Genome-wide association and gene enrichment analyses of meat tenderness in an Angus-Brahman cattle population

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Summary

The objective of this study was to identify genomic regions associated with meat tenderness related traits using a whole-genome scan approach followed by a gene enrichment analysis. Warner-Bratzler shear force (WBSF) was measured on 673 steaks, and tenderness and connective tissue were assessed by a sensory panel on 496 steaks. Animals belong to the multibreed Angus-Brahman herd from University of Florida and range from 100% Angus to 100% Brahman. All animals were genotyped with the Bovine GGP F250 array. Gene enrichment was identified in two pathways; the first pathway is involved in negative regulation of transcription from RNA polymerase II, and the second pathway groups several cellular component of the endoplasmic reticulum membrane.

Keywords: tenderness, gene enrichment, regulation of transcription, cell growth, cell proliferation

Introduction

Identification of quantitative trait loci (QTL) for any complex trait, including meat tenderness, is the first most important step in the process of understanding the genetic architecture underlying the phenotype. Given a large enough population and a dense coverage of the genome, a genome-wide association study (GWAS) is usually successful in uncovering major genes and QTLs with large and medium effect on these type of traits. Several GWA studies on *Bos indicus* (Magalhães et al., 2016; Tizioto et al., 2013) or crossbred beef cattle breeds (Bolormaa et al., 2011b; Hulsman Hanna et al., 2014; Lu et al., 2013) were successful at identifying QTL for meat tenderness; and most of them include the traditional candidate genes μ-calpain and calpastatin.

Genome wide association combined with gene enrichment analyses will not only identify genomic regions of large effect but have also the potential to help with the challenging small effect regions. The objective of this study was to apply this methodology to meat tenderness related traits in an Angus-Brahman cattle population.

Material and methods

Population and phenotypic data

Animals used in the study belong to the multibreed Angus-Brahman herd from University of Florida (Elzo *et al.*, 2012) born between 2007 and 2014. When steers reached 1.27 cm over the ribeye, they were transported to a commercial packing plant and harvested. Two 2.54 cm steaks

from the *Longissimus dorsi* muscle at the 12th/13th rib interface were sampled from each animal, transported to the Meat Science laboratory at University of Florida, aged for 14 days at 1-4°C, and then stored at −20°C.

Thawed steaks were cooked to an internal temperature of 71°C in an open-hearth grill. Objective tenderness was measured by Warner-Bratzler Shear Force (WBSF) on 673 steaks, and tenderness and connective tissue amount were measured through a trained sensory panel on 496 steaks.

Genome-wide association analysis

Animals were genotyped with the Bovine GGP F250 array (GeneSeek, Inc., Lincoln, NE, USA). Data processing and analysis were performed using the Genetics Q-K analysis workflow of JMP-Genomics 6.0 software (SAS Institute). Only animals with a calling rate higher that 85%, and autosomal markers with minor allele frequency higher that 5% were included in the analysis. The connective tissue variable was normalized using a Johnson normalizing transformation carried out with JMP-Genomics 6.0 software (SAS Institute). The mixed model K method including the genomic relationship matrix and fixed effects SNP and birth year was applied (Stich *et al.*, 2008; Stich & Melchinger, 2009; Yu *et al.*, 2006). The effective number of independent tests was calculated as described in Gao *et al.* (2008), and subsequently the Benjamini-Hochberg p-value correction was applied to correct for a genome-wide significance level.

Gene enrichment analysis

The methodology described by Baranzini *et al.* (2009) was slightly modified and carried out using an in-house scripts written in JAVA. All SNPs with uncorrected p-value ≥ 0.05 were included in the gene enrichment analysis. SNPs were assigned to genes if they were located inside the gene or located within 3 kbs upstream and downstream from a gene. Only genes reported by the EMBL-EBI Expression Atlas to be expressed in bovine skeletal muscle were included in subsequent analyses. The correlation between all the SNPs within each gene was calculated and SNPs were considered correlated when $r^2 > \pm 0.3$. Genes, whose SNPs are all correlated, were included once in the final gene list and genes with more than one uncorrelated SNP were included multiple times (as many as the number of uncorrelated SNPs) in the final gene list.

The GO terms molecular function, cellular component and biological process were included in the analysis, and 16,384 genes expressed in bovine skeletal muscle was used a background gene list. Gene enrichment was calculated using the hypergeometric test and applying Benjamini-Hochberg p-value correction to each pathway. A total number of 89, 70 and 76 GO terms for WBSF, connective tissue amount and tenderness respectively, were included in the analysis.

Results and Discussion

The means and standard deviations for WBSF, tenderness score and connective tissue were 4.3 ± .15, 5.5 ± 0.84 and 6.1 ± 0.82 kg, respectively. A total number of 5,980 markers assigned to 2,134 genes for WBSF; 5,844 markers in 2,191 genes for sensory panel tenderness; and 5,710 markers in 2,060 genes for sensory panel connective tissue were significant in the WGA studies, but none of them reached the whole genome significance for any trait.

All significant SNPs were initially included in the gene enrichment analysis and after the exclusion of genes without expression in skeletal muscle and analysis of correlated SNPs, the final gene lists had 1,503, 1,343, and 1,402 genes for WBSF, tenderness, and connective tissue, respectively.

Six pathways with gene enrichment were identified (Table 1). The pathway GO:0000122 was significant for connective tissue (adjusted p-value = 0.03). The pathway GO:0005789 was the second most significant pathway for connective tissue (adjusted p-value = 0.36). This GO:0005789 was also the most important pathway for WBSF (adjusted p-value = 0.19) and tenderness (adjusted p-value = 0.32), although was not statistically significant for any of these traits. The GO:0000122 pathway is involved in negative regulation of transcription from RNA polymerase II, and GO:0005789 groups several cellular components of the endoplasmic reticulum membrane (Table 2).

The gene TYRO3 is the "Tyrosine-Protein Kinase DTK" gene and may play a role in the regulation of cell proliferation and differentiation (www.genecards.org). The gene PIGN is the "phosphatidylinositol glycan anchor biosynthesis class N" and this protein may act as suppressor of replication stress and chromosome missegregation (www.genecards.org). Some other genes from this list are related to cancer in humans and have been stated as cell growth and proliferation regulatory proteins (www.genecards.org).

In conclusion, this study found an enrichment of genes in six GO terms and some of them are involved in negative regulation of transcription and cell growth and proliferation. These genes are related to phenotypic variation in WBSF, tenderness and connective tissue in the present population.

Table 1. List of enriched pathways for WBSF, tenderness, and connective tissue amount in Longissimus dorsi in an Angus-Brahman crossbred population.

Trait	GO term	GO term name	p-value[1]	Adjusted p-value[2]	Genes in gene list[3]	Genes GO term[4]
Tenderness	GO: 0005789	Endoplasmic reticulum membrane	4.17E-3	0.32	20	214
Connective tissue	GO: 0000122	Negative regulation of transcription from RNA polymerase II promoter	4.34E-4	0.03	19	225
Connective tissue	GO: 0005789	Endoplasmic reticulum membrane	1.03E-2	0.36	23	214
Connective tissue	GO: 0005743	Mitochondrial inner membrane	1.28E-2	0.3	17	168
Connective tissue	GO: 0045944	Positive regulation of transcription from RNA polymerase II promoter	1.95E-2	0.34	40	319
WBSF	GO: 0005789	Endoplasmic reticulum membrane	2.11E-3	0.19	22	213

[1] P-value without Benjamini-Hochberg adjustment
[2] P-value with Benjamini-Hochberg adjustment
[3] Number of genes in the final gene list that belong to the GO term
[4] Number of genes in the background gene list that belong to the GO term

Table 2. List of enriched genes in the GO:0005789 pathway for connective tissue amount, WBSF, and tenderness in Longissimus dorsi in an Angus-Brahman crossbred population.

Connective tissue amount		WBSF		Tenderness
LRRC8C	PIGW	RAB10	UFL1	FMO2
CYP1A1	SEC23B1	CLGN	RETSAT	PTGS1
SOAT1	PIGN2	TYRO3	SCD5	SACM1L
RTN4	SEC16B1	CD4	ERAP2	TMED10
RTN1	ITPR21	PAFAH2	ICMT	FA2H
LPCAT3	TMED31	ERGIC3	FDFT1	ERAP1
TM6SF2	REEP11	SLC8A3	ALOX5AP	TMEM147
MIA3	TMX31	MOXD1	NFE2L1	CYB5R3
TRIQK	SLC8A31	EXT2	DDRGK1	FKBPL
SCD5	ALG101	BCL2	SIGMAR1	LOC101907138
	ALG61		FKBP9	

[1] Enriched genes in connective tissue and tenderness pathway
[2] Enriched genes in connective tissue and WBSF pathway

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