Joint Analysis of the DGAT1, OPN and PPARGC1A Genes Effects on Variation of Milk Production and Composition in Holstein Cattle Population

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INTRODUCTION

Candidate gene approach and whole genome scans are two main strategies for QTL identification (Andersson, 2001). The candidate gene approach studies the relationship between the traits and known genes that may be associated with the physiological pathways underlying the trait (Liu et al. 2008). This approach has been successful to some extent. For example, several studies have identified QTLs for milk composition on chromosomes 6 and 14 (Riquet et al. 1999; Farnir et al. 2002; Olsen et al. 2005). The economic traits are polygenic. It means that they are controlled by many loci. Several studies indicated genetic variation in milk production traits cannot be explained by few candidate genes (Kaupe et al. 2007). Therefore, the effects of all candidate loci should be explored together in the same statistical model diacyl glycerol acyltransferase 1 (DGAT1) is located near the centromeric region of Bos taurus autosome 14 (BTA14). The first evidence for the effect of DGAT1 variation on milk yield and composition in Holstein cattle
was reported by Grisart et al. (2002). DGAT1 is considered as the key enzyme in controlling the synthesis rate of triglycerides in adipocytes. A non-conservative K232A substitution (conservation of alanine to lysine) in DGAT1 was associated with milk production and compositions in Holstein cattle (Thaller et al. 2003). Spellman et al. (2002) and Banos et al. (2008) reported that the K232A substitution in exon 8 of the DGAT1 gene was associated with increasing of milk fat yield and decreasing of milk production and protein yield. Some studies showed that there are significant associations between DGAT1 and milk, fat yield and protein yield. Bovine chromosome six (BTA6) harbors at least six QTLs influencing milk production traits of dairy cattle.

The osteopontin (OPN) and peroxisome proliferator activated receptor gamma co-activator 1 Alpha (PPARGC1A) are about 6 Mb apart, which is about 12 cM for this region of chromosome 6 (Olsen et al. 2005). OPN is a strong functional candidate for milk production and it is a highly phosphorylated glycoprotein (Leonard et al. 2005). Schnabel et al. (2005) reported an association between OPN and milk protein percentage in the North American Holstein population. PPARGC1A has main role in fat and glucose metabolism and plays a critical role in the activation of nuclear hormone receptors and transcription factors regulating energy homeostasis (Liang and Ward, 2006; Kowalewska-Luczak et al. 2010). Structure of PPARGC1A gene is made from 13 exons and expressed at different levels in a great number of tissues (Liang and Ward, 2006). Khatib et al. (2007) showed significant associations between PPARGC1A (c.3359) gene, milk yield, milk protein percentage, and somatic cell score in the North American Holstein population. The aim of this study was to investigate the joint effects of OPN, PPARGC1A and DGAT1 candidate genes on milk production traits in Iranian Holstein cattle population.

**MATERIALS AND METHODS**

**Animals and traits**

Totally 398 blood samples were collected from Holstein-Friesian cows of Iran, which were distributed in ten dairy herds in two provinces of Iran. The cows were under official milk recording of Animal Breeding Center (Karaj-Iran).

Finally 372 records for estimated breeding values for milk production adjusted for 305 days (EBV_M), fat yield (EBV_F) (kg) and fat percentage (EBV_PP) were obtained from the Animal Breeding Center for analyzing association between genotypes and economics traits. The EBVs were estimated by random regression test day model.

**DNA extraction, PCR amplification and SNPs genotyping**

DNA extractions were performed using standard salting out protocol (Miller et al. 1988). PCR reactions were performed using standard PCR (Thermo cycler, Biometra, Germany). More details about primers are shown in Table 1 (Kaupe et al. 2004; Weikard et al. 2005; Khatib et al. 2007).

PCR reaction for DGAT1 (GenBank: EU077528), OPN (GenBank: NW_255516) and PPARGC1A (GenBank: AY321517) loci were performed in a 25 µL volume using 100 ng genomic DNA, PCR buffer (1X), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.6 pmol of each primer and Taq polymerase enzyme (2U). All accession numbers are available in NCBI site. For DGAT1 gene, the addition of DMSO to the PCR reactions allowed an equal amplification of both alleles. The annealing temperature for DGAT1, OPN and PPARGC1A are considered as 60, 53 and 55 centigrade and finally 411, 290, 195 and 357 base pairs fragments were amplified for DGAT1, OPN, PPARGC1A (c.1892) and PPARGC1A (c.3359). The PCR products (5 µL) were digested using 2 units of the restriction enzymes (FERMENTAS, Lithuania) and separated on a 2% agarose gel. The gels were stained with ethidium bromide and visualized under UV light. Finally, SNPs were genotyped by PCR-RFLP technique. Table 2 shows more detail about restriction enzyme conditions.

**Gene and genotype frequencies**

The population genetic parameters including gene and genotypic frequencies, Hardy-Weinberg equilibrium (Chi-square test), Indices of genetic diversity in population (Nei (H) and Shannon (I)) were estimated using the PopGene software version 3.1d (Nei, 1977).

**Joint analysis of DGAT1, OPN and PPARGC1A variants**

The effects of genotypes were tested using the GLM procedure (Pillais trace test) of SPSS (2010) implementing the following fixed model:

\[
y_{ijkmn} = \mu + P_i + A_j + D_k + O_m + \varepsilon_{ijkmn}
\]

Where:

- \(y\): observation for each trait.
- \(\mu\): overall mean.
- \(P_i\), \(A_j\), \(D_k\) and \(O_m\): fixed effects of genotypes of PPARGC1A (c.1892), PPARGC1A (c.3359), DGAT1 and OPN genes.
- \(\varepsilon_{ijkmn}\): residual effect.

The mean comparisons were performed using the Tukey test for significant genotypes.

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**References**

- Grisart et al. (2002).
- Olsen et al. (2005).
- Spellman et al. (2002).
- Banos et al. (2008).
- Thaller et al. (2003).
- Kowalewska-Luczak et al. (2010).
- Schnabel et al. (2005).
- Khatib et al. (2007).
- Miller et al. (1988).
- Nei, 1977.
- PopGene software version 3.1d.
- SPSS (2010).
- Pi, Aj, Dk and Om: fixed effects of genotypes of DGAT1, OPN, PPARGC1A and DGAT1 candidate genes on milk production traits in Iranian Holstein cattle population.
The effects of allele substitutions on milk production traits were tested using the following multiple linear regression models (Knott et al. 1996):

\[
y_{ijkl} = \mu + b_i x_i + b_j x_j + b_k x_k + b_l x_l + e_{ijkl}
\]

Where:
- \( y \): observation for EBVM, EBVF and EBVFP traits.
- \( \mu \): overall mean.
- \( b_i, b_j, b_k, b_l \): regression coefficients representing the allelic substitutions for \((DGATK, OPNT, PPARGC1A)(c.3359)A, PPARGC1A(c.1892)T\).
- \( x_i, x_j, x_k, x_l \): indicator variables for genotypes of \(DGAT1, OPN, PPARGC1A(c.3359), PPARGC1A(c.1892)\) loci.
- \( e_{ijkl} \): residual effect.

### RESULTS AND DISCUSSION

The most extreme genotypes frequencies were estimated as 0.65 and 0.09 for \(PPARGC1A(c.1892)CT\) and \(DGAT1KK\) loci, respectively. Similar results were obtained about genotype frequencies in Holstein cattle population by Khatib et al. (2007), Thaller et al. (2003) and Komisarek and Dorynek (2009). In addition, the most and the least allele frequencies were calculated as 0.64 and 0.36 for \(A\) and \(C\) alleles of \(PPARGC1A(c.3359)\).

#### Table 1
<table>
<thead>
<tr>
<th>Loci</th>
<th>Primer sequence for PCR reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DGAT1</strong></td>
<td>F 5'-GCACCATCCTCTTCTCCAAG-3' R 5'-GGAAGGCTTTTCCGGATG-3'</td>
</tr>
<tr>
<td><strong>OPN</strong></td>
<td>F 5'-GCAATCAGAGTGTGATGAC-3' R 5'-CCAAGCCCAACGTGATGAG-3'</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.3359)</strong></td>
<td>F 5'-GCGAGCAGCGGTGTACATACTAAGGGAGGTGCGTAG-3' R 5'-GTTGTGTTGCACTCAAATGGAC-3'</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.1892)</strong></td>
<td>F 5'-CATAGCCCGCGGCCCCAGTAAGTACGTACGTTGGC-3' R 5'-CTGGTACTCTCCTGAGTGT-3'</td>
</tr>
</tbody>
</table>

**Table 2** Restriction enzymes and the digestion conditions

<table>
<thead>
<tr>
<th>Locus</th>
<th>Position</th>
<th>Enzyme</th>
<th>Digestion temperature (°C)</th>
<th>Digestion time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DGAT1</strong></td>
<td>K232A</td>
<td>CfrI</td>
<td>37</td>
<td>3 h</td>
</tr>
<tr>
<td><strong>OPN</strong></td>
<td>c.8514</td>
<td>BsrI</td>
<td>65</td>
<td>5 h</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.3359)</strong></td>
<td>c.3359</td>
<td>NheI</td>
<td>37</td>
<td>3 h</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.1892)</strong></td>
<td>c.1892</td>
<td>HaeIII</td>
<td>37</td>
<td>5 h</td>
</tr>
</tbody>
</table>

**Table 3** Summery of frequencies, H-W equilibrium and genetic variation indices

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele frequency</th>
<th>Genotype frequency</th>
<th>H-W equilibrium</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DGAT1</strong></td>
<td>K 0.37</td>
<td>A 0.63</td>
<td>0.09</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>OPN</strong></td>
<td>C 0.47</td>
<td>T 0.53</td>
<td>CC 0.19</td>
<td>CT 0.57</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.3359)</strong></td>
<td>A 0.64</td>
<td>C 0.36</td>
<td>AA 0.38</td>
<td>CA 0.52</td>
</tr>
<tr>
<td><strong>PPARGC1A(c.1892)</strong></td>
<td>C 0.56</td>
<td>T 0.44</td>
<td>CC 0.23</td>
<td>CT 0.65</td>
</tr>
</tbody>
</table>

* (P<0.05) and ** (P<0.01).
Joint Analysis of DGAT1, OPN and PPARGC1A for Milk Traits

Figure 1

Electrophoretic separation of DGAT1 (A), OPN (B), PPARGC1A (c.1892) (C) and PPARGC1A (c.3359) (D) genes PCR products

Figures a, c and d have a common ladder (PUC mix 8)

Figure b: ladder (gene ruler DNA ladder)

Table 4

<table>
<thead>
<tr>
<th>Locus</th>
<th>DGAT1</th>
<th>OPN</th>
<th>PPARGC1A (c.3359)</th>
<th>PPARGC1A (c.1892)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-value</td>
<td>3.19*</td>
<td>1.52</td>
<td>1.93</td>
<td>2.41*</td>
</tr>
</tbody>
</table>

*(P<0.05)
In addition, Leonard et al. (2005) showed significant association of OPN gene with milk protein percentage. Therefore, we suggest the further studies need to clarify the association between OPN, PPARGC1A (c.3359) and milk production traits in other populations. There are several possible reasons for different results of studies, including differences in allele frequency, the statistical models used to undertake the association analysis and genetic background of the animals in the study (Berry et al. 2010) and environmental circumstances where the animals were producing. The association between DGAT1 gene and EBVF was significant which may be due to critical role of DGAT1 gene in the synthesis rate of triglycerides (Grisart et al. 2002). Kadlecova et al. (2014) reported significant association between DGAT1 genotypes and fat percentage in primiparous Holstein cows. Anton et al. (2012) indicated the significant effects of the DGAT1 K232A polymorphism on milk yield, fat and protein percentage, as well. In addition, Fontanesi et al. (2015) illustrated that DGAT1 polymorphism was highly associated with fat yield and fat percentage in Reggiania dairy cows (local breed in north of Italy). The results of mean comparisons illustrated that the percentage in Reggiania dairy cows (local breed in north of Italy) showed significant association of DGAT1 gene and EBVF was significant which may be due to critical role of DGAT1 gene in the synthesis rate of triglycerides (Grisart et al. 2002). Kadlecova et al. (2014) reported significant association between DGAT1 genotypes and fat percentage in primiparous Holstein cows. Anton et al. (2012) indicated the significant effects of the DGAT1 K232A polymorphism on milk yield, fat and protein percentage, as well. In addition, Fontanesi et al. (2015) illustrated that DGAT1 polymorphism was highly associated with fat yield and fat percentage in Reggiania dairy cows (local breed in north of Italy).

The results of mean comparisons were confirmed by other studies. Winter et al. (2002) and Strzałkowska et al. (2005) showed that the DGAT1 allele has a positive effect on milk fat content in different cattle breeds.

Naslund et al. (2008) reported that DGAT1 allele was associated with an increase in milk fat and protein percentages but decrease milk yield compared with the DGAT1 variant. Similar results showed that the DGAT1 allele exceeds of DGAT1 allele, by (+0.34) percentage unit in fat (Grisart et al. 2002). The DGAT1 allele increases milk fat yield, whereas the DGAT1 allele increases both milk and protein yield (Kaupe et al. 2007; Thaller et al. 2003).

According to our finding PPARGC1A (c.1892) allele decreased the EBVF by -0.37 ± 0.01. In addition, an association of the PPARGC1A (c.1892) allele with higher fat yield has been suggested in German Holsteins (Weikard et al. 2005). Alim et al. (2012) indicated that PPARGC1A (c.1892) allele increased protein yield and protein concentration but there was no association between PPARGC1A (c.1892) allele and fat yield (% kg).

**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. Briefly, the results show that there is significant association between PPARGC1A, DGAT1 and EBVF trait. 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...

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**Table 5** The analysis results of between subjects effects (F-values)

<table>
<thead>
<tr>
<th>Locus/trait</th>
<th>DGAT1</th>
<th>OPN</th>
<th>PPARGC1A (c.3359)</th>
<th>PPARGC1A (c.1892)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV_m</td>
<td>2.12</td>
<td>1.18</td>
<td>0.44</td>
<td>1.89</td>
</tr>
<tr>
<td>EBV_r</td>
<td>1.40</td>
<td>1.20</td>
<td>0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>EBV_F</td>
<td>8.45**</td>
<td>1.98</td>
<td>2.07</td>
<td>4.79**</td>
</tr>
</tbody>
</table>

**Table 6** The results of mean comparisons for significant genes

<table>
<thead>
<tr>
<th>DGAT1</th>
<th>PPARGC1A (c.1892)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KK</td>
<td>AA</td>
</tr>
<tr>
<td>0.05±0.02^b</td>
<td>0.04±0.01^b</td>
</tr>
<tr>
<td>0.01±0.001^a</td>
<td>-0.008±0.001^a</td>
</tr>
<tr>
<td>0.03±0.01^a</td>
<td>0.09±0.02^b</td>
</tr>
</tbody>
</table>

The means within the same column with at least one common letter, do not have significant difference (P<0.05).

**Table 7** The effect of allele substitution for candidate genes

<table>
<thead>
<tr>
<th>Locus/trait</th>
<th>DGAT1</th>
<th>OPN</th>
<th>PPARGC1A (c.3359)</th>
<th>PPARGC1A (c.1892)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV_m</td>
<td>-76.75±42.54</td>
<td>37.25±108.05</td>
<td>-40.77±43.22</td>
<td>92.29±45.91</td>
</tr>
<tr>
<td>EBV_r</td>
<td>+0.75±1.32</td>
<td>1.97±1.26</td>
<td>-0.08±1.41</td>
<td>0.45±1.50</td>
</tr>
<tr>
<td>EBV_F</td>
<td>+0.04±0.01**</td>
<td>0.01±0.01</td>
<td>0.02±0.01</td>
<td>-0.37±0.01**</td>
</tr>
</tbody>
</table>

**Table 8** The analysis results of between subjects effects (F-values)

**Table 9** The results of mean comparisons for significant genes

**Table 10** The effect of allele substitution for candidate genes

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can be used for pre-selection of young bulls prior to progeny test.

ACKNOWLEDGEMENT
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REFERENCES


Kharrati Koopae et al.

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