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Revealing the Genetic Mutation in a Diprosopus Monauchenos Calf

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ABSTRACT

Diprosopus Monauchenos (*DM*) is one of congenital defect that can be caused by a genetical factor. This study was conducted to reveal the genetic mutation of *DM* cattle. Research showed that along 354 bp of partial BTA13 sequence (69,985,393th - 69,985,745th nucleotide) in *DM* and normal samples were obtained by sequencing analysis. Research showed that one insertion/deletion (indel) mutation at 28th and two transition mutations at 241th and 269th positions were detected in both samples. The obtained sequence in the present study was located at the intronic region of LPIN3 gene (69,972,281th - 69,991,534th nucleotide). In the future, a depth research to investigate the genetic mutation in *DM* animal with whole genome analysis is important to get the results accurately.

Keywords: cattle, *DM*, mutations, sequencing, BTA13

INTRODUCTION

Diprosopus or cranial duplication are structural and functional abnormalities that present at birth and is a rare craniofacial anomaly (Costa et al., 2013). They can affect a single structure or function, parts of various systems, or an entire system and may cause obstetrical problems (Long, 2001; Noden, 1985). The *Diprosopus* characterized by the presence of two faces with a single head on a single neck (Zaitoun *et al.,* 1999) which may be generally classified as attached symmetrical, or free or attached asymmetrical (Leipold *et al.,* 1972). These congenital defects are caused by genetic or environmental factors (infectious diseases, drugs, poisonings, plants, viruses, mineral salts and vitamins (A, D, E) deficiency, hormonal factors, and physical reasons) or by their interactions (Dennis & Leipold, 1986; Kaçar et al., 2008).

In human, *Diprosopus Menauchenos* (*DM*) syndrome can be caused by Sonic Hedgehog (SHH) gene (Vaidya et al., 2014). Recently, a molecular study to reveal the genetic mutation in *DM* cattle was not reported. Liu et al. (2019) reported that the copy number variance of SHH gene can be used for molecular selection because of highly correlated with body conformation traits in Chinese beef cattle. This study was carried out to reveal the genetic mutation in a *DM* Simmental calf born at 2/11/2019. The results study can be used as the early information to observe the genetic mutation in DM animal with an another advanced methods in the future.

MATERIALS AND METHODS

Animals Sample

Two DNA samples from positive (*DM*) and normal animals were used in this study for DNA analysis. Hence, the clinical observation in a *DM* sample was presented in Fig. 1. A *DM* sample in this study was occurred in the female Simmental calf,

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born on 2 November 2019 through artificial insemination (AI) at Imogiri District, Bantul Regency, Yogyakarta Province, Indonesia.

Fig. 1. The clinical observation in the *Diprosopus Monauchenos* calf (two face with one neck) with two eyes or retinas (R_1 and R_2) in the one eye socket (A). Each face has two nostrils (N), oral cavity (O), teeth (M) and tongue (T)

DNA Extraction

The blood sample (3-5 ml) was collected from jugularis vein of positive and normal samples using venoject tube and collected in a vaccutainer tube containing anticoagulant (K_3EDTA). The DNA extraction was performed with Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the producers instruction. Therefore, the extracted DNA was appropriately recorded and stored at -20°C for next analysis.

DNA Amplification

The DNA amplification was performed using a primer pairs of Forward: 5'- GAA CCA AGC TGG TGA AGG AC -3' and Reverse: 5'- TTG GTA GAG CAG CTC CGA GT -3'. This primer was designed using Primer 3 program (https://primer3plus.com.) based on the predicted *Bos taurus* SHH gene (GenBank: NC_037331.1). A PCR sample was conducted in 30 µL containing 7.8 µL of ddH_2O; 6 µL of DNA template; 0.60 µL of primer (1 pmol) and 15 µL of PCR kit Green Taq (Thermo Scientific, USA). The PCR program was performed in a Mastercycler gradient machine (Eppendorf, Germany). The PCR reaction was programmed with initial denaturation at 95°C for 2 min., and followed 36 cycles of denaturation at 95°C for 30 sec., annealing at 55°C for 1 min. 30 sec., extension at 72°C for 30 sec. and final extension at 72°C for 2 min. The PCR visualization was performed in the 1% of agarose gel with GelRed staining (Biotium, USA) and captured in GelBox Documentation system (Syngene, USA).

Sequencing and Sequence Analysis

Two PCR products (amplicons) from normal and positive (DM) sample were sequenced through commercial laboratory service (1st BASE Laboratory, Malaysia) using ABI Prism 96-capillary 3730xl DNA Analyzer (Applied Biosystems, USA). The identification of obtained sequence was performed using BLAST and Genome Data Viewer programs in NCBI (https://www.ncbi.nlm.nih.gov.). Thus, a molecular software of BioEdit package was used for alignment analysis between obtained sequences and reference sequence of GenBank

RESULTS AND DISCUSSION

Results

Along 400 bp of the amplicons in the normal and positive samples were obtained as ilustrated in Fig.2. One insertion/detetion (indel) mutation was detected at 28th position and two transition mutations at 241th and 269th positions were detected in the observed sequence (Fig. 3). A mutation point at 241th position of normal sample was detected as the heterozygote AG genotype (Fig. 4). Actually, About 98% of the observed sequences were identified as the partial *Bos taurus* chromosome13 (BTA13) sequence (GenBank: LR962743) from 69,985,393th to 69,985,745th nucleotide (Fig. 3). Furthermore, the obtained sequences were located at the intronic region of LPIN3 gene of BTA13 (69,972,281th- 69,991,534th nucleotide) based on the Genome Data View program (Fig.5).

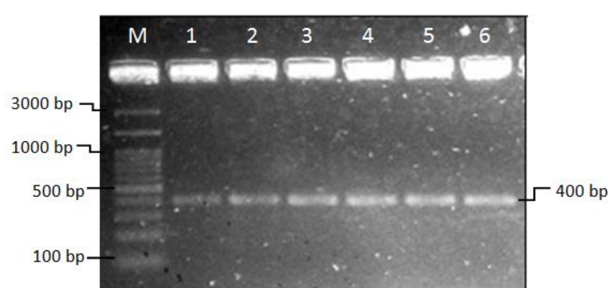

Fig. 2. The amplicons along 400 bp separated on 1% agarose gel in repeated normal sample (lane 1 & 2) and repeated positive sample (lane 3-6). M: DNA ladder 100 bp.

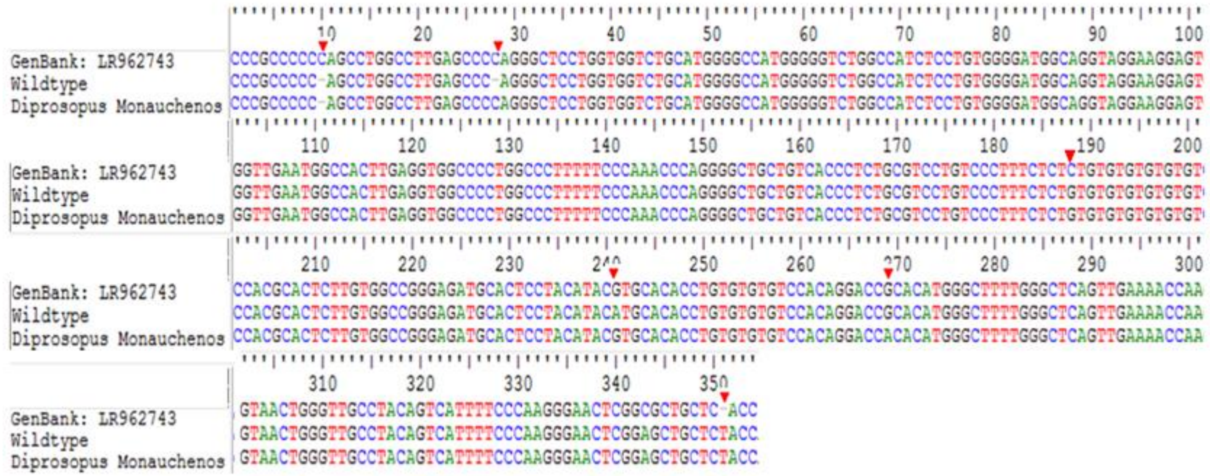

Fig. 3. The Sequence Alignment Along 354 bp in Positive and Normal Samples Compared to GenBank Showed Six Mutation Sites (arrow).

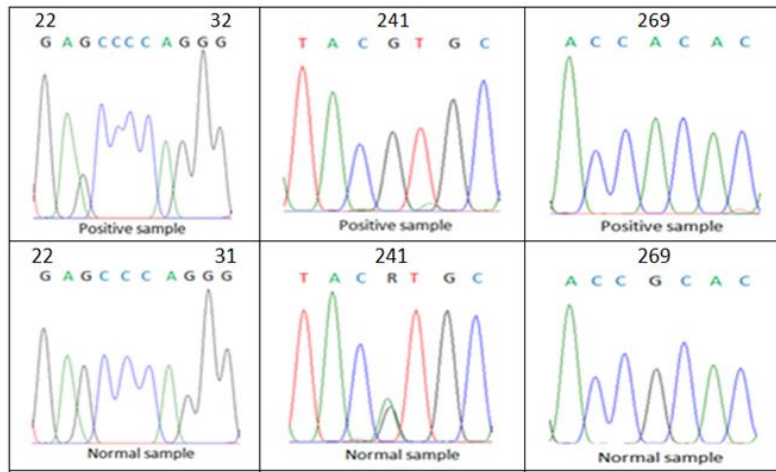

Fig. 4. Three Mutation Sites Were Detected in the Observed Sequences of Positive (above) and Normal (bottom) Samples. R: A/G

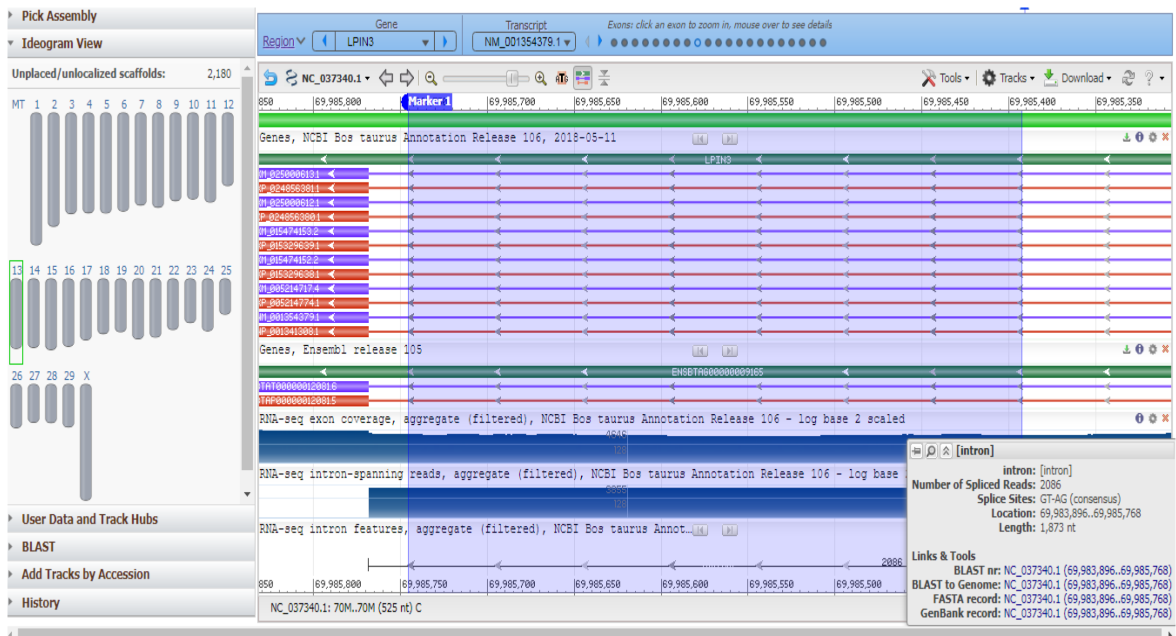

Fig 5. The Result of Genome Data Viewer Revealed That the Observed Sequences are Located at the Intronic Region of LPIN3 Gene (BTA13)

Discussion

The observed sequences in this study were located at *bovine* BTA13 based on the Genome Data View program. According to the NCBI information, the size of *Bovine* BTA13 was 83.47 Mb with 43.7% of GC ratio, 2,342 proteins, 1,185 genes and 105 pseudogenes. The observed mutation sites in the present study were occured in the LPIN3 gene at BTA13. This gene produces a Lipin protein that plays important role for cell metabolism. In the cytoplasm, Lipin is important to catalyze the dephosphorylation of phosphatidic acid to produce diacylglycerol, which is the precursor of both triglycerides and phospholipids. Lipin complexes are also thought to regulate gene expression as transcriptional co-activators in the nucleus (Han *et al.,* 2006; Donkor *et al.,* 2007). Recently, a privous study to detect the mutation site in LPIN3 gene of cattle was not reported.

In human, some mutation sites in the SHH gene were affected to *Holoprosencephaly* syndrome (Roessler *et al.,* 1996; Roessler *et al.* 1997; Odent *et al.* 1999; Nanni *et al.,* 1999; Schell-Apacik *et al.,* 2003; Aguinaga *et al.,* 2011). Despite of genetical factor, the craniofacial abnormalities in lamb can be born from ewe fed with highly alkaloid contain such as *Veratrum californicum* (Binns *et al.,* 1963). Fortunately, mutation at 250R can be detected using PCR-RFLP with *Nsp*I restriction enzyme (RCATG*Y). However, the case of *DM* can be prevented by good recording and feeding systems. Halverson *et al.* (2006) reported that the inbreeding level more than 12.5% included of high category. In Bali cattle (*Bos javanicus*), the inbreeding level of 12.5% has a negative effect such as low performance traits and still birth case (Putra and Muzawar, 2020).

CONCLUSION

The observed sequences in the present study were identified as the partial sequence of *bovine* BTA13 mainly at the intronic region of LPIN3 gene. A mutation site at 241th position can be detected with PCR-RFLP analysis with *Nsp*I restriction enzyme. In the future, the depth study with more sample of *DM* cattle with whole genome analysis are important to get the results accurately.

CONFLICT OF INTEREST

The authors whose names are listed have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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