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Polymorphism of SCD1 and DGAT1 gene in Isfahan Holstein cows

Hojjat Asadollahpour Nanaei *, Saeid Ansari Mahyari, Mohammad-Ali Edriss, Mahmood Rostami, Malihe Pirzad and Amir Boroushak

Dep. of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

ABSTRACT

In this study we estimated the allele and genotype frequencies of SCD1 and DGAT1 gene polymorphism. The analysis was conducted on 408 Holstein cows from five dairy herds in Isfahan province. Genotypes were identified using PCR-RFLP technique. Two genetic variants (A and V) of the SCD1 gene were observed in this experiment. The frequency of A-allele ranged from 0.49 to 0.66, while frequency of V-allele 0.34 to 0.51. Regards with DGAT1 gene, also two genetic variants (A and K) were determined that frequency of A-allele ranged from 0.54 to 0.68 and K-allele 0.32 to 0.46. It was found that these two genes were polymorphic in Isfahan Holstein cows, which suggested that could be associated with composition and production traits.

Key words: Holstein cow, SCD1 gene, DGAT1 gene, polymorphism, milk traits

INTRODUCTION

Gene mapping research has led to the discovery of many polymorphic sites throughout the dairy cattle genome that can serve as genetic markers for selection in breeding schemes. Among many different candidates, diacylglycerol acyltransfrase 1 (DGAT1) and stearoyl-CoA desaturase 1 (SCD1) gene, seems to be directly associated with production traits [12 and 15]. In dairy cows, DGAT1 gene is considered to be a very strong positional candidate gene for fat percent of milk. This gene is located on the centromeric end of the bovine chromosome 14 (BTA14), harboring the QTL with a large impact on milk production traits [3, 9 and 21]. DGAT1 encodes an integral microsomal membrane enzyme that catalyses the final step in triglyceride synthesis [14]. Milk fat contains approximately 98 percent triglycerides, so this is a key enzyme for milk triacylglycerol synthesis in the mammary gland [16]. Kaupe *et al*. [2004] studied polymorphism of this gene in Bos taurus and Bos indicus breeds. They claimed that K allele of DGAT1 gene is a wild type and the A allele substitution probably occurred after the divergence of Bos taurus and Bos indicus [10]. Recently, many studies showed a significant association between polymorphism of this gene and milk production traits [8, 12 and 20]. The SCD1 locus has been mapped on BTA 26 [2]. This gene encodes a key enzyme introducing a double bond between carbons Δ^9 and Δ^{10} in a large spectrum of medium and long-chain fatty acids. SCD1 plays a role in the endogenous synthesis of the conjugated

Corresponding Author E-mail: Sayare1@yahoo.com

linoleic acid (CLA) in ruminants. Also it was suggested that this protein plays an important roles in the regulation of fatty acid oxidation [4 and 6]. This gene is present in several tissues such as: adipose tissue and liver. And its expression is controlled by insulin, liver X receptor (LXR), sterol regulatory element binding protein (SREBP-1c), PPARs and leptin [18]. Additionally, there is a suggestion that the SCD1 protein involved in some aspects of energy homeostasis like: lipogenesis, lipid oxidation, and thermogenesis [4 and 6]. Based on these considerations, the aim of the present study was to estimate the allelic and genotypic frequencies of all two these genes polymorphism in Isfahan Holstein dairy cows.

MATERIAL AND METHODS

In this experiment, blood samples were collected from 408 Holstein dairy cows that randomly selected among 10000 cows from five dairy herds in Isfahan province. DNA was extracted from whole blood samples using standard salting out protocol [17], and these two genes were amplified with the standard polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The PCR amplification was performed in a 20-µL reaction volume, which included 25-50 ng genomic DNA, 2 µL 10x PCR buffer, 10 pmol of each primer of two genes (Table 1), 1.5 mM $Mgcl_2$, 200 µM dNTP, 1 unit of taq DNA polymerase and sterilized distilled water to make a final volume of 20 µL. Thermal cycling conditions were as follows: 94°C for 5 min; 30 cycles of 94°C for 30 s, annealing temperature (Table 1) for 30 s, and 72°C for 30 s, followed by a final step of 72°C for 5 min. Seven microliters PCR products was digested with five units of respective (Table 1) restriction enzymes (Fermentas Co.) in 20 µL of reaction volume at 37°C for 6 hours. The digested products plus 2 µL loading dye were run on 2% ethidium bromide-stained agarose gel for 3 h, and genotype bands were visualized under UV light.

RESULT AND DISCUSSION

Looking for candidate genetic markers of potential value in Marker Assisted Selection (MAS) in dairy cows is lately very popular. The PCR-RLFP method was useful for DGAT1 and SCD1 genotype marking and could be useful for selection in dairy cattle. In the population studied, the allelic and genotypic frequency of all two genes DGAT1 and SCD1 are given in table 2. Two genetic variant (A and K) of the DGAT1 polymorphism were observed in this experiment. The A and K alleles of this gene were identified based on amplification of a 411 bp fragment, followed by digestion with the restriction enzyme *Cfr1*. Genotype AA was determined by the present of a single 200-bp fragment, while genotype KK was characterized by a single 400-bp fragment. Also KA individuals present two fragments of 203 and 208 bp. The KA and KK genotypes were the most and least frequent in all herds, respectively (Table 2). The following DNA restriction fragments were obtained for SCD1 gene using *NcoI* enzyme. The fragments were 400 (no digestion) for the VV genotype, 200 and 400 bp for the AV and 200 bp for the AA genotype. Study on SCD1gene shown that frequencies for A and V alleles were 0.58 and 0.42 respectively (Table 2). The AV genotype showed the highest frequency (0.65), whereas the VV had the least frequent genotype (0.09).

Table 1. Selected PCR-RFLP conditions for the analyzed polymorphisms

gene	Primers (5'-3')	Annealing temp (°C)	PCR produc size (bp)	Restriction endonuclease
SCD1	F- CCC ATT CGC TCT TGT TCT GT R- CGT GGT CTT GCT GTC GAC	59	400	NcoI
DGAT1	F-GCACCATCCTCTTCCTCAAG R- GGAAGCGCTTTCGGATG	60	411	Cfr1

Table 2. Genotypic and allelic Frequency of SCD1 and DGAT1 gene in Holstein dairy cows

Gene	SCD1						DGAT1				
Herd	N	Genotypes			Alleles		Genotypes			Alleles	
		AA	AV	VV	A	V	AA	KA	KK	A	K
1	83	0.28 (23)	0.42 (35)	0.30 (25)	0.49	0.51	0.40 (34)	0.50 (41)	0.10 (8)	0.66	0.34
2	65	0.15 (10)	0.80 (52)	0.05 (3)	0.56	0.44	0.37 (24)	0.55 (36)	0.08 (5)	0.64	0.36
3	85	0.24 (21)	0.73 (62)	0.03 (2)	0.61	0.39	0.47 (40)	0.41 (35)	0.12 (10)	0.68	0.32
4	79	0.35 (28)	0.62 (49)	0.03 (2)	0.66	0.34	0.35 (28)	0.61 (48)	0.04 (3)	0.65	0.35
5	96	0.25 (24)	0.35 (67)	0.06 (5)	0.60	0.40	0.21 (20)	0.68 (65)	0.11 (11)	0.54	0.46
Total/ Average	408	0.26	0.65	0.09	0.58	0.42	0.36	0.55	0.09	0.63	0.37

There have been many studies of the allelic frequency of DGAT1 gene and influence of this gene polymorphism on milk production traits, especially on milk fat yield in different cattle breeds including: Jersey, Fleckvieh, Normande, and Angeln breed [7, 22, 23 and 24]. For example, the investigations functional polymorphism of the DGAT1 gene on milk composition have been reported in dairy cattle by Grisart *et al*. [2002] and Ripoli *et al*. [2006]. They showed significant effect of DGAT1 genotypes on determination of milk composition [8 and 20]. An association between the DGAT1 gene polymorphism and production traits in Holstein-Friesian and Jersey cattle has been investigated by Komisark *et al.* [2004], who claimed that homozygous KK animals produced milk with higher fat content and yielded more fat than other genotypes [13]. Recently, investigation of the same polymorphism in Iranian Holstein cattle reported by Kharrati Koopaei *et al.* [2012], they provided contrary results as the genotype KA animals showed the more milk fat than other groups [12]. Several studies have reported QTL located on BTA26 affecting milk, fat, and protein yields in Holstein dairy cows [1, 5 and 19]. It has been showed that some genes involved in lipid metabolism such as: SCD, LPF (gastric lipase), or GPAM (glycerol-3-phosphate acyltransferase mitochondrial), located on this chromosome. Some studies were performed about SCD1 gene polymorphism and its correlation with production traits in cattle. In Italian Holsteins, an association between SCD gene polymorphism and milk production traits (milk, fat, and protein yields, fat and protein contents) has been reported on a sample of 701 lactations of 313 cows. Macciotta *et al*. [2008] showed an effect of the SCD genotype on milk and protein yields. Who claimed that animals with VV genotypes producing more milk (about 2 kg/d) and protein (ar 0.07 kg/d) compared with AA cows [15].

These results suggest a possible use of these candidate markers in gene-assisted selection programs for improvement of milk production traits in dairy cattle, although large-scale studies in different breeds are required.

REFERENCES

[1] Boichard D, Grohs C, Bourgeois F, Cerqueira F, Faugeras R, Neau A, Rupp R, Amigues Y, Boscher M Y, Leveziel H. Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Sel. Evol. 2003; 35:77–101.

[2] Campbell EMG, Callagher DS, Davis SK, Taylor JF, Smith SB. Mapping of the bovine stearoyl-coenzyme A desaturase (SCD) gene to BTA26. J. Anim. Sci. 2001; 79:1954–1955.

[3] Coppieters W, Riquet J, Arranz JJ, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Nezer C, Simon P, Vanmanshoven P, Wagenaar D, Georges M. A QTL with major effect on milk yield and composition maps to bovine chromosome 14. Mammalian Genome. 1998; 9, 540-544.

[4] Dobrzyn A, Ntambi JM. The role of stearoyl–CoA desaturase in the control of metabolism. Prostaglandins Leukot Essent Fatty Acids. 2005; 73:35–41.

[5] Druet T, Fritz S, Boichard D, Colleau JJ. Estimation of genetic parameters for quantitative trait loci for dairy traits in the French Holstein population. J. Dairy Sci. 2006; 89:4070–4076.

[6] Flowers MT, Ntambi JM. Role of stearoyl–coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol. 2008. 19: 248–256.

[7] Gautier M, Capitan A, Fritz S, Eggen A, Boichard D, Druet T. Characterization of the DGAT1 K232A and variable number of tandem repeat polymorphisms in French dairy cattle. Journal of Dairy Science. 2007; 90, 2980-2988.

[8] Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M, Snell R. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Research. 2002; 12, 222-231.

[9] Heyen DW, Weller JI, Ron M, Band M, Beever JE, Feldmesser E, Da Y, Wiggans GR, Vanraden PM, Lewin HA. A genome scan for QTL influencing milk production in dairy cattle. Physiological Genomics. 1999; 1, 165-175.

[10] Kauppe B, Winter A, Fries R, Erhardt G. DGAT1 polymorphism in Bos indicus and Bos taursus cattle breeds. Journal of Dairy Research. 2004; 71, 182-187.

[11] Kgwatalala PM, Ibeagha-Awemu, EM, Mustafa, A F, Zhao, X. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. Animal Genetics. 2009; 5, pages 609–615.

[12] Kharrati Koopaei H, Mohammad Abadi MR, Ansari Mahyari S, Esmailizadeh Koshkoiyeh A, Tarang AR, Potki P. Effect of DGAT1 variants on milk composition traits in Iranian Holstein cattle population. Animal Science Papers and Reports. 2012; 3, 231-239.

[13] Komisarek J, Waskowicz K, Michalak A, Dorynek Z. Effects of DGAT1 variants on milk production traits in Jersey cattle. Animal Science Papers and Reports. 2004; 3, 307-313.

[14] Lehner R, Kuksis A. Biosynthesis of triacylglycerols. Progress in Lipid Research. 1996; 35, 169-201.

[15] Macciotta NPP, Mele M, Conte G, Serra A, Cassandro M, Dal Zotto R, Cappio Borlino A, Pagnacco G, Secchiari P. Association Between a Polymorphism at the Stearoyl CoA Desaturase Locus and Milk Production Traits in Italian Holsteins. J. Dairy Sci. 2007; 91:3184–3189.

[16] Marshall MO, Knudsen J. Biosynthesis of triacylglycerols containing short-chain fatty acids in lactating cow mammary gland. Activity of diacylglycerol acyltransferase towards shortchain acyl-CoA esters. Journal of Biochemistry. 1977; 81, 259-266.

[17] Miller SA, Dykes DD, Polesky HF. A simple salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research. 1988; 16, 1215-1219.

[18] Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol-Endoc M 297, E 28–E37. 2009 Doi: 10.1152/ajpendo.90897.2008, http: //ajpendo. physiology.org.

[19] Plante Y, Gibson JP, Nadesalingam J, Mehrabani-Yeganeh H, Lefebvre S, Vandervoort G, Jansen GB. Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. J. Dairy Sci. 2001; 84:1516–1524.

[20] Ripoli MV, Corva P, Giovambattita G. Analysis of a polymorphism in the DGAT1 gene in 14 cattle breeds through PCR-SSCP methods. Research Veterinary Science. 2006; 80, 287-290.

[21] Riquet J, Coppieters W, Cambisano N, Arranz JJ, Berzi P, Davis S, Grisart B, Farnir F, Karim L, Mni M, Simon P, Taylor J.F, Vanmanshoven P, Wagenaar D, Womack JE, Georges M. Identity-by-decent fine-mapping of QTL in outbred populations: Application to milk production in dairy cattle. Proceedings of the National Academy of Sciences, USA. 1999; 96, 9252-9257.

[22] Sanders K, Bennewitz J, Reinsch N, Thaller G, Prinzenberg EM, Kuhn C, Kalm E. Characterization of the DGAT1 mutations and the CSN1S1 promoter in the German Angeln dairy cattle population. Journal of Dairy Science. 2006; 89, 3164-3174.

[23] Spelman RJ, Ford CA, Mcelhinney P, Gregory GC, Snell RG. Characterization of the DGAT1 gene in the New Zealand dairy population. Journal of Dairy Science. 2002; 85, 3514-3517.

[24] Thaller G, Kramer W, Winter A, Kaupe B, Erhardt G, Fries R. Effects of DGAT1 variants on milk production traits in German cattle breeds. American Society of Animal Science. 2003; 81, 1911-1918.