Animal Science Papers and Reports vol. 28 (2010) no. 1, 5-11
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Association between polymorphism in *STAT5A* gene and milk production traits in Chinese Holstein cattle*

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(Received December 3, 2008; accepted December 10, 2009)

STAT5 is a key intracellular mediator of prolactin signaling and can activate transcription of milk proteins in response to prolactin. *STAT*5 genes are suggested to be candidate markers for milk protein yield and composition in dairy cattle. PCR-SSCP was applied to analyze the polymorphisms of two *loci* in *STAT*5A gene in 279 Chinese Holstein cattle. Genotype frequencies, allele frequencies and correlation coefficients between the polymorphic variants and milk production traits were estimated. Three genotypes were found at the two *loci*. At *locus* P_1 the frequencies of genotypes AA/GG/AG were 0.240/0.147/0.613 and those of alleles A and G were 0.547 and 0.453, respectively. The A/G genotypes had significant effect on milk yield and milk protein content in lactations 1 and 2. At *locus* P_2 the genotype frequencies of CC/TT/CT were 0.752/0.004/0.244 and C and T allele frequencies were 0.875 and 0.125, respectively. Different genotypes had remarkable effect on the milk protein content in lactation 2.

KEY WORDS: Chinese Holstein cattle / gene polymorphism / lactation / milk / SSCP / *STAT*5A

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*Partly supported by the National "863" Program of China (2006AA10Z197 2008AA101010), National Natural Science Foundation of China (No.30771544 30972080), National Key Technology R&D Programme (No.2006BAD01A10-5), Keystone Project of transfergene in China (2009ZX08009-157B, 2008ZX08007-002), "13115" Sci-Tech Innovation Program of Shaanxi Province (2008ZDKG-11), Program of National Beef Cattle Industrial Technology System the Key Discipline Fund of Jiangsu Province (No.SYC0701), and Natural Science Foundation of Xuzhou Normal University (No. 07XLA08; No. KY2007019).
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Signal transducers and activators of transcriptions are transcription factors family including STAT1, 2, 3, 4, 5A, 5B and 6. The DNA-binding activity of STAT5 was first identified in the mammary gland. STAT5 exists in two closely related forms, A and B, encoded by two separate genes [Darnell 1997]. STAT5 is a key intracellular mediator of prolactin signaling, and can activate the transcription of milk protein genes in response to prolactin [Wakao 1994]. STAT5 is also known as the major mediator of GH action on target genes [Argetsinger 1996]. STAT5 dimers bind to GAS sequences located in promoters of milk protein genes and activate their transcription. Therefore, they are suggested as candidate genes associated with milk protein yield and content in dairy cattle. In cattle the *STAT*5A gene has been assigned to chromosome 19q17 within 40 Kbp *STAT locus* containing also *STAT*3 and *STAT*5B genes [Seyfert *et al*. 2000, Moleenar *et al*. 2000]. *STAT*5A gene consists of 19 exons encoding a 794 amino acid protein [Seyfert *et al*. 2000].

Data on nucleotide sequence polymorphism in the bovine *STAT*5A gene are limited and additional polymorphisms are needed to investigate the effect of *STAT*5A variation on milk production traits in cattle. It is necessary to detect more polymorphisms to help the investigation of the role of *STAT*5A variation in the milk production traits in Chinese Holsteins.

Material and methods

Animals and data source

The dairy Holstein cattle with known pedigree from Xuzhou Dairy Farm were used. The 279 cows, born in 1999-2000, were daughters of 25 sires. Mean number of daughters per sire was 11; each of the sire group contained no less than six cows. Cows were fed *ad libitum* with a complete ration of artificially dried grass, corn silage and concentrates. Traits analysed were milk yield (kg), protein content (%) and fat content (%) in lactations 1 and 2. Milk yield was recorded measured twice a month, and at the same time milk components were determined. The data of milk yield and mean milk components of individuals were collected. The total milk yield was adjusted to 305 days standard lactation yield [Qiu 1995].

DNA samples and PCR

Blood samples were collected from jugular vein and DNA was extracted using a phenol-chloroform protocol [Sambrook *et al.* 2002]. PCR amplification was performed in 25-µl reactions containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 µM each of the four dNTP, 5 pmol of each primer and 1 U of Taq DNA polymerase (TaKaRa, China) under the following conditions: one cycle 94°C 3 min; 39 three-step cycles (94°C 60 s, 60°C 90 s and 72°C 60s) followed by a final extension for 10 min at 72°C. The primer sequences, position, PCR fragments size and the annealing temperatures of *STAT*5A P_1 and *STAT*5A P_2 are listed in Table 1.

Table 1. Primer sequences, PCR fragment sizes annealing temperatures and position of *STAT*5A P_1 and *STAT*5A P_2

Loci	Primer sequence	Size (bp)	Annealing temperature	Position	References
P_1	5´- ccagggtgcatacaggacag -3´ 5´- gcaggttacgaggactcagg -3´	224	60 °C	parts of intron 9 and exon 10	Brym [2004]
P_2	5'- agccctacagctccaatcct -3' 5' - gggtgtacccgctgcttag -3'	281	60 °C	parts of intron 15 and exon 16	Flisikowski [2003]

SSCP analysis

SSCP analyses were carried out using a DYCZ-24B electrophoresis apparatus (Liu yi Ltd., Beijing). The 10% polyacrylamide gel was prepared with a 0.5 x TBE buffer. Initial electrophoresis (without samples) was run for 1 h at 180 V, 80 mA, 15 W. Ten µl samples of PCR products were mixed with 6 µl of denaturation buffer (95% deionized formamide, 0.25% bromophenol blue, 0.5 M EDTA), denatured for 10 min at 95°C, rapidly chilled on ice, and then loaded onto the gel. The electrophoresis was run at 140V, 50mA, 8W, for approx 16 h. The gels were stained with $AgNO_3$.

Sequencing of PCR products

PCR products of different genotypes in the *STAT*5A gene were sent to Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, and automatically sequenced in an ABI377 sequencer (APPLIED BIOSYSTEMS, USA). The sequence was analysed using the DNAMAN3.0.

Statistical

Genotype and allele frequencies and the Hardy-Weinberg equilibrium were calculated with POPGENE 1.31 software (http://www.ualberta.ca/~fyeh). The significance of differences in allele and genotype frequencies was verified with the *chi-square* test. The analysis of associations between *STAT*5A genotypes and milk production traits was conducted with the GLM procedure (SPSS12.0) using the following model:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

where:

Y_{ijk} – analysed trait of cow k;

μ – overall mean;

G_i – effect of genotype;

S – effect of season;

e_{ijk} – random error.

Results and discussion

Three SSCP patterns of *STAT*5A P_1 were observed as reported by Brym [2004]. SSCP patterns of *STAT*5A P_2 are shown in Fig. 1. PCR products of different genotypes were sequenced and analysed. Two SNPs were found in *STAT*5A gene, of which that at P1 *locus*, the A9501G transition (according to GeneBank AJ237937) was detected before [Brym 2004]. A novel mutation, a C12735T transition (according to GeneBank AJ237937) was found in *STAT*5A gene *locus* P_2 (Fig. 2), and this mutation caused amino acid T→I substitution in the STAT5A protein. Allele and genotype frequencies for the two *loci* were analysed in Chinese Holsteins (Tab. 2). Some genotypes were found at low frequency in Chinese Holsteins, for example only 0.0036 for genotype TT at *STAT*5A *locus* P_2. The genotype frequencies of *STAT*5A P_2 in Chinese Holsteins were in accordance with Hardy-Weinberg equilibrium, whereas those at *STAT*5A P_1 *locus* were at Hardy-Weinberg disequilibrium ($P<0.05$).

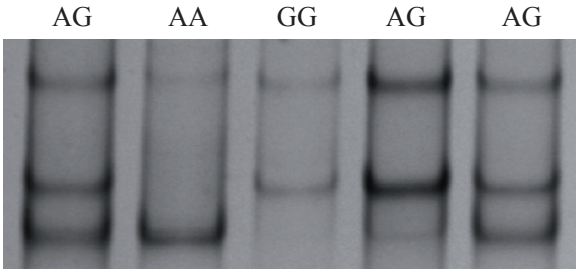

Fig. 1. SSCP patterns and partial sequence graph of STAT5A P_1. A – Three banding patterns (AA, AG, GG) were detected with SSCP.

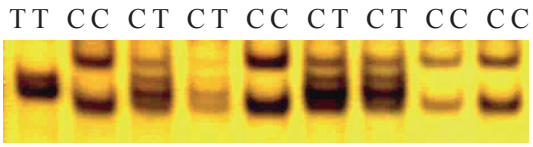

Fig. 2. SSCP patterns and partial sequence graph of STAT5A P_2. A – Three banding patterns (CC, CT, TT) were detected by SSCP.

Table 2. Genotype and allele frequencies at *STAT*5A P_1 and P_2 *loci*

Loci	Number	Genotype frequencies		Allele frequencies		*Locus* equilibrium *chi-square* test
P1	279	AA	0.2401	A	0.5466	Disequilibrium
		AG	0.6129	G	0.4534	
		GG	0.1470			
P2	279	CC	0.7527	C	0.8746	Equilibrium
		CT	0.2437	T	0.1254	
		TT	0.0036			

Significant differences were found for *STAT*5A P_1 for milk traits. Cows of the GG genotype showed higher milk yield (+945 kg) in lactation 1 compared to cows of the genotype AA (Tab. 3). Cows of genotypes AA and AG showed higher protein content compared to those of the GG genotype ($P<0.05$) in both lactations considered.

Table 3. Least squares means (LSM) and standard errors (SE) for milk yield and fat and protein content in different *STAT*5A P_1 genotypes

Lactation	Genotypes		305 d milk yield (kg)	Fat (%)	Protein (%)
1	AA (n=67)	LSM	7265.34[a]	3.55	2.28[b]
		SE	207.38	0.15	0.05
	AG (n=171)	LSM	6876.94[b]	3.66	3.02[a]
		SE	129.81	0.12	0.04
	GG (n=41)	LSM	6320.51[b]	3.72	3.05[a]
		SE	268.39	0.19	0.07
2	AA (n=67)	LSM	7087.49	3.27	2.49[b]
		SE	198.66	0.10	0.05
	AG (n=171)	LSM	6653.44	3.73	3.27[a]
		SE	124.35	0.08	0.04
	GG (n=41)	LSM	6904.10	3.76	3.67[a]
		SE	253.95	0.13	0.07

[ab]Within columns means bearing different superscripts differ significantly at $P<0.05$.

For *STAT*5A P_2 *locus* significant differences were only found in lactation 2 for protein content (Table. 4). Cows of genotype CT showed higher protein content than cows of the CC genotype ($P<0.05$).

Table 4. Least squares means (LSM) and standard errors (SE) for milk yield, and fat and protein content in different *STAT*5A P_2 genotypes

Lactation	Genotypes		305 d milk yield (kg)	Fat (%)	Protein (%)
1	CC (n=210)	LSM	6812.65	3.68	3.01
		SE	116.45	0.09	0.04
	CT (n=68)	LSM	6690.34	3.50	3.01
		SE	204.65	0.16	0.06
2	CC (n=210)	LSM	6836.75	3.19	2.64[b]
		SE	112.79	0.07	0.04
	CT (n=68)	LSM	6454.47	3.14	3.56[a]
		SE	198.20	0.12	0.06

[ab]Within columns means bearing different superscripts differ significantly at $P<0.05$.

McCracken *et al*. [1997] found TG repeats of different length within intron 12 of the bovine *STAT5*A gene. Antoniou *et al*. [1999] found two SSCP variants of the gene fragment that encodes the SH2 domain in bovine *STAT*5A protein. Flisikowski and Zwierzchowski [2003] identified a deletion of trinucleotide CCT at position 12549 in intron 15, and a T→C substitution at position 12743 in exon 16. The latter mutation deletes the cutting site for *Msl*I nuclease. In exon 7 a silent SNP was detected – C/T transition at position 6852 [Flisikowski and Zwierzchowski 2002]. That polymorphism has been used as a genetic marker of meat production traits in beef cattle. It was shown that cattle of the CC genotype have a higher body weight, better growth rate, higher dressing percentage, and better other productive and technological traits at the age of 9 and 15 months than cattle of the genotype CT. Homozygotes TT were found only in two Polish local breeds [Flisikowski *et al*. 2003a]. Flisikowski *et al*. [2003b] showed a T12743C mutation which caused amino acid substitution (V686A) and was located very close to tyrosine 694, which plays a key role in the phosphorylation, activation and dimerization of *STAT*5. Brym [2004] reported a A9501G transition and no relationship between the genotypes and milk production traits in Holsteins, while in the present study relationship between this polymorphism and milk production traits in Chinese Holsteins was found. This discrepancy may be due to several factors: (i) for a long time Chinese Holstein cattle have been intercrossed with other breeds; (ii) both populations of the dairy cattle in question are bred under different conditions, and iii) equations are corrected in different ways and ignore different factors.

A novel SNP, a C/T transition, was detected in exon 16 where many mutations have been reported. The CC and CT genotypes were shown to have a remarkable effect on the milk protein content in lactation 2 in Chinese Holsteins. The animals carrying the TT genotype were not included in the statistical evaluation because of the small number of observations. Before using the *STAT*5A gene as a marker of milk traits in mass selection of Chinese Holsteins further studies should be carried out to confirm the polymorphisms and their relationships on a larger number of animals.

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Związek polimorfizmu genu *STAT5A* z cechami produkcji mlecznej chińskiego bydła holsztyńsko-fryzyjskiego

S t r e s z c z e n i e

Czynniki STAT5 są ważnymi wewnątrzkomórkowymi mediatorami działania hormonów a STAT5A pośredniczy w działaniu prolaktyny na transkrypcję genów białek mleka. Gen *STAT*5A jest rozważany jako potencjalny marker wydajności i składu mleka bydła. Zastosowano techniki PCR-SSCP do analizy polimorfizmu genu w dwóch *loci* genu *STAT*5A u 279 chińskich krów rasy holsztyńsko-fryzyjskiej. Wyliczono frekwencje genotypów i alleli oraz korelacje pomiędzy wariantami genu *STAT5A* i cechami produkcji mlecznej krów. Dla obu *loci* znaleziono trzy genotypy. W *locus* P_1 frekwencja genotypów AA/GG/AG wyniosła odpowiednio 0.240/0.147/0.613 a frekwencja alleli A i G - 0.547 i 0.453. Wykazano, że genotypy A/G istotnie wpływały na wydajność mleczną i zawartość białka w mleku krów w pierwszej i drugiej laktacji. W *locus* P_2 frekwencja genotypów CC/TT/CT wyniosła odpowiednio 0.752/0.004/0.244, a frekwencja alleli C i T - 0.875 i 0.125. Genotypy C/T miały istotny wpływ na zawartość białka w mleku w drugiej laktacji.