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Effect of STAT1 variants on milk production traits in Esfahan Holstein Cows

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ABSTRACT

STATs are a family of latent transcription factors that reside in the cytoplasm of resting cells. The STAT1 transcription factor gene located on the chromosome 2 at interval 60 to 63 cM. This factor upon stimulation with INF gamma, dimerizes and translocate to the nucleus where mediates transcriptional regulation. In this study, we estimated the allele and genotype frequencies of STAT1/*Pag*1 gene polymorphism and examined the association whit milk yield (305-day milk yield) and milk component (fat and protein percentage) traits. DNA was isolated from 317 Holstein cows of five different herds. A 314 bp fragment in STAT1 gene was amplified and the animals were genotyped using RFLP-PCR technique. Seven genotypes including DD (89 animals), BB (25 animals), CC (11 animals), AC (15 animals), BC (9 animals), CD (164 animals) and BD (4 animals) were identified. Frequencies of A, B, C, and D alleles were estimated to be 0.021, 0.101, 0.332 and 0.546, respectively. Association of STAT1/*Pag*1 genotypes with percentage of fat in the milk was relatively high ($P < 0.05$); the DD genotype was superior to other groups. But corrected milk production for 305 days and protein percentage of the milk did not differ among all groups ($P > 0.01$). We concluded that this marker should be considered for milk component (fat percentage) selection in Holstein dairy cows.

Key words: Holstein cows / STAT1 gene / RFLP-PCR / fat / milk composition

INTRODUCTION

Gene mapping research has led to the discovery of many polymorphic sites throughout the cattle genome that can serve as genetic markers and that are related to important economic traits. STATs are a family of latent transcription factors that reside in the cytoplasm of resting cells (Levy and Gilliland 2000). Based on reports, bovine transcription factors family have seven members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 (Catlett-Falcone *et al*. 1999, Levy and Gilliland 2000, Cobanoglu *et al*. 2006). These factors based on their activation profiles in vitro classified into two groups. Four members of these proteins including STAT1, 3, 5a and 5b are activated by a large number of both extracellular and

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intracellular stimuli signals, while STAT2, 4 and 6 are primarily activated by only a single extra cellular factor (Catlett-Falcone *et al*. 1999). The STAT proteins have important biological roles in mammary gland, fertility and early embryonic development (Khatib *et al*. 2009, Klover *et al*. 2010).The STAT1 transcription factor gene located on the chromosome 2 at interval 60 to 63 cM (Band *et al*. 2000). This factor is central to mediating the effects of INF gamma and activated in response to many cytokines, growth factors and other signaling molecules. STAT1 upon stimulation with INF gamma, dimerizes and translocate to the nucleus where mediates transcriptional regulation (Gavrilescu *et al*. 2004). Recently Cobanoglu *et al*. (2006) reported a significant association between polymorphism of STAT1 gene and milk production traits in Holstein dairy cattle. They showed that allele C of this gene increases fat and protein percentage of milk, and claimed that genotypes CC and CT were associated with higher milk, fat and protein yield. In this experiment we estimated allele and genotype frequencies of STAT1gene polymorphism in Holstein cows and examined association between this polymorphism and milk production, as well as with milk components.

MATERIAL AND METHODS

Animals

For this experiment, blood was collected from 317 multiparous (N<5) Holstein cows, randomly selected among about 6000 cows from five dairy herds in Isfahan province. All animals were kept in identical environmental conditions and milked twice a day with the use of a pipeline milking machine. Accurate recorded data for three to four years for each cow was used for analysis of the production parameters.

Marker genotype determination

DNA was extracted from blood samples using standard salting out protocol (Miler *et al*. 1988) and STAT1 genotypes were identified with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. The PCR mixture contained 50 ng genomic DNA, 2 µL 10X PCR buffer, 10 pmoL of each primer, 0.7 mM $Mgcl_2$, 200 µM dNTP, 1 unit Taq polymerase and sterilized water to make a final volume of 20 µL. The sequences of primers were as follows:

Forward: 5’ – GCCTCAAGTTTGCCAGTGGC-3’

Reverse: 5’ – GGCTCCCTTGATAGAACTGT-3’

Conditions for PCR were 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 63°C for 30 s, and 72°C for 30 s. The final step was at 72°C for 10 min. The PCR products (5µL) were digested using 2 units of the *Page*1(Fermentas Co.) restriction enzyme for 12 hrs at 37°C and separated on a 2% agarose gel. The digested products plus 2 µL loading dye were run and the genotype bands were visualized under UV light.

Statistical analysis

The effect of STAT1 genotypes on interest traits were tested using the general linear model (GLM) procedure of SAS program (Statistical Analysis System, 1997), implementing the following statistical model:

$$Y_{ijkl} = \mu + G_i + HYS_j + S_k + b_1 (Z_{ijkl} - Z) + b_2 (N_{ijkl} - N) + e_{ijkl}$$

Where, Y_{ijkl} = value for each milk-related trait, μ= overall mean, G_i = fixed effect of the ith genotype, HYS_j = herd – year- season effect, S_k= random effect of sire (1,…,155), b_1 = the linear regression coefficient of 305-day milk yield, Z_{ijkl} = 305-day milk yield, Z = mean 305-day milk yield, b_2 = the linear regression coefficient of open days, N_{ijkl} = open days, N = mean open days and e_{ijkl} = random residual effect.Tukey test was used for pair wise comparisons of the means. The Hardy-Weinberg test, Allelic and genotypic frequencies for all animals were estimated using the Pop Gene software version 3.1d (Nei 1977).

RESULT AND DISCUSSION

Genetic research in farm animals focuses mainly on the identification of genes influencing economically important traits that could be useful in breeding programs (Asadollahpour Nanaei et al. 2013). Among very different candidates, the STAT1 gene was chosen because of its involvement in the development and differentiation of the mammary gland (Watson et al. 2008). Also results from some genomic studies on dairy cattle showed that quantitative trait loci (QTL) with major effect on milk composition traits located in the region of STAT1, interval 60 to 63 CM (Mosig et al. 2001, Ashwell et al. 2004, Ron et al. 2004). The current study focused on a single SNP of STAT1 gene and assessed it is associations with milk performance traits. The A, B, C and D alleles of the STAT1 gene were identified based on amplification of a 314 bp fragment, followed by digestion with the restriction enzyme *Pag*1 (table1). The fragments were 314 bp (no digestion) for the BB genotype, 201 and 113 bp for the AA, 260 and 54 bp for the CC, 201, 59 and 54 bp for the DD genotype. In the present study the frequency of A allele in two herds of 2 and 5 were 0.099 and 0.008 respectively. While this frequency in the other groups (1, 3 and 4) was zero. Seven genetic variants DD, BB, CC, AC, BC, DC and BD of the STAT1 polymorphism were observed. Genotypic frequencies of all animals are show in table 2. The CD and BD genotypes were the most and least frequent in all herds, respectively. The values of chi-square and likelihood ratio test are shown in table 3. Result of Chi-square test indicated that the population under study was not found in a Hardy-Weinberg equilibrium, as for years it has been under for milk production selection. The observed and expected heterozygosity, homozygosity and average heterozygosity of STAT1 gene are given in table 4. All seven genotypes were considered to be in the association analysis between STAT1 polymorphism genotypes and milk production traits. There was an association between genotypes and fat percentage of milk ($P < 0.05$), but corrected milk production for 305 days and protein percentage of the milk did not differ among all groups ($P > 0.01$). The least square means (LSM) results showed that the animals in DD genotype groups had higher milk fat percentage than the other animals (table 5). The significant association between polymorphism of this gene and milk fat percentage was not surprising because recent studies showed that the STAT genes might be important for the regulation of fat metabolism, and probably through the prolactin signal transduction pathway operating in the mammary gland (Mao *et al*.

2002). An association between the bovine STAT1 gene polymorphism and milk production traits has been investigated by Cobanoglu *et al*. (2006). Who claimed that animals with genotypes CC and CT have statistically significant increase in milk, fat, and protein yields compared with TT genotype. In conclusion, we showed that dairy cattle STAT1 gene polymorphism is distinguishable by examining STAT/*Pag*1, and that this polymorphism is correlated with milk fat percentage. This polymorphism could have a significant effect on gene expression pattern through the modification of DNA-protein interaction sites. The DD genotype of STAT1 polymorphism can be selected for genetic gains in milk fat percentage of the Holstein cattle.

REFERENCES

Asadollahpour Nanaei H, Ansari Mahyari S, Edriss MA, Pirzad M, Boroushak A (2013) Polymorphism of SCD1 and DGAT1 gene in Isfahan Holstein cows. Int J Adv Biol Biom Res, 1(7), 783-788.

Ashwell MS, Heyen DW, Sonstegard TS, VanTassell CP, Da Y, Vanraden PM, Ron M, Weller JI, Lewin HA (2004) Detection of quantitative trait loci affecting milk production,health, and reproductive traits in Holstein cattle. J Anim Sci 87, 468–475.

Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW, Donovan J, Windish R, Steining C, Mahyuddin P, Womack JE, Lewin HA (2000) An ordered comparative map of the cattle and human genomes. Genome Res10, 1359–1368.

Catlett-Falcone R, Dalton WS, Jove R (1999) STAT proteins as novel targets for cancer therapy.Curr Opin Oncol 11, 490 – 496.

Cobanoglu O, Zaitoun I, Chang YM, Shook GE, Khatib H (2006) Effects of the Signal Transducer and Activator of Transcription 1 (STAT1) Gene on Milk Production Traits in Holstein Dairy Cattle. J Anim Sci 89, 4433–4437.

Gavrilescu LC, Butcher BA, Delrio L, Taylor GA, Denkers EY (2004) STAT1 Is Essential for Antimicrobial Effector Function but Dispensable for Gamma Interferon Production during Toxoplasma gondii Infection. Infect Immun 72, 1257–1264.

Khatib H, Huang W, Mikheil D, Schutzkus V, Monson RL (2009) Effects of signal transducer and activator of transcription (STAT) genes STAT1 and STAT3 genotypic combinations on fertilization and embryonic survival rates in Holstein cattle. J Anim Sci 92, 6186-91.

Klover PJ, Muller WJ, Robinson GW, Pfeiffer RM, Yamaji D, Hennighausen L (2010) Loss of STAT1 from mouse mammary epithelium results in an increased Neu-induced tumor burden. Neoplasia 12, 899–905.

Levy DE, Gilliland DG (2000) Divergent roles of STAT1 and STAT5 in malignancy as revealed by gene disruptions in mice. Oncogene 19, 2505 – 2510.

Mao J, Molenaar AJ, Wheeler TT, Seyfert HM (2002) STAT5 binding contributes to lactational stimulation of promoter III expressing the bovine acetyl-CoA carboxylase α-encoding gene in the mammary gland. J Mol Endocrinol 29, 73–88.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting-out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 16, 1215-1219.

Mosig MO, Lipkin E, Khutoreskaya G, Tchourzyna E, Soller M, Friedmann A (2001) A whole genome scan for quantitative trait loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. Genetics 157, 1683–1698.

Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41, 225-233.

Ron M, Feldmesser E, Golik M, Tager-Cohen I, Kliger D, Reiss V, Domochovsky R, Alus O, Seroussi E, Ezra E, Weller JI (2004) A complete genome scan of the Israeli Holstein population for quantitative trait loci by a daughter design. J Anim Sci 87, 476–490.

SAS (Statistical Analysis Systems), 1997 – SAS-STAT User's Guide, SAS Inst. Inc. Cary. NC.
Watson C J, Neoh k (2008) The Stat family of transcription factors have diverse roles in mammary gland development. Semin Cell Dev Biol 19, 401-6.

Table 1. Frequencies of STAT1 alleles in the analysed population.

Herd	Allele frequencies			
	A	B	C	D
1	0.000	0.108	0.334	0.558
2	0.099	0.056	0.328	0.521
3	0.000	0.194	0.306	0.500
4	0.000	0.045	0.366	0.589
5	0.008	0.104	0.328	0.560
Average frequencies	0.021	0.101	0.332	0.546

Table 2. Genotype frequencies of STAT1 gene in Holstein dairy cows.

Herd	N	Genotypes						
		DD	BB	CC	CD	AC	BC	BD
1	51	0.294	0.059	0.040	0.509	0	0.079	0.019
2	71	0.324	0.056	0.028	0.394	0.198	0	0
3	61	0.260	0.147	0.049	0.449	0	0.065	0.030
4	67	0.268	0.045	0.045	0.642	0	0	0
5	67	0.254	0.089	0.015	0.597	0.015	0.015	0.015
Total/Average	317	0.280	0.079	0.035	0.518	0.043	0.032	0.013

Table 3. Chi-square values and Likelihood ratio test.

Test	Degree of freedom	Value	P-value
Chi-square	6	255.82	0.000001
Likelihood ratio	6	186.11	0.000003

Table 4. Summary of homozygosity and heterozygosity.

Gene	Sample size	Observed homozygosity	Observed heterozygosity	Expected homozygosity	Expected heterozygosity	Average heterozygosity
STAT1	634	0.4	0.6	0.42	0.58	0.56

Table 5. Least square means and standard errors (LSMean± SE) of the milk fat obtained from different genotypes of STAT1/*Page*1 polymorphism gene in Holstein dairy cows.

polymorphism	Genotype	N	Fat (%)
STAT1/*Page*1	DD	89	3.22 ± 0.08^{a}
	BB	25	2.80 ± 0.11^{b}
	CC	11	2.85 ± 0.05^{b}
	AC	15	3.14 ± 0.12^{ab}
	BC	9	2.91 ± 0.08^{b}
	DC	164	2.83 ± 0.10^{b}
	BD	4	2.89 ± 0.07^{b}
		317	$P < 0.05$

Regarding the P value, numbers with the same superscript letters in each column are not significantly different.