THE EXPRESSION OF GENES ENCODING LIPID STORAGE PROTEINS IS CORRELATED WITH INTRAMUSCULAR FAT PERCENTAGE IN BRAHMAN STEERS

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SUMMARY

In the beef industry, intramuscular fat percentage (IMF%) is an important trait due to its economic benefits and was the focus of this study. The mechanisms involved in determining IMF% are not entirely clear and we set out to investigate gene expression patterns associated with the variation in this trait. We profiled the genome-wide mRNA expression in LM biopsy samples from 48 Brahman steers by microarray and also measured IMF% at slaughter, two weeks later. We investigated the correlation between each of the probes on the array and the IMF% across the animals. Enriched amongst the genes whose expression levels were most positively correlated with IMF% were genes annotated to be involved in lipid metabolism. Of the lipid metabolism categories (e.g. synthesis and degradation), the genes whose expression was most correlated with IMF% represented the lipid *storage* category. The genes include CIDEA, ADIG, S100G, PCK1, PLIN1, FABP4, ADIPOQ, PSL1, AGPAT2, DGAT2, CIDEC and TUSC5. Therefore, this result supports the hypothesis that increased IMF% is primarily associated with the increased ability intramuscular adipocytes to store lipid, as opposed to increased synthesis or decreased degradation, which may occur in organs elsewhere.

INTRODUCTION

Marbling, as measured by intra-muscular fat percentage (IMF%), is a key determinant of juiciness and flavour in beef, two important drivers of consumer satisfaction (Platter *et al.* 2003). Combined with its economic relevance and reasonably moderate heritability ($h^2 \sim 0.37 - 0.46$), IMF% has been incorporated into several genetic evaluation programs world wide (Bertrand and Green 2001; Newman *et al.* 2002; Speidel *et al.* 2010). Variation in IMF% exists and often carcasses do not meet the threshold specifications for premium markets. Feed lotting of cattle, using high-energy concentrated nutrition, is generally preferred over pasture-based feeding in terms of increasing marbling (Hidirogloua *et al.* 1987). However, in some instances, regardless of animals going through these intensive feedlot periods, marbling market thresholds are not met and premiums are lost. The biological mechanisms underlying this variation at present are not entirely clear and predicting the marbling potential of animals prior to feedlot and/or slaughter is of continued interest. Methods to measure IMF% other than ultrasound are sought after as it has been shown to be unreliable (MacNeil *et al.* 2010), likely due to noise. In an attempt to predict marbling potential, several DNA markers, such as SNPs associated with diacylglycerol O-acyltransferase homolog 1 (*DGAT1*) and Thyroglobulin 5 (*TG5*), have been developed and are commercially available, however these explain little or no variation in independent datasets (Rincker *et al.* 2006; Graser 2008; Johnston and Graser 2010; Pannier *et al.* 2010). The markers were identified using GWAS strategies, which does not put great emphasis on considering the biology of the trait. Their failure may be due to the incomplete understanding of the biological basis of IMF% variation.

Previously, studies have highlighted the correlation between the expression of genes prior to slaughter in muscle biopsy samples and IMF% for genes including adiponectin, C1Q and collagen domain containing (*ADIPOQ*), stearoyl-CoA desaturase (*SCD*) and thyroid hormone responsive (*THRSP*) (Wang *et al.* 2009). Similar results have been reported in other livestock species including pigs (Gerbens *et al.* 1998; Damon *et al.* 2006) and chickens (Luo *et al.* 2006).

Our study focussed at the genomic level and considered the genome-wide expression levels of thousands of genes in skeletal muscle of cattle. Using Brahman steers raised in commercial conditions, we investigated the correlation between gene expression levels and IMF% as a means of gaining a better biological understanding of processes involved in determining the trait.

MATERIALS AND METHODS

Animal resources and experimental design. The phenotypic data used in this study originated from an animal resource described in a previous experiment (Cafe *et al.* 2010a; 2010b). Briefly, the subset of animals used consisted of 48 Brahman steers averaging 600 ± 67 days in age, which were feedlot finished. These animals included three factors: tenderness genotype, environment and hormone growth promotant (HGP) treatment.

The tenderness genotypes are based on the following three genes and SNP: Calpastatin: CAST3-84 (G/A in the 3' UTR of CAST) (Barendse 2002), calpain 3: CAPN3JK (T/G in an intron of CAPN3) (Barendse et al., 2008) and calpain 1:CAPN1-4751 (T/C in an intron of CAPN1) (White et al. 2005). The presence of two favourable alleles of each of these genes has previously been shown to be associated with an improvement in tenderness (Cafe, McIntyre et al. 2010). For the purpose of this experiment, a "tough" genotype has no favourable alleles for CAST3-84, CAPN3JK and CAPN1-4751, an "intermediate" genotype has two favourable for both CAST3-84 and CAPN3JK, and no favourable alleles for CAPN1-4751, while a "tender" genotype has two favourable alleles for CAST3-84 and CAPN3JK and one favourable allele for CAPN1-4751.

The environment contrast is between two finishing sites in Australia, New South Wales (NSW) and Western Australia (WA). The hormone growth promotant (HGP) treatment was the commercially available Revalor-H (Virbac, Milperra, NSW, Australia) which consists of 200mg trenbolone acetate and 20mg 17β estradiol. Each treatment contains 10 slow release pellets which are implanted in the ear of the animal according to the protocol. The average duration of the treatment was 68 ± 20 days.

Needle biopsy samples (~1g) were collected from the LM under local anaesthetic and immersed in RNAlater solution at -20^{o}C. Following removal of any visible subcutaneous fat, total RNA was extracted from ~20mg of tissue using TRIZOL (Invitrogen) and RNeasy Kits (Qiagen). Gene expression levels were measured using the Bovine Agilent 44K expression microarray platform (Agilent Technologies, Inc., Santa Clara, CA) representing 21,475 probes printed in duplicate. Following slaughter, samples of LM were collected and IMF% was determined by near infrared spectrophotometer methods following protocols previously described (Perry *et al.* 2001).

Analysis of gene expression data. As described in De Jager *et al.* (2011), we normalised the gene expression data by fitting a mixed-model that contained the fixed effects of finishing sites, HGP treatment and tenderness genotype, and the random effects of gene, gene × animal interaction and residual. For the present study, we considered the correlation between the gene expression pattern for each probe on the array and IMF%, across the 48 animals. Gene ontology analysis was carried out using a ranked list of genes (n = 19,265) based on the strength of their correlation with IMF% and processed through the GOrilla suite of tools (Eden *et al.* 2007; 2009). Finally, we investigated the effect that HGP treatment and site had on these correlations.

RESULTS AND DISCUSSION

Our analysis shows that the expression of genes involved in lipid storage, including adiponectin, C1Q and collagen domain containing (ADIPOQ), 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), cell death-inducing DFFA-like effector c (CIDEC), diacylglycerol O-acyltransferase homolog 2 (DGAT2), Fatty acid binding protein 4 (FABP4), phosphoenolpyruvate carboxykinase 1 (PCK1) and tumor suppressor candidate 5 (TUSC5), adipogenin (ADIG), cell death-inducing DFFA-like effector a (CIDEA), perilipin 1 (PLIN1), plastin 1 (PLS1) and S100 calcium binding protein G (S100G), whose biological roles are illustrated in Figure 1, is positively correlated with IMF%.

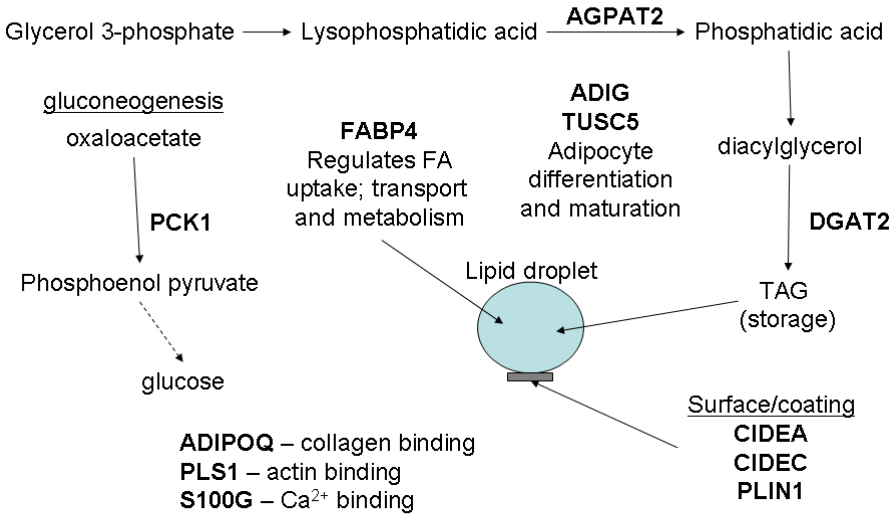

Figure 1. A set of genes primarily involved in lipid storage showing their relative functions. The genes are adiponectin, C1Q and collagen domain containing (ADIPOQ), 1-acylglycerol-3-phosphate O-acyltransferase 2 (AGPAT2), cell death-inducing DFFA-like effector c (CIDEC), diacylglycerol O-acyltransferase homolog 2 (DGAT2), Fatty acid binding protein 4 (FABP4), phosphoenolpyruvate carboxykinase 1 (PCK1) and tumor suppressor candidate 5 (TUSC5), adipogenin (ADIG), cell death-inducing DFFA-like effector a (CIDEA), perilipin 1 (PLIN1), plastin 1 (PLS1) and S100 calcium binding protein G (S100G).

The Brahman breed used in this study is not renowned for its marbling potential; however our results are particularly encouraging since correlations between IMF% and the expression of lipid storage genes are evident even at such low levels of IMF% variation. This relationship exists regardless of site or genotype and of the three factors investigated; HGP treatment had the biggest effect on the correlation between IMF% and expression of these lipid storage genes. This was not surprising since HGP treatment is associated with a decrease in IMF%.

While a number of these genes have previously been shown individually to have expression patterns that correlate with IMF%, for example ADIPOQ (Li *et al.* 2008; Wang *et al.* 2009; Zhao *et al.* 2009), the connection to lipid storage has not clearly been made. Furthermore, our findings based on gene expression data, in part, supports the view that there is an association between the increase in IMF% and with the filling of existing adipocytes during feed lotting (Luo *et al.* 2006). A subset of these genes were previously shown to have very similar expression patterns at ten time points during skeletal muscle development (Hudson *et al.* 2009). This co-expression suggests that

these genes may be co-regulated and in addition to their gene ontology, adds weight to the hypothesis that they are involved in a similar biological process.

CONCLUSIONS

We have shown that the expression of genes primarily involved in lipid storage is positively correlated with IMF% in cattle. This suggests that higher amounts of IMF% primarily results from an increased ability of intramuscular adipocytes to store lipid, not an increase in synthesis or decrease in breakdown. We hypothesise that single nucleotide polymorphisms associated with the expression levels of these genes may potentially be candidate markers for IMF%. Since the expression levels of these genes are strongly correlated with each other throughout development, it suggests that there may be a regulator or regulators in common and may be an area to be explored in the future. We conclude that our gene expression study supports the hypothesis that IMF% is largely associated with the ability of intramuscular adipocytes to store lipid, rather than regulate its synthesis or degradation.

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