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Allele distribution and testing for association between an oxygen dependent degradation domain SNP in *EPAS1* and pulmonary arterial pressures in yearling Angus cattle

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A B S T R A C T

At altitudes > 1500 m, measurements of mean pulmonary arterial pressures (mPAP; mmHg) are a measure of pulmonary hypertension (PH) in cattle. Genotypes of a G/A single nucleotide polymorphism (SNP; rs208684340) in the endothelial PAS domain protein 1 (*EPAS1*) gene were determined for mPAP categories (low, moderate, high) on Angus cattle. The A allele of this SNP was postulated to be associated with altitude-induced PH. The first objective was to estimate allele and genotypic frequencies in Angus cattle at high- (elevation 1850 to 2800 m) and low-altitude (elevation 91 to 1192 m) ranches (n = 118; random sample of 1275). Percent of cattle at these low elevation ranches with genotypes G/G, G/A, and A/A were 64.4, 33.9, and 1.7%, respectively, and a minor allele frequency (MAF; A allele) of 18.6%. Bulls, steers, and heifers (n = 691) from 4 high-altitude Angus herds in Colorado and Wyoming were genotyped. Percent of cattle with genotypes G/G, G/A, and A/A were 48.6, 34.3, and 17.1%, respectively, with a MAF of 34.2%. Average mPAP was 40.5 ± 8.8 mmHg. Low, moderate, and high-mPAP category MAF were similar (χ^2 = 3.03; P = 0.22), with values of 35.4, 31.0, and 37.8%, respectively. Additionally we sought to evaluate the genotype-to-phenotype association of this SNP with mPAP phenotypes (n = 532) from the Colorado State University Beef Improvement Center (elevation 2150 m) Angus herd. Linear mixed model analysis suggested genotype was not a predictor (P = 0.61) of mPAP. Results do not suggest the A allele of SNP in *EPAS1* is associated with high altitude-induced PH in yearling Angus cattle.

1. Introduction

Pulmonary arterial pressures are a measure of pulmonary hypertension (PH), which could be due to thickening of intimal, medial, and adventitial layers of the pulmonary artery from fibroblast proliferation as well as smooth muscle and endothelial cell hypertrophy (Pugliese et al., 2015a; Shimoda and Laurie, 2013; Stenmark et al., 2009). Workload of the right ventricle and blood pressure increases to overcome these physiological changes (Ryan et al., 2015). Due to its moderate heritability (h^2 = 0.26 to 0.34), mean pulmonary arterial pressures (mPAP) have been utilized in selection procedures of cattle at high altitudes to reduce the incidence of PH and potential for right heart failure (RHF) (Crawford et al., 2016; Shirley et al., 2008). Historically, RHF most commonly occurred in herds at high altitude (> 1500 m) with incidence of 3 to 5% in *Bos taurus* cattle native to high altitude (Holt and Callan, 2007).

Previous research by Newman et al. (Newman et al., 2015) studied cattle from high altitude with high and low mPAP measures. Two single nucleotide polymorphisms (SNP) within the oxygen dependent degradation domain (ODDD) of the endothelial PAS domain protein 1 (*EPAS1*) gene were discovered (Fig. 1) and suggested to be associated with PH. Due to linkage disequilibrium between the 2 SNP, we chose to study the population distribution of the G/A SNP (rs208684340) within this gene on *Bos taurus* chromosome 11, where the A allele was

Abbreviations list: ANOVA, analysis of variance; CSU-BIC, Colorado State University Beef Improvement Center; *EPAS1*, endothelial PAS domain protein 1 gene; HIF, hypoxia-induced factor; HWE, Hardy-Weinberg equilibrium; mPAP, mean pulmonary arterial pressure; NFW, nuclease free water; N-TAD, N-terminal transactivation domain; NTC, non-template control; ODDD, oxygen-dependent degradation domain; PCR, polymerase chain reaction; PH, pulmonary hypertension; RHF, right heart failure; RXN, reaction mix; SNP, single nucleotide polymorphism; TE, Tris-EDTA; QC, quality control

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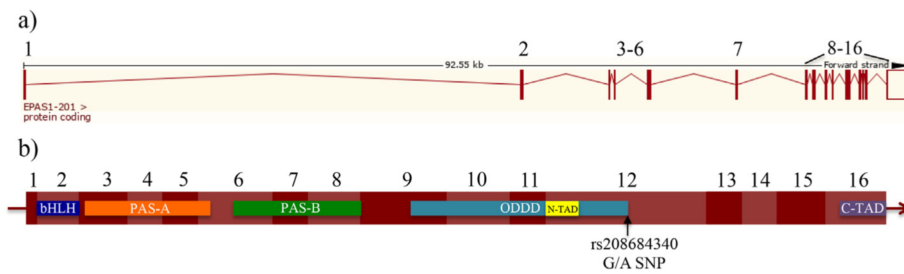

Fig. 1. Maps of protein coding (exon) regions of *EPAS1* located on *Bos taurus* chromosome 11. a) Ensemble image of the 16 exonic regions of *EPAS1*. b) Domain locations along the 16 exonic regions (light and dark red) of *EPAS1* and location of the G/A variant within the ODDD.
EPAS1, endothelial PAS domain protein 1 gene.
bHLH, Basic Helix-Loop-Helix (blue).
PAS, Per-Arnt-Sim domains (orange and green).
ODDD, oxygen-dependent degradation domain (turquoise).
N-TAD, N-terminal transactivation domain (yellow).
C-TAD, C-terminal transactivation domain (purple).
http://uswest.ensembl.org/Bos_taurus/Gene/Summary?db=core;g=ENSBTAG00000003711;r=11:28576347-28668899;t=ENSBTAT00000004836. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

proposed to be associated with PH. The objectives of this study were to survey genotypes in Angus cattle: 1) at high altitude ranches (elev. 1850 to 2800 m), 2) at low altitude ranches across the U.S. (elev. 91 to 1192 m), and 3) to assess the potential association of this SNP with mPAP phenotypes.

2. Materials and methods

2.1. Cattle and mPAP

Angus cattle from four beef production systems located at high altitude were used for the first objective. Mean PAP was measured by a state licensed veterinarian (Holt and Callan, 2007). Summary statistics for mPAP and number of sires for each high altitude ranch are described in Table 1. Additionally, a potential consequence high mPAP is High Mountain Disease. Cattle have been previously described as low- (< 41 mmHg), moderate- (41 to 49 mmHg), or high- (> 49 mmHg) risk candidates for this disease (Holt and Callan, 2007). Thus these categories were used in the first objective.

The second objective utilized Angus cattle from low altitude beef production systems in California, Missouri, Iowa, Texas, and New Mexico (elevation 91 to 1192 m). These states were chosen due to their populations of Angus cattle and the availability of DNA samples for analysis. Of the 1275 available DNA samples, a random sample of 118 cattle were genotyped. Randomization was performed by selecting every 8th DNA sample from those available. Mean PAP measures are not routinely collected for cattle residing at these altitudes, and were therefore unavailable for this objective.

Cattle ($n = 532$) from the Colorado State University Beef Improvement Center (CSU-BIC) were utilized in the third objective to determine if an association exists between this G/A SNP and mPAP phenotypes. The association was conducted only with samples from the CSU-BIC herd due to limited sire connectivity between the other high altitude herds.

2.2. Sample DNA extraction and genotyping

The genotyping procedure included: sample collection, DNA extraction, and a TaqMan genotyping assay. Blood cards ($n = 659$) and hair samples ($n = 235$) were obtained for DNA extraction. The DNA from GeneSeek blood cards was extracted with wash and incubation methods. In brief, a single Harris uni-core 3.0 mm hole punch was taken from each blood card and placed in a 96-well plate. Nuclease-free water (NFW) at 100 µl was added to each well and incubated at 25 °C (room temperature) for a minimum of 30 min until the punches were whitish in color. Liquid was then removed and 50 µl of Tris-EDTA (TE) buffer was added to each well. The 96-well plates were incubated in a thermocycler (SimpliAmp, Thermo Fisher) at 95 °C for 30 min. Extracted DNA samples were stored at 4 °C refrigerator until used for genotyping.

Deoxyribonucleic acid from hair was isolated with the protocol described by Locke et al. (Locke et al., 2002). This protocol used 5 to 7 bulbs of hair. Five microliters of Proteinase K (2 mg/ml) was added to each sample, followed by 100 µl of hair lysis buffer. The hair lysis buffer consisted of 10 µl 10 × PCR Buffer (100 mM Tris-HCl, pH 8.3 at 25 °C; 500 mM KCl; 15 mM $MgCl_2$; 0.01% gelatin; Sigma-Aldrich), 10 µl of 25 mM $MgCl_2$, 0.5 µl Tween 20, and 79.5 µl NFW per reaction. Samples were incubated in a thermocycler at 60 °C for 45 min, then at 95 °C for an additional 45 min to denature the Proteinase K. Extracted DNA samples were refrigerated and stored at 4 °C until analyzed.

A TaqMan assay was utilized for genotyping the G/A SNP (rs208684340) in *EPAS1*. The assay yielded a 70 bp amplicon (Fig. 1). Design details of the assay were described in Newman et al. (Newman et al., 2015). The PCR preparation involved a 1 × reaction mix (RXN) of 2.25-µl Taq Master Mix (catalog #4371355, Thermo Fisher) and 0.5-µl 20 × SNP assay (part #4332077, Thermo Fisher). A 40 × SNP assay was diluted to a 20 × with equal parts SNP assay and TE. The 1 × PCR

Table 1
Descriptive statistics[a] of mean pulmonary arterial pressure (mPAP, mmHg) and the number of sires associated with each ranch, for single nucleotide polymorphism genotyped Angus cattle at high altitude.

Ranch Location	Item	n	Minimum	Q1[b]	Mean	Median	Q3[b]	Maximum	Standard deviation
Southern Wyoming (CSU-BIC[c]), elevation 2150 m	mPAP	532	30	36	40	38.5	42	99	6.4
	Sires	69							
North Central Colorado, elevation 1950 m	mPAP	86	33	37	38.7	38.5	40	48	3.2
	Sires	–							
Southwest Colorado, elevation 2800 m	mPAP	41	34	39	45.7	41	45	114	15.9
	Sires	17							
North Front Range Colorado, elevation 1850 m	mPAP	32	28	38	48.7	40	47.3	126	23.1
	Sires	13							
Total	mPAP	691	28	36	40.5	39	42	126	8.8
	Sires	99							

[a] Median and quartiles included due to non-normality of mPAP.
[b] Q1 = Quartile 1 (25th percentile), Q3 = Quartile 3 (75th percentile).
[c] Colorado State University-Beef Improvement Center.

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