Quantitative trait loci and genes affecting beef tenderness

By

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DECLARATION

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List of Abbreviations

adjusted shear force	= best linear unbiased prediction which is effectively an average of shear force values (kgF) taken during 4 ageing days (1, 5, 12 and 26 days); derived from a multi-variate mixed model in which key effects were accounted
ageing rate	= amount of ageing in ln kg per 25 days; calculated as the difference between natural log shear force values after 1 and 26 days ageing
bp	= base pairs
BTA	= cattle chromosome
BLUP	= best linear unbiased prediction
CAPN1	= calpain 1; 80-kDa subunit for μ-calpain protease (by convention, italics = gene, no italics = protein)
clld	= cooking loss (%) measured in LD muscle
clst	= cooking loss (%) measured in ST muscle
cM	= centiMorgan; genetic map distance based on recombination rate where 1% = 1 cM
dNTPs	= deoxyribonucleotide triphosphates
HRM	= high resolution melt; genotyping method
in/del	= insertion/deletion; DNA variants involving nucleotide addition or removal
LD	= *M. longissimus dorsi* muscle
MSA	= Meat Standards Australia
Mb	= megabases; one million bases
MSTN	= myostatin; negative inhibitor of muscle growth (by convention, italics = gene, no italics = protein)
PCR	= polymerase chain reaction; method of amplifying specific DNA segments
pHld	= pH measured in LD muscle averaged across 4 ageing times
pHst	= pH measured in ST muscle averaged across 4 ageing times

QTL	=	quantitative trait loci; region of genome controlling trait
QTL peak	=	QTL maximum; location with highest probability of linkage
QTN	=	quantitative trait nucleotide; DNA variant generating QTL
SE	=	standard error
SNP	=	single nucleotide polymorphism; single base change with frequency > 1%
ST	=	*M. semitendinosus* muscle
ST_Compression	=	compression measurements (kgF) of ST muscle
ST_Hydroxyproline	=	hydroxyproline content of ST muscle (mg/g); measure of collagen
STR	=	simple tandem repeat; short tandem of less than 10 bases, also known as microsatellites
wbld_adjusted	=	BLUP adjusted shear force (kgF) for LD muscle derived from a multi-variate mixed model in which key effects were accounted
wbst_adjusted	=	BLUP adjusted shear force (kgF) for ST muscle derived from a multi-variate mixed model in which key effects were accounted
wbld1	=	LD Warner-Bratzler shear force (kgF) at day 1 of ageing
wbld26	=	LD Warner-Bratzler shear force (kgF) at day 26 of ageing
wbst1	=	ST Warner-Bratzler shear force (kgF) at day 1 of ageing
wbst26	=	ST Warner-Bratzler shear force (kgF) at day 26 of ageing

DEDICATION

I dedicate this work to my wife, Tzu, and my parents for their unlimited support with love and patience during the whole journey. Completing this research was not an easy job. Not only did I need to become accustom to the way of studying in Australia, but also we had to abandon what we had already had. My wife, Tzu, sacrificed her own job and had to live with me in a peaceful but monotonous place - a place with many lovely people but without suitable jobs for her. This work was done by Tzu and me. I could not get through the journey without her help. Therefore, I would like share this thesis with her.

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ABSTRACT

Tenderness is one of the major meat quality factors that affects the intent of consumers to re-purchase beef. Both genetic and non-genetic factors affect the quantitative trait of tenderness. Among the genetic factors, polymorphisms in key genes, such as the *myostatin* (*MSTN*) and *calpain 1* (*CAPN1*), play important roles on tenderness. However, these genes do not explain all the genetic variation associated with tenderness. The aim of this study was to discover additional genes associated with tenderness to help integrate genetic information into beef cattle breeding programmes and meat quality assurance programmes, such as Meat Standards Australia, and produce high quality tender meat for consumers. Discovery of such genes should also aid in the understanding of mechanisms underlying tenderness.

Backcross QTL mapping progeny based on crosses between two extreme *Bos taurus* breeds (Limousin and Jersey) were used in the study. There were four new traits created for the QTL mapping and association studies. Two of the traits (wbld_adjusted and wbst_adjusted) were based on Warner-Bratzler (WB) shear force measurements from the *M. longissimus dorsi* (LD) and *M. semitendinosus* (ST) muscles and were derived from a multi-variate mixed model in which the environmental effects, *myostatin* F94L genotype effect, ageing day effect and the interaction effects were accounted for. The adjusted shear force traits offered a more accurate prediction for average tenderness. The other new trait was the amount of ageing per 25 days (called “ageing rate” herein) for the two muscles, calculated as the difference between natural log shear force values after 1 and 26 days ageing.

Quantitative trait loci (QTL) mapping for these traits indicated there were 2 QTL (92 cM on BTA 5 and 52 cM on BTA 29) for adjusted shear force of the LD muscle, 3 QTL (96 cM on BTA 5, 36 cM on BTA 18 and 52 cM on BTA 29) for adjusted shear force of the ST muscle, 2 QTL (40 cM on BTA 4 and 0 cM on BTA 13) for ageing rate of the LD muscle and 2 QTL (48 cM on BTA 1 and 44 cM on BTA 19) for ageing rate of the ST muscle.

Twelve candidate genes were selected for further study based on their physiological functions and the QTL mapping results from herein and elsewhere. Twenty DNA variants in these candidate genes were chosen for the association studies. The analyses were conducted with and without three known tenderness related gene variants (*MSTN* F94L, *CAPN1*-SNP316 and *CAPN1*-SNP530). Variants in the candidate genes were discovered to be significantly associated with traits related to tenderness, most of which were muscle specific effects. Of note, the effects of *CAPN1*-SNP316 were muscle specific. The heterozygous genotype (GC) of *CAPN1*-SNP316 had the opposite effect on LD and ST muscles in that the G allele was dominant for the LD but recessive for the ST. Another variant of large effect, *MYO1G*-SNP2 (*myosin* 1G), showed an effect on ageing rate of the LD muscle but not the ST muscle.

Importantly, however, the interactions between gene variants frequently explained more of the genetic variation than the individual variants. For example, the interaction between the candidate gene variant *SNIP1*-SNP3 (*Smad nuclear interacting protein 1*) and the *CAPN1*-SNP316 explained more of the variation in the

adjusted shear force of the ST muscle than *CAPN1*-SNP316 alone (9.5% vs. 5.2%).

The studies also suggest that tenderness is not always affected by the genes that change the muscle weight or collagen content (eg. *insulin-like growth factor 1*). In fact, the results indicate that the effect of the *myostatin* gene on tenderness is not caused by the increased muscle mass or collagen changes associated with the *myostatin* F94L variant. Instead, most of the effect of *myostatin* on tenderness may be explained by a change in the muscle fibre types which affects calpain activity.

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