The association of SNP rs42688595 in LOC514211 gene with Indonesian-Holstein cow’s reproductive traits

A P Rahayu1,2,4, T Hartatik3, A Purnomoadi1 and E Kurnianto1

1Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia
2Permanent Address: Agriculture, Fisheries and Food Center of Semarang Regency, Ungaran, Indonesia
3Faculty of Animal Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: amaliapuirahayu.kablsmg@gmail.com

Abstract. The aim of this study was to study the association between SNP (single nucleotide polymorphism) rs42688595 in LOC514211 gene with the reproductive traits on Indonesian Holstein dairy cows. Blood samples and reproduction records from 89 cows were used. The DNA analysis was carried out using the PCR-RFLP method. Cows with AC genotypes showed the lowest days open (152.06 ± 20.54 days) and the lowest calving interval (427.06 ± 20.26 days) (P<0.05) compared to those of CC genotypes (236.46 ± 23.34 and 514.95 ± 22.86 days, respectively) and AA genotypes (270.14 ± 26.27 and 552.06 ± 26.29 days, respectively). The studied SNP was not associated with the age at first calving and service per conception (P>0.05). The LOC514211 rs42688595 shows a potency to be used as a genetic marker for a selection program to produce cows with better reproductive performance. Selection could be done by increasing the genetic frequency of heterozygous animals (AC).

1. Introduction

Improving the genetic quality of livestock through selection is one of the efforts to increase the productivity of dairy cows. In addition to selecting the characteristics of milk production, the selection is also needed in improving the genetic quality of reproductive traits. This needs to be considered because the reproductive traits also have economic value [1].

Molecular biology techniques can be used to access genomic information based on sequences of bovine genomes with differences in nucleotide sequences [2]. The difference in one single nucleotide is called SNP (single nucleotide polymorphism). SNPs can be used as selection markers to carry out genomic selection. Genomic selection is a revolution in dairy cow breeding that makes it possible to estimate breeding values based on the genotype of the SNP [3].

Junjing [4] and Anggraeni et al [5] found that SNP rs42688595 in the LOC514211 gene could be a potential marker for the characteristics of production and reproduction of dairy cattle in the Chinese Holstein cattle population. No other study has reported the effect of this gene on the reproductive traits of dairy cows. The research on SNPs on these genes are still limited. Meanwhile, to be defined as genetic markers, in-depth research needs to be done, so this research is important to be performed. Based on various studies of other genes [2,6], the different results of the genetic marker were known in various breeds. The purpose of this study was to examine the effect of LOC514211 rs42688595 SNP on Indonesian-Holstein cow population.
2. Materials and methods

2.1. Materials
The materials used in this study were the first lactation reproduction record of 89 Indonesian-Holstein cows at Baturraden Dairy Breeding and Forage Center (BBPTUHPT Baturraden). The materials used for DNA analysis were cow blood samples, DNA extraction kits, PCR kits, forward primers (F) 5'-acggttgggttcctgc-3' and reverse (R) 5'-ctgtctgccgtgttgcg-3', TaqI restriction enzymes (10 U/µL), agarose gel, reagents for electrophoresis, and 50 bp DNA markers. The DNA analysis was conducted in the Laboratory of Animal Breeding and Genetics, Universitas Gadjah Mada, Yogyakarta, Indonesia.

2.2. Methods
The method used was PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) based on Anggraeni et al [5] (modified). A total of 1-3 ml of blood samples were taken through the tail vein. Genomic DNA is extracted from the blood using a kit according to the manufacturing protocol. PCR mix was made by mixing 2 µl DNA templates, 12.5 µl PCR kits, 0.5 µl forward primers (10 pmol/µl), 0.5 µl reverse primer (10 pmol/µl) and 9.5 µl ddH2O, so the total volume was 25 µl. The mixture was put into a thermal cycler machine (Peqlab, Germany) in accordance with the program as follows: Predenaturation of 94°C for 10' followed by 36 cycles consisting of denaturation 94°C, 30”; annealing 58.8°C, 45”; extensions 72°C, 45”; and final extension 72°C, 25’. 4 µL of the PCR product was cut by adding 0.3 µl of the TaqI restriction enzyme; 1.5 µl TaqI Buffer and 9.2 µl ddH2O, then incubated 65°C for 3 hours. The RFLP results were then evaluated by 3% agarose gel electrophoresis using 50 V voltage for an hour. DNA bands were observed in UV transilluminator and compared with DNA markers.

2.3. Statistical analysis
Association analysis of the SNPs with traits was performed using the general linear model procedure in SPSS 20 program.

3. Results and discussions

Gene amplification was based on nucleotide sequences from GenBank no. access SNP rs42688595. The PCR product produced was 352 bp. PCR products were cut with restriction enzymes resulting in a different pattern of band lengths that indicate variation in one locus (figure 1). Mutations (SNP) occur in the +501 nucleotide sequence or the 238th order from the forward primer. Three genotypes were found, i.e. AA, AC, and CC. In the CC genotype, the C allele (normal allele) does not change so that the restriction enzyme can recognize the cutting site and cut it into two bands (97 bp and 237 bp). In the AA genotype, the C allele mutates into A allele so that the restriction enzyme cannot recognize the
cutting site and only form one band (334 bp). This point mutation is occurred because of transversal substitution from pyrimidine to purine bases cause changes in codons from CGA (arginine) to AGA (arginine). A change in a base pair that causes a change in genetic code but does not result in amino acid changes (synonymous) is categorized as a silent mutation [7]. The AC genotype contains A allele and C allele, so it formed 3 bands: 97 bp, 237 bp, and 334 bp.

The association of LOC514211 rs42688595 with reproductive traits are presented in table 1. Heterozygote animals (AC genotype) showed the lowest days open (DO) (152.06±20.54 days) compared to homozygous AA genotype (270.14±26.27 days) and CC genotype (236.46±23.34 days) (P=0.024). AC genotype also showed the lowest calving interval (CI) (427.06±20.26) compared to AA (552.06±26.29) and CC (514.95±22.86) (P=0.014). On the other hand, genotypes were not associated with age at first calving (AFC) and service per conception (S/C) (P>0.05).

| Parameter         | Genotype (Y±SEM) | P   |
|-------------------|------------------|-----|
| AFC (n=36)        | AA | 845.72±28.07   | 0.599 |
|                   | AC | 818.69±26.21   |     |
|                   | CC | 816.14±15.83   |     |
| DO (n=16)         | AA | 270.14±26.27   | 0.024 |
|                   | AC | 152.06±20.54   |     |
|                   | CC | 236.46±23.34   |     |
| S/C (n=37)        | AA | 2.89±0.30      | 0.176 |
|                   | AC | 1.94±0.27      |     |
|                   | CC | 2.86±0.33      |     |
| CI (n=37)         | AA | 552.06±26.29   | 0.014 |
|                   | AC | 427.06±20.26   |     |
|                   | CC | 514.95±22.86   |     |

AFC= age of first calving (days), DO= days open (days), S/C= service per conception, CI= calving interval (days). Different superscripts on same row show significant differences (P<0.05).

In the previous study [5], A allele was called T, so the TT and TC genotypes in that study were the same as AA and AC genotypes in this study. In contrast to this study, the genotypes in the previous study were not associated with DO and CI, both at parity 1, 2, and 3, but associated with days to first service (DFS). The AC genotype showed the lowest DFS compared to the other two genotypes at the second parity. This is in line with this current study that heterozygous genotypes show the best reproductive performance. If the DFS is low, the DO and CI can also be lowered, assuming if the S/C is the same value. The similarity of the two studies is that genotypes are not associated with the AFC.

Genotype does not affect the S/C, possibility because the S/C is more influenced by environmental factors. This is indicated by the generally small S/C heritability value. S/C heritability values from various studies including 0.001 [8]; 0.02 [9]; and 0.03 [10]. Low S/C heritability value means that genetic additives that affect S/C are small so that improvements through direct selection will be slow [11]. The intended environmental factors are feed (quality and quantity), the number of cows, reproductive disorders, and reproductive management including frozen semen handling, heat detection, skills, the optimum timing of insemination, etc [12,13].

The LOC514211 gene is located on chromosome 13 in the Bos taurus. Although its role has not been known with certainty but based on the GWAS (genome-wide association) study by Junjing [4], SNPs on this gene have a good effect on the production traits without worsening their reproductive performance. GWAS is an observational study of a genome-wide set of genetic variants to see if any variant is associated with a trait [14]. From various studies [15-17], there was an unfavorable genetic correlation between milk production and fertility, so improvements in production traits will generally have a negative effect on reproductive traits, and vice versa. However, with GWAS technology, where many traits can be analyzed at once, candidate genes that have beneficial effects on production and reproduction, can be chosen, including in this LOC514211 gene. Mutations that occurred in the SNP position were synonymous. However, Hunt et al [18] stated that synonymous mutations can still influence gene expression by affecting the enzymatic structure.

4. Conclusion
The LOC514211 rs42688595 is associated with Indonesian-Holstein cow’s reproductive traits. It
shows a potency to be used as a genetic marker for breeding selection program based on reproductive traits. Selection could be done by increasing the genetic frequency of heterozygous animals (AC) to reduce days open and calving interval. Exploration of SNP in other positions of this gene is a prospective study opportunity for cattle selection.

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