Identification Single Nucleotide Polimorphism of Melanocortin 4 Receptor Gene in Madura Cattle

Prihandini P.W.[1,2], Sumadi[1], I.G. Suparta Budisatria[1] and Dyah Maharani*[1]
[1]Departement of Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Beef Cattle Research Station, Indonesian Agency for Agricultural Research and Development, The Ministry of Agriculture, Indonesia
*Corresponding author: d.maharani@ugm.ac.id

ABSTRACT

Signaling by the Melanocortin 4 Receptor (MC4R) is important for mediation the effect of leptin on food intake, homeostasis energy which effects to economic traits such as body weight, EBV and carcass weight. MC4R gene plays a key role in the hypotalamic control of food intake and energy balance. The aim of this study was to identify Single Nucleotide Polimorphism (SNP) of Melanocortin 4 Receptor (MC4R) gene in Madura cattle. Twenty seven Madura cattle were used in this study. Primers were designed based on alignment 11 Genbank in both Bos taurus, Bos indicus and Bos grunniens. The forward primer: 5'-GTCGGGCGTCTTGTTCATC-3'and reverse primer: 5'-GCTTGTGTTTAGCATCGCGT-3' were used to amplify 493 bp of PCR product. Single nucleotide polymorphism was detected by means of DNA sequencing. As a result, this study detected two SNPs of MC4R in exon region (g. 1108 C>T and g. SNP 1133 C>G). In case of g. 1133 C>G was missense mutation and 1108 C>T was silent mutation. SNP g. 1133 C>G was changed of amino acid valin (V) to leucine (L). Based on restriction enzyme mapping, HpyCH4IV can recognize the SNP in region 1133 C/G. The HpyCH4IV enzyme may be used for digesting of the targeted gene using PCR-RFLP method. Next, the SNPs may be used as a marker to be associated with growth traits and feed intake in Madura Cattle in the future study.

Keywords: Melanocortin 4 Receptor (MC4R), Madura cattle, Single nucleotide polimorphism (SNP)

INTRODUCTION

Madura cattle as one of the local Indonesian cattle which should preserve its existence and we need to increase production to meet the needs of meat in Indonesia. The Madura cattle *(Bos indicus)*, is an Indonesia native cattle which is considered belong to the crossing Zebu cattle and Bison breeds. In Indonesia, consumer demands are driving efforts to increase meat production and produce higher quality meat. Breeding and selection of a company with high potential for meat production or meat quality is incorporating molecular approaches, in particular the identification of selection markers. Knowledge of genetic polymorphisms that are involved in different quantitative traits, and increased understanding of how these polymorphisms interact with the environment or with other genes affecting economic traits is essential. In particular, the identification of genetic markers associated with such traits could contribute to an increased rate of genetic gain in farmed animals (Seong *et al*., 2012).

Melanocortin-4 receptor (MC4R) is a G-protein-coupled receptor with seven transmembrane domains that is highly expressed in the hypothalamus, a region of the brain intimately involved in appetite regulation (Zhang *et al*., 2009). MC4R signaling is important for mediating the effect of leptin on food intake and energy homeostasis. It is associated with obesity, energy homeostasis and control of feeding behavior (Dubern, 2015). In bovines, *MC4R* is located on chromosome 24; the gene has a length of 1,808 bp and one exon (GenBank accession No. EU366350.1) (Zhang et al., 2009).

MATERIALS AND METHODS

Animals and data collection

Twenty seven Madura cattle were used in this study (Figure 1). The animals were collected from Madura cattle farmers: Beef Cattle Research Station, Pasuruan, East Java and Pamekasan Distric, Madura Island, East Java. The cattle were reared under the same condition in a tradisional management by local farmers.

Single Nucleotide Polymorphism (SNP) identification

Primers were used based on Seong *et al*. (2012), the forward primer: 5'-GTCGGGCGTCTTGTTCATC-3' and reverse primer: 5'-GCTTGTGTTTAGCATCGCGT-3' were used to amplify 493 bp of PCR product in MC4R Gene (Figure 2). Reference DNA sequence were used from research result on Brahman cattle (Bos indicus) was saved on National Center for Biotechnology Information (NCBI) website and was did alignment using 11 Genbank in both Bos taurus, Bos indicus and Bos grunniens.

Sequence analyses

The PCR products which represented different PCR-RFLP genotypes, including both homozygous and heterozygous genotypes were purified with PCR DNA Purification Kit and sequenced using the 1st BASE DNA Sequencing Division (www.base-asia.com). Sequences were read using BioEdit 7,5 and aligned using web based on CLUSTAL-W (http://www.ebi.ac.uk/clustalw/ index.html) program. Identify SNP and heterozigosity base on chromatogram sequence (Figur 3).

RESULTS AND DISCUSSION

The DNA samples from 27 unrelated cattle were amplified and sequenced for the bovine MC4R gene. Two polymorphic sites (SNPs) were identified by sequencing. Two SNPs (g. 1108 C>T and g. SNP 1133 C>G) were detected in the exon 1 region (Figur 4). In case of g. 1133 C>G was missense mutation and 1108 C>T was silent mutation. SNP g. 1133 C>G was changed of amino acid valin (V) to leucine (L) (Figure 5). Previous studies have detected several SNPs (C293G, A193T, T192G, A129G) (Zhang *et al*., 2009), T927C (Valle *et al*., 2004); SNP C1133G (Maharani *et al*., 2016); C1108G (Thue *et al*, 2001) C927T, C1069G (Zhang *et al*., 2006); Val145Ala and Ala172Thr (Haegeman *et al*., 2001), in the MC4R gene of cattle, and have linked the SNPs to growth traits. Based on restriction mappping, HpyCH4IV was detected to recognize SNP in region 1133 C/G (Figure 6).

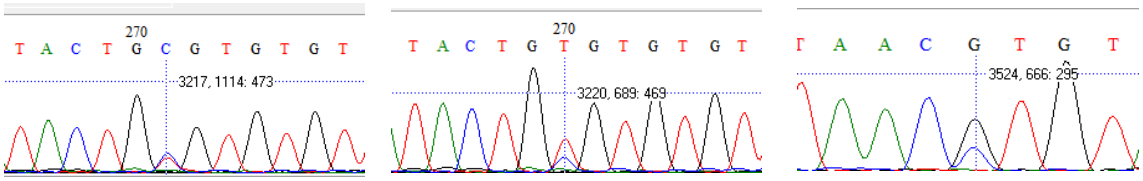


(a) **(b)** **(c)**

>MADURA 1

GTCGGGCGTCTTGTTCATCATTTACTCAGACAGCAGTGCTGTTATCATCTGCCTCATC
ACCGTGTTCTTCACCATGCTGGCTCTCATGGCGTCTCTCTATGTCCACATGTTCCTCA
TGGCCAGACTCCACATTAAGAGGATCGCGGTCCTGCCAGGTAGCGGCACCATCCGC
CAGGGCGCCAACATGAAGGGGGCGATTACCCTGACCATACTGATCGGGGTCTTTGTT
GTCTGCTGGGCCCCCTTCTTCCTGCACCTGATATTCTACATCTCTTGTCCCCAGAACC
CATACTGTGTGTGTTTCATGTCTCACTTTAACGTGTACCTCATCCTCATCATGTGCAA
TTCCATCATTGACCCTCTGATTTATGCCCTGCGGAGCCAAGAACTGAGGAAAACCTT
CAAAGAGATCATTTGTTGCTCTCCTCTAGGTGGCCTCTGTGATTTGTCTAGCAGATAT
TAAATGGGGACAAACGCGATGCTAAACACAAGC

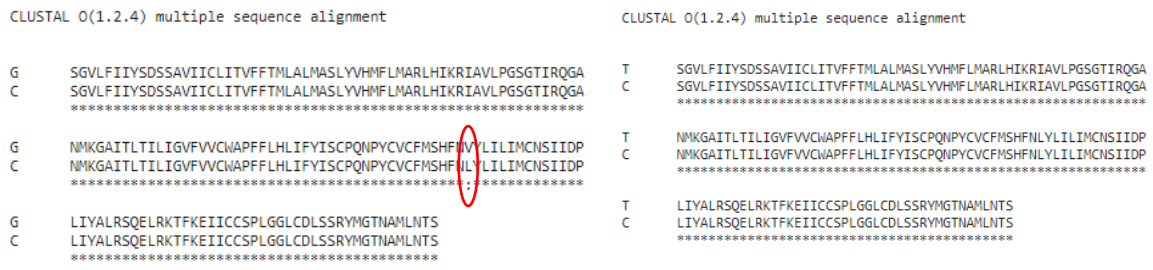
Figure 2. Target sequence result (493 bp) on MC4R gene



(a) **(a)** **(b)**

Figure 3. The chromatogram results: a. g SNP 1108 C>T, b. g SNP 1133 C>G with BioEdit programs

Figure 4. The SNP g. 1108 C>T dan g.1133 C>G detection using alignment method (Clustal W)



(a) (b)

Figure 5. The Amino Acid translation, a. In SNP g.1133 C>G (missense mutation Valin to Leusin) b. SNP g.1108 C>T (silent mutation)

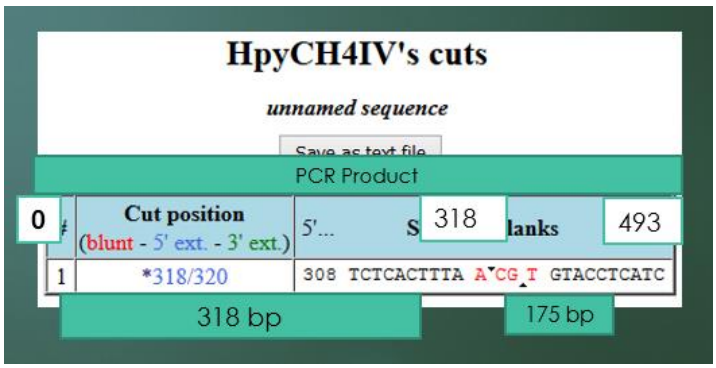


Figure 6. Restriction enzyme mapping (HpyCH4IV recognize SNP g.1133 C/G using NEBcutter ProgramsV2.0)

Restriction enzyme HpyCH4IV may be used for digesting with identifies the site 5'-A │CG │T-3' on both homologous chromosomal devices in PCR products that produce two GG alleles meant the animal having GG genotype. Individu with CC genotypes were marked with unbroken fragment size and remained at 493 bp while CG heterozygous animals were characterized by digested the fragments into 175, 318 and 493 bp.

CONCLUSIONS

Two SNPs (g. 1108 C>T and g. SNP 1133 C>G) of MC4R gene have been identified in Madura cattle. The HpyCH4IV restriction enzyme may be used for digesting the targeted gene using PCR-RFLP method. Moreover, the Single Nucleotide Polimorphisms identified may be used as a marker to genotype the Madura Cattle in the future study.

REFERENCES

Dubern B. 2015. MC4R and MC3R Mutations. In M.L. Frelut (Ed.), The ECOG's eBook on Child and Adolescent Obesity. Available on ebook. ecog-obesity. eu 2-7.

Haegeman, A., Coopman, F, Jacobs K, Mattheeuws M, Van Zeveren A and Peelman L. 2001. Bovine melanocortin receptor 4: cDNA sequence, polymorphisms and mapping. J. Anim. Genet. 32:189-192

Liu H, Tian W, Zan L, Wang H, Cui H (2010) Mutations of MC4R gene and its association with economic traits in Qinchuan cattle. J. Mol. Biol. Rep. 37(1):535-540

Maharani D., Sumadi, T. Hartatik, A. Fathoni dan M. Khusnudin. 2016. Asosiasi Gen MC4R terhadap Ukuran ukuran Tubuh Sapi Peranakan Ongole Kebumen Pada Sapi Lahir dan Sapih.. Fakultas Peternakan Universitas Gadjah Mada. Pros. ISSTAP (Indonesian Society For Sustainable Tropical Animal Production). ISBN 978-979-1215-28-2. Hlm: 65-70

Seong J., H. K. Lee, D. S. Suh, H. S. Kong and K. D. Park. 2012. Identification and analysis of MC4R polymorphisms and their association with economic traits of korean cattle (hanwoo). J. Mol. Biol. Rep. 5: 3597-3601.

Thue T.D., S.M. Schmutz and F.C. Buchanan. 2001. A SNP in the cattle MC4R gene is used to map MC4R to BTA 24. J. Anim. Genet. 32:390-391

Valle E., F.A Habermann, S.S. Moore, D.H. Crews, B.F. Benkel. 2004. Genomic localization and SNP discovery in the bovine melanocortin receptor 4 gene (MC4R). J. Anim.Genet. 35:350-359

Zhang CL., H Chen, YH Wang, XY Lan, L Zhang, Al Zhang. 2006. Association of a missense mutation of the MC4R gene with growth traits in cattle. Arch Tierz 49:515-516

Zhang, C. L., Y. H. Wang, H. Chen, X. Y. Lan, C. Z. Lei dan X. T. Fang. 2009. Association between variants in the 50-untranslated region of the bovine MC4R gene and two growth traits in Nanyang cattle. J. Mol. Biol. Rep. 36: 1839–1843