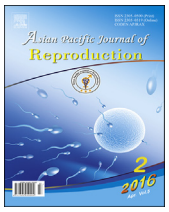
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2016.01.010

A new nucleotide variant G1358A potentially change growth differentiation factor 9 profile that may affect the reproduction performance of Friesian Holstein cattle

Afifi Inayah[1], Sri Rahayu[1], Nashi Widodo[1*], Widya Ayu Prasdini[2]

[1]*Biology Department, Mathematics and Science Faculty, Brawijaya University, Malang, East Java, Indonesia*

[2]*Training Center for Animal Husbandry, Batu, East Java, Indonesia*

ARTICLE INFO

Article history:
Received 1 Aug 2015
Received in revised form 10 Oct 2015
Accepted 12 Nov 2015
Available online 23 Jan 2016

Keywords:
BMP-15
Folliculogenesis
GDF-9
In silico
Docking molecule

ABSTRACT

Objective: To determine the polymorphism of *GDF-9* gene in FH cattle that may affect its interaction with the BMP-15 protein.
Method: Blood was taken from the jugular vein using 10 mL sterile syringes from ten Friesian Holstein cattle. The DNA were isolated from the whole blood and used as a template to amplify *GDF-9* gene. The Amplicon (PCR product) was sequenced to identify the new SNP. The three-dimensional structure of *GDF-9* protein was modeled by SWISS-MODEL. The characteristic of the protein structure was analyzed by using projectHOPE. The binding affinity *GDF-9* into MBP was examined by PatchDock and FireDock.
Results: The result indicates a new variant G1358A changed amino acid residue at position 435 from Arginine into Histidine (R453H). *In silico* analysis using projectHOPE predicted that the variant altered side chain of *GDF-9* and changed its interaction with BMP-15. Further study suggested that the polymorphism R453H also reduce the binding affinity of the *GDF-9* into BMP-15 from −85.58 kcal/mol (R453) into −80.79 kcal/mol (H453).
Conclusion: The new variant G1358A at an estrous FH a cattle has potency alter *GDF-9* profile that may affect to reproduction performance.

1. Introduction

GDF-9 is a member of Transforming Growth Factor β (TGF-β) superfamily [1,2] that has a significant role in follicle growth and development at all stages of folliculogenesis [3]. *GDF-9* could influence the initiation of primordial follicle [4] in rat [5] and human ovaries [6]. *GDF-9* promotes follicular survival by suppressing granulosa cell apoptosis and follicular atresia [7]. Therefore, *GDF-9* regulates proliferation, differentiation and cumulus expansion of granulosa cells [8,9] which can influence oocyte maturation. Also, *GDF-9* may also involve luteinization of the follicle at ovulation [10]. Knockout mice are lacking *GDF-9* lead to infertility [11].

GDF-9 gene consist of 2 exons and 1 intron [1,12,13]. Exon 2 encoding an entire mature peptide of protein [14] and mutation in the region may affect the reproduction performance in sheep. Polymorphism of *GDF-9* has lower ovulation rate in Moghani and Ghezel sheep [15]. Homozygote mutation S395F can lead to sterility in Cambridge and Belclare sheep [16]. Mutation S109R also has an effect on the fecundity of Thoka sheep [17]. Also, a mutation at position V371M has associated with litter size in Norwegian White Sheep.

The SNP is likely to affect the three-dimensional structure of the *GDF-9* protein [18] and reduce its binding capacity with other protein that may influence the phenotype [19]. *GDF-9* is important to interact with receptor or dimer protein that is BMP-15 protein [20] that related to fertility performance. The *GDF-9* gene can be a candidate gene for determining the fertility of livestock [12]. Therefore, polymorphism of *GDF-9* in exon 2 is important to be evaluated to explore biomarker of cattle fertility.

2. Material and methods

2.1. Amplification GDF-9 gene

This blood samples were collected from 10 Friesian Holstein (FH) cattle from Training Center for Animal Husbandry, Batu –

*Corresponding author: Nashi Widodo, Biology Department, Brawijaya University, Jl Veteran, Malang 65145, Indonesia
E-mail: Widodo@ub.ac.id
Peer review under responsibility of Hainan Medical College.

Indonesia. Three of samples have low performance that is anestrous while the rest of samples have normal performance. Then blood was taken from each FH cattle from the jugular vein using 10 mL sterile syringes. Obtained blood is placed in an EDTA-vacutainer and stored at −20 °C. The DNA was isolated using NORGEN kit (#46300). Amplification *GDF-9* gene exon 2 using forward primer 5′AAGACTCTCCCTAGAGCTCCATACTC3′ and reverse primer 5′TAGAACTGCAATTCCACCCAAG3′ [21]. The PCR product was purified and sequenced in 1st Base co. Ltd., Malaysia.

2.2. Variant analysis

The sequences of each sample were aligned with *GDF-9 Bos taurus* (M_174681.2) sequence to find SNP in the sequence of samples. Nucleotide sequence translated into an amino acid sequence by using MEGA5 software to determine the SNP may result in amino acid alteration.

2.3. Prediction of polymorphism's effect

Tertiary structure of *GDF-9* protein was modeled by using homology method (SWISS-MODEL) based on BMP-3 (2QCQ) as a template. The protein model was validated by using Ramachandran Plot. The effect of polymorphic on a characteristic of protein structure was analyzed by using projectHOPE web server, pyMol and YASARA software. The binding affinity of the protein with its protein partner (MBP) was done by PatchDock and FireDock web server.

3. Results

Amplification of *GDF-9* gene exon 2 using the primers yielded an 1100 bp DNA. Sequencing results of the PCR product of the tens samples have 99–100% similarity to *GDF-9* gene of *Bos taurus* (GQ922451.1; NM_174681.2). Further sequence Alignment analysis indicated that one of the ten samples has variant in position 1358 (Figure 1). The variant is not recorded yet in the Gene Bank (NCBI) and occur in the anestrus cattle.

In silico analysis revealed the substitution of guanine into adenine (G1358A) has changed amino acid Arginine into Histidine at position 435. The amino acid residue located at the surface of protein and lies on the relevant domain to carry out the primary activity of the *GDF-9* protein. The alteration of arginine into histidine residue (R453H) may result in a shift rotation some residues and this effect to the shape or difference protrusions of the protein surface (Figure 2). Also, the project-HOPE analysis predicted the arginine residue involved in the ionic reaction, the alteration of the residue likely to interfere protein activity.

Further docking analysis showed that the substitution of Arginine into Histidine reduce the binding affinity of *GDF-9* into BMP-15 (Figure 3). The binding energy of *GDF-9* wild type (R453) and mutant (H453) with BMP-15 are −85.88 and −80.79, respectively. The docking protein analysis suggested that Histidine residue altered the binding pattern of *GDF-9* with BMP-15 and caused loosing 24 binding sites.

4. Discussion

The new variant G1358A at gene *GDF-9* was found in one among three samples of anestrous cattle. The variant altered amino acid residue 453 from Arginine into histidine (R453H) of *GDF-9*. The arginine usually involved in the ionic reaction, alteration of the residue into histidine may disrupt *GDF-9* function. The histidine reduced protein hydrophobicity and shifted rotation side chain of some amino acid that detracted protrusion on the protein surface. Therefore, the amino acid substitution possibly changed character and function of *GDF-9*. The GDF9 mutation has an effect on fertility and ovulation rate of Moghani and Ghezel sheep in Iran and Turkey [15]. The *GDF-9* was expressed in ovaries that regulate folliculogenesis in domestic ruminants [1].

Further investigated, we found that the variation is resided in heterodimer binding domain of *GDF-9*, which is important to bind with BMP-15. Study on protein–protein interaction using molecular docking showed that variation R453H changed the binding affinity and pattern of the *GDF-9* and BMP-15 complex. The Histidine residue caused loosing 24 bonds between *GDF-9* and BMP-15 compared to Arginine. So as the alteration Arginine into Histidine reduced the binding affinity of *GDF-9* with BMP-15 from −85.88 kcal/mol and −80.79 kcal/mol, respectively.

The changing of binding affinity and pattern of the complex protein may interfere cellular cascade on oocyte maturation. This is due to the heterodimer of the *GDF-9* and BMP-15 complex is required for normal follicular development and ovulation that necessary to regulate of mammals fertility [20–22]. Moreover, *GDF-9* promote granulosa cell to produce luteinizing hormone receptor that is essential to pre-ovulatory process [23]. The bone BMP-15 and *GDF-9* play crucial roles in determining folliculogenesis, and oocyte-secreted factors, ovulation rate in

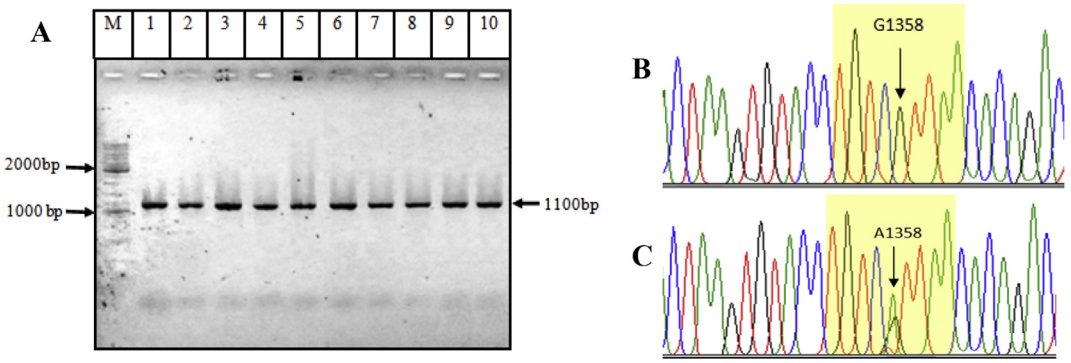


Figure 1. One of ten samples has new variant G1358A of *GDF-9* gene.
The PCR product of *GDF-9* gene (**A**) that have wild-type sequence (**B**) and new variant A1358 (**C**). M = DNA ladder 100–10 000 bp. Lane 1–10 = s PCR samples.

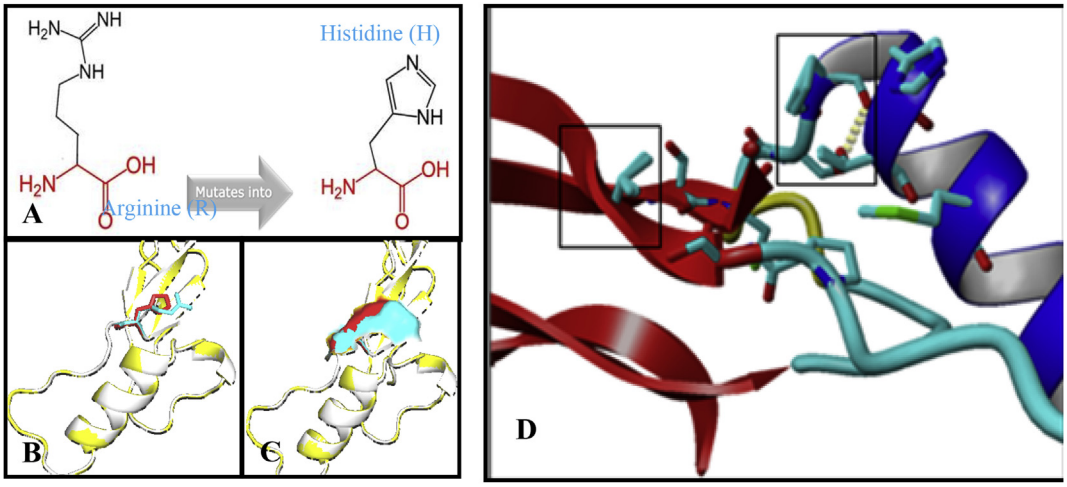
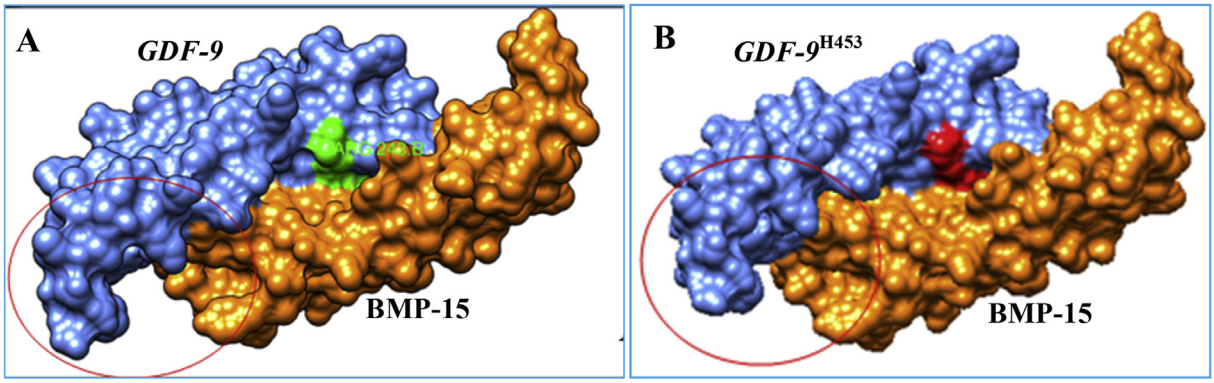


Figure 2. The variant G1358A of *GDF-9* gene changes Amino acid from Arginine into Histidine (**A**), that altered side chain (**B**) and the surface shape (**C**) of *GDF-9 wild type* (blue) into new variant (red). The amino acid residues substitution shifted the rotation of the two side chain (black box in panel D).



Parameters	*GDF-9 wild type*	*GDF-9 Variant*
Genotype	G1358 (Guanine)	A1358 (Adenine)
Residue	R453 (Arginine)	H453 (Histidine)
Residue Structure	Has no aromatic ring	Has aromatic ring
Charge of residue	Positive	Positive
Hydrophobicity (Kyte/ Doolittle scale)	-4,5	-3,2
Polar/Non-Polar	Polar	Polar
Binding Energy (kcal/mol)	-85,58	-80,79

Figure 3. The variant R453H changed binding pattern and affinity of *GDF-9* with BMP-15. *GDF-9 wild type* bound to BMP-15 (**A**) and *GDF-9*^{H453} (variant) bound to BMP-15 (**B**) that changed binding pattern (red circle) and energy (Table).

sheep and mice [25]. GDF9 and BMP15 form a complex intrafollicular regulatory system during folliculogenesis [24]. So the alteration on the BMP-15 binding domain of *GDF-9* may affect the fertility of FH.

The illustration is consistent with the data that all of normal FH in this study have not variant 1358A. Whereas the variant 1358A was found in anestrous FH, although only in one among three samples. The study suggested that the new variant G1358A has potentially influenced the fertility performance of FH cattle. The result warranted to be explored further for examining the potential of the variant as a biomarker in FH cattle reproduction performance.

The new variant G1358A at an estrous FH cattle more likely alter *GDF-9* profile and function that may affected to reproduction performance of FH cattle.

Declare of interest statement

The authors declare that there is no conflict of interests.

Acknowledgments

The author would like to thanks to Didik Huswo Utomo, who have helped in bioinformatics analysis.

References

[1] Bodensteiner KJ, Clay CM, Moeller CL, Sawyer HR. Molecular cloning of the ovine growth/differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. *Biol Reprod* 1999; **60**: 381-386.

[2] McGrath SA, Esquela AF, Lee SJ. Oocyte-specific expression of growth/differentiation factor-9. *Mol Endocrinol* 1995; **9**: 131-136.

[3] Otsuka F, McTavish KJ, Shimasaki S. Integral role of *GDF-9* and BMP-15 in ovarian function. *Mol Reprod Dev* 2011; **78**: 9-21.

[4] Vitt UA, McGee EA, Hayashi M, Hsueh AJW. In vivo treatment with *GDF-9* stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats 1. *Endocrinology* 2000; **141**: 3814-3820.

[5] Hayashi M, McGee EA, Min G, Klein C, Rose UM, van Duin M, et al. Recombinant growth differentiation factor-9 (*GDF-9*) enhances growth and differentiation of cultured early ovarian follicles. *Endocrinology* 1999; **140**: 1236-1244.

[6] Hreinsson JG, Scott JE, Rasmussen C, Swahn ML, Hsueh AJW, Hovatta O. Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. *J Clin Endocrinol Metab* 2002; **87**: 316-321.

[7] Orisaka M, Jiang JY, Orisaka S, Kotsuji F, Tsang BK. Growth differentiation factor 9 promotes rat preantral follicle growth by up-regulating follicular androgen biosynthesis. *Endocrinology* 2009; **150**: 2740-2748.

[8] Vitt UA, Hsueh AJ. Stage-dependent role of growth differentiation factor-9 in ovarian follicle development. *Mol Cell Endocrinol* 2001; **183**: 171-177.

[9] Wu X, Matzuk MM. *GDF-9* and BMP-15: oocyte organizers. *Rev Endocr Metab Disord* 2002; **3**: 27-32.

[10] Juengel JL, Hudson NL, Heath DA, Smith P, Reader KL, Lawrence SB, et al. Growth differentiation factor 9 and bone morphogenetic protein 15 are essential for ovarian follicular development in sheep. *Biol Reprod* 2002; **67**: 1777-1789.

[11] Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 1996; **383**: 531-535.

[12] Ghaffari M, Nejati-Javaremi A, Rahimi-Mianji G. Lack of polymorphism in the oocyte derived growth factor (GDF9) gene in the Shal breed of sheep. *South Afr J Anim Sci* 2009; **39**: 355-360.

[13] Tang KQ, Yang WC, Li SJ, Yang LG. Polymorphisms of the bovine growth differentiation factor 9 gene associated with superovulation performance in Chinese Holstein cows. *Genet Mol Res* 2013; **12**: 390-399.

[14] Aono T, Sugino H, Vale WW, (eds.). *Inhibin, activin and follistatin: regulatory functions in system and cell biology*. Norwell, Massachusetts: Springer Science & Business Media; 2012. Available at: https://books.google.com/books?id=7Iu1BwAAQBAJ&pgis=1 [Accessed on 15 August 2015]

[15] Barzegari A, Atashpaz S, Ghabili K, Nemati Z, Rustaei M, Azarbaijani R. Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. *Reprod Domest Anim* 2010; **45**: 666-669.

[16] Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, et al. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol Reprod* 2004; **70**: 900-909.

[17] Nicol L, Bishop SC, Pong-Wong R, Bendixen C, Holm LE, Rhind SM, et al. Homozygosity for a single base-pair mutation in the oocyte-specific GDF9 gene results in sterility in Thoka sheep. *Reproduction* 2009; **138**: 921-933.

[18] Wang TT, Ke ZH, Song Y, Chen LT, Chen XJ, Feng C, et al. Identification of a mutation in GDF9 as a novel cause of diminished ovarian reserve in young women. *Hum Reprod* 2013; **28**: 2473-2481.

[19] Våge DI, Husdal M, Kent MP, Klemetsdal G, Boman IA. A missense mutation in growth differentiation factor 9 (GDF9) is strongly associated with litter size in sheep. *BMC Genet* 2013; **14**: 1.

[20] Peng J, Li Q, Wigglesworth K, Rangarajan A, Kattamuri C, Peterson RT, et al. Growth differentiation factor 9:bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions. *Proc Natl Acad Sci U S A* 2013; **110**: E776-E785.

[21] Santos-Biase WKF, Biase FH, Buratini J, Balieiro J, Watanabe YF, Accorsi MF, et al. Single nucleotide polymorphisms in the bovine genome are associated with the number of oocytes collected during ovum pick up. *Anim Reprod Sci* 2012; **134**: 141-149.

[22] Knobil Ernst, Neill Jimmy D. *Knobil and Neill's physiology of reproduction*. Oxford: Gulf Professional Publishing; 2006.

[23] Elvin JA, Clark AT, Wang P, Wolfman NM, Matzuk MM. Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Mol Endocrinol* 1999; **13**: 1035-1048.

[24] Silva JRV, Van Den Hurk R, Van Tol HTA, Roelen BAJ, Figueiredo JR. Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. *Mol Reprod Dev* 2005; **70**: 11-19.

[25] Moore RK, Erickson GF, Shimasaki S. Are BMP-15 and *GDF-9* primary determinants of ovulation quota in mammals? *Trends Endocrinol Metab* 2004; **15**: 356-361.