, which might influence the signal transduction pathway of the hormone (Liefers *et al*., 2004). In the presence of prolactin, leptin has been shown to increase fatty acid synthesis in the mammary gland (Feuermann *et al*., 2004). Furthermore, in support of the authors' hypothesis, it has been demonstrated that leptin, acting through its receptor, modulates some functions of *DGAT1* (Chen *et al*., 2002) as a key enzyme in triacylglycerol synthesis (Schennink *et al*., 2008), though the underlying physiological mechanisms remain unclear. The threonine → methionine amino acid substitution in the intracellular domain of *LEPR* may change its signalling capability. Hence, the *LEPR*-T945M can be supposed to be the causal mutation underlying the QTL affecting fat yield mapped in close vicinity of the *LEPR* gene (Rodriguez-Zas *et al*., 2002). In contrast, an effect of this polymorphism on fat and protein percentages was reported in Jersey cows (Komisarek & Dorynek, 2006). In that study, animals with the TT genotype were characterized by the lowest values for both traits. Similarly, an association of this polymorphism with fat and protein percentages was found in Jersey and Polish Holstein-Friesian, respectively (Suchocki *et al*., 2010). Interestingly, opposite effects were observed for fat percentage in Holstein-Friesians (allele C increased fat content) and in Jerseys (allele C decreased fat content) in the latter study. In agreement with the researchers' results, no association of the *LEPR*-T945M with fat percentage (Glantz *et al*., 2011; Glantz *et al*., 2012), protein percentage (Glantz *et al*., 2011; Glantz *et al*., 2012), milk yield (Komisarek & Dorynek, 2006; Banos *et al*., 2008; Suchocki *et al*., 2010; Glantz *et al*., 2011; Glantz *et al*., 2012) and SCS (Komisarek, 2010) was reported in various cattle populations.

The current study indicated an effect of the *SCD1*-A293V genotype on 305-day milk yield. Animals of the AA genotype had lower 305-day milk yield in comparison with those of genotype AV ($P$ <0.01). As presented in Table 4, substituting the A allele with the V allele increased 305-day milk yield by 290.5 kg. Association of the *SCD1*-A293V with 305-day milk yield in the present study was in line with the results from a study on Italian Holsteins with a higher milk yield for the VV genotype than the AA genotype (Macciotta *et al*., 2008). In contrast, a positive effect of the A allele on increased 305-d milk yield was revealed in a Canadian Jersey population (Kgwatalala *et al*., 2009). Contrary to the current study, no significant effect of this SNP on milk yield was found in Dutch Holstein-Friesian (Schennink *et al*., 2008), Polish Holstein-Friesian (Komisarek & Dorynek, 2009) and four breeds of Friesian, Jersey, Piedmontese and Valdostana (Signorelli *et al*., 2009), respectively. The current result also revealed an effect of the *SCD1*-A293V polymorphism on protein percentage. Cows homozygous for the V allele showed higher protein percentage in their milk compared with the AA and AV genotypes ($P$ <0.01) and substituting the A allele with the V allele increased protein percentage by 0.02% (Table 4). Findings of other studies showed no association of the SNP with this trait (Schennink *et al*., 2008; Kgwatalala *et al*., 2009; Komisarek & Dorynek, 2009; Signorelli *et al*., 2009). However, an effect of this polymorphism on protein yield was reported in a Canadian Jersey population (Kgwatalala *et al*., 2009) and in Italian Holsteins (Macciotta *et al*., 2008). Several studies have mapped QTLs for milk yield and composition traits on chromosome 26 and in close proximity of the *SCD1* gene (Plante *et al*., 2001; Boichard *et al*., 2003; Jiang *et al*., 2005). It can therefore be postulated that the *SCD1*-A293V SNP may be the causal mutation or a marker in linkage disequilibrium with an unknown causal mutation underlying the QTLs. No significant relationship was found between the *SCD1*-A293V genotypes and breeding value for milk and fat yield, fat percentage and SCS ($P$ >0.05). Similar to the current findings, no association has been reported between the *SCD1*-A293V and fat yield and percentage (Macciotta *et al*., 2008; Schennink *et al*., 2008; Kgwatalala *et al*., 2009; Komisarek & Dorynek, 2009; Signorelli *et al*., 2009) and SCS (Komisarek & Dorynek, 2009).

## Conclusions

In conclusion, the present study demonstrated the association of polymorphisms at the *ABCG2*-Y581S, *LEPR*-T945M and *SCD1*-A293V loci with milk production traits in Iranian Holstein population. The C variant of the *ABCG2*-Y581S was associated with higher fat and protein percentages and lower milk yield. Furthermore, the T allele of the *LEPR*-T945M showed a significant effect on increased fat yield. Additionally, the V allele of the *SCD1*-A293V was revealed to be associated with higher 305-day milk yield and protein percentage. Regarding the associations reported in this study, these three polymorphisms have the potential to be used as quantitative trait nucleotides (QTNs) for selection in favour of these traits through genomic selection.

## Acknowledgement

The authors gratefully thank Isfahan University of Technology for supporting this research financially. The authors also acknowledge the Vahdat Industrial Agriculturists & Dairymen Cooperative of Isfahan province of Iran for supplying data for milk-related traits.

## Authors' contributions

MS-G analysed the data and prepared the manuscript with contributions from other co-authors. SA-M supervised the experiment. MR and SG-B designed and carried out the experiment. MAE supervised the experiment.

## Conflict of interest declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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