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Association of a sequence nucleotide polymorphism in exon 16 of the *STAT5A* gene with milk production traits in Polish Black-and-White (Polish Friesian) cows*

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Signal transducer and activator of transcription 5 (STAT5) is a transcription factor that mediates signals from prolactin and growth hormone, the main regulators of lactation and growth, respectively. Therefore, *STAT5A* gene is a candidate marker for quantitative traits in farm animals. Earlier, a nucleotide sequence polymorphism of the bovine *STAT5A* gene was found – the T→C transition at position 12743 in exon 16 coding for the transactivation domain SH2 of the STAT5A. This mutation has already been shown to modify the STAT5A DNA-binding properties. In the present paper, an association is reported between the *STAT5A* RFLP-*Msl*I polymorphism and milk production traits in Polish Black-and-White (Polish Friesian) cattle.

Cows of the *STAT5A TC* genotype produced daily significantly (P≤0.05) more milk and FCM than *TT* homozygotes, with higher (P≤0.05) content of lactose. Moreover, the daily yield of VCM, milk total solids, solids-non-fat, protein, and lactose was higher (P≤0.01) in cows of *TC* than of *TT* genotype. No relationship was found beetwen the *STAT5A* RFLP-*Msl*I genotype and protein and fat content of milk or the daily milk fat yield.

KEY WORDS: cattle / gene polymorphism / milk production traits / *STAT5A* gene

Signal transducers and activators of transcription (STATs) are key intracellular signal transducers of hormone and cytokine action, from cell surface receptors to nucleus. STAT5 is the major mediator of prolactin signalling, that activates transcription of milk

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protein genes in response to prolactin [Wakao *et al*. 1994]. STAT5 is also a main mediator of growth hormone (GH) action on target genes [Argetsinger and Carter-Su 1996]. STAT5 exists in two isoforms, A and B, differing by few amino acids in the carboxylic end of the protein molecule, and encoded by separate genes. In cattle the *STAT5A* gene has been mapped to chromosome 19q17 within 40 Kpz *STAT locus* which also contains *STAT3* and *STAT5B* genes [Seyfert *et al*. 2000, Moleenar *et al*. 2000].

Due to its mediatory function in prolactin and GH actions, STAT5A and its gene are promising candidate markers for quantitative traits in farm animals. Several nucleotide sequence polymorphisms have been detected in the bovine *STAT5A* gene [McCracken *et al*. 1997, Antoniou *et al*. 1999]. In our earlier studies [Flisikowski and Zwierzchowski 2002, 2003, Flisikowski *et al.* 2003a, Flisikowski *et al*. 2004] several new nucleotide sequence polymorphisms were reported within the bovine *STAT5A* gene. One of them, the C→T transition at position 6853 in exon 7, showed a significant relation to the beef production traits. Another detected nucleotide sequence polymorphism, the T→C transition at position 12743 [Flisikowski *et al.* 2003b], was found to be located in exon 16 coding for the transactivation domain SH2 of the bovine STAT5A factor. The latter mutation was shown to modify DNA-binding properties of the STAT5A In the DNA-protein binding assays (electrophoretic mobility shift assay – EMSA) nuclear proteins extracted from livers of *CC* genotype animals always showed less DNA protein complexes than those of animals *TT* [Flisikowski *et al*. 2003b].

In the present paper, we report on the association between the T→C polymorphism at position 12743 in exon 16 of the *STAT5A* gene and milk production traits in Polish Black-and-White (Polish Friesian) cattle.

Material and methods

Animals

One hundred and fifty dairy cows were genotyped in an experimental dairy herd of the Institute of Genetics and Animal Breeding, Jastrzębiec. The animals belonged to Polish Black-and-White (Polish Friesian) breed with more than 80% of Holstein-Friesian blood and were daughters of 48 sires.

All cows were kept in a loose barn and fed *ad libitum* the Total Mixed Ration based on corn silage, wilted grass silage, and concentrates, supplemented with minerals and vitamins. Cows were milked twice a day. Milk yield from both milkings was recorded individually and milk samples were collected once a month. Mean lactation yield of the herd rose from 7142 kg milk with 4.34% fat and 3.51% protein in year 2001 to 8244, 3.75% and 3.37%, respectively, in year 2003. Productivity data were recorded for each cow over three consecutive lactations.

In order to make statistical evaluations easier a record was included into analyses only when both following conditions were fulfilled: number of cows of a given genotype was higher than 20 and a cow had at least one paternal half-sister within the data. As

a consequence, in the final dataset used for analyses at least 20 cows and not less that two bulls represented each genotype. The dataset consisted of 2411 test-day records for 102 cows (72 of *TT* and 30 of *TC* genotype), daughters of 22 sires.

All procedures carried out with the use of animals were approved by the Local Ethics Commission, permission No 67/2001.

DNA isolation from whole blood

Blood samples were withdrawn from the jugular vein on K_2-EDTA by an authorized veterinarian and stored at -25°C for a few weeks or at -75°C up to several months. The isolation of DNA from whole blood was done with a rapid method by Kanai *et al.* [1994].

Determination of STAT5A polymorphism

The T→C polymorphism at position 12743 in exon 16 of the bovine *STAT5A* gene was determined as previously described [Flisikowski *et al*. 2003b]. The following PCR primers were used:

STAT1 – 5'-AGCCCTACAGCTCCAATCCT-3' and

STAT2 – 5'-GGGTGTACC-CGCTGCTTAG-3

to amplify a 281-bp PCR fragment, encompassing parts of intron 15 and exon 16 of the *STAT5A* gene. The polymerase chain reactions (PCR) were performed using a PCR-mix with: primers STAT1 and STAT2 each at a final concentration of 2 pmol/µl, 1 U Taq polymerase (SIGMA), 1 µl Taq polymerase buffer, dNTPs of 2.0 mM/µl, ca. 100 ng of genomic DNA, and H_2O up to 10 µl. The following PCR protocol was used: 1 min at 94°C, 1 min at 61°C, and 1 min at 72°C – 34 cycles. The yield and specificity of the PCR reactions were both evaluated by electrophoresis of the products in 2% agarose gel (GIBCO BRL) with ethidium bromide.

The PCR products were digested in 10-µl aliquots with 10 U of *Msl*I restriction nuclease (BioLabs, New England, USA) for 3 hours at 37°C. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gels (GIBCO, BRL) in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). The gels were examined under UV light and documented in a FX Phosphoimager apparatus (Bio-Rad).

Analysis of milk composition

The fat, protein and lactose contents (%) were determined in fresh milk using MilkoScan 104A/B. Content (%) of total solids in each sample was expressed as a sum of per cent of fat, total protein, lactose and minerals, while the solids-non-fat were calculated as total solids minus fat. The concentration of minerals was calculated according to the Sherman equation, as follows: P = 0.1 × percent of total protein + 0.38. The value corrected milk (VCM) indicator was calculated as follows:

0.05 × daily milk yield (kg) + 8.66 × fat (kg) + 25.98 × protein (kg) – Arbel *et al*. [2001]

Statistical

The data were analysed with univariate linear repeatability mixed models. Preliminary analyses showed the significant effect of the test-day, lactation parity and lactation stage. As an interaction between lactation parity and lactation stage was proved significant, in final analyses lactation curves for considered traits were fitted within the parity.

The model used for all traits and all considered polymorphisms was:

$$y_{ijklmn} = Gen_i + Parity_j + (\sum_{r=1}^{5} b_r LP_r) + tday_k + sire_l + cow_m + (cow \times parity)_{mj} + e_{ijklmn}$$

Where: y – the individual measure of considered trait in given test-day. The fixed effects in the model were: *Gen* – the considered polymorphism (with either 2 or 3 levels) and at 3 *Parity* subclasses.

LPs are Legendre polynomials of standardized days-in-milk (lactation days), which were fitted as fixed covariates within each *Parity* subclass, in order to represent changes of considered traits due to stage of lactation. Fixed regressions were fitted up to the 5th power of LPs (r=1, …5).

Legendre polynomials are commonly used for test-day models and were shown to be more satisfactory than e.g. logarithmic polynomials [Kettunen *et al*. 2000].

The effect of date of test-day (*tday*), with 50 levels, was considered as random. Animal effects were *cow*, cow-by-parity (*cow* × *parity*) as specific effect of n-th cow in her j-th lactation, and *sire* of a cow. Size of the dataset was too small to consider genetic variance in analyses, and thus genetic relationships between animals were ignored. All random effects were assumed to be uncorrelated and follow a normal distribution.

Procedure MIXED in SAS (SAS Institute Inc., 1999) was used for computations.

Results and discussion

The RFLP-*Msl*I genotypes of the STAT5A gene (the T→C transition in exon 16) were estimated for 150 animals of dairy Black-and-White (Friesian) cattle. The estimated frequencies of *TT*, *TC*, and *CC* genotypes were 0.72, 0.26, and 0.02, respectively. The *T* and *C* alleles were represented at the frequency of 0.85 and 0.15, respectively (figures not tabulated).

The effects of *STAT5A* genotypes on milk yield and composition and on the yield of milk constituents are shown in Table 1 and 2, respectively. Due to the very low frequency of the *CC* genotype (0.02), only *TT* and *TC* genotypes were considered in the calculations.

Both milk and FCM yield as well as lactose content of milk were higher (P≤0.05) in cows of the *TC* than of *TT* genotype (Tab. 1). Cows of the *TC* genotype yielded daily more VCM (P≤0.05), solids-non-fat, total solids, protein and lactose (P≤0.01)

Table I. Least square means (LSM) and their standard errors (SE) for daily milk yield, FCM yield, milk energy and major milk constituents contents as affected by STAT5A genotype

STAT5A genotype	n[a]	Milk (kg)		Fat-corrected milk (kg)		Gross energy (MJ)		Total solids (%)		Solids-non-fat (%)		Fat (%)		Protein (%)		Lactose (%)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
TT	1675	11.7[a]	0.50	12.9[a]	0.65	71.5	0.04	11.5	0.12	9.06	0.05	4.45	0.08	3.51	0.01	4.78[a]	0.01
TC	716	12.1[b]	0.64	13.1[b]	0.5	71.2	0.01	11.5	0.13	9.11	0.07	4.39	0.10	3.51	0.01	4.82[b]	0.04

[a]Number of milk samples analysed
[a,b]within columns means bearing different superscripts differ significantly at P<0.05

Table II. Least square means (LSM) and their standard errors (SE) for daily yield of major milk constituents as affected by STAT5A genotype

STAT5A genotype	Number of animals	FCM (kg)		Solids-non-fat (g)		Total solids (g)		Fat (g)		Protein (g)		Lactose (g)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
TT	1675	10.1[a]	0.55	1019[a]	4.7	1041[a]	5.1	999	5.5	788[a]	1.8	1068[a]	3.6
TC	716	11.1[b]	0.75	1191[b]	1.8	1316[b]	4.1	1040	4.1	841[b]	2.1	1176[b]	7.1

[a,b]within columns means bearing different superscripts differ significantly at: small letters – P<0.05, capitals – P<0.01

than the *TT* homozygotes (Tab. 2). These results suggest either beneficial influence of the *C* allele, or unfavourable effect of the allele *T* on majority of milk production traits considered in the present report. No association was found beetwen the RFLP-*Msl*I polymorphism and protein or fat content of milk (Tab. 1) and milk fat yield (Tab. 2).

Hormones, growth factors, and other regulatory proteins associated with so called "somatotropic axis" are candidate markers for quantitative traits in farm animals [Parmentier *et al*. 1999]. Genes encoding growth hormone (GH), GH receptor (GHR), transcription factor Pit-1 (activating expression of GH and prolactin genes in the anterior pituitary), insulin-like growth factor-I (IGF-I), and perhaps presently still unknown genes coding for GH signal transduction pathways, could contribute to the progress in genetic selection of farm animals.

The STAT5 transcription factors are important participants of the somatotropic axis. They mediate actions of the pituitary hormones – GH hormone and prolactin – within the target cells. Therefore, genes encoding for *STAT5A* and *STAT5B* are candidate markers for quantitative traits in the cattle. Only in few cases nucleotide sequence polymorphism has been detected in the bovine *STAT5A* gene, but no studies were devoted to date on the effect of the *STAT5A* gene polymorphism on production traits in cattle or of any other farm animal species. In the earlier study [Flisikowski and Zwierzchowski 2002] we reported on 6 different SSCP patterns in a fragment of the *STAT5A* gene encompassing parts of intron 6 and exon 7. Then, we precisely identified the polymorphism as the C→T transition at position 6853 within the exon 7 that codes for the DNA-binding domain of STAT5A protein. Moreover, we showed for the first time, an association between *STAT5A* gene polymorphism and meat production traits in cattle [Flisikowski *et al*. 2003a, Oprządek *et al*. 2003]. Our results demonstrated the value of the *STAT5A locus* as a marker for growth performance, feed intake and conversion, and carcass traits in the beef cattle.

Later on, using SSCP, RFLP, and sequencing methods we found two other polymorphisms in the coding region of the *STAT5A* gene – a CCT deletion/insertion in intron 15, and a T→C transition in exon 16 [Flisikowski *et al*. 2003b]. The latter mutation changes an amino acid sequence in the STAT5A protein, resulting in the Val→Ala substitution at position 686 within the transactivation SH2 domain. Moreover, this mutation was shown to change the DNA-binding capacity of the STA5A transcription factor [Flisikowski *et al*. 2003b]. We performed electrophoretic mobility shift assay (EMSA) using proteins extracted from the cell nuclei of liver tissues derived from bulls of different *STAT5A* genotypes. Significant ($P<0.05$) differences in nuclear protein binding to DNA were observed between genotype *TT* and *CC* – nuclear proteins derived from *CC* animals always showed less DNA protein complexes than those from *TT* animals. The EMSA competition experiments confirmed that STAT5 transcription factors take part in the formation of the DNA-protein complexes. Therefore, we considered the T→C transition in 12743 in exon 16 as a "functional polymorphism" since it could modify the transactivation properties of the STAT5A transcription factor.

Candidates for genetic markers are usually identified based on mathematically

calculated correlations between polymorphic variants of a gene and a production trait. However, such correlation says nothing about a causative relation between genotype and a trait. Based on our previous results and on the results presented here we suggest the C→T polymorphism in exon 16 of the *STAT5A* gene be considered as a causative mutation for certain dairy traits in cattle. It changes the amino acid sequence of the coded protein within the functional SH2 domain and modifies the DNA-binding capacity of the STAT5A transcription factor. Moreover, as shown in the present paper, in Polish Black-and-White (Polish Friesian) cows the T→C transition in exon 16 of the *STAT5A* gene significantly influenced the daily milk yield, VCM, and daily yield of milk total solids, solids-non-fat, protein, and lactose. However, it should be stressed that in this study only a small cattle population was used without family structure. Therefore, the present result can be interpreted only as an association between the marker and production trait at **this** time and in **this** population. In order to confirm these results, further investigations including bigger cattle populations of different breeds are necessary.

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Wpływ polimorfizmu sekwencji nukleotydowej w 16 eksonie genu *STAT5A* na cechy produkcji mlecznej krów rasy czarno-białej

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

Czynniki transkrypcyjne STAT5 pośredniczą we wpływie prolaktyny na ekspresję genów białek mleka i w aktywowaniu docelowych genów przez prolaktynę i hormon wzrostu, które są głównymi regulatorami laktacji i tempa wzrostu zwierząt. Z tej racji gen *STAT5A* jest kandydatem na marker cech ilościowych u zwierząt gospodarskich. We wcześniejszych badaniach wykryto polimorfizm sekwencji nukleotydów – tranzycję T→C w pozycji 12743 w 16 eksonie, kodującym domenę transaktywacyjną SH2 bydlęcego czynnika transkrypcyjnego STAT5A. Mutacja ta modyfikuje zdolność wiązania STAT5A z DNA.

Przedstawiono wpływ mutacji T→C w 16 eksonie genu *STAT5A* na cechy mleczności polskiego bydła czarno-białego z ponad 80% dolewem krwi hf. Krowy o genotypie *TC STAT5A* charakteryzowały się istotnie większą (P≤0.05) dzienną wydajnością mleka, wydajnością FCM oraz zawartością w mleku laktozy (%) w porównaniu ze zwierzętami o genotypie *TT*. Ponadto, dzienna wydajność suchej masy, suchej masy beztłuszczowej, białka i laktozy była większa (P≤0.01) w mleku krów o genotypie *TC* niż o genotypie *TT*. Nie stwierdzono zależności między genotypem *STAT5A* a zawartością białka i tłuszczu w mleku, jak również dzienną wydajnością tłuszczu.