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Evaluation of associations of the polymorphism in the placenta-specific promoter 1.1 of the *CYP19* gene in Black-and-White and Jersey cattle with milk production traits (short communication)

Abstract

The relationship between the SNP of the cytochrome P450 gene (*CYP19-Pvu*II) and milk production traits of Black-and-White and Jersey cattle were analysed. A total of 437 cows were included in the study. A PCR-RFLP was used to genotype. The frequencies of genotypes and alleles for the Black-and-White cows were as follows: 0.8985 - *AA*, 0.0977 - *AB*, 0.0038 - *BB*, and 0.9474 - $CYP19^{A}$, 0.0526 - $CYP19^{B}$. In the Jersey, all cows were genotyped as $CYP19^{AA}$ (no polymorphism). There weren't any associations between *CYP19-Pvu*II polymorphism and milk production traits of the investigated cows.

Key Words: oestrogens, aromatase cytochrome P450, gene *CYP19*, milk production traits, dairy cattle

Zusammenfassung

Titel der Arbeit: **Polymorphismus des plazentaspezifischen Promotors 1.1 der *CYP19*-Gene beim Schwarzbunten und Jersey-Rind sowie seine Zusammenhänge mit Milchleistungsmerkmalen** (Kurzmitteilung)

Zusammenhänge zwischen dem Polymorphismus des Gens von Aromatase Cytochrom P450 (*CYP19-Pvu*II) und den Milchleistungsmerkmalen beim Schwarzbunten und Jersey-Rind wurden untersucht. Die Untersuchungen umfassten insgesamt 437 Kühe. Zur Genotypfeststellung wurde die PCR-RFLP-Methode angewandt. Für das Schwarzbunte Rind wurden folgende Genotyp- und Allelfrequenzen ermittelt: 0,8985 - *AA*, 0,0977 - *AB*, 0,0038 - *BB*, und 0,9474 - $CYP19^{A}$, 0,0526 - $CYP19^{B}$. Bei der Rasse Jersey wiesen alle Kühe den Genotyp AA (kein Polymorphismus) auf. Es wurden keine Zusammenhänge zwischen dem *CYP19-Pvu*II-Polymorphismus und den Milchleistungsmerkmalen nachgewiesen.

Schlüsselwörter: Östrogene, Cytochromaromatase P450, Gen *CYP19*, Milchleistungsmerkmale, Milchrind

Introduction

It is known that conversion of androgens into oestrogens is catalyzed by aromatase cytochrome P450 enzyme (VANSELOW et al., 1999) encoded by the *CYP19* gene (FÜRBASS et al., 1997; KALBE et al., 2000), which was mapped to band q2.6 on chromosome 10 (VANSELOW et al., 2000). Characteristic for *CYP19* is that it utilises different promoters in tissue-specific expression. Products of that gene are present in placenta, ovary, testes, adipose, bone (CONLEY and HINSHELWOOD, 2001). Different promoter regions correspond to different 5'-UTR transcripts but the coding region is identical for all tissues (KALBE et al., 2000). For example in placenta expression of aromatase is mainly driven by P1.1, a distal promoter (FÜRBASS et al., 2001) and P1.2, a promoter which is also active in ovary and brain (FÜRBASS et al., 1997; VANSELOW et al., 2000).

Candidacy of *CYP19* for milk production traits arise from the fact that oestrogen is involved (not directly) in lactogenesis but it influences mammary cells by increasing numbers of prolactin and growth hormone receptors. The level of oestrogen is associated with the activity of aromatase and *CYP19* gene expression.

The aim of this study was to identify the *CYP19-Pvu*II gene polymorphism in placental specific promoter P1.1 in Black-and-White (B&W) and Jersey cattle and its associations with milk production traits.

Materials and Methods

A total of 266 B&W cows with diverse proportion of Holstein Fresian (HF) genes (166 cows with 100% of HF and 100 cows with less than 100% of HF; in this group HF proportion ranging from 50 to 99%) and 171 pure-breed Jersey cows were genotyped using PCR-RFLP. Holstein Fresian breed have been introduced to the herd. The genomic DNA was isolated using *Master Pure*^{TM} Kit (Epicentre Technologies). The analysed fragment of *CYP19* gene was amplified using forward 5'-CTCTCGATGAGACAGGCTCC-3' and reverse 5'-ACAATGCTGGGTTCTGGACT-3' primers (VANSELOW et al., 1999). The PCR reactions contained approx. 100 ng of genomic DNA, 0.5µM of each primer, 1xPCR buffer, 1.5 mM $MgCl_2$, 200µM dNTP, 0.5 units of *Taq* polymerase (MBI Fermentas) and deionized water up to 20 µl. PCR product was digested with *Pvu*II enzyme (Fermentas) at 37 ^{o}C/3 hours. The digestion products were separated by horizontal electrophoresis (120V, 50 minutes) through 2% agarose gels (PRONA) in 1xTBE and 1,0 µM ethidium bromide.

The data of milk production traits in the first lactation were obtained from the farm documentation. Type III ANOVA was used (STATISTICA, 2005). Differences of means were tested with the multiple Duncan test. The following model was used:

$$Y_{i\,j\,k\,l\,mn} = \mu + G_i + S_j + HF_k + YS_l + A_m + E_{i\,j\,k\,l\,mn}$$

$Y_{i\,j\,k\,l\,m\,n}$ - 305-day milk production record at 1st lactation of cow o, µ- the overall mean, G_i –the fixed effect of CYP19 genotype (i = 1,2), S_j- the fixed effect of sire (j = 1, …74), HF_k -the fixed effect of percentage of HF genes (k = 1,2)), YS_l – the fixed effect of year-season of calving class (l = 1, …6), A_m *– age of calving, $E_{i\,j\,k\,l\,m\,n}$ - the random error*

Results

In the group of Black-and-White cows the following DNA restriction fragments were obtained for the *CYP19-Pvu*II polymorphism: 405 bp for the *AA* genotype (no digestion), 405, 327 and 78 bp for *AB* and 327 and 78 bp for *BB* genotype (Fig.). All Jersey cows were genotyped as *AA* (Table 1).

Table 2 shows influence of genotypes (*CYP19-Pvu*II polymorphism) on milk production traits in B&W cattle. The *BB* individual was excluded from the statistical analysis.

Table 1
Frequencies of genotypes and alleles of the *CYP19-Pvu*II (Frequenz von Genotypen und Allelen von *CYP19-Pvu*II)

Breed	n	Genotype			Alleles	
		AA	*AB*	*BB*	*CYP19^A*	*CYP19^B*
B&W (94% of HF)	266	0.898 (n=239)	0.098 (n=26)	0.004 (n=1)	0.947	0.053
100% HF	166	0,886 (n=147)	0.108 (n=18)	0.006 (n=1)	0.940	0.060
<100% HF (83,6% of HF)	100	0.920 (n=92)	0.080 (n=8)	-	0.960	0.040
Jersey	171	1.0000 (n=171)	-	-	1.0000	-

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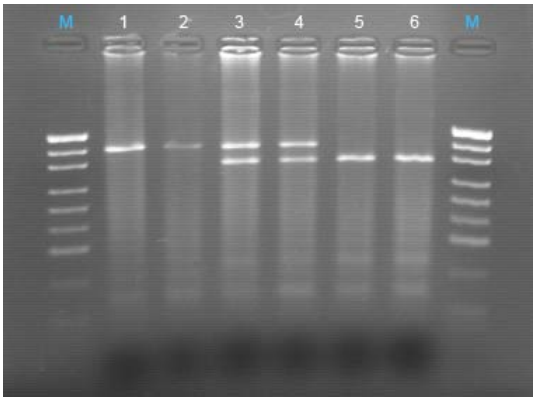

Figure: Representative results of *CYP19-Pvu*II analysis detected by agarose gel electrophoresis (Elektrophoretische Trennung von Restriktionsfragmenten *CYP19-Pvu*II)

M- DNA marker (pUC19/*Msp*I); 1,2 - *AA* genotypes, 3,4 - *AB* genotypes, 5,6 - *BB* genotypes

Table 2
Least square mean and standard deviation of milk production traits in B&W cows with different *CYP19* genotypes (Mittelwerte und Standardabweichungen der Milchleistungsmerkmale bei B&W Kühen mit verschiedenen Genotypen *CYP19-Pvu*II)

Genotype	n	Milk yield (kg)	Fat		Protein	
			kg	%	kg	%
AA	239	6618.8 (1173.3)	244.2 (49.2)	3.69 (0.46)	228.9 (40.1)	3.45 (0.23)
AB	26	6819.8 (1007,4)	247.6 (65.8)	3.59 (0.53)	241.5 (46.6)	3.54 (0.20)

Discussion

Nowadays breeding and selection is complemented by the application of DNA marker information (COPPIETERS et al.,1999). In cattle a number of candidate genes and QTL regions for milk yield and composition have been detected (DYBUS, 2002; DYBUS et al., 2004; FREYER et al., 2003; GRUPE and SCHWERIN, 1998).

Oestrogen together with other hormones stimulates mammary gland proliferation and causes the ducts lengthening and branching during first half of gestation. With progesterone oestrogen establish the conditions needed for geometric cell multiplication and are absolutely essential for mammary epithelial development and differentiation (TOPPER and FREEMAN, 1980). Placenta plays an important role in mammary gland development during the second half of pregnancy. This organ is one of the origins of oestrogen and activity of the product of *CYP19*, aromatase, is absolutely essential for conversion androgens to oestrogens. Expression of that gene in placenta is dependent on promoter 1.1, which is specific for this organ and it is hypotesed present that variation in the promoter region (P1.1) of *CYP19* may affect milk yield.

Frequencies of *CYP19-Pvu*II alleles obtained in the present study were 0.9474 ($CYP19^A$) and 0.0525 ($CYP19^B$) for Black-and-White cattle and were similar to the frequencies obtained by KOMISAREK and DORYNEK (2002) for HF cattle. Higher frequencies of the $CYP19^B$ (0.12) was noticed by VANSELOW et al. (1999). In this study no statistically differences between individuals of different *CYP19* genotypes were found.

In the present study, no associations between *CYP19-Pvu*II gene and milk production traits were found for Black-and-White and Jersey cattle. However the distribution of

genotypes was unfavourable to detect association with one allele showing very low frequency. Moreover, the gene effects may be small allowing to obtain significant association in large numbers of animals only. The influence of $CYP19^{AB}$ and $CYP19^{BB}$ variants on the milk production and reproduction traits should be evaluated further using other population and higher animal numbers.

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