

Inconsistency of associations between growth hormone receptor gene polymorphism and milk performance traits in Polish Holstein-Friesian cows and bulls*

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The aim of the study was to evaluate the significance of associations between missense mutation S555G in bovine *GHR* gene and two sets of data: milk performance data of cows and breeding values of bulls. To generate genotypes the polymorphic region of *GHR* exon 10 (S555G) was amplified and genotyped using PCR-RFLP method. 395 Polish Holstein-Friesian and 477 Polish Holstein-Friesian bulls were screened giving the following frequencies of alleles: A – 0.832 and 0.891 and G – 0.168 and 0.109 for cows and bulls, respectively. With the use of the Linear Mixed Model analysis it was shown that A allele has positive effect on milk performance traits in cows and breeding value of bulls. The A allele is significantly related to fat yield (by 18.554±5.24 kg; P<0.0005), protein yield (by 9.072±3.643 kg; P<0.01) and fat content (by 0.1±0.05%; P<0.05). The A allele significantly increases bulls' breeding value for protein content (by 0.044%±0.011, P<0.0002). The results show inconsistency of associations between cow and bull data signalling that careful consideration has to be undertaken before final approval of SNP as effective marker used in dairy cattle selection.

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QTL mapping experiments showed that BTA 20 harbours at least one QTL with major effect on milk yield and composition at about 50 cM [Mosig *et al.* 2001, Viitala *et al.* 2003], confirmed by meta-analysis [Khatkar *et al.* 2003]. Ge *et al.* [2000] found 4 SNPs in exon 10 of Angus breed. Two of them are missense mutations: A541S (G200A) and S555G (A257G) – Viitala *et al.* [2006] – and were found in Holstein cattle. Blott *et al.* [2003] characterized 10 SNPs across the GHR gene. Two of them were missense mutations (F279Y and N528T in exons 8 and 10, respectively), the remaining eight being silent mutations or mutations located in introns, or non-coding part of the gene. All of known polymorphisms in the coding part of the gene in question is shown on Figure 1.

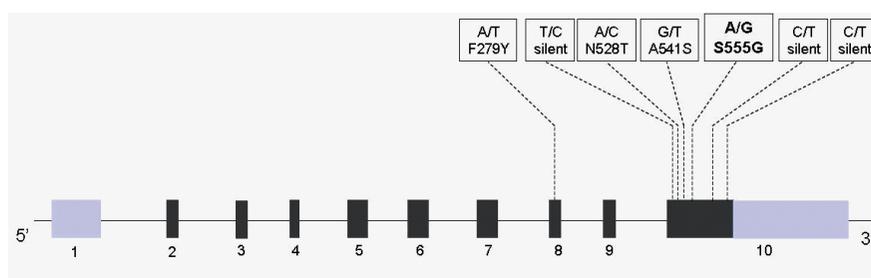


Fig. 1. Schematic representation of polymorphisms within growth hormone receptor gene. Dark grey colour marks coding part, and light grey – 5' and 3' untranslated region. SNP genotyped is bolded.

Linkage disequilibrium studies showed that F279Y polymorphism is a casual mutation for BTA20 QTL effect on milk performance traits. QTL harboured on BTA 20 has got significant influence on the genetic variation of milk performance traits, especially protein content (13,5 %). For other dairy traits this influence varied from 4 % (protein yield) to 7,8 % (fat content) – Druet *et al.* [2006]. QTL effects are mostly estimated in cows. For most of such studies there is no data showing whether this effect will have similar importance in bull's breeding value. This gap encouraged the authors to select one of GHR missense mutations and check its effect on both types of data. Instead of GHR F297Y polymorphism which showed very unbalanced allele frequency (data not shown), another GHR SNP – S555G was used to find associations with milk performance traits of cows and breeding value of bulls in Polish Holstein-Friesian cattle.

Material and methods

An analysis included 395 Polish Holstein-Friesian cows, the daughters of 5 unrelated sires. The cows were in the first lactation and in the same season (year 2003). The analysis involved also 477 Polish Holstein-Friesian active bulls born between 2000 and 2002.

Genomic DNA was isolated from blood with MasterPure DNA Purification Kit (EPICENTRE) and from the half of semen commercial straw with the use of Wizard Plus Megapreps DNA Purification System (PROMEGA).

Based on the genomic sequence available in GenBank (AF140284), the following PCR primers were designed by Primer 3 programme (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi):

GHR2 SNP88 forward: 5' ACCCTGCCAAGCTAACTTCA 3';

GHR SNP88 reverse: 5' TGGCATGATTTTGTTCAGTTGGTCTG 3'.

Primers were tested for genomic uniqueness with BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>).

PCR was carried out in 25 µl containing: 1.5 µl 20xPCR Buffer (400 mM (NH₄)₂SO₄, 1.0 Tris-HCl, pH 9.0, (EPICENTRE), 2.0 µl dNTP (2.5 mM each), 5.0 µl 10xEnhancer (EPICENTRE), 20 pM of each primer, 0.8 U MasterAmp™ Tfl Polymerase (EPICENTRE), average 100 ng of genomic DNA and water to 25 µl. The PCR program consisted of an initial denaturation (94°C/3 min), 35 cycles of denaturation (94°C/30 s), annealing (60°C/30 s), elongation (72°C/30 s) and final synthesis (72°C/5 min). The specificity and yield of the PCR product were evaluated by electrophoresis in 1,5% agarose gel (PRONA) stained with ethidium bromide.

To determine genotypes, RFLP method was used. Eight µl of the PCR product were mixed with 1,3 µl of 10x Y⁺/Tango Buffer and 10 U of AluI enzyme (FERMANTAS) and incubated for 3 h at 37°C. The genotypes were observed after electrophoresis in 2,5% agarose gel (PRONA). The results were observed, analysed and documented with the use of Fluor-S Multimager (BIO-RAD).

Genetic equilibrium was estimated based upon the Hardy-Weinberg principle and the chi-square test.

The effect of GHR alleles on milk production traits of cows representing 5 half sib families and on the breeding values of bulls were analysed with the following Linear Mixed Model (SAS 9.1, SAS Inc.):

$$Y = Z\alpha + X\beta + e$$

where:

Y – vector of estimated bull's breeding values for one of the milk performance traits or cows milk performance traits (milk, fat and protein yield, fat and protein content);

$\alpha \sim N(0, A\sigma_a^2)$ – vector of random additive polygenic effects of a bulls or a cows with A being an additive polygenic relationship matrix and σ_a^2 being an additive variance;

β – fixed additive effect of the A/G GHR polymorphism;

$e \sim N(0, D\sigma_e^2)$ – residual effect with D being a diagonal matrix with elements on diagonal representing the reciprocal of the accuracy of the estimated breeding value for each bull or cow;

σ_e^2 – residual variance;

Z and $X \in \{-1, 0, 1\}$ – design matrices for α and β , respectively.

Parameters of this model were estimated by solving Mixed Model Equations [Henderson 1984] with variances assumed as known and amounting to $\sigma_a^2 = 0.3\sigma_y^2$ and $\sigma_e^2 = 0.7\sigma_y^2$ (where σ_y^2 represents a phenotypic variance). To evaluate significance of differences between groups, test t was used. Bulls' breeding values and their reliability were obtained from publicly available database and corresponded to the national genetic evaluation release from January 2008.

Results and discussion

PCR yielded the specific product of expected 349 bp. Digestion with Alu I enzyme revealed three genotypes: AA (188, 100, 50, 11 bp), AG (188, 150, 100, 50, 11 bp) and GG (188, 150, 11 bp) – Photo 1. 395 Polish Holstein-Friesian cows and 477 Polish Holstein-Friesian bulls were screened by RFLP method. The following frequencies of genotypes and alleles were obtained. For cows: AA – 0.699, AG – 0.266, GG – 0.035 and alleles: 0.832 and 0.168 for A and G, respectively. For bulls: AA – 0.791, AG – 0.203, GG – 0.006, and alleles: 0.891 and 0.109 for A and G, respectively. Analyzed populations were in the Hardy-Weinberg genetic equilibrium ($P < 0.01$).

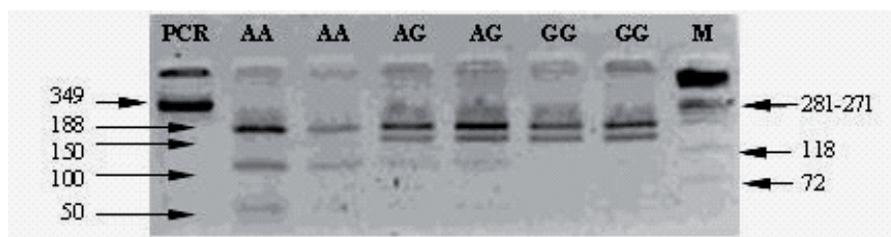


Photo 1. Genotyping of SNP S555G. Genotypes: AA (188, 100, 50, 11 bp), AG (188, 150, 100, 50, 11 bp) and GG (188, 150, 11 bp). PCR marks non-digested product, M – molecular marker Φ X 174/*Hae*III. 11 bp DNA band – not visible because of DNA diffusion.

Table 1 shows the effect of the GHR alleles on cows' performance traits and Table 2 on bulls' breeding values for these traits. The A allele (coding the G protein variant) significantly increased cow's fat yield (by 18.554 ± 5.24 kg; $P < 0.0005$), protein yield (by 9.072 ± 3.643 kg; $P < 0.01$) and fat content (by $0.1 \pm 0.05\%$; $P < 0.05$), protein yield (by 9.072 ± 3.643 kg; $P < 0.01$) and fat content (by 0.1 ± 0.05 pp; $P < 0.05$). The A allele significantly increased bulls' breeding value for protein content (by $0.44 \pm 0.044\%$; $P < 0.0002$). The obtained results partially confirm the report by Viitala *et al.* [2006] who showed that SNP S555G polymorphism influences milk yield, protein yield and protein content of milk in first lactation. The present results indicate the distinct inconsistency: relations revealed on cows' data were not confirmed by bull breeding value. The most significant effect of allele A on cow's fat yield (Tab. 1) disappeared when computed with bull's breeding value (Tab. 2). And opposite, the only significant

Table 1. Estimated effect of A/G alleles of growth hormone receptor gene on milk performance traits of Polish Holstein-Friesian cows

Trait	Estimated effect	SE	P value	Allele
Milk yield (kg)	178.67	111.70	0.1106	G
Fat yield (kg)	18.554	5.24	0.0005	A
Protein yield (kg)	9.072	3.643	0.0132	A
Fat content (%)	0.1	0.05	0.0453	A
Protein content (%)	0.033	0.022	0.1360	A

Table 2. Estimated effect of A/G alleles of growth hormone receptor gene on Polish Holstein-Friesian bulls' breeding value

Trait	Estimated effect	SE	P value	Allele
Milk yield (kg)	45.657	32.891	0.1360	G
Fat yield (kg)	1.163	1.393	0.4045	A
Protein yield (kg)	1.628	0.89	0.0679	A
Fat content (%)	0.042	0.022	0.0610	A
Protein content (%)	0.044	0.012	0.0002	A

effect of the same allele on bull's breeding value for protein content (Tab. 2) turned to be not valid for milk performance data in cows (Tab. 1). Inconsistencies may come from different datasets (direct milk performance records *vs.* highly overcalculated breeding values generated by animal model). The results exemplify general problem in assessing usefulness of single SNP in marker-assisted selection – their slight and unstable effect on polygenic trait.

Concluding, our results indicate that candidate SNPs in dairy cattle should be verified on the base of both data sets: milk performance traits and their breeding values. Inconstancies of effects may suggest low response in MAS.

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