Candidate genes and biological pathways associated with carcass quality traits in beef cattle

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[1]Livestock Gentec and the Department of Agricultural, Food and Nutritional Science, 4.10 Agriculture Forestry Center, University of Alberta, Edmonton, Alberta, Canada T6G 2P5; [2]Montana State University, Department of Animal and Range Sciences, Bozeman MT 59717, USA; and [3]The University of Queensland, Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, St. Lucia, 4072, Queensland, Australia.
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Karisa, B. K., Thomson, J., Wang, Z., Bruce, H. L., Plastow, G. S. and Moore, S. S. 2013. **Candidate genes and biological pathways associated with carcass quality traits in beef cattle**. Can. J. Anim. Sci. **93**: 295–306. The objective of this study was to use the candidate gene approach to identify the genes associated with carcass quality traits in beef cattle steers at the University of Alberta Ranch at Kinsella, Canada. This approach involved identifying positional candidate genes and prioritizing them according to their functions into functional candidate genes before performing statistical association analysis. The positional candidate genes and single nucleotide polymorphisms (SNP) were identified from previously reported quantitative trait loci for component traits including body weight, average daily gain, metabolic weight, feed efficiency and energy balance. Positional candidate genes were then prioritized into functional candidate genes according to the associated gene ontology terms and their functions. A total of 116 genes were considered functional candidate genes and 117 functional SNPs were genotyped and used for multiple marker association analysis using ASReml®. Seven SNPs were significantly associated with various carcass quality traits ($P \leq 0.005$). The significant genes were associated with biological processes such as fat, glucose, protein and steroid metabolism, growth, energy utilization and DNA transcription and translation as inferred from the protein knowledgebase (UniprotKB). Gene network analysis indicated significant involvement of biological processes related to fat and steroid metabolism and regulation of transcription and translation of DNA.

Key words: Beef cattle, carcass traits, gene networks

Karisa, B. K., Thomson, J., Wang, Z., Bruce, H. L., Plastow, G. S. et Moore, S. S. 2013. **Gènes candidats et voies biologiques associés avec les critères de qualité de la carcasse chez les bovins de boucherie**. Can. J. Anim. Sci. **93**: 295–306. L'objectif de cette étude était d'utiliser l'approche du gène candidat pour identifier les gènes associés avec les critères de qualité de la carcasse chez les bouvillons de boucherie du ranch de l'Université de l'Alberta à Kinsella, Canada. Cette approche a consisté en l'identification de gènes candidats positionnels et leur classement par ordre de priorité selon leurs fonctions en gènes candidats fonctionnels avant d'effectuer l'analyse statistique d'association. Les gènes candidats positionnels et les SNPs ont été identifiés à partir de QTLs reportés précédemment pour les critères incluant le poids corporel, le gain moyen quotidien, le poids métabolique, l'efficacité alimentaire et le bilan énergétique. Les gènes candidats positionnels étaient alors classés en gènes candidats fonctionnels par ordre de priorité selon les termes d'ontologie qui leur sont associés et leurs fonctions. Un total de 116 gènes ont été considérés gènes candidats fonctionnels et 117 SNPs fonctionnels ont été génotypés et utilisés pour une analyse d'association de marqueurs multiples avec ASReml®. Sept SNPs étaient associés significativement avec divers critères de qualité de la carcasse ($P \leq 0{,}005$). Les gènes significatifs étaient associés avec des processus biologiques tels que le métabolisme du gras, du glucose, des protéines et des stéroïdes, la croissance, l'utilisation de l'énergie, la transcription et la traduction de l'ADN tel qu'inféré à partir de la base de connaissance des protéines (UniprotKB). L'analyse des réseaux de gènes a indiqué une participation significative des processus reliés au métabolisme du gras et des stéroïdes et la régulation de la transcription et la traduction de l'ADN.

Mots clés: Bovin de boucherie, qualité de la carcasse, réseaux de gènes

Economic success, in terms of profitability, in beef production relies on producing a product of high economic value (output) at the lowest cost possible (input) (MacNeil et al. 1997). One of the options to increase profitability is to increase the meat quantity and/or quality (outputs) through selection for carcass quality traits such as backfat (BF) thickness, marbling

Abbreviations: **ABTB2**, ankyrin repeat and **BTB** domain containing gene; **BF**, backfat; **CMAR**, carcass marbling; **CREA**, carcass ribeye area; **DGKD**, diacyglycerol kinase delta; **GHR**, growth hormone receptor precursor; **GRDFT**, grade fat; **HSD17B12**, 17-beta-hydroxysteroid dehydrogenase 12; **LMY**, lean meat yield; **LRP4**, lipoprotein receptor-related protein 4; **MAS**, marker-assisted selection; **NDUFS3**, NADH dehydrogenase iron sulfur protein 3; **QGRD**, quality grade; **QTL**, quantitative trait loci; **RFI**, residual feed intake; **RN**, Rendement Napole; **SLC45A2**, solute carrier family 45, member 2; **SNP**, single nucleotide polymorphism; **STK10**, serine/threonine kinase 10; **UMAR**, ultrasound marbling; **UREA**, ultrasound ribeye area; **YGRD**, yield grade

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and ribeye area. Alternatively, beef producers may select cattle for increased feed efficiency using traits such as residual feed intake (RFI) (Koch et al. 1963). Some of these economically important traits are correlated, for example average BF thickness was reported to be highly correlated with yield grade ($r = 0.86$) (Rios-Utrera et al. 2005), both intramuscular and inter-muscular fat were negatively correlated with lean meat yield and feed efficiency (Basarab et al. 2003) and lean meat yield was positively correlated with RFI (Richardson et al. 1998; Herd and Bishop 2000; Basarab et al. 2003). Mirzaei et al. (2011) reported several significant correlations between some growth traits, such as body weight and growth, with carcass quality traits, such as hot carcass weight, carcass ribeye area and fat depth. These correlations may result in antagonistic effects on the breeding goal resulting in a dilemma that requires producers to be certain of what traits they wish to select and to be aware of what other traits will be affected and in which direction the secondary effects will occur.

The traditional selection practiced by most breeders is based on the quantitative genetic approach (Dekkers and Hospital 2002). This approach relies on the availability of phenotypes, the heritability of the traits and their genetic correlations. More recently, molecular markers have gained importance in the evaluation and ranking of candidates for selection. The use of molecular markers in selection relies on the ability to determine the genotypes of individuals for the mutations associated with the traits of interest (Dekkers and Hospital 2002).

Molecular markers such as single nucleotide polymorphisms (SNP) have great potential for use in marker-assisted selection (MAS) in beef cattle, especially for carcass quality traits because these traits have a larger impact on profitability. In addition to profitability, carcass quality traits can only be measured after the animal has been slaughtered, which removes its genetics from the breeding population unless semen or oocytes were collected and stored for future use. In addition, beef producers invest significant expense into herd sires which may ultimately have undesirable carcass traits that are discovered late after the purchase when progeny carcass characteristics are realized.

Another advantage of selecting animals using molecular markers relies on the overlap between quantitative trait loci (QTL) associated with multiple traits. Markers located in these QTL can be used to select for one trait and also to predict pleiotropic effects of the markers on other economically important traits. This can be done using bioinformatics tools or where data are available, by calibrating the markers for effects on all economically relevant traits. For example, the calpastatin gene (CAST) was reported to be associated with multiple traits such as RFI in cattle (McDonagh et al. 2001), meat quality in cattle (Morgan et al. 1993; McDonagh 1998; Barendse 2002; Casas et al. 2006) and meat quality in pigs (Ciobanu et al. 2004). Due to this pleiotropy, beef producers who wish to embrace MAS should be able to predict the effects of genetic markers on traits other than those under primary selection.

In this study, we use the candidate gene approach to identify the genes associated with carcass quality traits in beef cattle steers at the University of Alberta Ranch at Kinsella, Canada. We also reconstruct a gene network using the significant genes to analyze the gene interactions and biological processes associated with the various carcass traits.

MATERIALS AND METHODS

The animals were managed and cared for according to the guidelines of the Canadian Council on Animal Care (1993) and the research was approved by the animal care and use committee at the University of Alberta.

Phenotypic data were obtained from 531 beef cattle at the University of Alberta Ranch at Kinsella, Alberta, Canada. The breed composition of this herd was described in detail by Goonewardene et al. (2003), Nkrumah et al. (2007) and Mujibi et al. (2010, 2011a). Cows and heifers were bred on pasture in a multiple-sire breeding system and the sire of each calf was later determined in a parentage test by using a panel of bovine microsatellite markers (Nkrumah et al. 2007). The steers were managed and tested under feedlot conditions using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, AB). A detailed review of the GrowSafe system can be found at Nkrumah et al. (2004).

Phenotypic Data

Carcass traits were collected on steers raised at the University of Alberta Ranch at Kinsella, Canada. There were two batches of steers tested each year (Mujibi et al. 2010, 2011b), and carcass quality traits were collected as described by Nkrumah et al. (2004) and Nalaila et al. (2011). The carcass traits considered in this analysis included average BF, grade fat (GRDFT), carcass ribeye area (CREA), ultrasound ribeye area (UREA), carcass marbling (CMAR), ultrasound marbling (UMAR), yield grade (YGRD), quality grade (QGRD) and lean meat yield (LMY). Lean meat yield as a percentage was estimated using the equation:

$$\begin{aligned}\%\ \text{lean meat yield} &= 57.96 + (0.202 \times \text{cm}^2\ \text{l. thoracis area}) \\ &\quad - (0.027 \times \text{kg warm carcass weight}) \\ &\quad - (0.703 \times \text{mm average BF thickness}).\end{aligned}$$

Yield grade, the proportion of lean meat, was classified as follows: $1 = \geq 59\%$; $2 = 54$ to 58%; and $3 = < 54\%$ (Basarab et al. 2003).

The Candidate Gene Approach

Identification of Positional Candidate Genes

Two sets of positional candidate genes were utilized in this study. The first set consisted of 1100 candidate genes positioned within a range of 500 kbp on either side

of 203 QTL reported in the Bovine QTL database (cattleQTLdb) (Hu et al. 2007). These QTL were associated with some component traits associated with carcass quality including body weight, average daily gain, feed efficiency, dry matter intake, metabolic weight and energy balance. The second set of positional candidate genes consisted of 1018 genes positioned within a range of 500 kbp on both sides of 310 SNPs previously reported to be significantly associated with RFI in a GWAS using the 50 K bovine SNP chip (Mujibi et al. 2011). A total of 2118 positional candidate genes were identified in this study.

Single Nucleotide Polymorphism Detection

The SNPs located in the positional candidate genes were identified from the NCBI SNP database (dbSNP) (Sherry et al. 2001) and by comparing cDNA sequences generated from liver samples from steers at the University of Alberta Ranch at Kinsella, AB, with reference sequences from Ensembl version 57 (Hubbard et al. 2009).

To generate the cDNA library, RNA was prepared from pooled liver samples using TRIzol® reagent (Invitrogen, Carlsbad, CA) using the protocol described in the TRIzol® reagent user manual (Conolly et al. 2006). The quality and quantity of RNA were determined using a NanoDrop 2000 spectrophotometer (Nanodrop technologies, Wilmington, DE) (Gallagher and Desjardins 2007). The liver cDNA library was constructed according to the TruSeq® RNA and DNA sample preparation kit (Illumina, USA, v2 published by Illumina Inc. 2011, publication number 970-2009-039) and cDNA sequencing was performed on the Genome Analyzer II using the TruSeq® RNA and DNA sample preparation kit, (Illumina, USA, v2 published by Illumina inc 2011, publication number 970-2009-039).

To generate a list of putative SNPs, Maq (version 0.7.1) (Li et al. 2008) was used to map DNA sequencing reads and the bovine transcript sequences from Ensembl version 57 (Hubbard et al. 2009) were used as reference sequences. The SNPs returned by Maq's SNPfilter command were annotated using NGS-SNP (Grant et al. 2011) by assigning a functional class to each SNP (e.g., nonsynonymous) and then providing NCBI, Ensembl identifiers and gene ontology terms for the affected genes, when applicable. Only the non-synonymous SNPs were considered functional candidate SNPs and were retained if the minor allele frequency was greater than 10%.

The positional candidate genes containing functional SNPs were then prioritized according to their functions and gene ontology terms (Ashburner et al. 2000) in relation to the biological processes associated with feed efficiency (Herd and Arthur 2009). Genes with functions related to metabolism of carbohydrate, lipid and protein, growth, ATP and body temperature regulation were considered functional candidate genes and a final set of 116 genes was identified spread across 12 chromosomes with some chromosomes containing larger numbers of functional candidate genes than others. The non-synonymous SNPs from each gene were prioritized based on the expected effect of the amino acid change, such that among several SNPs, a non-conservative amino acid change was considered of higher priority than a conservative amino acid change in the same gene. A total of 117 SNPs were selected within the 116 genes, one SNP from each gene and two SNPs from the CAST gene, and genotyped at GeneSeek® using DNA samples obtained from steers at the University of Alberta Ranch at Kinsella, AB.

Association Analysis

Association analysis was performed using ASReml 3.0 software (Gilmour et al. 2009). The homozygous genotype containing the two alleles with lower frequency in the population was coded 0, the homozygous genotype with two alleles of higher frequency was coded 2 and the heterozygote genotypes were coded 1. Of the 117 functional SNPs, 113 were successfully genotyped and 39 SNPs were monomorphic, leaving 74 polymorphic SNPs to be used in the analysis.

The multiple marker association analysis was performed using the animal model:

$$Y_{ij} = \mu + X_{1i}\beta + \sum_{j=1}^{74} X_{2j}g_j + Za + e \quad (1)$$

where Y_{ij} represents the specific carcass trait being studied for animal i which has j SNPs ($j = 74$ SNPs), μ is the population mean, β is the vector of fixed effects associated with animal i, in this case the breed of the sire and the batch (Mujibi et al. 2010), $\Sigma_{j=1}^{74} X_{2j}g_j$ is the sum of the SNP effects (g), X_2j is a design matrix relating an observation (y) to one of the genotypes 0, 1 or 2 at the jth SNP, a is a vector of random additive effects [exclusive of SNP g effect(s) in the model] constructed using each animal's pedigree information to construct the relationship matrix A. It was assumed to be normally distributed with a mean of 0 and variance of $A\sigma_{a.}^2$

e was the vector of random residual effects assumed to be normally distributed with a mean of 0 and a variance of $I\sigma^2$ where I was an identity matrix.

The significance criteria was a P value corrected for nine tests using the same model for nine different traits in this study; therefore SNPs were considered significant if $P < 0.005$.

A multivariate analysis was performed for significantly correlated ($P < 0.05$) traits (Table 1) using ASReml 3.0 to identify markers that were significantly associated with variation in multiple traits simultaneously. Traits in this analysis were BF, UREA, CREA, CMAR, UMAR, YGRD, QGRD, GRDFT and LMY.

Reconstructing Gene Networks

Gene interaction networks were reconstructed and biological processes were identified using IPA software (Ingenuity® Systems, www.ingenuity.com) for the genes that were significantly associated with single

Table 1. Phenotypic and (genetic)[z] correlations between carcass merit traits in cattle from University of Alberta Kinsella Ranch[y]

Traits	BF	GRDFT	CREA	UREA	CMAR	UMAR	YGRD	QGRD	LMY
BF	1.00	0.76[x] (0.69)[x]	0.01 (0.01)	0.35[x] (0.08)	0.47[x] (0.18)[x]	0.47[x] (0.14)[x]	0.64[x] (0.32)[x]	−0.06 (0.04)	−0.66[x] (−0.78)[x]
GRDFT		1.00	−0.09 (−0.12)	0.22[x] (−0.16)[x]	0.53[x] (0.24)[x]	0.43[x] (0.32)[x]	0.76[x] (0.36)[x]	−0.03 (0.08)	−0.87[x] (−0.62)[x]
CREA			1.00	0.67[x] (0.80)[x]	−0.07 (−0.01)	−0.13[x] (−0.08)	−0.30[x] (−0.12)[x]	−0.30[x] (0.45)[x]	0.45[x] (0.56)[x]
UREA				1.00	0.13[x] (0.11)	0.11[x] (0.13)[x]	0.06 (0.02)	−0.40[x] (0.52)[x]	0.03 (0.58)[x]
CMAR					1.00	0.55[x] (0.75)[x]	0.41[x] (0.65)[x]	0.03 (0.38)[x]	−0.54[x] (−0.22)[x]
UMAR						1.00	0.39[x] (0.54)[x]	0.13[x] (0.15)[x]	−0.44[x] (−0.24)[x]
YGRD							1.00	0.06 (0.12)[x]	−0.81[x] (0.19)[x]
QGRD								1.00	−0.08 (0.06)
LMY									1.00

[z]Genetic correlations are in parentheses.
[y]The phenotypic and genetic correlation between various carcass merit traits, including backfat (BF), grade fat (GRDFT), carcass ribeye area (CREA), ultrasound rib eye area (UREA), carcass marbling (CMAR), ultrasound marbling (UMAR), yield grade (YGRD), quality grade (QGRD) and lean meat yield (LMY).
[x]Significant correlation ($P < 0.05$).

carcass traits. The IPA software was selected because it offered a large knowledge base and can model relationships between genes, proteins, and metabolites, and can be used to identify biological pathways and interaction complexes (Ingenuity® knowledge base). To reconstruct the gene network, a list of significant genes and their corresponding P values were imported into the IPA software and the parameters were set to allow the network to include indirect relationships between the imported genes and genes that were in the database. Indirect relationships would assist in the identification of other genes that were not among the genes analyzed, but may be associated with carcass traits. The IPA algorithm generates gene networks by mapping each gene identifier to its corresponding gene in the IPA Knowledge Base. The genes are then overlaid onto a global molecular network developed from information contained in the Knowledge Base. The networks are generated based on their connectivity such that each network has a maximum of 35 imported genes. Each network is assigned a significance score, which represents the likelihood that the imported genes within the network are found therein by random chance. A high number of imported genes within a dataset leads to a higher network score. The network score is calculated as the negative of the exponent of the P value such that a score of 25 will be equal to a P value of 10E-25 (Calvano et al. 2005), and therefore larger scores correspond to high significance.

RESULTS

Correlation Analysis

Phenotypic correlation analysis indicated significant correlations between multiple carcass traits. Average BF was significantly correlated with ultrasound ribeye area, ultrasound marbling, carcass marbling, yield grade, grade fat and lean meat yield. A summary of the correlations is shown in Table 1.

Association Analysis for Individual Carcass Traits

Seven genes were significantly associated with various carcass traits at $P < 0.005$. Three more genes with $P < 0.05$ were considered to show a trend of association because they had been reported to be associated with carcass traits in previous studies. The amount of phenotypic variation explained by the significant SNPs for each trait is discussed in subsequent sections. These genes, their allele substitution effect and their functions are summarized in Table 2, and Table 3 shows the details of the SNPs and amino acid changes.

The SNPs located in the diacylglyerol kinase gene (DGKD), serine/threonine kinase 10 (STK10) and the Ankyrin repeat and BTB domain containing gene (ABTB2) were associated with slaughter weight at $P = 0.005$, 0.0027 and 0.0047, respectively. Two SNPs located in the 17-beta-hydroxysteroid dehydrogenase 12 (HSD17B12) gene and the UBXN4 domain-containing protein 4 were significantly associated with carcass quality grade ($P = 0.0006$ and 0.0048, respectively). The SNP in the NADH dehydrogenase iron sulfur protein 3 (NDUFS3) was associated with carcass ribeye area ($P = 0.0005$) with an effect of -2.66 cm^2. A SNP located in the low-density lipoprotein receptor-related protein 4 (LRP4) was significantly associated with yield grade ($P = 0.0008$).

The three genes showing a trend of significance were the growth hormone receptor precursor (GHR) associated with grade fat and average BF thickness ($P = 0.03$ and 0.025, respectively) and the solute carrier family 45, member 2 (SLC45A2) associated with grade fat ($P = 0.01$) and ARHGAP1 protein containing fragment (ARHGAP1) associated with carcass weight ($P = 0.008$).

Multivariate Analysis

A multivariate analysis was performed in ASReml 3.0 (Gilmour et al. 2009) using the significantly correlated traits to identify genes that were significantly associated with all traits. The highly correlated traits were: BF,

Table 2. SNPs and genes significantly associated with various carcass traits, the position of the SNPs and the functions of the genes[z]

Gene symbol	Gene name	Associated trait	*P* value	Number of individuals per genotype 0, 1, 2			Allele effect	Gene function[y]
UBA5	Ubiquitin-like modifier activating enzyme 5	Multiple traits	0.0003	4	113	408	–	Ubiquitin-like post-translational modifier protein
UBXN4	UBX domain-containing protein 4	Quality grade	0.0048	57	236	230	−0.1676	Involved in endoplasmic reticulum-associated protein degradation thus regulation of phenotypic expression
DGKD	Diacylglycerol kinase	Slaughter weight	0.005	6	160	356	−12.43 kg[w]	Involved in glycerolipid and glycerophospholipd metabolism and phosphatidylinositol signaling system
ABTB2	Ankyrin repeat and BTB (POZ) domain containing 2	Slaughter weight	0.0047	132	238	160	11.63 kg[w]	Suggested to be involved in DNA and protein binding. However, the functions of this gene remain largely unknown.
PDHX	Pyruvate dehydrogenase complex (complex X)	Multiple traits	0.0023	125	264	137	–	The pyruvate dehydrogenase complex catalyzes the conversion of pyruvate to acetyl CoA.
HSD17B12	17-beta-hydroxysteroid dehydrogenase 12	Quality grade	0.0006	34	160	327	0.1951	Catalyzes the transformation of estrone (E1) into estradiol (E2), suggesting a central role in estrogen formation. Also has 3-ketoacyl-CoA reductase activity, reducing both long chain 3-ketoacyl-CoAs and long chain fatty acyl-CoAs, suggesting a role in long fatty acid elongation
ARHGAP1	ARHGAP1 protein Fragment	Carcass weight	0.0083[x]	60	213	250	12.11 kg[w]	One of cytoskeleton regulators and the ontology terms associated with this gene were protein binding and regulation of GTPase activity. Has been associated with reduction in body mass, adipose tissue and osteoporosis
LRP4	Low density lipoprotein receptor-related protein 4	Yield grade	0.0008	23	27	471	−0.4533	Plays a key role in the formation and the maintenance of the neuromuscular junction. It has also been proposed to function as a cell surface endocytic receptor binding and internalizing extracellular ligands for degradation by lysosomes In humans and cattle this gene is involved in bone development and growth. May also be involved in cholesterol metabolism.
NDUFS3	NADH dehydrogenase	Carcass ribeye area	0.0005	30	200	294	−2.663 (cm^2)[w]	Involved in a complex of reactions in the electron transport chain and oxidative phosphorylation in the mitochondria. Therefore plays a role in energy metabolism.
STK10	Serine/threonine kinase 10	Slaughter weight	0.0027	22	215	284	18.14 kg[w]	One of serine threonine kinase genes. Their roles are in cell cycle arrest, protein amino acid phosphorylation and regulation of fatty acid oxidation
SLC30A5	Zinc transporter (solute carrier family 30) member 5	Multiple traits	0.004	80	265	171	–	May be involved in zinc transport into cells to form insulin crystals
GHR	Growth hormone receptor Precursor (GHR)	Grade fat	0.03[x]	65	204	255	1.59	This gene encodes the receptor that binds the growth hormone and activates intercellular signals that lead to growth.
GHR	Growth hormone receptor Precursor (GHR)	Average back fat	0.025[x]	65	204	255	1.626 cm[w]	Encodes the receptor that binds the growth hormone and activates intercellular signals that lead to growth.
SLC45A2	Solute carrier family 45, member 2	Grade fat	0.0126[x]	7	60	461	0.9835	Related to melanin production and in humans the gene is associated with hair, skin and eye pigmentation. In animals it is also involved in pigmentation, lipid metabolism and growth.

[z]Details of SNP position and type are shown in Table 3.
[y]Referred from genecards (Rebhan et al. 1997), gene ontology databases (Ashburner et al. 2000).
[x]Author?
[w]Author?

Table 3. Details of significant SNPs associated with carcass traits

Gene symbol	SNP ID[z]	SNP Chr:bp	Type of SNP	Position of SNP in gene	AA change	Position of AA in gene
UBA5	NF	1:139081617	A/T[y]	231	Glu > Val	13
UBXN4	rs208513069	2:61935185	T/A[y]	591	Leu > His	195
DGKD	NF	3:120460419	A/G[x]	3067	Thr > Ala	1023
ABTB2[w]	rs211653218	15:65558664	A/C[y]	2339	Glu > Ala	780
PDHX	rs211170349	15:66290876	A/T[y]	782	Tyr > Phe	255
HSD17B12	rs109711563	15:74828355	A/G[x]	816	Arg > Gly	243
ARHGAP1	NF	15:76986446	C/T[x]	235	Pro > Ser	79
LRP4	NF	15:77158469	T/C[x]	3410	Met > Thr	1137
NDUFS3	NF	15:77730420	C/A[y]	168	Ala > Asp	55
STK10[w]	rs136660541	20:3772213	C/T[x]	2226	Thr > Met	728
SLC30A5	rs136504424	20:10487612	A/G[x]	1666	Ser > Gly	556
GHR	NF	20:33915503	T/A[y]	873	Phe > Tyr	279
SLC45A2	NF	20:42286376	G/A[x]	718	Ala > Thr	240

[z]SNPs with NF in their ID did not match any SNP in the SNP database.
[y]Transversion.
[x]Transition.
[w]The SNP reported in the SNP database differed from the SNP alleles in the population we studied but they were located at the same position in the transcript and amino acid (AA) sequence.

UREA, CREA, CMAR, UMAR, YGRD, QGRD, GRDFT and LMY.

Four SNPs located in the 17-beta-hydroxysteroid dehydrogenase 12 (HSD17B12), pyruvate dehydrogenase complex (PDHX), solute carrier family 30 (zinc transporter), member 5 (SLC30A5) and ubiquitin-like modifier activating enzyme 5 (UBA5) genes were significant ($P < 0.005$). The SNP in the HSD17B12 that was significant in the multivariate analysis was the same as the one that was significantly associated with quality grade, which was significantly correlated with CREA, UREA and UMAR.

Gene Network and Biological Pathways

Using the 13 significant genes imported into IPA, three networks were reconstructed in IPA. The first network had the highest score of 23 and out of the 13 significant genes, this network consisted of nine genes including ABTB2, HSD17B12, LRP4, DGKD, STK10, GHR, UBXN4, SLC30A5 and PDHX genes as shown in Fig. 1a. The second gene network had a score of 6 and out of the 13 significant genes this network consisted of three genes including ARHGAP1, NDUFS3 and UBA5 (Fig. 1b). The third network had a score of 3 and out of the 13 genes this network contained only one gene, SLC45A2. Several other genes that formed indirect relationships with these genes were also included in the networks. However, only the first and the second gene networks, which included more than one of the significant genes, will be discussed further.

The gene network with the highest score had several hubs as shown in Fig. 1a.

The Ins1 hub was composed of several interactions with the insulin 1 gene, which encodes the insulin hormone. The insulin hormone plays a role in decreasing blood glucose concentration by increasing cell permeability to glucose. It also increases cell permeability to amino acids and fatty acids. In addition, it accelerates glycolysis, the pentose phosphate cycle and glycogen synthesis in the liver (Rebhan et al. 1997).

An additional hub was centered on the NFkB (complex) transcription factor, which is involved in several processes, such as cellular growth, immune and inflammatory responses and developmental processes. A complete review of the NFkB complex was published by Gilmore (2006). There were several interactions including three genes involved in estrogen metabolism. These genes are also involved in lipid metabolism and fatty acid biosynthesis.

The second gene network was centered at the Ubiquitin C (UBC) gene (the UBC hub) and also consisted of several minor hubs associated with the NDUF genes. The UBC gene also interacted with NDUFS3 and the several minor hubs associated with the NDUF genes. The UBC gene was not analyzed in this study, but its location on the network and its multiple interactions with the significant genes indicate that it may influence their function and thereby potentially influence carcass traits.

Several genes were included in the gene networks and may be associated with carcass traits including AGRN, MAPK8IP1, CDKN1A, RANBP9, the NDUF genes and several additional genes as shown in Fig. 1a and 1b.

The biological processes represented by the UBC hub relate to regulation of gene expression, and include degradation of proteins in the endoplasmic reticulum, lysosomal degradation, and protein degradation via the proteasome, activation of transcription factor NF-kappa-B, cell signaling and DNA repair. Therefore, this hub represents genes and biological processes that influence variation in phenotypes through processes that regulate the levels of gene expression and protein function such as DNA transcription and protein degradation respectively.

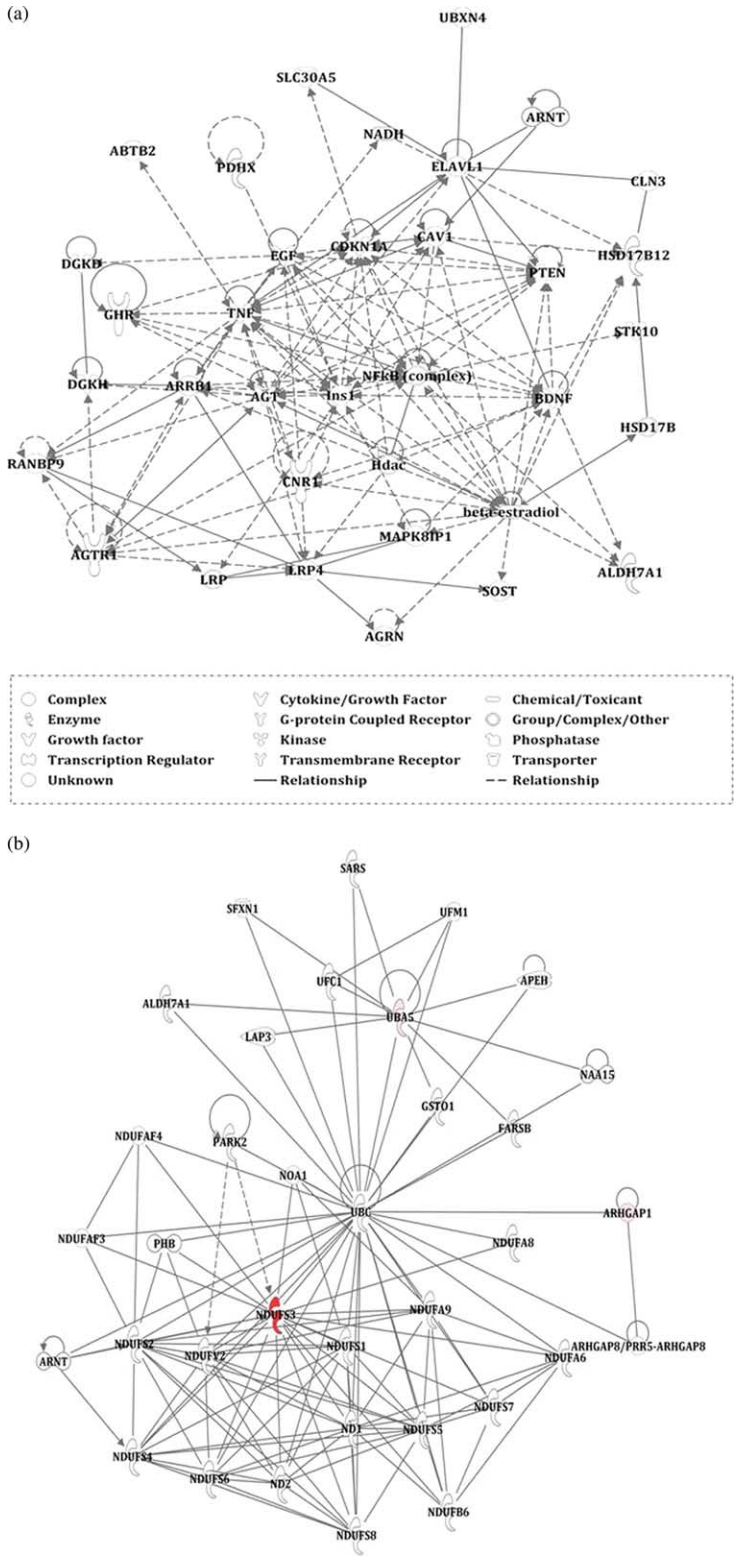

Fig. 1. (a) Gene interaction network 1 associated with carcass quality genes studied in beef cattle. Genes in the network that were significantly associated with various carcass traits were: PDHX, DGKD, LRP4, ABTB2, SLC30A5, HSD17B12, UBXN4, GHR and STK10. (b) Gene interaction network 2 associated with carcass quality genes studied in beef cattle. Genes in the network that were significantly associated with various carcass traits were: NDUFS3, ARHGAP1 and UBA5.

The NDUF hubs are associated with energy production and utilization, which is also related to fat and steroid metabolism.

Other important biological processes identified in this pathway analysis included acetyl-CoA biosynthesis, androgen and estrogen biosynthesis, phospholipid degradation, glycerophospholipid metabolism and cytokine signaling.

DISCUSSION

Protein structure and function have been shown to be influenced by amino acid properties such as polarity and their interaction with water. In most cases, polar amino acids are found located on the outside of the protein and interact closely with tissue fluids because they are hydrophilic (Branden and Tooze 1999). Mutations that cause a change from a polar to a non-polar amino acid may result in a major change in the structure of the protein and as a result affect its function. In this study, all the significant SNPs resulted in amino acid whose polarity was different from the amino acid in the reference sequence (Table 3).

The genes highly associated with carcass traits ($P < 0.005$) are discussed here.

Diacyglycerol Kinase Delta (DGKD)

In this study, DGKD was significantly associated with slaughter weight and explained 0.4% of the phenotypic variation in slaughter weight. DGKD also had the effect of increasing the average BF thickness and a reduction in carcass weight, though these effects were not significant ($P = 0.05$ and $P = 0.02$, respectively). The function of DGKD is to catalyze the conversion of diacylglyerol to phosphatidic acid with ATP as the phosphate group donor. Both diacylglyerol and phosphatidic acid are lipid signaling molecules and DGKD acts as the switch by terminating the signaling of one lipid, while simultaneously activating signaling by another lipid (Merida et al. 2008). According to the gene ontology database (Ashburner et al. 2000), and the UniProt knowledgebase (UniProt Consortium 2012), the functions of the DGKD gene relate to fat metabolism, and include glycerolipid and glycerophospholipid metabolism and the phosphatidylinositol signaling system, both of which may influence the accumulation of BF. Although there are no other studies reporting the association between this gene and carcass traits the gene has a role in lipid metabolism, explaining the effect it has on average BF, and we hypothesize that this may secondarily affect the carcass weight. Further studies are required to validate this association in other populations.

17-beta-hydroxysteroid Dehydrogenase 12 (HSD17B12)

17-beta-hydroxysteroid dehydrogenase 12 catalyzes the transformation of estrone (E1) into estradiol (E2), suggesting a central role in estrogen formation. It also has 3-ketoacyl-CoA reductase activity, reducing both long-chain 3-ketoacyl-CoAs and long-chain fatty acyl-CoAs, suggesting a role in long fatty acid elongation (Moon and Horton 2003). In general, the enzyme is involved in lipid metabolism, fatty acid biosynthesis and steroid, especially estrogen, synthesis. In this study, this gene was associated with quality grade ($P = 0.0006$) and explained 1.36% of the phenotypic variation. HSD17B12 also showed a trend of association with marbling ($P = 0.02$, data not shown). Although there has been no previous report on its association with quality grade and marbling, we hypothesize that its role in biological processes related to lipid metabolism may influence the fat levels in meat resulting in variation in marbling and consequently carcass quality grade.

Low-density Lipoprotein Receptor-related Protein 4 (LRP4)

The LRP4 gene was significantly associated with yield grade, explaining about 5.5% of the phenotypic variation. LRP4 is a potential cell surface endocytic receptor, and has been associated with functions such as calcium ion binding, anatomical structure development, cell differentiation and bone formation (Ashburner et al. 2000; Uniprot Consortium 2012; Rebhan et al. 1997). A SNP in the LRP4 gene was significantly associated with bone mineral density and limb development in humans (Unnur et al. 2008). A mutation in this gene caused syndactyly in Holstein cattle (Duchesne et al. 2006). LRP4 also plays a role in lipid and cholesterol metabolism as a member of the low-density lipoprotein receptor gene family, which is involved in reducing cholesterol levels in blood (Brown and Goldstein 1997). The role of this gene in anatomical development and bone mineralization may have an impact on the weight of the carcass. In addition, based on its role in cholesterol metabolism, we hypothesize that it may consequently affect yield grade, which is estimated from a combination of carcass weight, fat content and muscle development.

NADH Dehydrogenase (NDUFS3)

The NDUFS3 gene was associated with CREA ($P = 0.0005$), and explained 3.2% of the phenotypic variation in CREA. The gene also showed a trend of association with YGRD ($P = 0.03$, data not shown). The enzyme NADH dehydrogenase (NDUFS3) is involved in a complex of reactions in the electron transport chain and oxidative phosphorylation in the mitochondria (Ashburner et al. 2000; UniProt Consortium 2012). Other gene ontology terms associated with NDUFS3 are protein binding, apoptosis and negative regulation of cell growth (Ashburner et al. 2000). In a previous study, the NADH dehydrogenase 2 was significantly associated with marbling fat content in the loin muscle (Kim et al. 2009). We hypothesize that its role in energy metabolism in the electron transport chain may influence traits related to growth and fat deposition thereby influencing marbling. Further research is needed to validate the

association between this gene and carcass ribeye area in other populations of cattle.

Serine threonine kinase 10 (STK 10)

STK10 was significantly associated with slaughter weight ($P = 0.02$) and explained 0.62% of the phenotypic variation in SLTWT. It also showed a trend of association with carcass weight ($P = 0.03$), yield grade ($P = 0.04$) and ultrasound ribeye area ($P = 0.04$) (data not shown). Serine/threonine kinase 10 belong to the family of serine/threonine kinases and their functions are to phosphorylate and activate members of the AMPK-related subfamily of protein kinases (Baas et al. 2003). In pigs, the protein kinase AMP-activated γ3 subunit gene, *PRKAG3*, which encodes the γ3 isoform of AMPK was identified by positional cloning as the causative gene for the Rendement Napole (RN) phenotype (Andersson 2003). The RN phenotype is common in Hampshire pigs and is characterized by a 70% increase in skeletal muscle glycogen content, decreased post mortem muscle pH and water content and increased lean meat content (Andersson 2003). There is evidence that the RN phenotype is caused by a missense mutation (Arg to Gln) in *PRKAG3*. In sheep, AMPK was shown to be negatively correlated with muscle adipogenesis (Tong et al. 2008). In beef cattle, a SNP marker in PRKAG3 position T2885C has been significantly associated with meat tenderness (Wu-Feng et al. 2012). We hypothesize that the effect of STK10 on the carcass traits may be through the activation of the PRKAG3 gene and consequently have effects on multiple carcass traits. Additional studies are required to validate this hypothesis.

Genes Significant in Multivariate Analysis

The genes significant in multivariate analysis were HSD17B12 (see above), PDHX, SLC30A5 and UBA5. The multiple traits considered in this analysis were either related to fat content in meat, such as carcass and ultrasound marbling, average BF thickness and grade fat, or they had components that were calculated from fat-related traits such as yield grade, which is estimated from fat content, carcass weight, and muscle development.

The PDHX is a component of the pyruvate dehydrogenase enzyme complex, which catalyses the conversion of pyruvate into acetyl CoA which enters the citric acid cycle (cellular respiration) producing energy for cellular processes (Rebhan et al. 1997). A previous study reported significant association between a SNP in the PDHX gene and body weight and body mass index in humans (Fox et al. 2007). The biological relevance of this gene in energy metabolism may subsequently affect fat and growth in steers causing variation in fat content, growth and carcass weight. We therefore hypothesize that the gene may play a similar role in cattle as that reported in humans by Fox et al. (2007) resulting in variation in carcass weight. There were no studies that reported association between the SLC30A5 and UBA5 and carcass traits; however, we recommend further studies to validate the significant associations observed.

Genes Showing a Trend of Association

The GHR gene codes for the receptor that binds growth hormone and other peptide hormones. In this study, the SNP in GHR gene showed a trend of association with both average BF thickness and grade fat explaining 8.8% of the phenotypic variation in grade fat and 9.0% of the phenotypic variation in average BF. Mogens et al. (1993) showed that growth hormone significantly reduced the fat content and trim on meat. Another allele on the GHR gene has been significantly associated with mean differences in final weight, eye muscle area, marbling score and fat color, but the same allele was not associated with carcass weight, BF thickness and final meat quality grade or meat color (Han et al. 2009). A different SNP in the 4th intron of the GHR gene was significantly associated with body weight and feed efficiency in beef cattle (Sherman et al. 2008).

The SLC45A2 gene showed a trend of association with grade fat explaining 5% of the phenotypic variation. The functions of the SLC45A2 gene are more related to melanin production, and in humans the gene is associated with hair, skin and eye pigmentation. Polymorphisms in the same gene have been associated with silver and white color phenotypes in chickens (Gunnarsson 2007) and using the comparative functional genomics approach, similar effects may be anticipated in beef cattle. The relationship between coat color and growth in cattle was investigated by Finch et al. (1984) who showed that color had significant effects on growth, with white steers gaining 0.13 kg more per day than dark steers. They also showed that coat color affected the feeding behavior of steers, with light-colored steers spending more time in the sun grazing than dark ones. The relationship between coat color, obesity and adiposity has also been illustrated with the agouti gene in mice and its interactions with the melanocortin receptors, with Mc4r knockout mice showing obesity. Mc3r has also been implicated in body weight regulation where antagonists of Mc4r result in increased fat mass, reduced lean meat and increased feeding (Voisey and Van Daal 2001). In addition, a SNP located in a highly conserved region of the MC4R gene was found to be significantly associated with BF thickness, growth rate and appetite in pigs (Kim et al. 2000; Fan et al. 2009). Therefore, although a direct link between the SLC45A2 and carcass traits has not been reported, we hypothesize that it may interact with the pigment genes and melanocortin receptors and consequently influence fatness, growth rate and appetite.

The ARHGAP1 gene is a member of the cytoskeleton regulator family associated with protein binding and regulation of GTPase activity. Although the ARHGAP1 gene has not been linked to carcass traits before, a SNP in the promoter region of this gene has been associated with osteoporosis in mice (Duncan and Brown 2010).

In another study, ARHGAP1 knockout mice tended to be weaker and most died during their neonatal period. ARHGAP1 knockout mice that survived had a short lifespan, showed premature ageing phenotypes, such as reduction in body mass, loss of sub-dermal adipose tissue and osteoporosis (Wang et al. 2007). In this study, the ARHGAP1 gene was associated with carcass weight and accounted for 1.2% of the phenotypic variation. Although there is a lack of previous reports of association between ARHGAP1 and carcass traits, we hypothesize that variation in this gene may have effects similar to the ones reported by Wang et al. (2007) resulting in lower body mass and sub-dermal adipose tissue, which would result in reduced carcass weight in beef cattle.

Although two SNPs in the Calpastatin gene were included in this study there was no significant effect on carcass quality traits. Previous studies have reported significant association between CAST and feed efficiency and carcass quality (see Introduction), the main effect is on beef tenderness (Barendse 2002; Casas et al. 2006).

When all the genes significantly associated with specific traits were considered together, DGKD, ABTB2 and STK10 accounted for about 5% of the phenotypic variation in slaughter weight and UBXN4 and HSD 17B12 accounted for about 14% of the phenotypic variation in quality grade. To be cost effective, a marker set needs to account for at least 10–15% of the genetic variance (Crews et al. 2006); therefore, the two markers associated with quality grade may be validated and incorporated into a MAS panel for beef quality grade. Although these genes were initially selected as positional candidate genes for traits that may also influence feed efficiency, none of the genes that accounted for more than 10% of the phenotypic variation in a carcass trait was also significantly associated with feed efficiency (data not shown) indicating that their use in MAS will not have significant effects on feed efficiency traits.

Gene Networks and Biological Processes

The gene networks and biological processes were reconstructed using IPA® (Ingenuity® systems) software. There were few interactions between the significant genes, which may indicate a possibility that their effects are relatively independent, and only a few genes interact with each other directly. However, these genes may interact indirectly depending on the hub they belong to and the genes that are involved in that hub.

The major hubs identified were associated with the insulin (Ins1) gene in the first network and the UBC gene in the second network. There were several hubs associated with other genes including the NDUF genes.

The Ins1 hub corresponded with biological processes associated with the metabolism of glucose, sterol and lipids. Other genes in the first network were involved in sterol metabolism and the regulation of transcription of several genes involved in several biological processes including cellular growth, immunity and cellular development.

The Ubiquitin C (UBC) hub corresponded to biological processes including degradation of proteins in the endoplasmic reticulum, lysosomes and the proteasome, and activation of transcription factor NF-kappa-B, cell signaling and DNA repair.

The NDUF hubs represent biological processes involved in energy production and utilization and, by extension, lipid metabolism. These processes are more specific to carcass traits, especially the traits related to fat content in meat, such as marbling and average BF thickness.

Other important biological processes identified by the gene network analysis were acetyl-CoA biosynthesis, estrogen biosynthesis and cytokine signaling. Acetyl CoA is important in energy and lipid metabolism and may have an effect on fat traits in carcasses. Estrogen is also involved in lipid and cholesterol metabolism and growth in complex biological pathways that will not be discussed in detail here, but can be found in a review by Berthezène (1999). These processes may be implicated in its role in influencing carcass traits in cattle.

CONCLUSIONS

We have reported 10 SNPs in 10 genes associated with various carcass traits in beef cattle with significant effects on slaughter weight, carcass weight and average BF thickness. These genes need to be validated across other diverse breeds and populations in other geographical locations to assess the reproducibility of the results in other populations. If these markers show consistent results across different populations then SNP panels for MAS could be developed from these markers for selection of carcass traits in diverse beef populations.

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