Linkage mapping of the Endothelin-1 gene to chicken chromosome 2

Mapa de ligamiento del gen Endotelina-1 en el cromosoma 2 del pollo

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Resumen

El objetivo de este estudio fue asignar por mapa ligamiento el gen Endotelina-1 (END1) al cromosoma 2 del pollo (GGA2). Los microsatélites MCW0314, MCW0245, ADL0164, MCW0264 y el diseño de cebadores fueron usados para la amplificación de secuencias específicas de END1. La técnica PCR-SSCP y el uso de geles denaturantes permitió la tipificación de los genotipos. Para la construcción del mapa se usaron los genotipos identificados en una población de 200 pollos Cobb500 utilizando el programa Mapmaker® version 3.0b. El resultado es un mapa del GGA2 donde END1 está ligado a los microsatélites reportados en un locus de un carácter cuantitativo (QTL) para susceptibilidad de los pollos de engorde a desarrollar el síndrome de hipertensión pulmonar.

Palabras claves: *síndrome de hipertensión pulmonar, pollos de engorde, microsatélites.*

Abstract

The aim of this study was assigned by linkage map the Endothelin-1 gene (END1) to the Chicken chromosome 2 (GGA2). The microsatellites MCW0314, MCW0245, ADL0164, MCW0264 were used and primers for amplification of specific sequences END1 were designed. The PCR-SSCP technique, and the use of denaturing gels allowed the characterization of the genotype. The genotypes identified in 200 broilers Cobb500 and Mapmaker® version 3.0b program were used in the map construction. The result, a map in which END1 is linked to microsatellites that it was reported in quantitative trait loci (QTL) for susceptibility of broilers to develop pulmonary hypertension syndrome in the GGA2.

Keywords: *Pulmonary Hypertension Syndrome, broilers, microsatellites.*

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Introducción

Pulmonary Hypertension Syndrome (PHS) affects approximately 25% of broilers production around the world (Hassanzadeh et al., 2014). Syndrome includes heart failure, ascites and develop pulmonary arterial hypertension by vasoconstriction and vascular remodeling of small pulmonary arteries (Buzala et al., 2015). Broilers breeders are more predisposed for intensive genetic selection of economically important production traits like feed efficiency and carcass quality (Kalmara et al., 2013; Hassanzadeh et al., 2014; Resnyk et al., 2015). PHS is considered polygenic resulting from the susceptibility genes and environmental factors combination (Binder, 2007; Wideman et al., 2013). Candidates genes selected to study the PHS are physiologically implicated in blood pressure regulation *e.g*. Endothelin-1 (EDN1) (Gomez et al., 2007; Flores, 2013). In broilers, EDN1 gene is found on chromosome two (GGA2; Gene ID: 420854) (Moncaleano-Vega et al., 2013). The detection of sequence changes in this candidate gene will lead the identification of polymorphisms and it is used in marker linkage analysis, characterization of economic importance loci and detection of mutation causing disease in domestic livestock species. The PCR coupled with SSCP (single strand conformation polymorphism) analysis and denaturing condition gels can be used to exclude defined mutation from large family groups (Ariza et al., 2001) or non- family groups (Inesta et al., 2005). The aim of this study was assignment by linkage mapping the END1 with quantitative trait loci (QTL) ´s microsatellites reported for PHS susceptibility broilers to GGA2.

Materials and methods

DNA of 200 broilers Cobb500 line (DNA bank of Cytogenetic laboratory, Faculty of Veterinary

Medicine and Zootechnics, Universidad Nacional de Colombia, Bogotá, Colombia), oligonucleotide primers of END1 (intron 1th) were derived from sequences present in the Ensembl database (http://www.ensembl.org/Gallus_gallus/Gene/Summary?db=otherfeatures;g=420854;r=2:62883381-62888254;t=XM_418943.4), it was design with tool web free primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) and used in the SSCP analysis. The microsatellites primers were taking from Rabie et al. (2005) and used denaturants gels to analysis (Table 1).

Table 1. Sequence of primers for PCR amplification of 1 locus on GGA2

Marker	Longitude pb	Primer forward 5′-3′	Primer reverse 5′-3′	Tm
***MCW0314**	278-84	GCCAGGCTACACCTCTTCTAG	GTTGGTATGATGGTATGATGC	58.5°C
***MCW0245**	284-90	ATCTATGGCCACCTCAAACTG	GATCTGTGCTGAACACAGCAG	61°C
***ADL0164**	150-200	TCCTCAGGCCTTTCAACATA	GGTAGCATGAACAAAGCATC	58°C
***MCW0264**	208-63	AGACTGAGTCACACTCGTAAG	CTTACTTTTCACGACAGAAGC	56°C
****ET1**	150	AGAGACAACATGAAAGTCACC	TGCGGGATCATCAGAAGTAG	56°C

*Rabie et al. (2005).
**Primers were design with intron 1th sequences stored in the Ensembl database using primer3 web tool.

Polymerase chain reaction were performed in a volume of 12.5 ul, using 25 ng of chicken genome DNA, 20 ng of each of the forward and reverse primers, 12.5 mM of each of dATP, dGTP, dTTP, dCTP and 0.3 U Taq polymerase. PCR amplifications were obtained using the following protocol: 95 °C for 3 min, then 30 s at 95°C, 60 s between 56 and 61°C, 90s at 72°C for 30 cycles, finally 72°C for 10 min. Products were assayed by electrophoresis in 6% Bis-acrylamide nondenaturing and denaturing. DNA fragments can be visualized silver straining method (Figura 1).

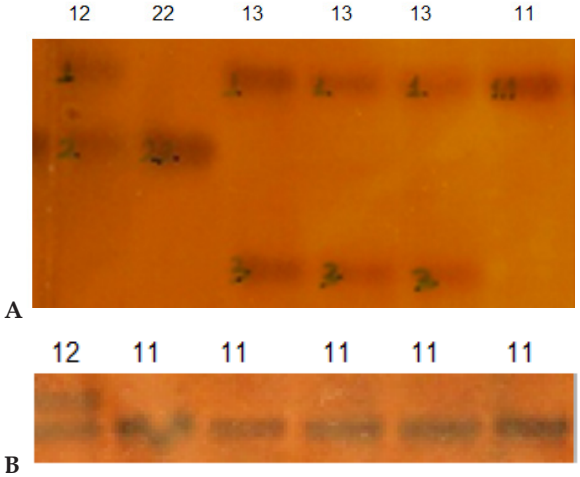

Figure 1. (A) END1 Gel conditions for the SSCP technical were 5% of glycerol, 49:1 bis-acrylamide 6% concentration and 8 Watts during 15 h. Genotypes 12, 22, 13 and 11. (B) Gel condition denaturant for microsatellites, 29:1 bis-acrylamide 6% concentration and 65 – 75 Watts during 2.3 - 3.3 h. Genotypes 11 and 12. Silver straining.

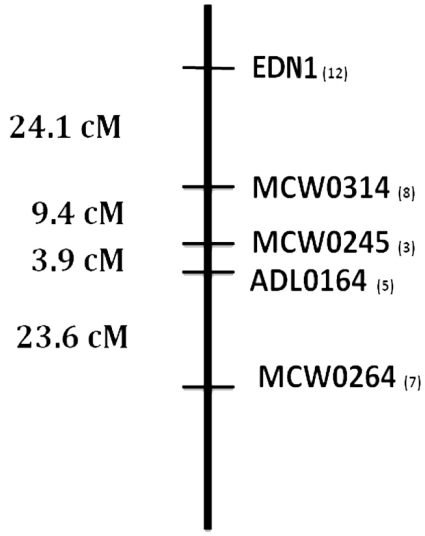

Figure 2. The Endothelin-1 gene (END1) was linked to MCW0314, MCW0245, ADL164 and MCW0264 microsatellites located at the end of chromosome 2 (GGA2). The markers have been ordered and the number to left indicates their position in distance Kosambi cM. These possible locations of the loci whose order is supported by LOD score > 3.0 and $\theta < 0.5$.

The MAPMAKER® version 3.0b program was used to determine locus order and map linkage on GGA2 with a Lod score greater than 3.0 and recombination frequency less 50 cM ($P < 0.001$) for each locus.

Results and discussion

The END1 gene was linked to MCW0314, MCW0245, ADL164 and MCW0264 microsatellites

on GGA2 ($p < 0.001$). The small linkage group of the consensus map is showing in Fig. 2. The MCW0314, MCW0245, ADL164 and MCW0264 microsatellites have been reported by Groenen et al. (2000), Schmid et al. (2000), and used to build QTL genetic map on GGA2 in broiler whit susceptibility to develop the PHS (Rabie et al., 2005). In human, the mutations in this gene are associated with blood pressure increment, and two SNP (single nucleotide polymorphisms are related: G and T (Tanaka et al., 2004; Lee et al., 2016). The polymorphism at codon 198 in exon 5 interacts with body mass index in association with BP (Jin et al., 2003).

The orthologous endothelin-1 gene human is known and conserved homology between chicken and human gene sequence (http://www.ensembl.org/Gallus_gallus/Gene/Compara_Ortholog). The increment expression END1 gene, mapping on chicken chromosome 2 in this study, has been associated at pathophysiology of PHS in broilers (Gomez et al., 2007; Wideman et al., 2013; Hassanzadeh et al., 2014; Buzala et al., 2015). However, many genes influence blood pressure, we hope to sequent SNP in this candidate gene and other like NOS3 and HIF1A genes become to association genetic with the PHS and suggested the specific combinations of alleles maybe contribute to the control this disease in broilers.

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