Genetic Variation of the IGF1 and OPN Genes in Holstein-Friesian Dairy Cattle of Historical and Non-Historical Twins

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Abstract

The application of DNA molecular techniques can be used to determine the mutation cases at DNA fragments associated with fertility traits in cattle. This study was aimed to identify genetic variants of Insulin Like Growth Factor 1 (IGF-1) and Osteopontin (OPN) genes to be considered as candidate genes controlling fertility traits in HF cattle of historical twins (27 heads) and non historical twins (15 heads) from West Java, Indonesia. Historically twinning cattle was defined as either the cows ever calved twins (more) or their offspring (female and males). Investigation of genetic variants was done by applying PCR-RFLP method by using restriction enzyme of SnaB1 for The IGF-1 gene and Bsr1 for the OPN gene. Amplification produced DNA products of 249 bp (IGF-1 intron-1) and 290 bp (OPN intron-4). Genotyping on the IGF-1 gene locus Snab1 produced only one DNA fragment, meaning all the cows having the BB genotype (249 bp). Monomorphic of the IGF-1 gene was probably due to no mutation (C/T) at the nucleotide as a cutting site. Instead, it was discovered genetic variants of the OPN gene locus Bsr1, resulting three DNA banding patterns, these genotypes were successively CC (200 and 90 bp), CT (290, 200, 90 bp) and TT (290 bp). The proportion of CC, CT and TT genotypes in historical twinning cattle (30%: 56%: 14%) differed to those of non historical twinning one (20%: 40%: 40%). Former cattle had the amount of CC and CT genotypes higher than the latter uterine milk uterus fertility for embryo growth in HF dairy cows observed.

Keywords: Hostein Friesian, IGF-1 gene, OPN gene, PCR-RFLP, twinning

Introduction

Productivity of dairy cows is strongly determined by the level of their fertility. The potency of a cow to give twin births needs to be studied to get information on how far this trait can be inherited. The inheritance of twin births in cattle had a similar pattern to quantitative traits, it was controlled by many genes and interacted with environment (Lien *et al*., 2000). DNA molecular techniques focusing on genomic analysis can be used to examine the insidences of mutation of the sequences of DNA fragments related to the changes in breeding values or performances of valuable traits.

Genetic polymorphisms of two fertility genes in cattle, including the IGF1 and OPN genes, using the method of restriction fragment length polymorphisms (RFLP) or others have been studied. The IGF1 gene in cattle is located in the chromosome 5 (BTA5), of which containing QTL regions of controlling twin (multiple) births. So this gene was possible to be used as a candidate gene to increase the genetic potency of cows to calve twin or multiple (Lien *et al*., 2000). The IGF1 gene played an important role in regulating folliculogenesis and possibly be involved also in regulating multiple ovulations in cows (Kim *et al*., 2009). Therefore, the IGF1 gene could be used as a positional candidate gene and intron 2 IGF1 gene was highly significant (P= 0.003) associated with twinning traits in cattle (Kim *et al*., 2009).

Osteopontin (OPN) gene in cattle is located in the chromosome 6 (BTA6), closely located to the QTL genes of milk production (Leonard *et al*., 2005). The bovine OPN gene consisted of 6 exons with the size of about 7 kb from genomic DNAs (Gen Bank accession number: NW_255516) and encoded a 278-AA protein (Kerr *et al*., 1991). Regulation and immediately functional implication of the conditions involving the OPN gene gave temporary and partial possibilities as the result of joining activities in developing and ensuring the maintenance of a pregnancy (Johnson *et al*., 2003). The objective of this study was to determine genetic polymorphisms of the IGF1 and OPN genes in HF cattle with historical twin and non-historical twin raised by small dairy farmers in Lembang District, West Java Province.

Materials and Methods

Blood and DNA Samples

A total blood sample number of 42 heads of HF dairy cattle consisting 27 heads of historical twin birth cattle and 15 heads of non-historical twin birth ones as the control. Genomis DNA was extracted from fresh blood sampes by using standard phenol-chloroform protocol (Sambrook and Russel, 2001).

Amplification and Polymorphism Identification

Amplification of IGF-1 and OPN gene fragments was done by using poly-

merase chain reaction (PCR) methods. Reagents were used for amplification of both fragments are 2 µl of DNA sample, 25 pmol of each primers (Table 1), 200 µM of dNTPs mixture, 1 mM of $MgCl_2$, and 0.5 unit of DreamTaq™ DNA polymerase with its buffer (Fermentas) in 25 µl of total solutions. Amplification process was running within GeneAmp® PCR system 9700 (Applied Biosystems™) with the condition of pradenaturation at 95°C for 5 minutes, 35 cycles consisting of denaturation at 95°C for 30 second, primer annealing at 60°C for 45 second and extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes.

Polymorphism identification both in IGF-1 and OPN gene fragments were detected by restricted fragment length polymorphism (RFLP) methods. The restricted enzyme which used for IGF-1 gen fragment was SnaBI (New England Biolabs) and for OPN was BsrI with following manufacture's instructions. The product of RFLP methods were visualized on 2% agarose gel (w/v) which stained by EtBr (ethidium bromide). Allele identification was followed Siadkowska *et al*. (2006) for IGF-1 gene and Leonard *et al*. (2005) for OPN gene.

Table 1. Primers information were used

Gene	Sequence (5'-3')	PCR Product	Restriction enzyme	Reference
IGF-1	F: ATT ACA AAG CTG CCT GCC CC	249 bp	SnaBI	Siadkowska *et al.*, 2006
	R: ACC TTA CCC GTA TGA AAG GAA			
OPN	F: GCA AAT CAG AAG TGT GAT AGA C	290 bp	BsrI	Leonard *et al*., 2005
	R: CCA AGC CAA ACG TAT GAG TT			

Statistical Analysis

Genotype frequency represents the ratio of a genotype to total population. Allele frequency is a ratio of an allele to the overall allele at a locus in the population. Mathemathics model genotype and allele frequency (Nei and Kumar, 2000) is represented as follows:

$$X_{ii} = \frac{n_{ii}}{N} \; x \; 100\%$$

$$X_i = \frac{(2n_{ii} + \sum_{i \neq j} n_{ij})}{2N}$$

Note :

χii = iith genotype frequency
nii = number sample of ii genotype
nij = number sample of ij genotype
N = total sample
χi = ith allele frequency

Results and Discussion

Amplification and Genotyping of the IGF1 Gene Fragment

Amplification of IGF-1 gene fragment was successful to amplified the 249 bp fragment were located in intron 1 of IGF-1 gene. Genetic polymorphism of the IGF-1 gene was detected by PCR-RFLP method by Siadkowska *et al*. (2006) through the examination of the presence of a C/T base transition at the 472 nucleotide position in non-codign region of *Bos taurus* IGF-1 gene. The substitution of C to T produced a new SnaBI (IGF-1|SnaBI) restriction site. The animals were genotyped by follow Siadkowska *et al*. (2006). Animal with homozigot TT was indicated by the presence of two fragments i.e. 223 and 26 bp, while the genotype homozigot CC was indicated by absent of SnaBI restriction site and showed only one fragment i.e. 249 bp. The heterozigot CT was indicated by the presence of three fragments i.e. 249, 223 and 26 bp.

The RFPL analysis samples show that the genotype of HF cattle was homozigot CC. The allele was found are allele C. This result caused the frequency of the CC genotype obtained are 100%, regardless of the CT and TT genotype are 0%. This result was contrast to those of some previous studies by detecting the presence of genetic polymorphisms in the bovine IGF-1 gene. Polymorphism short tandem repeat (STR) in the 5'flanking region of intron 3 on IGF1 gene was identified by Kirkpatrick (2001). Single strand conformation polymorphism (SSCP) in the 5' flanking region of intron 1 on IGF-1 gene was also identified as a transition of T/C known as RFLP|SnaB1 (Ge *et al*., 2001). Two polymorphisms of the IGF1 gene, the insertion/dilesi TTTG (InDel) in intron 4 and RFLP|DpnI in intron 5 were found in Norway cattle (Lien *et al*., 2000).

Amplification and Genotyping of the OPN Gene Fragment

Amplification of the intron 4 OPN gene which located at the chromose 6 (BTA6) investigated in HF cattle resulted in a fragment length of 290 bp. The amplification product was then restricted by BsrI enzyme to detect the presence of point mutation in the intron 4 OPN gene. Genetic polymorphism of the OPN gene in this study followed the methods of Leonard *et al*. (2005) which examined the transition C/T in the 5' non-code area in the intron 4 of *Bos taurus* OPN gene .

The substitution of C to T produced a new BsrI (OPN|BsrI) restriction site. Animal with homozigot CC was indicated by the presence of two fragments i.e. 200 and 90 bp, while the genotype homozigot TT was indicated by absent of BsrI restriction site and showed onlyone fragment i.e. 290 bp. The heterozigot CT was indicated by the presence of three fragments i.e. 290, 200 and 90 bp.The analysis of RFLP on the OPN|SbrI within HF cattles with historical twin from Pangalengan district show that there was found three genotypes, namely the CC genotype, CT, and the TT genotype.

Table 2. Genotype and allele frequency of the OPN gene in HF historical twin and control

Population	Cattle (Head)	Genotype Frequency (%)			Allele Frequency (%)	
		CC	CT	TT	C	T
Pangalengan	Non-twin (0)	-	-	-		-
	Sub total (17)	24 (4)	53 (9)	24 (4)	50	50
	Twin (10)	40 (4)	60 (6)	0 (0)	70	30
Lembang	Non-twin (15)	20 (3)	40 (6)	40 (6)	40	60
	Sub total (25)	28 (7)	48 (12)	24 (6)	52	48
Total	Twin (27)	30 (8)	56 (15)	14 (4)	57	43
	Non-twin (15)	20 (3)	40 (6)	40 (6)	40	60
	Total (42)	26 (11)	50 (21)	24 (10)	51	49

Note: (….) was blood samples

The frequencies of the occurrences of CC, CT and TT genotypes of the OPN gene of HF historical twin cattles from Pangalengan ditrict were 24, 52 and 24% repectivelly. For HF cattles from Lembang district were found some interesting things. For non-historical twinning cattles as the controls were identified three genotypes, namely CC, CT and TT genotypes, with the frequencies of the occurences of the respective genotypes were succesively 20, 40 and 40%y. For historical twin animals in this location were found none animal having the TT genotype (0%), so those historical twin cattle had only two genotypes of CC (40%) and CT (60%) respectively.

Conclusion

Genotyping on the intron 1 region of IGF-1 gene in the BTA5 in HF cattles of both historical twin and non-historical twin resulted in no genetic polymorphism (monomorphic) as the DNA fragment representing solely the CC genotype. This is as indication of the C/T substitution in the intron1 IGF1 gene might be disappearence, so this gene was unable to be functioned as a candidate gene in studying twinning traits in HF cattles.

Genotyping on the intron 4 of OPN gene in HF cattles with historical twins and non-historical twins resulted in three genetic variance, providing CC, CT, and TT genotypes, but their frequencis was varied. This result proved that the C/T transition in the non-code area on intron 4 of OPN gene could be used as an early indicator as a candidate gene to study its control on milk uterus secretion to mediate twinning birth in in HF cattle.

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