Polymorphisms in *CGIL4*, breeding value for somatic cell count and resistance to mastitis

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ABSTRACT: The relationship between a potential marker for mastitis, *CGIL4*, and the breeding value for somatic cell count (SCC) was analysed in a panel of Czech Simmental and German Holstein sires. Genotyping was done by PCR/RFLP. The analysis did not reveal a significant difference in breeding values for SCC depending on the genotype of *CGIL4*, even though other authors confirmed the relationship between this marker and clinical mastitis. Further investigations will be necessary to clarify the relevance of the marker for selection against mastitis.

Keywords: mastitis; *CGIL4*; somatic cells; Czech Simmental cattle; German Holstein cattle

The increase in milk performance is coupled with many problems. Many authors adverted to the relationships among milk yield, milk component content, udder health, and fertility (Řehák et al., 2009; Hanuš et al., 2010; Yazgan et al., 2010). In this context, the most important problems are as follows: an increase in mastitis genetic susceptibility, incidence of mastitis and higher somatic cell counts in milk. Mastitis is one of the most frequent and economically most expensive diseases of dairy cattle (Kossaibati et al., 1998). Mastitis causes heavy financial losses, both directly (e.g. depression in milk yield, earlier culling of dairy cows from herd, or higher antibiotic treatment costs) and indirectly (e.g. by a decrease in milk quality or causing residual devalued milk).

Mastitis is caused by many factors. Klastrup et al. (1987) estimated that 25% of the susceptibility to mastitis was a result of environmental factors, 20% was a result of genetic factors and 50% was a result of herd management. From genetic aspects, susceptibility to mastitis is considered to be a complex feature that is affected by a number of genes (Sharma et al., 2006).

Due to the economic importance of mastitis, resistance to mastitis is one of the primary goals of breeding. Breeding mastitis-resistant cattle is complicated by the low heritability of the trait (Heringstad et al., 2000; Hansen et al., 2002; Carlén et al., 2004). Norberg et al. (2009) found out the heritability of somatic cell score of 0.14–0.15, and of clinical mastitis of 0.03–0.05. The breeding values for somatic cell count (SCC) in milk and somatic cell score (SCS) are used as indicators of susceptibility to mastitis (De Jong and Lansbergen, 1996). The use of these indicators is based on studies that have demonstrated a genetic correlation between clinical mastitis (CM) and SCC. The correlation of these two values was estimated to be from 0.37 up to 0.90 (Pösö and Mäntysaari, 1996; Heringstad et al., 2000). In Czech Holstein cows, Wolf et al. (2010) found out the genetic correlation of 0.80.

Sharma et al. (2006) tried to identify a genetic marker for resistance to mastitis. They screened 200 cows: 100 of them were clinically highly resistant and 100 of them were highly susceptible to mastitis. AFLP analysis revealed 27 significant

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AFLP markers. The allele frequencies of one of these markers, *CGIL4*, were significantly different between resistant and susceptible individuals. Sequencing revealed a single nucleotide polymorphism, an A to G mutation. Their analysis demonstrated a higher frequency of allele $CGIL4^A$ in the clinically resistant group.

Of course, current genome-wide analyses are a more effective tool, but at the end of these analyses, the respective loci must be described. Other authors searched for a relation between genetic markers and milk performance (Matějíček et al., 2008; Kowalewska-Łuczak et al., 2010; Manga and Dvořák, 2010). The aim of our study was to evaluate the relationship between a potential marker for resistance to mastitis, *CGIL4*, and breeding values for SCC in German Holstein and Czech Simmental sires.

MATERIAL AND METHODS

A panel of 110 Czech Simmental sires born in 1999–2003 and 104 German Holstein sires born in 1998–2003 was used in this analysis. DNA was isolated from whole blood or semen using commercial kits (NucleoSpin Blood and NucleoSpin Tissue from Macherey-Nagel) according to the manufacturer's protocols. The quality of isolated DNA was verified by electrophoresis on 1.5% agarose gels and by spectrophotometry using a Helios Zeta instrument (Thermo Scientific). To detect polymorphisms, the PCR/RFLP method described in Sharma et al. (2006) was used. The sequences of the primers were as follows: E155F (forward) 5'-TGA CGC AGA ATC CAA AGT TAA AAC A-3' and E155R (reverse) 5'-GAG GAG GTG GCC GGT TCA GA-3'. The reaction mixture was made up of 2.0 µl 10× Taq buffer, 2.0 µl $MgCl_2$, 2.5 µl dNTPs, 1.0 µl of each primer, 1.5 µl Taq polymerase (1 IU), 2.0 µl DNA and 13.0 µl H_2O. The PCR cycle consisted of 95°C for 2 min, 35 cycles of 95°C for 45 s, 60°C for 1 min and 72°C for 1 min, and 72°C for 5 min. The presence of a 399-bp PCR fragment was detected by electrophoresis on a 3.5% agarose gel. The PCR fragment was subsequently restricted using restriction endonuclease *Taq*I. The presence of the $CGIL4^A$ allele was detected as two fragments, one of 125 bp and the other of 274 bp in length. For the $CGIL4^G$ allele, three fragments (39 bp, 125 bp and 235 bp) were found.

Breeding values for the somatic cell count were obtained from the websites of breeding associations. The differences in the breeding values for SCC among genotypes were tested by the analysis of variance (ANOVA) using STATISTICA software. The Hardy-Weinberg equilibrium was tested by the χ^2 test, and the differences in the frequencies between breeds were tested by the test of difference in relative frequencies.

RESULTS AND DISCUSSION

In all, 104 German Holstein sires and 110 Czech Simmental sires were genotyped. The predominance of individuals of the $CGIL4^A/CGIL4^G$ genotype was found in German Holstein sires and in Czech Simmental sires. The frequencies of the

Table 1. Genotype and allele frequencies

Breed		Genotype frequencies			Allele frequencies	
		$CGIL4/^ACGIL4^A$	$CGIL4^A/CGIL4^G$	$CGIL4^G/CGIL4^G$	$CGIL4^A$	$CGIL4^G$
Czech Simmental	absolute	10	87	13	107	113
	relative %	9.1	79.1	11.8	48.6	51.4
	theoretical %	23.6	50.0	26.4		
	χ^2		33.919***			
Holstein	absolute	17	76	11	110	98
	relative %	16.3	73.1	10.6	52.9	47.1
	theoretical %	28.0	49.8	22.2		
	χ^2		21.851***			

Differences in the genotype and allele frequencies between breeds were not significant

***$P < 0.001$

Table 2. Breeding values for somatic cell count (SCC)

Breed		Genotype			Alleles	
		$CGIL4/^{A}CGIL4^{A}$	$CGIL4^{A}/CGIL4^{G}$	$CGIL4^{G}/CGIL4^{G}$	$CGIL4^{A}$	$CGIL4^{G}$
Czech Simmental	$\bar{x}$	100.90	102.61	98	102.29	101.55
	$s_{\bar{x}}$	9.34	11.26	14.36	60.69	65.45
Holstein	$\bar{x}$	98.44	99.89	101.97	99.39	100.88
	$s_{\bar{x}}$	9.28	11.76	11.19	79.37	94.54

Differences among genotypes and alleles were not significant

$CGIL4^{A}$ and $CGIL4^{G}$ alleles were almost equal, as shown in Table 1. The obtained data did not agree with findings of Sharma et al. (2006), who reported a higher frequency of the $CGIL4^{A}$ allele in the resistant group. As the Czech Simmental cattle were bred for dual-purpose performance and were believed to be more resistant to mastitis, a difference in the frequencies was expected. However, the frequency of the $CGIL4^{A}$ allele in this group was slightly lower than the frequency of the $CGIL4^{G}$ allele. The allele frequencies in Czech Simmental and German Holstein cattle were not significantly different. The observed and theoretical genotype frequencies were not in Hardy-Weinberg equilibrium in either breed, and the empirical frequencies were deflected in favour of heterozygotes.

The breeding values for SCC in milk for the genotypes and alleles of *CGIL4* are summarized in Table 2. None of the differences was significant, thus the locus did not influence the breeding value for somatic cells in this analysis. Apparently, further investigations will be necessary to elucidate the relevance of the marker. As for other genetic markers, Skelding et al. (2010) did not find out any association between SNPs in interleukin-12 and interleukin-23 receptor and breeding values for somatic cell score, similarly like Sender et al. (2010) for SCC and polymorphisms of lactoferrin gene. However, according to Baes et al. (2010) the QTL in BTA27 was estimated to be responsible for 18% of the genetic variation in somatic cell score, and Tal-Stein et al. (2010) found out that 22 QTL explained most of the observed variation in estimated breeding value for somatic cell score. Therefore, the search for relevant genetic markers was meaningful. Oltenacu and Broom (2010) confirmed the profitability of including resistance to mastitis in the breeding objective while proper selection would result in genetic improvement of cell counts (Montaldo et al., 2010).

CONCLUSIONS

The influence of the *CGIL4* marker on breeding value for somatic cell count was not confirmed. Further investigations will be necessary to clarify the relevance of the marker for selection against mastitis.

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