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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Polymorphism of stearoyl-coenzyme A desaturase 1(SCD1) gene in Iranian holstein dairy cattle

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Abstract

The aim of this study was to investigate the polymorphism of SCD1 gene in Holstein dairy cattle. The identification of alleles of loci associated with important function in animal metabolism could assist in recognizing potential molecular marker for dairy cattle milk production traits. Milk is essential for energy, protein, minerals and vitamins for the young stock in the early phase of their life. The SCD1 gene, expressed in adiposities mammary glands and also in other tissues, encodes a key enzyme which is responsible for converting palmitic and stearic acids into their monounsaturated forms. Additionally, the SCD1 protein was shown to be involved in some aspects of energy homeostasis e.g. lipogenesis, lipid oxidation, and thermo genesis. The SCD1 gene is located on chromosome 26 in cattle. The blood samples of 394 dairy cattle from four farms were used for extracting genomic DNA. A 400 bp fragment of exon 5 of SCD1 gene was amplified by standard PCR, and digested with NcoI enzyme for recognizing the SNP (C/T). The cytosine to thymine substitution causes Alanine to change to Valine amino acids on protein (A293V). The frequency of AA, AV and VV were 0.6015, 0.3173 and 0.0812 respectively. The frequency of alleles A and V were 0.76 and 0.24 respectively. The results of chi square test showed that the population was not under Hardy Weinberg equilibrium.

Keywords: Dairy cattle; Polymorphism; stearoyl-coenzyme A desaturase 1 gene

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Introduction

The recent studies have shown that polymorphism of candidate gene have had an effect on the composition of milk fat. Selection based on different genotype could therefore be useful for changing composition of milk fat. Milk fat has been characterized by a high amount of saturated fatty acids and a low amount of (poly) unsaturated fatty acids. With regard to human health aspects, increasing the amount of unsaturated fatty acids in milk is an important selection objective (Taniguchi et al., 2004; Schennink et al., 2008; Kgwatalala et al., 2009). Stearoyl-CoA desaturase (SCD) is an enzyme that plays an important role in the biosynthesis of fatty acids. This enzyme belongs to the large family of enzymes that are involved in the synthesis of saturated fatty acids and found in both animals and plants. Stearoyl-CoA desaturase (SCD) is the enzyme responsible for conversion of saturated fatty acids into 9-mono-unsaturated fatty acids in mammalian adiposities (Campbell et al., 2001; Taniguchi et al., 2004). Two SCD isoforms have been 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). Several researches have shown significant effects of genotypes SCD1A293V on the composition of fatty acids in milk and the carcass (Moioli et al., 2007; Kgwatalala et al., 2009; Clark et al., 2010). The purpose of this study was to investigate the SCD1 gene polymorphism in Holstein dairy cows.

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Materials and Methods

Samples: 394 Holstein dairy cattle belonging to four dairy farms located in Khorasan Razavi province were subjected to genotyping for SCD1 gene. Approximately 5 ml of blood was collected from each animal from the jugular vein, on K2EDTA tubes. The aliquots of whole blood were stored at -20 °C.

DNA extraction: The genomic DNA from blood samples were extracted based on GuSCN-Silica Gel method and standard protocol with commercial kit of DIAtom DNA Prep (Biokom). The quality and quantity of DNA were examined by way of agarose gel electrophoresis and JENWAY spectrophotometer. 5µl of DNA was diluted with 100 µl of dH2O.

PCR-RFLP analysis: The 400bp fragment of SCD1 gene was amplified with standard PCR using a Biometra thermo cycler using forward (5´-CCC ATT CGC TCT TGT TCT GT-3´) and reverse (5´-CCC ATT CGC TCT TGT TCT GT-3´) primers. The total volume of reaction was 25 µl that contained one unit (0.2 µl) of Taq polymerase , 200 µM (0.5 µl) of each dNTP, 200 mM $MgCl_2$, 10-20 pM (3 µl) primer mixed and 50-100 ng (5 µl) DNA and 2.5 µl standard Buffer in 13.8 µl dH_2O.

The thermal program of PCR reaction was initial denaturation at 94ºC for 3 min, cyclic denaturation at 94 ºC for 45s, cyclic annealing of primers at the temperature at 54 ºC for 30s , cyclic elongation at 72 ºC for 90s (for 34 cycles) and final elongation at 72 ºC for 10 min (Kgwatalala et al., 2009) . The PCR products were separated by electrophoresis in 2% agarose gel and visualized on UVP gel documentation system. The PCR product was digested with restriction enzyme NcoI (Fermentas). 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 ºC . 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 were analyzed with Chi square test using PoP-Gen software version 1.31 (Yeh and Yong, 1999).

Results

The fragment of 400bp of SCD1 gene was amplified with PCR (Fig 1). PCR-RFLP using NcoI restriction enzyme revealed three band patterns in 2% agarose gel in all the 394 DNA samples analyzed. Band pattern AA was observed when the 400 bp amplified fragment was digested at one site and yielded two overlapping bands of 200 bp thus showing a single band of 200 bp. Pattern VV was observed when the amplified fragment was not digested at all due to absence of restriction site for NcoI thus yielding only one band of 400 bp. A heterozygote AV was also observed clearly showing one 400 and another 200 bp band indicating one strand of DNA having presence and the alternate strand lacking the restriction site of NcoI. All the three band patterns have been presented in Fig. 2).

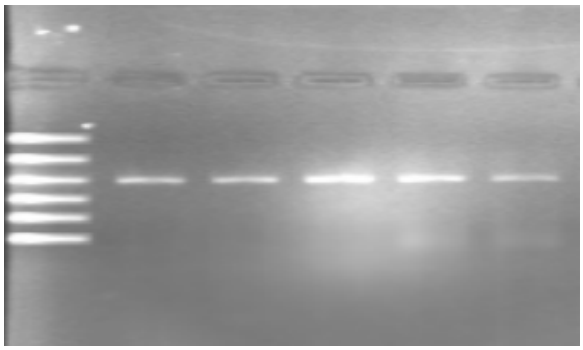

Fig 1: PCR products with molecular marker (M100)

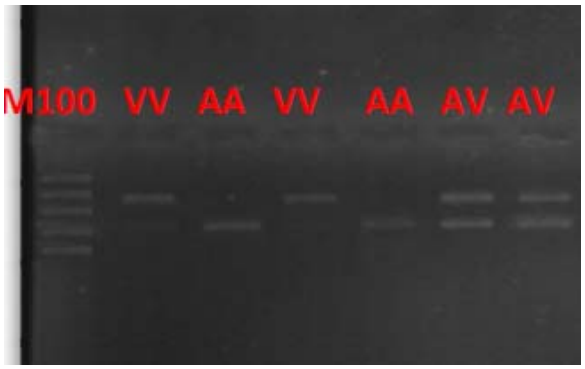

Fig 2: Genotype AA, AV and VV and molecular marker (M100)

Table 1: Chi square test of data

Genotype	Observed	Expected	$(O-E)^2 / E$
AA	237	227.6656	0.3827
AV	125	143.6688	2.4259
VV	32	22.6656	3.8442
sum	394	394	6.6528

Gene and genotypic frequency

Number of cows with AA, AV and VV genotype in this study were 237, 125 and 32 respectively. The genotypic frequency of AA, AV and VV were 0.6015, 0.3173 and 0.0812, respectively. Frequency of allele A and V were 0.76 and 0.24 respectively. Hardy Weinberg equilibrium investigated with chi square test (Table 1). The value of Chi square in this study was 6.6528 (Table 1), which compared to standard Chi

Table 2: Summarized of homozygosity and hetrozygosity of SCD1

Gene	Expected heterozigoty	Expected homozygoty	Observed heterozygoty	Observed homozygoty	Sample size
SCD1	0.3646	0.6827	0.3173	0.6354	394

square (5.991) was bigger hence the sample under study was not in Hardy Weinberg equilibrium (P<0.01).

Observed and expected homozygosity and hetrozygosity of SCD1 gene in this study are shown in Table 2.

Discussion

In this study, frequency of allele A and V were 0.76 and 0.24 respectively. The genotype frequencies were not in Hardy-Weinberg equilibrium. A higher frequency of the A allele is in agreement with results reported by other studies. Schennink et al. (2008) investigated the effect of SCD1 A293V polymorphism when 1725 cows were genotyped. The frequency of allele A and V were 0.73 and 0.27 respectively .The genotypes were in Hardy-Weinberg equilibrium. Kgwatalala et al. (2009 genotyped 525 Canadian Jersey cows for the SCD1 gene and reported that the genotypic frequencies were 0.686, 0.244, and 0.070 for the AA, AV, and VV genotypes, respectively. The frequency of the A allele was 0.808 and that of V allele was 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 an allele was reported 0.94 (Jersey), 0.65 (Valdostana) and 0.42 for Valdostana that the frequency of allele A is lower thanV allele (Moioli et al., 2007).

Clark et al. (2010) investigated the effect of Ala293Val SNP in SCD1 gene on conjugated linoleic acid concentration in milk fat of dairy cows, so the 143 and 215 cows in two studies were genotyped. The distribution of genotypes among 143 dairy cows evaluated was 72 AA, 60 AV, and 11 VV animals. Therefore, the respective allele frequencies were f (A)=0.71 and f(V)=0.29 In study 2, the distribution of genotypes among 215 dairy cows evaluated was 111 AA, 85 AV, and 19 VV animals. Allele frequencies were f (A)=0.71 and f(V)=0.29.

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. Genotypes were not in Hardy-Weinberg equilibrium (P<0.05). The higher frequency of the A allele (0.57) compared to allele V (0.43) is the same with result of this study (Mele et al., 2007).

In conclusion, the genotype of 237 individual of 394 cattle were AA so the frequency of allele A was 0.76 in studied sample of Iranian Holstein dairy cattle. Based on positive influence of A allele on unsaturated fatty acid in milk, we concluded that frequency of favorable allele A of SCD1 gene in studied sample is proper, This study suggests that the genotyping of the SCD1 gene could be used as a marker for genetic selection for increased unsaturated fatty acid.

References

Campbell, E.M.G., Gallagher, D.S., Davis, S.K., Taylor, J.F. and Smith, S.B. 2001. Mapping of the bovine stearoyl coenzyme A desaturase (SCD) gene to BTA26. *Journal of Animal Science*, 79: 1954-1955.

Clark, L.A., Thomson, J.M., Moore, S.S. and Oba, M. 2010. The effect of Ala293Val single nucleotide polymorphism in the stearoyl-CoA desaturase gene on conjugated linoleic acid concentration in milk fat of dairy cows. *Canadian Journal of Animal Science*, 90: 575-584.

Kgwatalala, P.M., Ibeagha-Awemu, E.M., Mustafa, A.F. and Zhao, X. 2009. Influence of stearoyl-coenzyme a desaturase 1genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. *Journal of Dairy Science*, 92: 1220-1228.

Mele, M., Conte, G., Castiglioni, B., Chessa, S., Macciotta, NP., Serra, A., Buccioni, A., Pagnacco, G. and Secchiari, P. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. *Journal of Dairy Science*, 90: 4458-4465.

Moioli, B., Contarini, G., Avalli, A., Catillo, G., Orru, L., De Matteis, G., Masoero, G. and Napolitano, F. 2007. Short communication: effect of stearoyl-coenzyme a desaturase polymorphism on fatty acid composition of milk. *Journal of Dairy Science*, 90: 3553-3558.

Schennink, A., Heck, JM., Bovenhuis, H., Visker, MH., van Valenberg, H.J. and van Arendonk, J.A. 2008. Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-coA desaturase (SCD1) and acyl CoA: diacylglycerol acyltransferase 1 (DGAT1). *Journal of Dairy Science*, 91: 2135-2143.

Taniguchi, M., Utsugi, T., Oyama, K., Mannen, H., Kobayashi, M., Tanabe, Y., Ogino, A. and Tsuji, S. 2004. Genotype of stearoyl-Co A desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, 14: 142-148.

Yeh, F.C., Yang, R.C. and Boyle, T. 1999. POPGENE. Microsoft Windows-Based Freeware for Population Genetic Analysis. Release 1.31. University of Alberta, Edmonton, Canada.