Archiv Tierzucht 52 (2009) 6, 647-649, ISSN 0003-9438

Amplification created restriction sites for genotyping SNPs in the bovine ABCG2 and its association with milk production traits (Brief Report)

Amplification erstellt Restriktionsschnittstellen zur Genotypisierung SNPs in der Rinder-ABCG2 und ihre Verbindung mit Milchleistungsmerkmalen (Brief Report)

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Background

ABCG2 (ATP-binding cassette, subfamily G, member 2) belongs to the superfamily of ATP-binding cassette (ABC) transporters. In ATP-dependent processes, ABCG2 is responsible for transporting xenobiotics and cytostatic drugs across various cellular membranes. The *ABCG2* gene is expressed in the apical membrane of alveolar mammary epithelial cells and is responsible for the active secretion of substrates into mouse milk. Other members of the ABC subfamily G are sterol transporters. It therefore appears that ABCG2 might transport cholesterol into milk (COHEN-ZINDER *et al.* 2005). In the study by COHEN-ZINDER *et al.* (2005), several SNPs were detected in the *ABCG2* gene but only two were genotyped – in exon 14 and in intron 3. In the case of SNP *A/C* in exon 14 resulting in an amino acid change Y581S, it was demonstrated that this substitution affects milk yield and composition. To detect theses polymorphisms in our study we used a new PCR-RFLP method based on an amplification created restriction site (ACRS). This method has been frequently used by various researchers in recent times (e.g. ZYCH *et al.* 2007). The aim of this study was to estimate the frequencies of genotypes and alleles and to investigate possible associations between *ABCG2* polymorphisms and milk production traits in Jersey cows.

Procedures

Genomic DNA samples were obtained from Jersey cows. The analysis covered 181 cows kept on a farm located in the Wielkopolska region in Poland. All the studied animals were born between 1990 and 1998 and came from 19 sires. The animals were kept in identical environmental conditions. They were fed standard feed rations and seasonally (in spring and summer) put out to pasture. The cows were milked twice a day with the use of a pipeline machine. The herd's milk yield was evaluated with the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR). To determine genotypes at the ABCG2 locus, the sequence reported by COHEN-ZINDER *et al.* (2005) (acc. no. AJ871176) was used for primer design. The naturally occurring nucleotides were substituted by mismatched nucleotides (underlined) in order to introduce a recognition site for the restriction enzyme (Table 1). The PCR was performed in a total volume of 25 µl containing 50 ng template DNA, 200 µM of each dNTP, 10 pmol

each primer, 1.5 mM MgCl2, PCR buffer and 1.0 U Taq (MBI Fermentas) DNA polymerase. The PCR products were digested by using a suitable restriction endonuclease (Table 1). The restriction-digested fragments were separated on 2% agarose gels.

Table 1
PCR-RFLP conditions
PCR-RFLP Bedingungen

	Exon 14	Intron 3
primers	5′-CAG TAT TCA CGA GAC TTC AGG GA-3′; 5′-CAC GGT GAC AGA TAA GGA GAA CAT ACC TCA-3′	5′-AAG AAG GAA GGG GAT AAA ATT GAA AGT-3′ 5′-CTT TTT AAA AAT TAT ATG CTA TAA TCA-3′
cycling protocol	5 min at 94 °C; 30 cycles of: 94 °C/30 s, 56 °C/45 s, 72 °C/30 s; final extension 5 min at 72 °C	5 min at 94 °C; 30 cycles of: 94 °C/30 s, 50 °C/30 s, 72 °C/30 s; final extension 5 min at 72 °C
Restriction endo-nuclease	*Hpy*188I (MBI Fermentas)	*Nla*III (MBI Fermentas)

Results

After the PCR product was restriction-digested with *Hpy*188I enzyme, only two genotypes were detected: *AA* (200 bp) and *AC* (200, 172, 28 bp). The frequencies of the genotypes and alleles were as follows: *AA* – 0.61, *AC* – 0.39 and *A* – 0.80, *C* – 0.20, respectively. This result is similar to the frequencies obtained by other researchers - allele *A* was more frequent in all studied populations (RON *et al.* 2006, OLSEN *et al.* 2007). On the other hand, amplicon digestion with restrictase NlaIII revealed only one genotype. However in Holstein-Friesian cattle all three genotypes (*AA*, *AT*, *TT*) and presence of alleles *A* and *T* were detected.

The analysis of associations between the *ABCG2/Hpy*188I genotypes and milk production traits was performed according to the GLM procedure. In the statistical model were taken following factors: genotype, number of lactation, season of lactation (spring, summer, autumn, winter) and consecutive month of lactation. A statistical analysis of associations between the different *ABCG2/Hpy*188I genotypes and milk production traits - daily milk yield (kg), fat and protein content (%), showed statistically significant differences ($P \leq 0.01$) only in the case of fat content in milk – cows with the *AC* genotype had a higher fat content (+0.20%). As regards other milk production traits, their values were also found to be higher in individuals with the *AC* genotype, but these differences were not statistically confirmed. The results of this study show that the *ABCG2/Hpy*188I polymorphism can be used in the selection for milk traits in cattle.

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Received 8 April 2009, accepted 14 July 2009.

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