Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

SHORT COMMUNICATION

Polymorphic Sites within a Bov-A2 Element in the Promoter Region of the Steroid 21-Hydroxylase Gene in Cattle

Magdalena Jedrzejczak

West Pomeranian University of Technology, Laboratory of Molecular Cytogenetics, Doktora Judyma 10, Szczecin 71-460, Poland
*Corresponding author: mjedrzejczak@zut.edu.pl

ARTICLE HISTORY

Received: January 05, 2013
Revised: June 10, 2013
Accepted: July 10, 2013

Key words:
Beef cattle
Bov-A2 element
CYP21 gene
Dairy cattle

ABSTRACT

SINE elements are frequently studied in the context of their location, evolution and hypothetical functions. In the present paper, the T→C transition at nucleotide position 1564 located within the Bov-A2 SINE element in the promoter region of the *CYP21* gene was investigated in seven cattle breeds including Polish Holstein-Friesian, Jersey, Montbeliard, Charolais, Angus, Hereford and Limousin. The frequency of homozygotes (*AA*) and heterozygotes (*AB*) was similar for the Polish Holstein-Friesian breed, whereas a complete monomorphism was found in Jersey cattle. Moreover, only two genotypes (*AA* and *AB*) were observed in Limousins. For the remaining breeds, the frequency of homozygotes (*AA*) was the highest ranging from 0.680 for Charolais to 0.865 for Montbeliard.

To Cite This Article: Jedrzejczak M, 2013. Polymorphic sites within a Bov-A2 element in the promoter region of the steroid 21-hydroxylase gene in cattle. Pak Vet J, 33(4): 523-525.

INTRODUCTION

The *CYP21* gene is a member of the *CYP* gene family and encodes the steroid 21-hydroxylase (P450c21) enzyme connected with the microsomal P450 cytochrome. It is essential to corticosteroid metabolism and involved in steroidogenesis in the adrenal cortex (Miller and Auchus, 2011). Bovine *CYP21* can be considered as a candidate gene due to several quantitative trait loci (QTL) for reproduction (Szatkowska *et al*., 2011) and production traits (Jedrzejczak *et al*., 2011; Silva *et al*., 2011b). The bovine *CYP21* gene containing 10 exons was mapped on chromosome 23 and is highly polymorphic in different local cattle breeds. The Bov-A2 SINE, which is a homodimer of 115 bp segments (named Bov-A), is located in the promoter region of the bovine *CYP21* gene (Damiani *et al*., 2000a; Damiani *et al*., 2000b). This SINE overlaps a putative Sp1 binding site (Silva *et al*., 2011a) and contains the T→C transition at nucleotide position 1564, recognized by the *Hpa*II enzyme, which was investigated in the present study in seven cattle breeds.

MATERIALS AND METHODS

A total of 309 individuals of seven breeds (three dairy cattle breeds: Polish Holstein-Friesian, Jersey, Montbeliard and four beef cattle breeds: Charolais, Angus, Hereford and Limousin) were included in the study. The Polish Holstein-Friesian, Montbeliard, Charolais, Angus, Hereford and Limousin cows were kept in the West Pomeranian region of Poland, whereas Jersey cows came from the central part of the country. Genomic DNA was extracted from peripheral blood samples using the Master Pure™ DNA Purification Kit for Blood Version II (Epicentre®, Madison, WI, USA). In the present study, the fragment of promoter region of the *CYP21* gene was amplified using the primer sequences (CCCACCGAGTCCTGCCAC-forward and GTTGAAG GACTTAAAGGAGA-reverse) designed by Damiani *et al*. (2000a). Restriction analysis of PCR products was performed with RFLP method using *Hpa*II restrictase (Fermentas UAB, Vilnius, Lithuania). The PCR products with different restriction patterns were sequenced using an ABI3730 Genetic Analyzer (Applied Biosystems™, Foster City, CA, USA) and the ABI Prism™ BigDye™ Terminator Cycle Sequencing Kit at the Institute of Biochemistry and Biophysics (Polish Academy of Sciences).

RESULTS AND DISCUSSION

In the promoter region of the *CYP21* gene, the *CYP21*/*Hpa*II polymorphism was found in six breeds - Polish Holstein-Friesian, Montbeliard, Angus, Hereford, Limousin and Charolais. Two alleles (*A* and *B*, without and with the *Hpa*II site, respectively) and three genotypes

(*AA*, *BB*, *AB*) were identified. The locus turned out to be monomorphic in Jersey cattle (Table 1), whereas the *BB* homozygotes were absent among Limousin cows. Genotype and allele frequencies for all breeds are shown in Table 1. The new sequences containing the Bov-A2 repeat element, which were identified in this study, were deposited in the GenBank NCBI database (GenBank accession numbers JX481166-JX481169 for Charolais, Polish Holstein-Friesian, Jersey and Montbeliard, respectively, JX867721-JX867722 for Hereford and Limousin, and JX905359 for Angus). The sequencing of PCR products revealed several polymorphisms (Table 2).

Table 1: Frequencies of genotypes and alleles of *CYP21-Hpa*II in dairy cattle breeds

Dairy cattle breeds (n=309)	Genotypes			Alleles	
	AA	*AB*	*BB*	*CYP21*^{A}	*CYP21*^{B}
Polish HF (n=40)	0.375	0.400	0.225	0.575	0.425
Jersey (n=25)	1.000	-	-	1.000	-
Montbeliard (n=74)	0.865	0.122	0.013	0.926	0.074
Charolais (n=50)	0.680	0.240	0.080	0.800	0.200
Angus (n=40)	0.725	0.200	0.075	0.825	0.175
Hereford (n=40)	0.775	0.175	0.050	0.863	0.137
Limousin (n=40)	0.875	0.125	-	0.9375	0.0625

In the promoter region of the *CYP21* gene, polymorphic sites for the Bov-A2 element were identified by the PCR-RFLP method using restriction enzyme *Hinf*I at position 1406 (adenine in the M11267 sequence but cytosine for all the analyzed breeds). The results were confirmed by sequencing analysis (Table 2). At position 1540 in the analyzed sequence, the *A*1540*G* transition was identified in Angus cattle by sequencing, which was also verified by the PCR-RFLP method using the *Nco*I restriction enzyme. This transition should be investigated in future research.

Many studies have been focused on the short interspersed nuclear elements (SINEs) in vertebrate genomes. Consequently, it was estimated, inter alia, that SINEs constitute approximately 8-22% of the total analyzed genomes and are specific for particular mammalian orders or families (Lenstra *et al*., 1993). In the *Bovidae* genomes, several SINEs were localized and analyzed. Three different short interspersed nuclear elements: Bov-A2, Bov-tA and Bov-B, which occupy about 1.8%, 1.6% and 0.5% of the bovine genome, respectively were found (Lenstra *et al*., 1993). The Bov-A2 SINE, which is a homodimer of 115 bp segments (named Bov-A), was found in the promoter region of the bovine *CYP21* gene (Damiani *et al*., 2000a; Damiani *et al*., 2000b). Several authors have examined this region in different local cattle breeds (Italian Holstein-Friesian, Grey Alpine, Friuli Red Pied, Reggio and Nellore) and have shown that Bov-A2 is a highly polymorphic element (Silva *et al*., 2011b). The SINE sequences may be used for evolutionary studies and help in understanding how microsatellites were formed and dispersed in the cattle, sheep and goat genomes (Lenstra *et al*., 1993). On the other hand, the bovine *CYP21* gene was mapped to the bovine chromosome 23 and may be considered as a candidate gene due to the QTLs for production (Jedrzejczak *et al*., 2011; Silva *et al*., 2011b) and reproduction traits (Szatkowska *et al*., 2011). In humans, mutation in this gene is associated with congenital adrenal hyperplasia (Damiani *et al*., 2000b; Miller and Auchus, 2011), whereas in pigs, this gene was used to analyze associations between the *CYP21*/*Hsp92*II genotypes and litter-size parameters (Buske *et al*., 2006).

The location of Bov-A2 in the promoter region may affect *CYP21* gene expression and provoke epigenetic and phenotypic variability (Druker and Whitelaw, 2004). Using allele-specific methylation assay it was demonstrated, that all examined heterozygous animals showed the presence of a methylated allele C (Silva *et al*., 2011a). The T→C transition located at nucleotide position 1564 could also create a methylated CpG motif. Such motifs present in the regulatory regions of genes may play a crucial role in the establishment of tissue-specific transcription (Silva *et al*., 2011a; Zemojtel *et al*., 2011). It is also known that the variability in these regions could affect the binding of transcription factors. In the case of the *CYP21* promoter region, the putative Sp1 site was found using bioinformatics tool, namely Searching Transcription Factor Binding Sites. It was shown that *CYP21*/*Hpa*II is overlapped by a putative Sp1 transcription factor binding site and that this polymorphic site provides protection against methylation (Silva *et al*., 2011a).

This preliminary study on the *CYP21*/*Hpa*II polymorphism presents allele and genotype frequencies for the three dairy (Polish Holstein-Friesian, Jersey and Montbeliard) and four beef (Charolais, Angus, Hereford and Limousin) cattle breeds. Genotypic frequencies in Polish Holstein-Friesians were the same for homozygotes (*AA*) and heterozygotes (*AB*). In the Charolais, Montbeliard, Angus, Hereford and Limousin breeds, homozygotes (*AA*) were the most frequent. Similar results were obtained for Nellore cattle (local beef cattle breed)

Table 2: Polymorphic sites of Bov-A2 SINE in the *CYP21* gene

Position within a Bov-A2 element of the *CYP21* gene	*CYP21* gene (M11267)	*CYP21* gene (AF163767)	*CYP21* gene (AF163098)	Sequences						
				Polish HF	Jersey	Montbeliard	Charolais	Angus	Hereford	Limousin
1346	T	C	C	C	C	C	C	C	C	C
1348	T	G	G	G	G	G	G	G	G	G
1375	A	G	G	G	G	G	G	G	G	A
1406	A	C	C	C	C	C	C	C	C	C
1408-1409	-	C	C	C	C	C	C	C	C	C
1420	T	C	C	C	C	C	C	C	C	T
1429	T	G	G	G	G	G	G	G	G	G
1456	C	A	A	A	A	A	A	A	A	A
1467	A	-	-	-	-	-	-	-	-	-
1521-1522	-	C	C	C	C	C	C	C	C	C
1540	A	G	A	A	A	A	A	G	A	A
1564	T	T	C	C	T	C	T	C	T	T
1567	A	A	A	A	A	A	A	G	A	A

(Silva *et al*., 2011b). The highest frequencies for homozygotes (*AA*) in the group of Grey Alpine cattle and the lowest for this genotype in the group of Friuli Red Pied were demonstrated. The analysis of Bov-A2 sequences showed several polymorphisms (Damiani *et al*., 2000a). The new polymorphic sites were also found in the present study but their role and association with epigenetic and phenotypic aspects need to be further investigated.

Conclusion: The results presented in this preliminary study confirmed existence of the *T*1564*C* polymorphism in the promoter region of the *CYP21* gene in the six breeds-Polish HF, Montbeliard, Angus, Hereford, Limousin and Charolais. Two other polymorphic sites for *Hinf*I restrictase at position 1406 and for *Nco*I at position 1540 were found. Analysis of those changes in SINE Bov-A2 element located in the promoter region of the *CYP21* gene may be used for association studies between polymorphic variants and milk and beef production traits. Presented results may be also useful to molecular evolution studies to prove if those polymorphic sites are involved in the selective differentiating processes in the bovine breeds.

REFERENCES

Buske B, I Sternstein, M Reiβmann and G Brockmann, 2006. Detection of novel single-nucleotide polymorphisms (SNPs) in the *CYP21* gene and association analysis of two SNPs for *CYP21* and *ESR2* with litter size in a commercial sow population. J Anim Breed Genet, 123: 343-348.

Damiani G, S Florio, E Budelli, P Bolla and A Caroli, 2000a. *Hpa*II PCR-RFLP within a Bov-A2 element in the promoter of the bovine *CYP21* (steroid 21-hydroxylase) gene. Anim Genet, 31: 154-154.

Damiani G, S Florio, E Budelli, P Bolla and A Caroli, 2000b. Single nucleotide polymorphisms (SNPs) within Bov-A2 SINE in the second intron of bovine and buffalo k-casein (*CSN3*) gene. Anim Genet, 31: 277-279.

Druker R and E Whitelaw, 2004. Retrotransposon-derived elements in the mammalian genome: a potential source of disease. J Inherit Metab Dis, 27: 297-330.

Jedrzejczak M, W Grzesiak, I Szatkowska, A Dybus, M Muszynska and D Zaborski, 2011. Association between polymorphisms of *CYP19*, *CYP21* and *ER1* genes and milk production traits in Black-and-White cattle. Turk J Vet Anim Sci, 35: 41-49.

Lenstra JA, JAF van Boxtel, KA Zwaagstra and M Schwerin, 1993. Short interspersed nuclear element (SINE) sequences of the *Bovidae*. Anim Genet, 24: 33-39.

Miller WL and RJ Auchus, 2011. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev, 32: 81-151.

Silva AM, MAR Freitas, AFL Rios, A Renzi, RB Lôbo, MAV Galerani, RA Vila and ES Ramos, 2011a. Identification of a DNA methylation point in the promoter region of the bovine *CYP21* gene. Genet Mol Res, 10: 1409-1415.

Silva AM, AFL Rios, ES Ramos, RB Lôbo, HN Oliveira and MAR Freitas, 2011b. Association between *IGF2* and *CYP21* gene polymorphisms and characteristics of economic interest in Nellore cattle. Genet Mol Res, 10: 2140-2147.

Szatkowska I, W Grzesiak, M Jedrzejczak, A Dybus, D Zaborski and D Jankowiak, 2011. An analysis of *CYP19*, *CYP21* and *ER* genotypes in Polish Holstein-Friesian cows with regard to the selected reproductive traits. Acta Vet Brno, 80: 65-71.

Zemojtel T, SM Kielbasa, PF Arndt, S Behrens, G Bourque and M Vingron, 2011. CpG deamination creates transcription factor-binding sites with high efficiency. Genome Biol Evol, 3: 1304-1311.