J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Penguatan Risbang No. 60/E/KPT/2016

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
43(3):195-200, September 2018
DOI: 10.14710/jitaa.43.3.195-200

Identification of KLF3 gene polymorphism in Indonesian Friesian Holstein cattle

P.P. Agung[1,*] and F. Laoditta[2]

[1]Research Center for Biotechnology, Indonesia Insitute of Sciences,
Jl. Raya Bogor KM. 46, Cibinong 16911, West Java - Indonesia
[2]Graduate School of Surya University,
Jl. M.H. Thamrin KM. 27, Tangerang 15810, Banten - Indonesia
**Corresponding E-mail: paskah_partogi@yahoo.com*

Received March 20, 2018; Accepted June 05, 2018

ABSTRAK

Gen KLF3 merupakan salah satu gen potensial untuk dikaji sebagai kandidat marker genetik karena keterlibatannya dalam berbagai proses biologi seperti proliferasi sel, diferensiasi sel, dan apoptosis. Penelitian ini dilakukan untuk mengidentifikasi keragaman gen KLF3 pada sapi FH di Indonesia. Sebanyak 302 sampel darah sapi FH yang berasal dari Jawa Barat (n=138), Jawa Tengah (n=34), dan Jawa Timur (n=130) digunakan dalam penelitian ini untuk memperoleh sampel DNA. Analisa sekuen DNA dilakukan terhadap 9 sampel darah (masing-masing daerah pengambilan sampel diwakili 3 sampel) sebagai tahap awal identifikasi keragaman dan dilanjutkan dengan analisa PCR-RFLP terhadap seluruh sampel DNA. Terdapat dua SNP pada daerah intron gen KLF3 yaitu SNP g.59607486delC dan SNP g.59607554A>G. Kejadian SNP g.59607554A>G pada populasi sapi FH dapat dideteksi menggunakan metode PCR-RFLP menggunakan enzim restriksi HpyCH4IV dan menghasilkan tiga genotipe gen KLF3 (AA, AG, dan GG) pada populasi sapi FH di Indonesia namun memiliki nilai heterozigositas yang rendah. Genotipe AA merupakan genotipe yang memiliki frekuensi tertinggi yaitu sebesar 0.73. Sementara genotipe AG dan GG berturut-turut memiliki frekuensi genotipe sebesar 0,24 dan 0,03. Gen KLF3 pada sapi FH di Indonesia bersifat polimorfik dan dapat dijadikan dasar bagi studi berikutnya untuk mempelajari hubungannya dengan produksi susu dan kandungan protein susu.

Kata kunci: identifikasi, keragaman, KLF3, sapi perah, Indonesia

ABSTRACT

The KLF3 gene was a potential genetic marker candidate due to its involvement in many biological processes such as cell proliferation, cell differentiation, and apoptosis. This study was conducted to identify the KLF3 gene polymorphism in the Indonesian FH cattle. A total of 302 individual cattle blood samples from West Java (n=138), Central Java (n=34), and East Java (n=130) provincies were used to obtain DNA samples. The DNA sequencing was performed using 9 samples (each location of sampling represented by 3 samples) for initial identification of the KLF3 gene polymorphism and followed by PCR-RFLP analysis using all DNA samples. There were two SNPs identified in the intron region of the KLF3 gene i.e. SNP g.59607486delC and SNP g.59607554A>G. The SNP g.59607554A>G could be detected using PCR-RFLP method with HpyCH4IV restriction enzyme and resulted three genotypes of the KLF3 gene (AA, AG, and GG) but its heterozygosity value was low in the Indonesian FH cattle. The AA genotype has the highest frequency (0.73), while the AG and GG genotypes frequency were 0.24 and 0.03, respectively. Information about the KLF3 gene polymorphism in this study can be considered for further study to analyse its association with milk and protein yield traits.

Keywords: identification, KLF3, polymorphism, dairy cattle, Indonesia

INTRODUCTION

The Friesian Holstein (FH) cattle breed is the most famous cattle breed in dairy industry. There were 0.5 million heads of FH cattle that spread over in Indonesia and it was concentrated in Java Island especially in West Java, Central Java, and East Java Provinces (Ministry of Agriculture of the Republic of Indonesia, 2017). Milk and protein yield were substantial traits in dairy cattle industry. Based on several studies, the milk and protein yield of the FH cattle in Indonesia still low-medium levels (Lestari *et al*., 2015; Martono *et al*., 2016; Widyobroto *et al*., 2018) and lead the high demmand of the imported milk. The FH cattle that have good quality in milk production trait can be generated through the breeding program that assisted by molecular technology due to the molecular information of specific genes which highly associated with economic traits can be identified and used as genetic markers in cattle breeding programs (Ribeca *et al*., 2014).

KLF3 gene is a member of the Kruppel-Like Factors (KLFs) family of zinc finger proteins which are involved in many biological processes such as cell proliferation, differentiation, apoptosis, and neoplastic transformation (Eaton *et al*., 2008). In addition, KLF3 gene was important component of transcriptional control of adipogenesis (Sue *et al*., 2008; Bell-Anderson *et al*., 2013) and regulated muscle specific gene expression and synergizes with serum response factor on KLF binding sites (Himeda *et al*., 2010). In dairy cattle breeding, KLF3 gene could be used for marker assisted selection program as a genetic marker for milk and protein yields (Yin *et al*., 2010).

The KLF3 gene polymorphism information in Indonesian dairy cattle was unknown. In other hand, information about polymorphism in certain genetic marker candidates was required to design a strategy of cattle breeding program. This study was conducted to identify the KLF3 gene polymorphism in the Indonesian FH cattle using polymerase chain reaction-sequencing-restriction fragment length polymorphism analysis.

MATERIALS AND METHODS

Blood Samples and DNA Extraction

A total of 302 heads of FH cattle consist of 138 cattle from West Java, 34 cattle from Central Java, and 130 cattle from East Java were included in this study to obtain blood samples. Blood samples (3-5 mL) were taken from *coccygeal* vein using *Venoject* and collected in *Vaccutainer* tubes containing an anticoagulant. DNA was extracted from blood samples using the Genomic DNA Mini kit (Geneaid Biotech Ltd., Taiwan) following the manufacturer's protocol.

Amplification and Sequencing

The PCR reaction was performed in a Mastercycler® gradient (Eppendorf, Hamburg, Germany) with a pair of primer to amplify the KLF3 gene that recommended by Yin *et al*. (2010) i.e. forward primer 5'-gccagtgttgttaatgctt-3' and reverse primer 5'-tggtaactttcaagatgcct-3'. The expected size of the KLF3 gene in this study was 329 bp. The PCR reagents composed of: 6.25 µL KAPA2G Fast Ready Mix PCR Kit 1X (Kapa Biosystems, Cape Town, South Africa), 0.5 µL forward and reverse primers (0.2 µM final concentration each), 1 µL DNA samples (5-50 ng>µL), and ddH_2O up to 12.5 µL final volume. The optimum condition for PCR programme was as follows: initial denaturation at 94°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing 55°C for 45 seconds, extension at 72°C for 45 seconds; and terminated by a final extension at 72°C for 5 minutes. The PCR products were visualized by electrophoresis process (1% agarose gel, SyBr® staining, and captured in GBOX documentation System (Syngene, UK)).

The DNA sequencing was performed using 9 PCR products (3 samples represented each location of sampling) for initial identification of the KLF3 gene polymorphism. The PCR products from individual cattle were sequenced using the ABI Prims 3100-Avant Genetic Analyzer in the 1st BASE Laboratory, Malaysia. The FH cattle KLF3 gene sequences were aligned and compared with the *Bos taurus* KLF3 gene sequence available in the GenBank (NCBI) database with accession number AC_000163 using MEGA ver 6.0 programs (Tamura *et al*. 2013). The position of the sequence in this study was relative to *Bos taurus* KLF3 gene sequence (AC_000163).

RFLP and Data Analysis

Identification of the KLF3 gene polymorphism in the Indonesian FH cattle was performed by RFLP method using HpyCH4IV restriction enzyme (Biolabs Inc., New England) with restriction site of 5'...A||CGT...3' (temperature and incubation time following the producer's guidelines). The PCR and RFLP

products were visualized were visualized by electrophoresis process. Individual cattle genotype in this study was determined based on the differences in the number and size of the visualized bands. The genotype frequency (equation *a* and *b*), allele frequency (equation *c*), observed heterozygosity (equation *d*), and expected heterozygosity (equation *e*) were calculated based on Nei and Kumar (2000):

a. $\chi_{ii} = (n_{ii}/N)$;

b. $\chi_{ij} = (n_{ij}/N)$;

c. $\chi_i = (2n_{ii} + \Sigma n_{ij})/(2N)$;

d. $H_o = n_{ij}/N$;

e. $H_e = 1 - \sum_{i=1}^{n} p_i^2$

where: χ_{ii} = frequency of A_iA_i genotype (homozygote); χ_{ij} = frequency of A_iA_j genotype (heterozygote); χ_i = frequency of ^{i}th allele; n_{ii} = number of individuals with A_iA_i genotype; n_{ij} = number of individuals with A_iA_j genotype; H_o = observed heterozygosity; H_e = expected heterozygosity; N = number of samples. The polymorphism information content (PIC) was calculated based on Botstein *et al*. (1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

where: p_i= allele frequency of A_i; p_j =allele frequency of A_j .

RESULTS AND DISSCUSION

The KLF3 gene in the Indonesian FH cattle was successfully amplified using a pair of primer designed by Yin *et al*. (2010). The results indicated that amplification fragment had good specificity (Figure 1), which could proceed directly to sequencing process. The PCR products of the KLF3 gene in this study indicated the same size as reported by Yin *et al*. (2010) i.e. 329 base pair (bp) approximately.

The DNA sequence alignment analysis revealed that there are two SNPs found in the Indonesian FH cattle KLF3 gene (Figure 2), i.e. SNP g.59607486delC and SNP g.59607554A>G. Based on the KLF3 gene sequence available in the GenBank (AC_000163), two SNPs that occurred in the Indonesian FH cattle were located in the intron region. In consequence, the KLF3 gene polymorphism in this study was intron region polymorphism. The SNP g.59607554A>G in the Indonesian FH cattle population can be detected by using HpyCH4IV restriction enzyme in the PCR-RFLP analysis. In previous study, Yin *et al*. (2010) have identified one single nucleotide polymorphism (SNP) in the fourth exon of the KLF3 gene i.e. SNP g.59607644G>C that was associated to milk production traits in Chinese Holstein, but this was not found in the Indonesian FH cattle.

The result of PCR-RFLP analysis revealed that the KLF3 gene in the Indonesian FH cattle were polymorphic with three genotypes (AA, AG, and GG). Each genotype was identified based on the differences in size and the number of bands (RFLP product) that appear in the visualization process (Figure 3). On the A allele, the base A at the position of 59607554 were not changed so that the HpyCH4IV enzyme could not recognize the restriction site and could not cuts the DNA fragments. While on the G allele, the HpyCH4IV enzyme recognize the restriction site at the position of 59607554 due to base substitution (A to G) and cuts the DNA fragments into two fragments (60 and 290 bp).

The allele and genotype frequencies were calculated (Table 1) and found that the highest

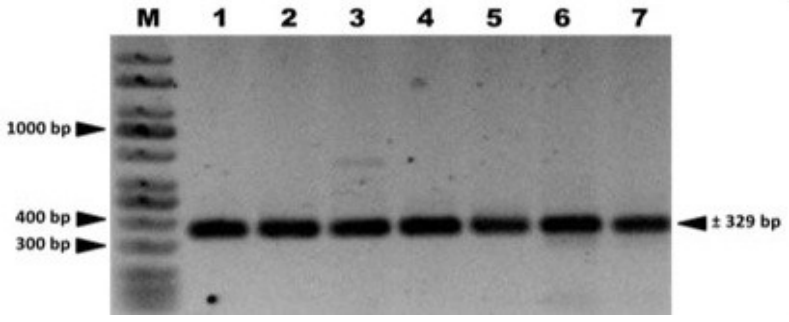

Figure 1. Photograph of Gel Electrophoresis of the PCR Product (M=100 bp Ladder Size Standard; 1-7=samples)

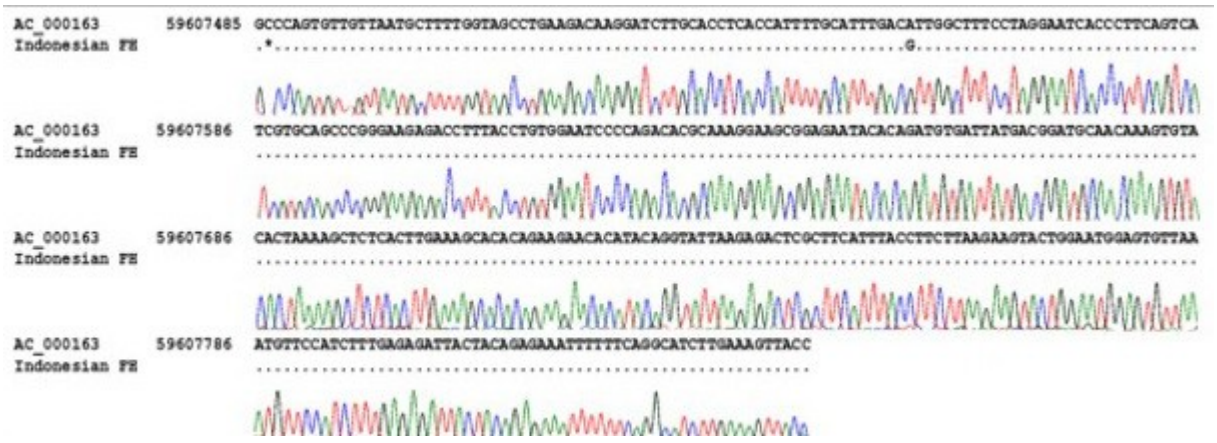

Figure 2. The Nucleotide Differences Based on the 2 SNPs between the Indonesian FH Cattle KLF3 Gene and *Bos taurus* (Hereford) KLF3 Gene Sequence in the GenBank with Accession Number AC_000163; [.] Symbol in the Indonesian FH Cattle Sequence Represent the Same Nucleotide with GenBank sequence; [*] Symbol Represent the Insertion or Deletion

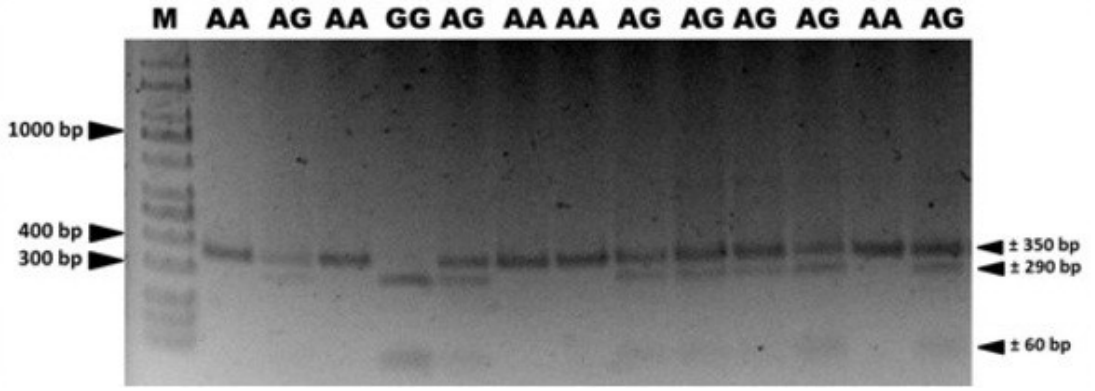

Figure 3. Photograph of Gel Electrophoresis of the RFLP Product (M=100 bp Ladder Size Standard; AA=Genotype AA, GG=Genotype GG, AG=Genotype AG).

genotype frequency in the Indonesian FH cattle was AA (0.73) while the lowest was GG (0.03). In general, the H_o value of the KLF3 gene was lower than the H_e value (0.24<0.25), indicating that the genetic diversity in the Indonesian FH cattle KLF3 gene is quite low and the population was not in *Hardy-Weinberg* equilibrium. Based on PIC value, diversity level of the KLF3 gene in this study was also in low level (PIC value=0.22). The low level of diversity of the KLF3 gene in this study can be caused by several factors, including selection and assortative mating (Cervini *et al*., 2006), limitation in the number of sires that used in the farms (Agung *et al*., 2017), and also *inbreeding* factor (Loeschche *et al*., 1994). In addition, the low frequency of GG genotypes and also G allele in this study can be an indication there has been a selection process in the Indonesian FH cattle population. However, the H_o value was higher than H_e value in West Java subpopulation. This condition can be an indication that random mating still happened in the FH cattle population in West Java.

The utilization of genetic markers in order to achieve breeding objectives was highly dependent on its polymorphism and its association with dairy cattle productivity parameters (e.g. milk yield, protein, fat, total solid, total solid non-fat, and

Table 1. The allele and Genotype Frequencies of the KLF3 Gene in the Indonesian FH Cattle

Samples	n	Genotype Frequency			Allele Frequency		H_o	H_e	PIC
		AA	AG	GG	A	G			
WJ	138	83 (0.60)	50 (0.36)	5 (0.04)	0.78	0.22	0.36	0.34	0.28
CJ	34	21 (0.62)	10 (0.29)	3 (0.09)	0.76	0.24	0.29	0.37	0.30
EJ	130	117 (0.90)	12 (0.09)	1 (0.01)	0.95	0.05	0.09	0.10	0.10
Total	302	221 (0.73)	72 (0.24)	9 (0.03)	0.85	0.15	0.24	0.25	0.22

WJ = West Java; CJ = Central Java; EJ = East Java; n = individuals genotyped; H_e= expected heterozygosity; H_o= observed heterozygosity; PIC = polymorphism information content; n = number of samples

lactose percentage). Therefore, validating the genetic markers of milk production traits is the initial step to establish the marker assisted selection (MAS) programme in the Indonesian FH cattle. In order to ensure the KLF3 gene utilization in the future, the genotypes in this study can be used for conducting the association study in larger cattle populations.

CONCLUSION

It can be concluded that the KLF3 gene was polymorphic with two SNPs were found in the intron region (SNP g.59607486delC and SNP g.59607554A>G). The genotypes of KLF3 gene based on SNP g.59607554A>G can be detected using RFLP method with HpyCH4IV restriction enzyme and resulted three genotypes (AA, AG, and GG) but its heterozygosity value was low in the Indonesian FH cattle. Information about the KLF3 gene polymorphism in this study can be considered for further study to analyse its association with milk and protein yield traits.

ACKNOWLEDGMENTS

This research was funded by Research Center for Biotechnology-Indonesian Institute of Sciences through DIPA LIPI scheme (0363/IPH.2/HK.03.01/2014). Authors would like to thank all the breeding staff in PT. KAR farm (West Java), the farmers in Boyolali (Central Java) and Malang (East Java) for providing the samples of animal. The authors are grateful to H. Nugroho and W. A. Septian for their excellent assistance.

REFERENCES

Agung, P.P., S. Anwar, W.P.B. Putra, M.S.A. Zein, A.S. Wulandari, S. Said and A. Sudiro. 2017. Association of growth hormone (gh) gene polymorphism with growth and carcass in sumba ongole (so) cattle. J. Indonesian Trop. Anim. Agric. 42(3):153-159.

Bell-Anderson, K.S., A.P. Funnell, H. Williams, H.M. Jusoh, T. Scully, W.F. Lim, J.G. Burdach,K.S. Mak, A.J. Knights, A.J. Hoy, H.R. Nicholas, A. Sainsbury, N. Turner, R.C. Pearson and M. Crossley. 2013. Loss of krüppel-like factor 3 (klf3/bklf) leads to upregulation of the insulin-sensitizing factor adipolin (FAM132A/CTRP12/ C1qdc2). Diabetes 62: 2728–2737.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Cervini, M., F. Henrique-Silva, N. Mortari and E. Matheucci Jr. 2006. Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). Genet. Mol. Biol. 29:486-490.

Eaton, S.A., A.P. Funnell, N. Sue, H. Nicholas, R.C. Pearson, and M. Crossley. 2008. A network of Kruppel-Like factors (Klfs). Klf8 is represses by Klf3 and activated by Kif1 in vivo. J. Biol. Chem. 283:26937-26947.

Himeda, C.L., J.A. Ranish, R.C. Pearson, M. Crossley and S.D. Hauschka. 2010. KLF3

regulates muscle-spesific gene expression and synergizes with serum response factor on KLF binding sites. Mol Cell. Biol. 30:3430-3443.

Lestari, D.A., L. Abdullah and Despal. 2015. Comparative study of milk production and feed efficiency based on farmers best practices and national research council. Media Peternakan 38(2):110-117.

Loeschcke, V., J. Tomiuk, and S.K. Jain. 1994. Conservation Genetics. Birkhauser Verlag. Berlin.

Martono, S., W. Negara, R.A. Gopar and M.N. Rofiq. 2016. Combination effect of feed supplements on milk yield and milk quality of dairy cattle. Journal of Advanced Agricultural Technologies 3(2):136-139.

Ministry of Agriculture of the Republic of Indonesia. 2017. Livestock and Animal Health Statistics 2017. Direktorat Jenderal Peternakan dan Kesehatan Hewan. Jakarta.

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press. New York.

Ribeca, C., V. Bonfatti, A. Cecchinato, A. Albera, L. Gallo and P. Carnier. 2014. Effect of polymorphisms in candidate genes on carcass and meat quality traits in double muscled Piemontese cattle. Meat Sci. 96: 1376-1383.

Sue, N., B.H. Jack and M. Crossley. 2008. Targeted disruption of the basic kruppel-like factor gene (klf3) reveals a role in adipogenesis. Mol. Cell. Biol. 28:3967-3978.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.

Widyobroto, B.P., Rochijan, C.T. Noviandu and A. Astuti. 2018. Dairy cows productivity and sosio-economic profile of dairy smallholder's communities in Yogyakarta, Indonesia. Proceedings, International Ruminant Seminar: "Eco-friendly livestock production for sustainable agriculture", Semarang, 24 October 2017. P. 1-6

Yin, Z., G. Mei, Y. Liu, X. Ding and Q. Zhang. 2010. Polymorphism identification and association with milk production traits of klf3 gene in a Chinese holstein population. J. Anim. Vet. Adv. 9(22): 2784-2787.