TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2010; 34(6): 493-496
© TÜBİTAK
doi:10.3906/vet-0707-30

Polymorphism of the *CYP19* gene and milk production traits of dairy cattle

Inga KOWALEWSKA-ŁUCZAK*

Department of Genetics and Animal Breeding, Westpomeranian University of Technology, Szczecin - POLAND

Received: 30.07.2007

Abstract: The aim of this study was to estimate the relations between *CYP19*/*Cfr*13I and *CYP19*/*Pvu*II genotypes and milk production traits. The study was carried on 915 Black-and-White cows kept in the Western Pomerania region of Poland. The *CYP19*/*Cfr*13I and *CYP19*/*Pvu*II polymorphisms were detected using the PCR-RFLP method. Statistically significant ($P \leq 0.05$) associations between *CYP19* genotypes and several milk production traits were found. These traits were significantly ($P \leq 0.05$) higher in the *CYP19*/*Cfr*13I *BB* and *CYP19*/*Pvu*II *AA* genotypes.

Key words: Aromatase cytochrome P450, *CYP19* gene, dairy cattle, milk production traits

Introduction

Estrogen is an important endocrine, paracrine, and autocrine acting hormone involved in the regulation of male and female reproduction and metabolic processes like fat deposition and growth. This hormone induces lactogenesis in many species with well-developed mammary glands. The role of estrogen in lactogenesis is an indirect one. Estrogen stimulates secretion of prolactin and possibly other hormones from the pituitary gland (1). The *CYP19* gene encodes aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis (2). In cattle, this gene has been mapped to band q2.6 of chromosome 10 (3). Bovine *CYP19* is transcribed from 6 different promoter regions (P1.1, P1.2, P1.3, P1.4, P1.5, and P2) that show organ-specific activities. In combination with alternative splicing these result in the generation of *CYP19* transcript variants with different 5' untranslated regions but identical coding sequences (4,5). P2 is mainly active in ovarian granulosa cells, P1.4 in the brain and P1.1 within the placenta (6).

Within the CYP19 gene in cattle, several single nucleotide polymorphisms (SNP) were found, which were located mainly in the promoter regions. Namely, the P1.1 region contains 3 SNPs detectable by restriction enzymes, namely *Pvu*II, *Cfr*13I, and *Bse*NI, whilst the P1.2 region contains 2 SNPs detectable by enzymes, namely *Bse*NI and *Tai*I (7,8).

The aim of this study was to estimate the frequencies of *CYP19*/*Pvu*II and *CYP19*/*Cfr*13I genotypes and alleles and to investigate to possible associations between polymorphisms of *CYP19* promoter region P.1.1 and milk production traits of Polish Holstein-Friesian strain Black-and-White cows.

* E-mail: Inga.Kowalewska-Luczak@zut.edu.pl

Materials and methods

The analysis covered 915 Polish Holstein-Friesian strain Black-and-White cows kept at 5 farms in the Western Pomerania region of Poland. The statistical analysis included the cows with at least the first lactation completed (915 cows with lactation I, 652 cows with lactations I and II, and 403 with lactations I, II, and III).

DNA was isolated from blood samples collected on K_3EDTA. The isolation of DNA was performed with using a *Master Pure* kit (Epicenter Technologies, Madison, WI, USA) according to the manufacturer's instructions. Genotypes analyses were performed using the PCR-RFLP method.

Two polymorphisms in the promoter region of the aromatase gene were analyzed. Both polymorphic sites are situated in promoter region P1.1 (EMBL accession no. Z69241). There are: the G1044A transition recognized by *Pvu*II and the G1902A transition recognized by *Cfr*13I. Two *CYP19* gene fragments (named *CYP19*/*Pvu*II and *CYP19*/*Cfr*13I) were amplified using the PCR with primer sequence reported by Vanselow et al. (7). Amplification reactions were conducted in a final volume of 20 μL, 0.2 mm of each dNTP, 10 pmol of each primer (forward and reverse), and 50-100 ng of bovine genomic DNA, containing 1 unit of *Taq* DNA polymerase in a standard PCR buffer and sterile water.

The DNA amplification was performed using an initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 30 s and extension at 70 °C for 2 min, ending with a final extension for 5 min at 72 °C.

The restriction fragments obtained were analyzed on a 3% agarose gel stained with ethidium bromide. Gels were visualized under UV light and documented (Vilber Lourmat).

The analysis of associations between genotypes of *CYP19* and milk performance traits was conducted with the use of the GLM procedure of the SAS program (9). The following model was used:

$$Y_{ijklmno} = \mu + M_i + O_j + R_k + S_l + G_m + (RSG)_{klm} + G_n + e_{ijklmno}$$

where $Y_{ijklmno}$ = observed value; μ = trait mean; M_i = share of dam of cow hf gene effect (i = 1, 2, …., 53); O_j = share of sire of cow hf gene effect (j = 1, 2, …, 21); R_k = year of birth effect (k = 1, …, 10); S_l = month of birth effect (l = 1,…, 12); G_m = herd effect (m = 1,..., 5); $(RSG)_{klm}$ = year of birth × month of birth × herd interaction effect; G_n = genotype effect; $e_{ijklmno}$ = random error.

Differences between mean values of the traits were tested with Duncan's multiple range test. Data on milk production traits in lactations I, II, and III, including milk yield (kg), protein and fat yield (kg), and protein and fat content in milk (%), were obtained from the farm records.

Results

The following restriction fragments were obtained for *CYP19*/*Cfr*13I polymorphism: 235 and 48 bp (genotype *AA*); 283, 235, and 48 bp (genotype *AB*); and no digested fragment 283 bp (genotype *BB*). The frequency of allele *A* was higher than that of allele *B*.

For *CYP19*/*Pvu*II polymorphism the following restriction fragments were obtained: no digested 288 bp fragment (genotype *AA*); 288, 197, and 91 bp (genotype *AB*); and 197 and 91 bp (genotype *BB*). The frequency of allele *A* was again higher than that of allele *B*.

Effects of genotypes of *CYP19*/*Cfr*13I gene on milk production traits in 3 consecutive lactations are given in Table 1. The analysis data showed a tendency for *BB* genotype individuals to have the highest milk, milk fat, and milk protein yield in all lactations. However, statistically significant differences ($P \leq 0.05$) in milk, milk fat, and milk protein yield between *BB* individuals and the rest were confirmed only in I and III lactations.

Table 2 shows the effect of the *CYP19*/*Pvu*II genotype on milk production traits in the cows studied. The analysis data with respect to milk, milk protein, and milk fat yield, and milk protein and milk fat content showed that *BB* genotype individuals had the lowest yield in all 3 lactations. Differences in milk yield, milk protein, and milk fat yield between cows with the *BB* genotype and the rest of the genotypes were confirmed statistically ($P \leq 0.05$). Cows with the *AA* genotype in all 3 lactations were characterized by the highest values of all analyzed milk performance

Table 1. Mean and standard deviation (SD) of studied traits in references to the *CYP19*/*Cfr*13I genotype.

Lactation	Genotype	n	Milk yield [kg]		Protein				Fat			
					yield [kg]		content [%]		yield [kg]		Content [%]	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
I	AA	668	5186^{a}	1355	164.6^{b}	46.0	3.17	0.19	216.8	61.7	4.17^{ab}	0.44
	AB	222	5290^{b}	1465	165.9^{a}	49.1	3.13	0.21	215.0	64.6	4.07^{ac}	0.43
	BB	25	5686^{ab}	1490	177.0^{ab}	47.3	3.11	0.12	225.4	63.4	3.98^{bc}	0.47
	Total	915	5225	1389	165.2	46.8	3.15	0.19	216.6	62.4	4.14	0.44
II	AA	474	5646	1433	182.5	49.0	3.22	0.23	238.4	71.4	4.20	0.56
	AB	163	5773	1383	185.4	47.6	3.20	0.20	242.8	70.9	4.19	0.52
	BB	15	5740	1641	185.8	55.0	3.24	0.23	242.9	77.8	4.24	0.64
	Total	652	5680	1425	183.3	48.8	3.22	0.22	239.6	71.3	4.19	0.55
III	AA	278	6080^{ac}	1608	194.6^{a}	54.5	3.18	0.21	254.2^{ac}	79.5	4.15	0.56
	AB	116	5843^{bc}	1336	185.2^{b}	42.6	3.17	0.17	243.8^{bc}	69.0	4.14	0.56
	BB	9	6543^{ab}	1446	211.5^{ab}	48.9	3.23	0.09	272.4^{ab}	92.5	4.09	0.73
	Total	403	6022	1534	192.3	51.4	3.18	0.20	251.6	76.9	4.15	0.56

n – number of observations within lactations;
$^{a, b}$ Means in the column with the same superscripts differ significantly ($P \leq 0.05$).

Table 2. Mean and standard deviation (SD) of studied traits in references to the *CYP19*/*Pvu*II genotype.

Lactation	Genotype	n	Milk yield [kg]		Protein				Fat			
					yield [kg]		content [%]		yield [kg]		Content [%]	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
I	AA	801	5234^{a}	1387	165.8^{a}	46.7	3.16	0.19	217.5^{a}	62.2	4.15	0.44
	AB	102	5215^{b}	1409	163.2^{b}	47.9	3.11	0.20	212.3^{b}	64.9	4.06	0.44
	BB	12	4668^{ab}	1264	145.9^{ab}	41.9	3.11	0.15	189.2^{ab}	50.3	4.07	0.40
	Total	915	5225	1389	165.2	46.8	3.15	0.19	216.6	62.4	4.14	0.44
II	AA	579	5700^{a}	1436	184.1^{a}	49.0	3.25	0.22	240.4^{a}	72.2	4.20	0.56
	AB	64	5634^{b}	1354	180.4^{b}	48.1	3.17	0.22	238.6^{b}	65.3	4.20	0.49
	BB	9	4684^{ab}	817	151.2^{ab}	25.0	3.22	0.25	189.8^{ab}	40.1	4.04	0.45
	Total	652	5680	1425	183.3	48.8	3.22	0.22	239.6	71.4	4.19	0.55
III	AA	352	6045^{a}	1567	193.9^{ab}	52.7	3.18	0.20	252.5^{ab}	77.9	4.16	0.52
	AB	46	5923^{b}	1290	183.2^{ac}	40.6	3.12	0.15	248.5^{ac}	68.4	4.15	0.55
	BB	5	5260^{ab}	1149	166.6^{bc}	37.2	3.17	0.09	219.8^{bc}	87.1	4.07	0.78
	Total	403	6022	1534	192.3	51.4	3.18	0.20	251.6	76.9	4.15	0.56

n – number of observations within lactations;
$^{a, b}$ Means in the column with the same superscripts differ significantly ($P \leq 0.05$).

traits. However, differences between the *AA* genotype and the other genotypes were statistically non-significant.

Discussion

The placenta produces several distinct estrogens. Two of the principle effects of placental estrogens are to stimulate growth of the myometrium and antagonize the myometrial-suppressing activity of progesterone and stimulate mammary gland development. Estrogens are one in a battery of hormones necessary for both ductal and alveolar growth in the mammary gland. The enzyme aromatase is responsible for conversion of androgen precursor steroids to estrogens and may, thus, have an indirect effect on mammary gland development and milk production traits.

In the studied herd of Polish Holstein-Friesian strain Black-and-White cows we identified 2 alleles and 3 genotypes of the analyzed *CYP19* gene fragments. The frequencies of *CYP19/Cfr*13I alleles were 0.86 for allele *A* and 0.14 for allele *B* (10). A similar frequency of occurrence of *CYP19/Cfr*13I alleles was observed in the German Holstein breed (7): allele *A* 0.87 and allele *B* 0.13. A higher frequency of allele *A* (0.96) was observed in the Jersey breed (11).

For *CYP19/Pvu*II polymorphism the frequencies of *CYP19/Pvu*II alleles were: *A* 0.91 and *B* 0.9 (10). A lower frequency of allele *A* was observed in the German Holstein breed (*A* 0.88) by Vanselow et al. (7). In the Jersey breed a higher frequency of allele *A* was observed, 0.98 (11).

The present study showed cows with the *BB CYP19/Cfr*13I genotype and cows with the *AA CYP19/Pvu*II genotype are characterized by significantly ($P \leq 0.05$) highest values of several milk production traits.

Further studies are necessary to verify associations between polymorphism within *CYP19* promoter region P1.1 and milk production traits. Moreover, the obtained results should be confirmed in a greater number of cows representing all possible genotypes.

Acknowledgment

This work was financially supported by the State Committee for Scientific Research (grant No. 2 P06D03526).

References

1. Akers, R.M.: Selection for milk production from a lactation biology viewpoint. J. Dairy Sci., 2000; 83: 1151-1158.
2. Simpson, E.R., Mahendroo, M.S., Means, G.D., Kilgore, M.W., Hinshelwood, M.M., Graham-Lorence, S., Amarenh B., Ito, Y., Fisher, C.R., Michael, M.D., Mendelson, C.R., Bulun, S.E.: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocrinology 1994; 15: 342-355.
3. Goldammer, T., Guérin G., Brunner, R.M., Vanselow, J., Fürbass, R., Schwerin, M.: Chromosomal mapping of the bovine aromatase gene (CYP19) and an aromatase pseudogene to Chromosome 10 and syntenic group U5. Mamm Genome., 1994; 5: 822-823.
4. Vanselow, J., Fürbass, R., Rehbock, F., Klautschek, G., Schwerin, M.: Cattle and sheep use different promoters to direct the expression of the aromatase cytochrome P450 encoding gene, Cyp19, during pregnancy. Domest Anim Endocrinol. 2004; 27: 99-114.
5. Vanselow, J., Fürbass, R., Zsolnai, A., Kalbe, C., Said, H.M., Schwerin, M.: Expression of the aromatase cytochrome P450 encoding gene in cattle and sheep. J Steroid Biochem Mol Biol. 2001; 79: 279-88.
6. Fürbass, R., Kalbe, C., Vanselow, J.: Tissue-specific expression of the bovine aromatase-encoding gene uses multiple transcriptional start sites and alternative first exons. Endocrinology, 1997; 138: 2813-2819.
7. Vanselow, J., Kühn, C., Fürbass, R., Schwerin, M.: Three PCR/RFLPs identified in the promoter region 1.1 of the bovine aromatase gene (CYP19). Anim. Genet., 1999; 30: 232-233.
8. Vanselow, J., Kühn, C., Fürbass, R., Schwerin, M.: Isolation of the bovine CYP10 promoter 1.2 and identification of genetic variants. Anim. Genet., 2000; 31: 337-338.
9. SAS/STAT, User's Guide, SAS Institute Inc., Cary, NC, USA, 1990.
10. Kowalewska-Łuczak I.: Study of the genetic structure of dairy cattle based on polymorphism within the aromatase gene. Genetika, 2009; 45: 926-931.
11. Kowalewska, I., Kmieć, M., Kulig, H., Sobek, Z., Terman, A.: The polymorphism in the promoter region of the bovine cytochrome P450 aromatase gene (CYP19/Cfr13I & CYP19/PvuII) in Jersey breed. In: Proceedings of the XXI Genetic Days. Wrocław (Poland) 1-3 September 2004, MG-28.