Candidate Gene Effects on Beef Quality

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Abstract

The contribution of five candidate genes to the variation in meat tenderness, pH, colour, marbling and water holding capacity (WHC) was analysed in muscle samples from 243 young bulls of Angus, Charolais, Hereford, Limousin, or Simmental breed, raised in Swedish commercial herds. The animals were genotyped for single nucleotide polymorphisms (SNPs) in the genes encoding calpain 1 (CAPN1:c.947G>C), calpastatin, (CAST:c.155C>T), diacylglycerol O-acyltransferase 1 (DGAT1), leptin (UASMS2C>T) and stearoyl-CoA desaturase 1 (SCD1).

The CAPN1:c.947G>C SNP showed associations with marbling and meat colour, and to some extent also tenderness. The CAST:c.155C>T T allele, which was the most common allele, showed a favourable association with Warner Bratzler shear force (WBSF) and compression tests. The K232A polymorphism at the DGAT1 gene was associated with level of beef marbling. An association was observed between UASMS2C>T and compression tests and meat colour. The SCD1 SNP was associated with variation in meat colour traits after 6 days with access to oxygen. There was no association of the tested SNPs with WHC traits and pH value.

Our results show that gene effects are of importance for quality of meat from Swedish young bull of beef breed and Swedish beef can therefore be improved by including beef quality and DNA-tests in the breeding program. The CAST:c.155C>T SNP proved to be a good marker for tenderness in Swedish beef.

Keywords: Beef meat quality, candidate genes, marbling, colour, water holding capacity, single nucleotide polymorphisms, genetic marker.

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Dedication

To the Swedish Beef Farmers

Fear gives wings.

Romanian proverb

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ekerljung, M., Li, X., Lundén, A., Lundström, K., Marklund, S., & Näsholm, A. (2012). Associations between candidate SNPs in the calpastatin, calpain 1 and leptin genes and meat tenderness among Swedish beef populations. Submitted.

II Li, X., Ekerljung, M., Lundström, K., Lundén, A. (2012). Association of polymorphisms at DGAT1, leptin, SCD1, CAPN1 and CAST genes with colour, marbling and water holding capacity in meat from beef cattle populations in Sweden. Submitted.

Abbreviations

ATP	Adenosine triphosphate
CAPN1:c.947G>C	SNP in the calpain 1 gene
CAST:c.155C>T	SNP in the calpastatin gene
DGAT1	Diacylglycerol acyltransferase
EU	European Union
KAP	Kött Avel Prodution (the Swedish beef recording scheme)
LSM	Least squares mean
LT	*M. Longissimus thoracic*
MetMb	Metmyoglobin
RTPCR	Real time polymerase chain reaction
SCD1	SNP in the Stearoyl-CoA desaturase1gene
SE	Standard error
S-EUROP	Carcass classification system in Europe based on conformation
SJV	Statens Jordbruksverk (Swedish Board of Argriculture)
SNP	Single nucleotide polymorphism
UASMS2C>T	SNP in the promoter region of the leptin gene
WBSF	Warner-Bratzler shear force

Introduction

Beef quality

During 2008 the Swedish government presented a new vision and Sweden wants to become Europe's new culinary nation with high standards for food safety and quality for the benefit to consumers (SJV, 2012). In line with the vision it is important that the beef produced in Sweden meet consumer demand. Meat tenderness, marbling (juiciness), and colour stability are important parts of beef quality (Wood et al. 1999; Seyfert et al. 2006; Legrand et al. 2012). Meat quality is a complex concept and thus has a variety of definitions of which one is the attractiveness of meat to the consumer. Consumers have an ambiguous relationship to meat; on the one hand they want to eat a fat and tasty steak at the restaurant and on the other hand they search for the leanest piece of beef in the shop. Retailers want a hygienic and safe meat product that is saleable, while beef farmers want animals that are healthy, fast growing, feed efficient, and that achieve high grading on the conformation scale. Animal welfare and nutrient and health aspects of eating beef are other important aspects. International studies have shown that beef eating quality is assessed fairly consistently by consumers worldwide (Polkinghorne & Thompson 2010).

Swedish beef

The beef industry in Sweden

The number of cattle in Sweden has decreased with 25 % between 1980 and 2011 (SJV, 2012). Even if the total number of cows (about half a million today) has decreased, the cows for beef production have increased almost three times and are today more than one third of all cows. The average beef cattle herd had increased from 6 heads in 1980 to 17 heads in 2011. The beef industry in Sweden produced almost 140 000 ton of beef (including bone) during 2011 and the degree of self-sufficiency of beef was 56 % in 2011 (SJV, 2012).

The most common beef breeds in Sweden are Charolais (more than 5000 recorded cows), Hereford (more than 3000 cows), Simmental (about 2500 cows), Limousin (about 1300 cows), and Angus (about 1000 cows) (KAP 2011). None of these breeds are of Swedish origin. Angus came to Sweden in the early 1940's, Hereford in the early 1950's, Charolais by the end of the 1960's, Limousine in 1970, and Simmental in 1974 (Swedish Beef Farmers Association 2012).

In Sweden, unlike many other countries, lots of intact bulls for slaughter are used. The slaughter category 'young bull' is a large group, about 40% of all slaughtered cattle, not counting calves (KAP 2011). It is well known that bulls are less tender than other gender (Jeremiah et al. 1991; Zhang et al. 2010), and when such a large proportion of the meat comes from young bull there is a risk that the consumers will not get the quality they want when purchasing beef at the store.

Tenderness

Most consumers agree that tenderness is a major characteristic in determining beef quality (Koohmaraie, 1994). There are many factors that contribute to the tenderness, like physical constraints due to the meat, or rather skeletal muscle structure, activity and availability of endogenous proteases, the environment in muscle cells such as temperature and pH value. All these features combine together in the tenderizing process (Huff Lonergan et al. 2010) and the process must extend over a time period which may not be too short to get optimal results. Tenderness is measured at a stage when it is too late for improvement. The high variation in tenderness is a major challenge to the beef industry. One problem is that the variation in tenderness is significant even though the cutting parts, e.g. striploin, are the same. The degree of tenderness depends on how the carcass is treated after slaughter. Toughness in European cattle breeds are due to differences in fibre type and proteolytic enzyme activity (Christensen et al. 2011). Feeding and handling before slaughter is also very important.

Marbling

Fat in the beef is one important property in eating quality (Thaller et al. 2003). Fat within the muscle is called marbling or intramuscular fat (IMF). IMF can vary from very small lipid cells, not visible to the naked eye, to snowflake-like fat structures or heavily marked fat islands in the muscle. Most consumers agree that well-marbled beef is both juicy and tasty. Fat content in the meat and tenderness are related (Christensen et al. 2011).

Water holding capacity

Water constitutes 75% of the meat weight. A high water holding capacity of beef is desired both for economic and palatability reasons (Huff-Lonergan & Lonergan, 2005). The continuously lowered pH after slaughter reduces the

charge of the proteins and causes water to move from intercellular to extracellular spaces whereby the water can be lost.

Meat colour stability

The colour of the meat after packing and storage is an important property and most consumers believe that discoloured meat is synonymous with inferior quality (Mancini & Hunt, 2005). The colour in beef is due to presence of myoglobin, which is an oxygen carrier with an iron ion (Fe^{2+} or Fe^{3+}) as a ligand (Mancini et al. 2003). Allowing oxygen access to the meat the surface will 'bloom' (i.e. turn red in colour) and gets an attractive appearance within an hour. In this oxymyoglobin state oxygen molecular is attached to the ligand. When the meat is vacuum-packed (i.e. no oxygen present) it attains a purple/lilac colour and a water molecule is attached to the ligand (deoxymyoglobin). After several days in oxygen the meat will attain an unattractive brown or gray colour, due to the formation of metmyoglobin resulting from oxidation of deoxymyoglobin or oxymyoglobin (Jeremiah, 2001). The miscoloured meat has hydrogen attached to the ligand and the iron ion is oxidised from Fe^{2+} to Fe^{3+}.

There are differences in colour stability between muscles of which *LT* has comparatively high colour stability (Lindahl, 2011). Lindahl (2011) also found that a steak from *LT* that had not gone through aging resulted in a product with lower colour stability, compared to aged beef cuts from the same muscle. The meat surface colour can be measured as: lightness (L*), redness (a*), yellowness (b*), intensity of the colour (chroma), and the hue of the colour (hue angle). The larger the hue angle the less red beef (Lee et al. 2003).

Candidate genes

Some genes have been selected for in thousands of year through breeding for particular phenotypic characteristics as growth and milk yield (Andersson et al. 2001). When it is discovered that genes are linked to the properties, they are called candidate genes. Candidate genes hypothetically regulate phenotypic variation of interest and are typically identified based on some prior knowledge about the gene function and/or observed association between the phenotypic variation and DNA variation (polymorphism) or differential gene expression. Candidate genes for the genetic regulation of phenotypic variation of interest such as beef quality can have the causative mutations in protein-coding sequences or in noncoding sequences that may affect gene expression, mRNA splicing or post-translational modification of the encoded protein. Polymorphisms in the DNA can affect the translation to amino acids building

up all the proteins the body needs. Mutations that alter the protein composition by an amino acid replacement may affect the structure of the vital three-dimensional folding, and thereby affect the protein function. If the polymorphism is limited to one nucleotide it is called a single nucleotide polymorphism (SNP). The SNP genotypes of individual animals can be determined and hypothetic associations between different SNPs and phenotypic observations can be tested. The use and interest of DNA tests as replacements or complements to phenotypic evaluation has increased since the late 1900's and the benefits are many. For example beef animals with desired genotypes can be selected for further breeding. Also, interesting studies on genetic historical relationships between cattle populations can be done with SNP analysis.

Calpain

Calpains are a big family of enzymes that are well conserved in vertebrates (Croall et al. 2007). Calpain was discovered in 1964 (Sorimachi et al. 2011) and is an endogenous enzyme with many physiological functions in the body. One function is as intracellular cysteine proteases. Calpain 1 is a variant of the calpain enzymes that attacks structural proteins in the meat and thereby make the meat tender. Calpain 1 is activated by calcium and inhibited by the enzyme calpastatin. There are two variants of calpain 1, m-calpain 1 and µ-calpain 1.The m-calpain 1 is activated by millimolar calcium ions (0.3 mM for half maximal activity) whereas µ-calpain 1 is activated by micromolar calcium iones (around 50 µM for half maximal activity). Both iso-enzymes of calpain can convert to the other variant when needed.

The calpain 1 gene is located on Bos Taurus chromosome 29 (BTA 29) and the enzyme is considered the most important in the beef tenderization process (White et al. 2005; Mullen et al. 2006). Barendse et al. (2007) reported an association between tenderness and the CAPN1:c.947G>C SNP of the calpain 1 gene, resulting in an amino acid substitution from glycine to alanine. The Gly316Ala substitution means that the small amino acid glycine is switched to alanine. Alanine is not much bigger but has a hydrophobic side chain, which glycine is lacking. The same SNP has also been denoted CAPN1-316 (Page et al. 2002) and is included in commercial DNA-tests (Van Eenennaam et al. 2007).

Calpastatin

Calpastatin is an endogenous enzyme and was purified as a Ca^{2+}-dependent proteinase in 1987 by Otuka and Goll (1987). Calpastatin is an inhibitor of the enzyme calpain. Calpain and calpastatin are active in the muscle of the living animal and also after slaughter in the tenderization of meat, during aging, as the calpastatin inhibit calpain and thereby resist the process.The calpastatin gene is located on BTA 7. Among several analyzed SNPs in the calpastatin gene the strongest association with meat tenderness was reported for the CAST:c.155C>T SNP (Barendse et al. 2007). According to the author´s knowledge the CAST:c.155C>T SNP is not included in any commercial DNA-tests. This amino acid substitution, where proline, having a ring structure, is replaced by an amino acid having a hydrophobic side chain, may have an impact on protein function.

DGAT1

Diacylglycerol acyltransferase 1 (DGAT1) catalyzes the triacylglycerol synthesis from acyl-CoA and 1, 2-diacylglycerol. This enzyme was found 1998 by Cases et al (1998). It has been shown that the dinucleotide mutation resulting in an amino acid substitution from lysine (K) to alanine (A) at position 232 (exon 8) in the gene encoding DGAT1is related to the amount of intramuscular fat in cattle (Thaller et al. 2003). Lysine has a positively charged side chain and alanine, which is a smaller amino acid, has a hydrophobic side chain.

The proposed ancestral K allele has been associated with higher activity of the enzyme (Grisart et al. 2002). In the study by Kaupe et al. (2004) the A allele was found to be the most frequent allele in the majority of the analysed beef breeds.

Leptin

Leptin was discovered by Halaas et al. (1995). The Greek word leptos means thin, despite the leptin gene being called the obese gene. It is a hormone that plays a key role in metabolism, the function is to inhibit appetite, such as when leptin is unable to act, the feeling of hunger increases. The leptin hormone is produced by fat cells, and the variation of its concentration in plasma is related to variations in body fat in sheep (Delavaud et al. 2000). In cattle serum

concentration of leptin has been reported to be correlated with marbling score and body fat (Geary et al. 2003). The leptin gene on BTA 4 encodes leptin, a hormone regulating the amount of back fat (Buchanan et al. 2002; Nkrumah et al. 2004). The T allele of the UASMS2C>T SNP in the leptin gene has been associated with ultrasound marbling score in a study by Nkrumah et al. (2005) and also with overall liking in a test panel evaluating tenderness and flavour (Gill et al. 2009). This SNP does not affect the translation, to mRNA, because it is located in a promoter region, but may still be important because such a region may regulate gene expression.

SCD1

Stearoyl-CoA desaturase 1 (SCD1) is a well conserved enzyme in mammals (Milanesi et al. 2008), responsible for conversion and regulation of the process that desaturate saturated fatty acids to monounsaturated in mammalian tissues SCD1 is also called delta 9-desaturase because the double bond is attached on the ninth carbon from the omega end of the molecule. This amino acid replacement (Ala293Val) in SCD1.878 G>A does not seem to make a difference on the protein structure as judged by the size and side chains. Both are fairly small and have hydrophobic side chains. Yet this SNP has showed effect on the amount of intramuscular fat (Wu et al. 2012).

From muscle to meat

Muscles

Skeletal muscles are complex structures made for locomotion, stability, and balance of the body (Hopkins 2006). The main functions are to be able to pull and push and also relax at rest. Both for muscle contraction and relaxation energy is needed. In a muscle there are structures in a hierarchy order from the entire muscle to bundles of fibres and to the small units' myofibrils (Figure 1). The structures of actin and myosin filaments in myofibrils are responsible for muscle contraction (Figure 2).

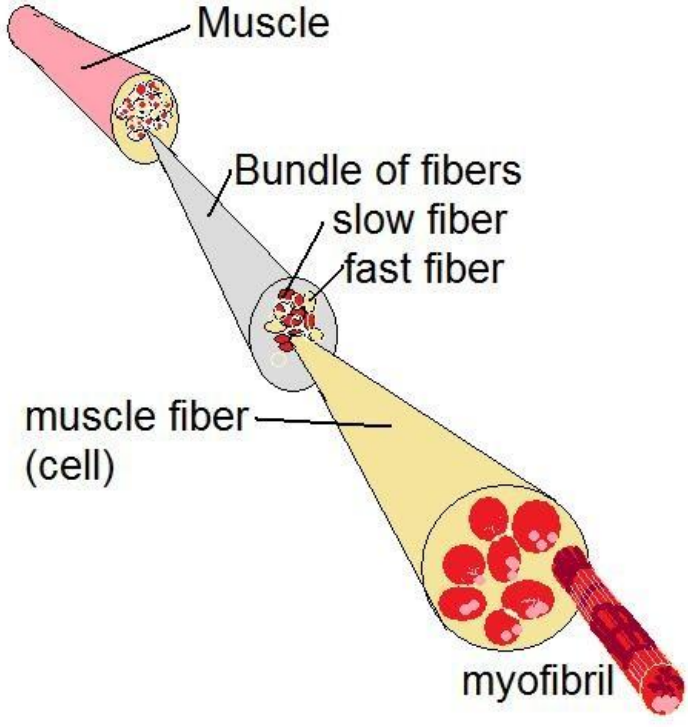

Figure 1. The structures in a muscle, the entire muscle, bundles of fibers, the muscle fiber and myofibril.

The most important proteases in muscles are calpain and calpastatin which both are depended on the calcium ion concentration in the muscle (Goll et al. 1992). When the calpain 1 activity is high, the growth rate is low and when the calpain 1 activity is low, the growth rate becomes high. Goll (et al. 1992) reported that calpastatin increased muscle weight as it prevented calpain 1 to break down the muscle. An intact bull is growing fast and has higher calpastatin activity (Morgan et al. 1993) during the first seven days of aging, than cow, heifer and steer. The high calpastatin level and activity in bulls at the time of slaughter prevents the calpain 1 enzyme to tenderize the muscle which may underlie the less tender meat from bulls.

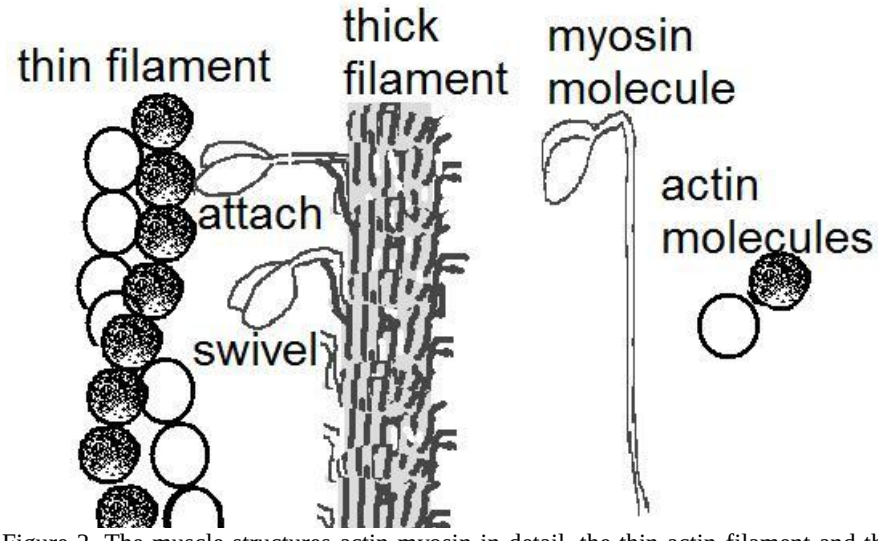

Figure 2. The muscle structures actin myosin in detail, the thin actin filament and the thick myosin filament. The myosin heads attach to the actin filament.

Meat

Conversion of muscle to meat is a complex process (Kemp et al. 2012; Thornton et al. 2012). The slaughter of bulls includes stunning and bleeding. The muscle of the slaughtered animal tries to maintain homeostasis despite the drastic change of the physiological environment with sudden lack of energy, oxygen and nutrients. When death occurs, the cell can initiate a process similar to the apoptotic process (i.e. systematic cell death that may occur in a living individual, see Figure 3). This process starts as a result of the environmental changes in energy, oxygen, and nutrients in the cells at death. Systematic cell death is a strictly regulated and highly conserved process. Calcium is crucial for controlling the process and for the activation of calpain (Ouali et al. 2006; Orrenius et al. 2003).

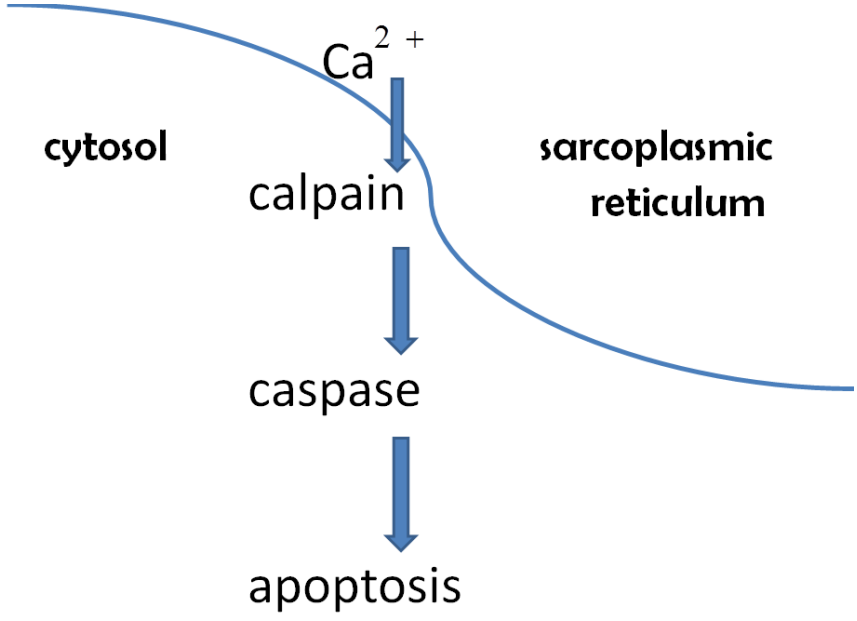

Figure 3. Calcium ions from the sarcoplasmic reticulum activate calpain, which in turn activates cysteinyl aspartate-specific proteinases caspase that starts apoptosis (systematic cell death) (after Pinton et al. 2001).

When the rigor phase starts it is very important that the carcass has already been taken care of in a proper way. Important for beef quality is carbohydrate stored in the muscles, that temperature falls in a certain rate, extension of the muscles and that the proteolyses of the muscles starts (the tenderness process). When the blood flow ends, after death, the muscle becomes anaerobic but glycolysis in the cell continues to produce ATP and the waste product lactate. Lactate can no longer be transported from the muscle by the blood, instead it accumulates in the muscle cells (Bendall et al. 1978). The amount of lactate in the muscle is dependent on the amount of glycogen. Amount of glycogen may be insufficient if the animals suffer from peri-mortal stress. Mixing bulls from different boxes or herds before slaughter is an example of a stress factor (Lacourt & Tarrant 1985). The adrenaline released during stress breaks down glycogen (Maltin et al. 2003). It is important to avoid stressing cattle before slaughter. If the carcass lacks lactate, due to stress, the muscle pH will not fall, and as a result the meat will become dark firm and dry (DFD) with shorter shelf-life and lower product quality (Dunne et al. 2011). Lactate in the cell makes pH to fall from 7 in the living animal to below 5.7 24 hours post mortem (Maltin et al. 2003).

Optimal pH for active µ-calpain is 6.5 but a too rapid drop of pH can lead to µ-calpain inactivation (Kemp et al. 2012). The temperature of the carcass should

not drop too fast when ATP is still present, as this will make muscles to contract strongly. Electrical stimulation is used to empty the carcass of ATP, to avoid irreversible cross-bridges within muscles. It is essential that the carcass is hanging or in some other ways make the muscles to stretch out as much as possible, before rigor sets in. The stretching of the muscle will increase the sarcomere length (Thompson et al. 2006) (Figure 4).

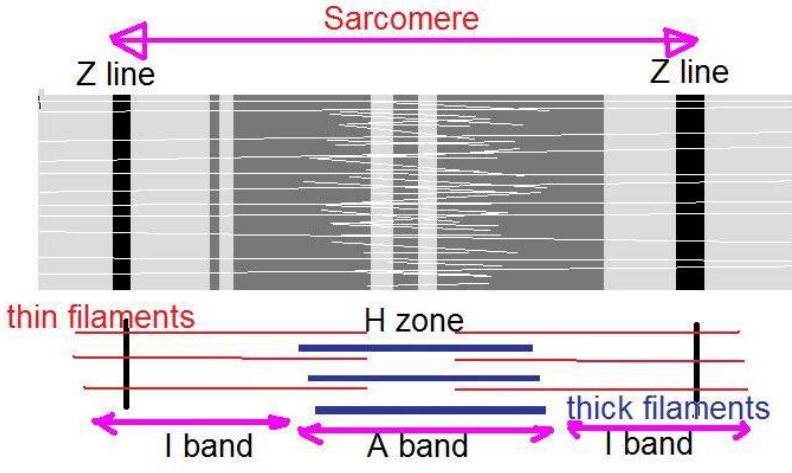

Figure 4. The sarcomere needs to be stretched out to avoid cold shortening. This cross-striations is the reason for the pattern of band that is well known in skeleton muscles.

Otherwise the muscles will contract as the actin and myosin will form cross-bridges which becomes irreversible when ATP runs out at the onset of rigor (Huff Lonergan et al. 2010) because the ATP is needed to release the actin-myosin formation.

If additional cross-bridges have been formed because of the shortage, nothing can make the meat tender. If the muscle gets shortened both toughness and drip losses will increase (Honikel et al. 1983). Calcium ions are released from the sarcoplasmic reticulum into the cytoplasm as the muscle fibre approaches rigor mortis (Ouali et al. 2006) and activate the calpains. The calpain system has been identified to be the most important contributor to post-mortem proteolysis (Kemp et al. 2012) and µ-calpain 1 is the first to degrade muscle protein (Figure 6).

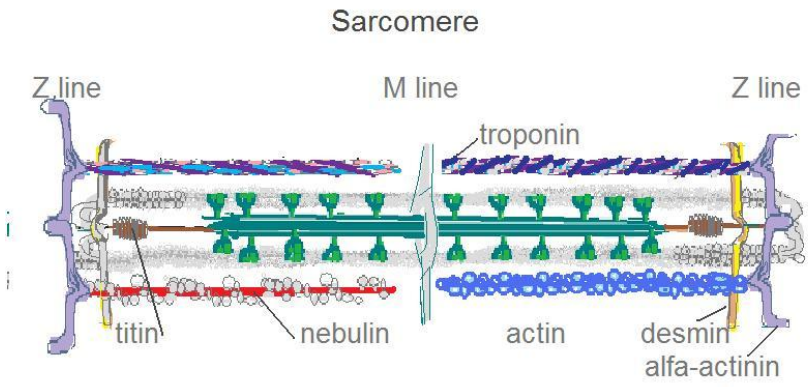

Figure 5. The sarcomere in detail. Calcium binds to troponin and an actinmyosin complex is formed as the actin is connected to the Z-band the sacromere (myofibril) will shortened and contract the muscle.

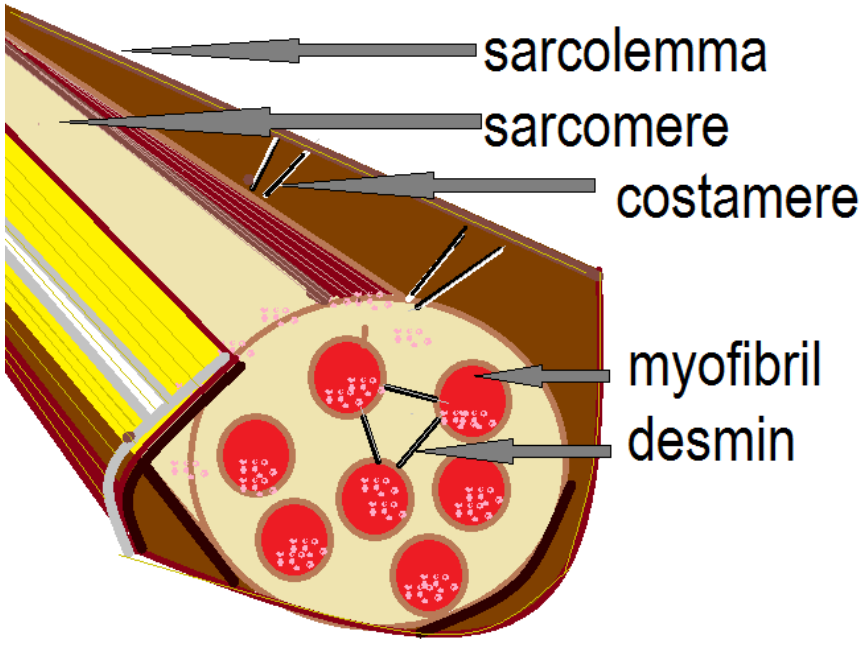

Figure 6. µ-calpain 1 is thought to attack the costamere and desmin during post-mortem proteolysis. Costamere and desmin are structural proteins which act as cohesive units in the muscle. The costamere attaches the sarcolemma to the sarcomere and desmin attaches the myofibrils with each other (adapted from Maltin et al. 2003).

Aims

The aim of the study was to examine possible associations between candidate genes and beef quality in purebred Swedish beef cattle. The intention was to investigate if commercial DNA marker-based tests for meat quality are applicable to beef cattle under Swedish conditions. In paper I candidate SNPs in the calpain 1, calpastatin, and leptin genes were investigated with respect to their influence on tenderness in meat samples from young bulls of Angus, Charolais, Hereford, Limousin, and Simmental breeds. In paper II the effects of candidate SNPs in the calpain 1, calpastatin, leptin, DGAT1, and SCD1 genes on colour, marbling, and water holding capacity were studied.

Summary of investigations

Materials

The project started in autumn 2008 and is based on purebred young bulls slaughtered in Swedish commercial beef herds during the years 2008, 2009, and 2010. A total of 243 *M. longissimus thoracis* (*LT*) meat samples from Angus, Charolais, Hereford, Limousin, and Simmental young bulls slaughtered at eight different Swedish abattoirs were collected. Average carcass weight for the breeds varied from 316 kg for Hereford to 386 for Charolais and average age at slaughter varied from 12.7 months for Simmental to 14.6 months for Hereford (Table 1). In the statistical analyses 14 meat samples with pH higher than 5.8, were excluded. In paper I meat samples cut from the wrong part of the *LT* muscle were also excluded, together with samples from Simmental bulls which were too few. In total 200 samples from four breeds were used in paper I and in paper II 229 samples from all five breeds were used.

Table 1. Mean by breed with standard deviation as subscript for carcass traits and measurements [1] analyzed in paper I

	Angus	Charolais	Hereford	Limousin	Simmental
Number of observations (n=205)	35	97	34	29	10
Carcass traits					
Slaughter age (months)	15_{2}	14_{1}	15_{1}	14_{1}	13_{1}
Carcass weight (kg)	319_{43}	386_{27}	316_{35}	368_{23}	368_{31}
EU-conformation (15 classes)	$7.2_{1.7}$	$10.2_{1.4}$	$6.9_{1.1}$	$12.5_{1.4}$	$9.2_{1.4}$
Carcass fatness (15 classes)	$8.0_{1.3}$	$7.0_{1.1}$	$7.7_{1.8}$	$6.5_{0.7}$	$7.9_{1.3}$
Warner Bratzler shear force					
Peak Force (N)	$37.1_{9.7}$	$45.5_{13.7}$	$49.8_{13.0}$	$51.3_{19.7}$	$46.6_{13.6}$
Shear firmness (N/mm)	$4.4_{1.1}$	$5.9_{1.8}$	$6.4_{1.7}$	$6.2_{2.2}$	$6.2_{1.6}$
Total energy (Nmm)	260_{58}	300_{72}	334_{67}	319_{104}	301_{62}
Compression variables					
Hardness (N)	93_{9}	117_{28}	125_{29}	126_{23}	120_{26}
Compression energy (Nmm)	439_{67}	563_{137}	644_{139}	535_{126}	585_{97}

[1] Peak force = force needed to shear through the meat; Shear firmness = the slope between the origin and the peak of the curve; Total energy = total energy needed to shear through the meat. Hardness = peak of first compression curve; Compression energy = area under the first curve.

Methods

Meat sample collection

A 15 cm length of *LT*, close to the 11th-12th ribs, was excised at random side from each animal. Meat samples were vacuum-packed, stored, and transported at around 4° C. The samples reached the laboratory within seven days after slaughter. On day seven, pH was measured and the meat samples were cut according to the scheme in Figure 7. Initially, the rough end was cut off to make a clean and nice cut. The next 2 cm slice was used twice, first a photo was taken on both sides for marbling measurements and then it was used for colour measurements. For DNA isolation, a small piece of meat from the inner part of the muscle was chopped, packed in sterile microfuge tubes, and stored at -80 °C. The remaining part of the meat sample (at least 7 cm) was vacuum-packed and stored at -20 °C until tenderness measurements were made.

Genotyping

Allele and genotype frequencies were calculated using data from all 243 slaughtered bulls. DNA was extracted from muscle tissue using the Omega Bio-Tek Tissue DNA Kit. Allelic discrimination was carried out using Real-Time PCR with TaqMan chemistry (Applied Biosystems StepOnePlus™) with primers and probes given in Table 2.

Beef quality measurements

Meat colour
After blooming with access to oxygen for 1.5 hour at day 13 post mortem colour measurements were carried out on the 2 cm thick slice using a Minolta CM-600d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan).

Tenderness analyses
The meat samples were thawed and after the meat had reached room temperature, it were cooked in a 72°C water bath until a core temperature of 70°C was reached thereafter cooled down until it reached room temperature. From each muscle sample, twelve 3-cm long strips with a 10×10 mm cross-sectional area were cut out, following the direction of muscle fibres parallel to the strip.

Table 2.Primer and probe sequences used for genotyping Swedish young beef bulls for candidate genes for meat tenderness

	Canditate genes for				
	Calpain [1]	Calpastatin [1]	DGAT1 [2]	SCD1 [3]	LEP [4]
SNP name	*CAPN1*:c.947	*CAST*:c.155	DGAT1	SCD1.878	*UASMS2*
Allele subst.	G>C	C>T	AA>GC	G>A	C>T
Translation	Gly316Ala	Pro52Leu	K232A (Lys232Ala)	Ala 293Val	promoter region
Forward primer	GGCTGGGCAGGTCAGT	AACAAGCCTTGGGAGCAGT	CGCTTGCTCGTAGCTTTGG	CCCCGAGAGAATATTCTGGTTTC	AGGTGCCCAGGGACTCA
Reverse primer	AGCTGCTCCCGCATGTAAG	AAAGTAGGTGTCTTTTCATGTCCA	CGCGGTAGGTCAGGTTGTC	CCACTAGACGTGGTCTTGCT	CAACAAAGGCCGTGTGACA
VIC probe	CCACGGCGTTCCA	AAAAAGCCCCGGTCC	CGTTGGCCTTCTTAC	CTTACCCGCAGCTCC	CAAGCTCTAGAGCCTGTGT
FAM probe	CCACGCCGTTCCA	AAAAAGCCCTGGTCC	TTGGCCGCCTTAC	ACTTACCCACAGCTCC	AAGCTCTAGAGCCTATGT
Genebank	AF252504	NM_174003.2	AY065621	AY241932.1	AB070368

[1] Barendse et al. (2007).

[2] Grisart et al. (2002).

[3] Taniguchi et al. (2004).

[4] Nkrumah et al. (2005)

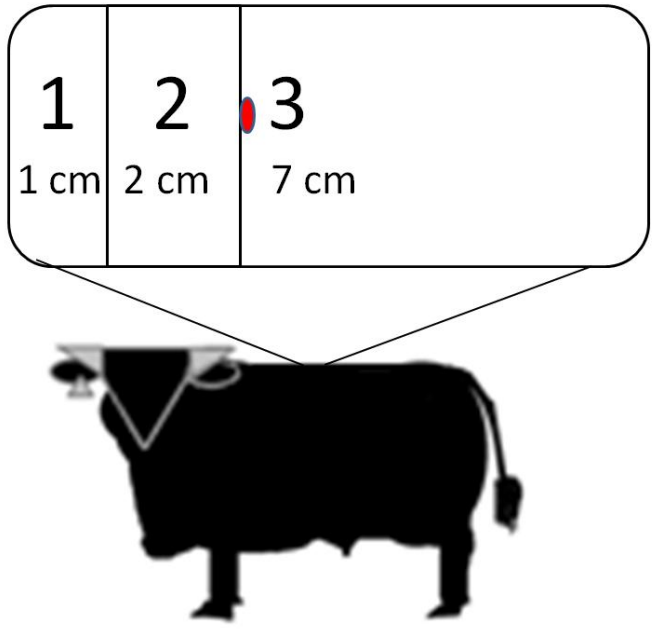

Figure 7. Illustration on utilization of the 10-15 cm long meat sample cut out close to the 12th rib the seventh day after slaughter. The carcass was split in four parts, first it was split along the spine and then across the body. From the back-part at random size, the first cut of the LT was our meat sample. 1) This part was used for pH measurements and after that 1 cm was cut off. 2) A 2 cm thick slice was photographed for marbling and then also used for colour measurements 3) a 7 cm (or more) long slice used for all tenderness measurements was vacuum-packed and put in a freezer at -20°C. A small amount of meat, marked with a red dot, from the inner part of the meat cut, was chopped and stored in -80° C for DNA-extraction.

Meat tenderness was measured on these strips as Warner Bratzler shear force (WBSF) by using Stable Micro Systems Texture Analyser HD 100 (Godalning, UK), equipped with a WB shear force blade with a rectangular shaped cutting area of 11 mm × 15 mm. WBSF was recorded as peak force (the highest force needed to shear through a standardized meat sample, N), shear firmness (the slope of a line drawn from the origin of the curve to its peak, N/mm), and total energy (the area under the curve, Nmm). Compression was recorded as hardness (the peak of first compression curve, N) and compression energy (the area under the first compression curve, Nmm) (Honikel, 1998). Mean values were calculated for all strips within sample and were used in statistical analyses. Pearson product-moment correlations between the five tenderness measurements varied between 0.77 and 0.98 (average 0.87).

Marbling measurements
An image analysis program analyzed the proportion of white and red pixels from the image of the meat sample (Figure 3, slice 2).The white pixels were used as an estimation of the IMF (Garcia et al. 2006). The subjective marbling score method used the same images. Two trained panellists scored the amount of visible fat within the meat. The work was done individually. Both sides of each slice of meat were used for both measurement methods. Five photographs with different levels of IMF were selected as reference samples. The scale ranges from 1 point = no visible IMF, to 5 = medium amount of IMF since no individual with high marbling was found in the group of young bulls.

Water holding capacity
Water holding capacity was measured as percentage of weight loss of the meat sample during thawing and cooking. Total loss of water was calculated as sample weight before freezing minus sample weight after cooking in percentage of sample weight before freezing.

Statistical analyses

To test the effects of the SNPs on the tenderness measurements a mixed linear model was used within the Statistical Analysis System computer package (SAS 2010) that included fixed effects of the SNPs (two or three genotypes for each SNP) breed of animal, random effect of slaughterhouse within breed, and age within breed as covariate. To correct for unequal variances between the four breeds, Satterthwaite's approximation method was implemented using breed as "group" in the "repeated" statement.

For each of our response tenderness measurements we were interested in making 9 comparisons (three SNPs and three genotypes) and we therefore adjusted with this number in the Bonferroni method.

Results

The animals

For the WBSF and compression variables (Table 1) the lowest averages were found for meat from Angus bulls, whereas the highest values were generally found for meat from Limousin and Hereford bulls. Mean and standard deviation for colour stability, pH, marbling, and water loss traits are presented in Table 3. For a* at day 0 (day 7 post mortem) meat from Hereford had the highest value i.e. most red, and Limousin the lowest. For hue angle the lowest

Table 3. Mean by breed, with standard deviation within brackets, for traits [1] analyzed in paper II

		Angus	Charolais	Hereford	Limousin	Simmental
Number of observations (n=233)		38	109	35	32	19
Number of observations (n=218)		37	100	35	29	17
Colour measurements (n=233)						
Day 0 in air [2]	L*	35.3 (2.3)	35.9 (2.1)	32.8 (2.1)	33.2 (2.5)	34.3 (1.8)
	a*	14.9 (2.5)	13.8 (2.1)	15.5 (1.5)	12.4 (1.6)	15.3 (2.3)
	b*	14.7 (1.4)	14.6 (1.3)	14.1 (1.4)	12.9 (1.4)	15.0 (1.5)
	Chroma	21.0 (2.6)	20.1 (2.2)	20.9 (1.9)	17.9 (2.0)	21.5 (2.6)
	Hue angle	44.8 (3.5)	46.7 (3.0)	42.4 (1.5)	46.1 (2.1)	44.6 (2.5)
	DeoxyMb	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)
	MetMb	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)
	OxyMb	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)	0.6 (0.0)	0.7 (0.0)
Day 6 in air [2]	L*	36.8 (2.6)	36.9 (2.2)	34.2 (1.7)	33.4 (2.5)	35.5 (1.8)
	a*	15.2 (2.4)	14.1 (2.8)	15.8 (1.7)	7.6 (3.2)	15.8 (3.0)
	b*	15.2 (1.9)	15.4 (1.4)	15.3 (1.1)	11.7 (1.8)	15.6 (1.6)
	Chroma	21.6 (2.7)	21.0 (2.7)	22.0 (1.9)	14.0 (3.2)	22.3 (3.1)
	Hue angle	45.2 (4.1)	48.1 (4.8)	44.2 (2.4)	58.2 (6.4)	45.1(4.8)
	DeoxyMb	0.6 (0.0)	0.6 (0.0)	0.6 (0.0)	0.7 (0.1)	0.6 (0.0)
	MetMb	0.7 (0.1)	0.6 (0.1)	0.6 (0.1)	1.0 (0.2)	0.6 (0.2)
	OxyMb	0.7 (0.0)	0.7 (0.1)	0.7 (0.0)	0.5 (0.1)	0.7 (0.1)
pH (n = 233)		5.6 (0.0)	5.6 (0.1)	5.6 (0.0)	5.6 (0.1)	5.6 (0.1)
Marbling (n = 233)						
	Score	2.9 (0.8)	2.0 (0.5)	2.3 (0.6)	1.8 (0.4)	2.3 (0.6)
	IMF (%)	3.6 (1.6)	1.9 (0.6)	2.1 (0.8)	1.8 (0.7)	2.3 (0.8)
Water holding capacity (n = 218)						
	Thawing loss (%)	4.7 (1.1)	4.6 (1.4)	5.2 (1.2)	3. 9 (1.7)	5.2 (1.0)
	Cooking loss (%)	21.0 (2.6)	22.0 (2.2)	22.2 (2.6)	18.7 (1.6)	22.9 (2.5)
	Total loss (%)	24.5 (3.5)	25.6 (2.6)	26.2 (2.7)	21.9 (2.3)	27.0 (2.4)

[1] L*(lightness), a* (redness), b* (yellowness) Deoxymyoglobin (DeoxyMb) is shown as (1.5 - K/S ratio), oxymyoglobin (OxyMb) as (1 - K/S ratio) and metmyoglobin (MetMb) as (2 - K/S ratio). IMF (%): intramuscular fat content, estimated as percentage of white pixels from image analysis. Marbling score: subjective assessment ranging from 1 (low marbling) to 5 (high marbling).

[2] Day0 and Day6 are 7 and 13 days post mortem, respectively.

value, i.e. the most red colour, was observed for meat from Hereford bulls. For oxymyoglobin at day 0 Limousin bulls got the lowest value, i.e. the most pale meat. After 6 days with oxygen supply the meat from Limousin still had the lowest value for a* and oxymyoblogin and Hereford the lowest for hue angle. The marbling measurements showed the highest averages for Angus and lowest for Limousin. For water holding capacity meat from Limousin bulls had the least amount of fluid lost of all five analysed breeds.

The candidate genes

CAPN1:c.947G>C

For CAPN1:c.947G>C the G allele was the most common in all breeds and for Hereford 97% of the animals carried at least one copy of the G allele (Table 4). Averaged over all breeds, the proportion of the G allele was 83%. The most common genotype was GG (69%) for all breeds except for Angus in which CG was the most frequent. In the present study the CC genotype was rare and was not found at all in the Hereford, Limousin, and Simmental breeds. The SNP showed significant effects for tenderness, colour and marbling (Table 5). The minor allele, C, was favourable for tenderness, marbling and colour (Table 6). Beef from animals with the CC genotype was more tender than beef from those with GG genotype and meat from the heterozygous animals was intermediate to the meat from homozygous. Meat from individuals with the CC genotype showed more marbling (fat content and marbling score) than the CG and GG genotypes, which did not differ. The genotype CC also showed better colour stability, according to the hue angle value (the lower the better) but the CC genotype was not significantly different from any of the other genotypes. There was no effect of the SNP on water holding capacity.

CAST:c.155C>T

The T allele of CAST:c.155C>T had a higher frequency than the C allele in all breeds except Simmental (Table 4). All three genotypes were present in all breeds except in Simmental, where no animal had the genotype TT. The heterozygous genotype was the most common in the Charolais, Hereford and Limousin breeds whereas in Angus TT was most common, and in Simmental CC was most common. The SNP showed an association with all tenderness measurements studied (Table 6). The lower peak force, shear firmness, total energy, hardness, and compression energy values observed for genotypes including the T allele indicated a favourable effect of this allele on tenderness. The effects of the CT and TT genotypes were not different which indicate a dominant effect of the T allele. For colour stability, IMF and water holding capacity no effect of CAST:c.155C>T SNP was observed.

Table 4. Allele and genotype frequencies (%) for SNPs in candidate genes for beef quality traits in Swedish young beef bulls of different breeds

		CAPN1:c.947G>C					*CAST*:c.155C>T					*DGAT1 K>A*					*SCD1.878G>A*					*UASMS2*C>T				
		Genotype			Allele		Genotype			Allele		Genotype			Allele		Genotype			Allele		Genotype			Allele	
Breed	N	CC	CG	GG	C	G	CC	CT	TT	C	T	KK	KA	AA	K	A	GG	GA	AA	G	A	CC	CT	TT	C	T
Angus	43	11	49	40	36	64	5	32	63	21	79	2	33	65	19	81	51	47	2	74	26	37	42	21	58	42
Charolais	109	4	21	75	14	86	18	50	32	43	57	0	21	79	11	89	39	50	11	64	36	76	23	1	88	12
Hereford	35	0	6	94	3	97	14	54	31	41	59	0	0	100	0	100	60	34	6	77	23	54	29	17	69	31
Limousin	35	0	31	69	16	84	17	52	31	43	57	0	11	89	6	94	31	54	14	59	41	31	63	6	63	37
Simmental	21	0	33	67	17	83	52	48	0	76	24	0	0	100	0	100	38	38	24	57	43	43	48	9	67	33

DGAT1 K232A

The DGAT1 K232 allele was not common in our study; overall frequency was 7%, with a range from 0 to 19%. The K allele frequency for Angus was 19, Charolais 11, Hereford 0, Limousin 6 and Simmental 0%. For the breeds Hereford and Simmental the DGAT1 232A allele was fixed. The KK genotype was not found in any of the breeds except Angus. The DGAT1 K232A showed effects for marbling (Table 5). The heterozygote genotype KA had the highest marbling both in the image analysis and in the subjective marbling score measurement. The AA allele was the favourable allele for marbling in the beef.

SCD1.878 G>A

For SCD1.878 G>A the G allele was the most common allele in all breeds with a frequency between 57 to 74% and with an average of 66%. Angus and Hereford had a similar distribution of the three genotypes, with a lower frequency for AA genotype (2 and 6% respectively,), whereas the other three breeds had a more even distribution for the genotypes. The average frequencies over breeds were 44% for GG, 45% for GA, and 11% for AA genotype. The SNP SCD1.878 G>A showed affects for colour traits (Table 5) and specific associations with colour parameters after 6 days in air (Table 6). Genotypes including the A allele had higher a*, b*, chroma value and OxyMb relative content in meat after 6 days with oxygen supply. There were no differences between AA and AG that indicate that the A allele is dominant.

UASMS2C>T

For UASMS2C>T, the C allele, was the most common in all breeds (Table 4). All genotypes were represented in all breeds but the TT genotype occurred in low frequency at average in 11%. In Charolais and Hereford the CC genotype was more common than CT whereas the reverse was true for the other three breeds. The SNP UASMS2C>T showed association for the traits; tenderness and colour (Table 5). For hardness and compression energy with a non adjusted comaprison between genotypes differ CC and CT, even CT and TT but not CC and TT from each other (data not shown). However, CT shows the lowest values for tenderness for hardness and compression energy. The C allele showed to be the most colour stable allele, at day 13 post mortem, as chroma (colour intensity) and there was no difference between the CC and CT. But the opposite was true for the amount of deoxymyoglobin where the genotype TT was higher than CC and CT.

Table 5. Effects of SNP polymorphisms for beef quality traits in Swedish young beef bulls of different breeds given as *P*-values (only traits for which significant SNP-effects were found in the study, are included in the table)

Trait [1]	SNP [2]					Breed [3]
	CAPN1	CAST	DGAT1	SCD1	UASMS	
WBSF						
Peak Force (N)	0.079	**<0.001**			0.156	0.060
Shear firmness (N/mm)	**0.005**	**<0.001**			0.082	0.144
Total energy (Nmm)	0.096	**<0.005**			0.134	**0.028**
Compression variables						
Hardness (N)	0.346	**0.001**			**0.039**	0.172
Compression energy (Nmm)	**0.012**	**<0.001**			**0.015**	0.091
Colour day 0 in air [4]						
L*	0.282	0.553	0.501	0.924	0.176	**0.002**
Hue angle	0.085	0.519	0.441	0.612	0.543	**0.012**
Colour day 6 in air [4]						
L*	0.693	0.261	0.213	0.964	0.169	0.065
a*	0.150	0.606	0.751	**0.003**	0.053	0.621
b*	0.876	0.439	0.612	**0.001**	0.085	0.973
Chroma	0.536	0.541	0.991	**0.001**	**0.040**	0.860
Hue angle	**0.006**	0.680	0.392	0.334	0.485	0.350
DeoxyMb	0.343	0.501	0.743	0.498	**0.027**	0.225
OxyMb	0.357	0.490	0.867	**0.002**	0.054	0.454
pH	0.438	0.416	0.520	0.479	0.278	0.565
Marbling						
Score	**0.028**	0.516	**0.021**	0.561	0.404	0.692
IMF (%)	**0.043**	0.104	**0.017**	0.942	0.460	**0.039**
Water holding capacity						
Total loss (%)	0.169	0.969	0.734	0.856	0.950	**0.015**

[1] For description view Table 1 and 3.

[2] SNPs CAPN1=CAPN1:c.947, CAST=CAST:c.155, DGAT1=DGAT1 K>A, and SCD1=SCD1.878G>A. DGAT1 K>A and SCD1.878G>A were excluded in paper I because of no significance with any of the tenderness traits.

[3] Breed for tenderness traits, was analyzed without the SNPs.
P-values lower than 0.05 are in bold.

Table 6. Significant effects of SNP polymorphisms and LSM for beef quality traits in Swedish young bulls

SNP	Trait [1]	n	Genotype [2]			*P*-value
			11	12	22	
CAPN1:c.947G>C	*WBSF*	200				
	Shear firmness (N/mm)		5.0 ± 0.4[a]	5.9 ± 0.2 [ab]	6.4 ± 0.2[b]	0.005
	Compression variables	200				
	Compression energy (Nmm)		494 ± 34	547 ± 20	583 ± 17	0.012
	Colour	229				
	Hue angle (day 6 in air)		46.8 ± 1.82[ab]	49.1 ± 1.36[a]	47.3 ± 1.32[b]	0.006
	Marbling	229				
	Marbling score		2.90 ± 0.22[a]	2.31 ± 0.12[b]	2.38 ± 0.10[b]	0.028
	IMF (%)		3.32 ± 0.31[a]	2.54 ± 0.16[b]	2.63 ± 0.14[b]	0.043
CAST:c.155C>T	*WBSF*	200				
	Peak force (N)		53 ± 3[a]	43 ± 2[b]	43 ± 2[b]	<0.001
	Shear firmness (N/mm)		6.8 ± 0.3[a]	5.3 ± 0.2[b]	5.1 ± 0.2[b]	<0.001
	Total energy (Nmm)		330 ± 16[a]	284 ± 12[b]	287 ± 12[b]	<0.005
	Compression variables	200				
	Hardness (N)		129 ± 6[a]	112 ± 5[b]	112 ± 5[b]	0.001
	Compression energy (Nmm)		607 ± 27[a]	519 ± 19[b]	497 ± 20[b]	<0.001
DGAT1 K>A	*Marbling*	229				
	Marbling score		-	2.64 ± 0.13[a]	2.42 ± 0.11[b]	0.021
	IMF (%)		-	2.99 ± 0.18[a]	2.67 ± 0.15[b]	0.017
SCD1.878G>A	*Colour*	229				
	a* (day 6 in air)		13.2 ± 0.75[b]	14.1 ± 0.74[a]	14.4 ± 0.83[a]	0.003
	b* (day 6 in air)		14.3 ± 0.47[b]	14.8 ± 0.47[a]	15.1 ± 0.51[a]	0.001
	Chroma (day 6 in air)		19.6 ± 0.76[b]	20.6 ± 0.75[a]	20.9 ± 0.83[a]	0.001
	OxyMb (day 6 in air)		0.66 ± 0.02[b]	0.68 ± 0.02[a]	0.69 ± 0.02[a]	0.002
UASMS2C>T	*Compression variables*	200				
	Hardness (N)		120 ± 5	113 ± 5	121 ± 5	0.039
	Compression energy (Nmm)		543 ± 19	507 ± 21	574 ± 26	0.015
	Colour	229				
	Chroma (day 6 in air)		20.9 ± 0.75[a]	20.6 ± 0.76[a]	19.6 ± 0.84[b]	0.040
	DeoxyMb (day 6 in air)		0.59 ± 0.01[b]	0.59 ± 0.01[b]	0.60 ± 0.01[a]	0.027

[1] For description view Table 1 and 3.

[2] Genotype allele 1 and allele 2; CAPN1:c.947G>C: C/G, CAST:c.155C>T: C/T , DGAT1 K>A: AA/GC, SCD1.878G>A: G/A, UASMS2C>T: C/T.

[ab]Least squares means within gene and measurement with different letters differ at $P < 0.05$ after Bonferroni correction

Discussion

For CAPN1:c.947G>C the frequencies in our study were consistent with results from other studies (Johnston & Graser, 2010; Van Eenennaam et al. 2007; Page et al. 2004). The CC genotype was rare or was not found at all in the Hereford, Limousin, and Simmental breeds. Page et al. (2002) also reported that the CC genotype was lacking in the Simmental breed. It could be noted that the G allele was almost, but not completely, fixed in a Brahman population reported by White et al. (2005). The CAPN1:c.947G>C SNP is included with the name T2 in the Gene STAR test (Johnston & Graser, 2010). Our study showed that CAPN1:c.947G>C was associated with marbling and colour stability, but this marker in the calpain gene was not a strong SNP for beef tenderness as expected.

For CAST:c.155C>T, the T allele was the most common in all breeds except in Simmental. Our results are in accordance with those in the study by Barendse et al. (2007), where the T allele was favourable. These results indicate that this SNP is a useful marker for predicting tenderness in meat from young bulls. Most breeds had a range from 57 to 79% of the T allele of CAST:c.155C>T, but Simmental had just 24%. In the Australian study by Barendse et al. (2007) frequencies of the T allele in CAST:c.155C>T varied between 38% and 77% for seven different breeds. Schenkel et al. (2006) showed polymorphism for a SNP in the calpastatin gene between different breeds. The somewhat contradictory results for Simmental in our study compared to the results for the other breeds, with T being the rare allele, are in agreement with results by Schenkel et al. (2006), where the common allele of the CAST/RsaI marker (AY_008267.1) located in intron 5, for Simmental was the opposite to the common allele for Angus, Charolais and Limousin.

The DGAT1 K232 allele was not common in our study and is in line with the frequency between 1-34% of the K allele for beef cattle breeds given by Kaupe (2003). Ripoli et al. (2006) reported that the European breeds have frequencis of the K allele on Angus 9.3, Hereford 0.0 and Charolais 15.0%. It may be noted that the likely original allele K is rare in the European breeds (Winter et al. 2002). High frequency of the K allele was reported by Lacorte et al. (2006), in four Bos taurus indicus breeds, there were in the frequency range 96 to 100%. A similar frequency of the K allele as in our study, were obtained by Pannier (et al. 2010) for Angus 18%, Charolais 18% Limousin 12% and for Simmental 6%. In Hereford the A allele was fixed in the study by Pannier et al. (2010), which is in agreement with thepresent study. The DGAT1 K232A mutation probably occurred after Bos separated into Taurus and Indicus with the K allele as the ancestral allele (Kaupe et al. 2003).

For SCD1.878 G>A the G allele was the most common in all breeds and the average for the frequency was 66%. That allele frequency is in accordance with Komisarek & Dorynek (2009) that showed an allele frequency of 70% for the G allele, in a study with 453 Polish Holstein-Friesian bulls. A similar frequency of 64 % was reported in a study by Milanesi (2008) for 281 Italian beef and dairy breeds. A bit lower frequency was reported in a study on 370 Fleckvieh, in Czech Republic, where the G allele frequency was 56% (Barton et al. 2010). SCD1 was associated with colour traits, the G allele showed higher colour value with oxygen supply on the sixth day and consistent results was found in a study by Reardon et al. (2010) already after 3 hour with oxygen supply. In a study by Wu et al. (2012) the G allele showed higher IMF than the A allele while our study was unable to show any effect of SCD1 on marbling trait.

For UASMS2C>T, the C allele, was the most common (69%) and the result are similar to other studies. Gill et al. (2009) showed frequencies of the C allele of 63% for crossbreed animals with purebred Angus as sire and Nkrumah et al. (2005) showed a frequency range of 74 to 89% for a hybrid experimental population. Schenkel et al. (2005) reported allele frequencies of the C allele for the breeds; Angus, Charolais, Limousin and Simmental to be 73, 77, 66 and 70 %, respectively. The SNP UASMS2C>T showed effects for tenderness and colour (Table 5). The results for tenderness tend to differ between the heterozygote genotype CC and CT also between CT and TT this it is difficult to assess because the differences was not between the alleles (11 and 22), then it is not possible to use this marker in a breeding program.

Interesting about the marbling traits is that the image analyses had a greater standard deviation than the subjective measurement, then the subjectively marbling scores set by two different persons. The correlation between IMF content and subjective marbling score was 0.77 ($P< 0.05$).

None of the tested SNPs had a significant effect on water holding capacity and pH. Breed differences were noted for total loss of water and meat from Limousin, which had the highest water holding capacity of all five breeds and with the least amount of fluid lost. There was not any differences in final pH measured the seventh day post mortem between any of the breeds.

The *LT* muscle do not differ in calpain activity between bulls and steers, however there is difference in calpastatin activity (Morgan et al. 1993) which make the bulls less tender. Probability of getting a though beef when buying Swedish meat is considerable, because a large proportion of beef in Sweden comes from intact bulls. The data in this study have limitations in numbers of

animals, difficulties with the large number of slaughterhouses and confounding between breeds and slaughterhouses. Any further adjustments to the data had to be done, some animals were DFD and from others, we received from the slaughterhouse, the wrong cut of the *longissimus dorsi*. Since different parts of the same muscle have different Shear Force (Bratcher et al. 2005) therefore the wrong cuts could not be used in the analyses.

Conclusions

Our results show that gene effects are of importance for quality of meat from Swedish young bull of beef breed and Swedish beef can therefore be improved by including beef quality and DNA-tests in the breeding program. Although the study was conducted on a limited material, it showed similar results as in studies on other cattle populations.

- The CAST:c.155C>T SNP showed clear effects on tenderness.
- The CAPN1:c.947G>C SNP showed associations with marbling and to some extent also tenderness.
- For meat colour stability there were effects of the CAPN1:c.947G>C, SCD1.878 G>A and UASMS2C>T SNPs.
- There was no association of the tested SNPs with WHC traits and pH value.

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Sammanfattning

Kandidatgeners effekt på nötköttets kvalitet

Mörhet, marmorering (intramuskulärt fett), färgstabilitet och vattenhållande förmåga är typiska köttkvalitetsegenskaper. Djurets ålder, kön och köttets mörningstid är faktorer kända för att påverka köttets kvalitet. Även hur djurets gener ser ut har betydelse. En kandidatgen är en gen som man har goda skäl att tro har effekt på en viss egenskap. Genom mutationer har det uppstått variation i genernas DNA. Proteaser är enzymer som bryter ner strukturer i köttet och på det sättet gör det mört. Calpain 1-enzymet anses vara det viktigaste enzymet i köttets mörningsprocess. Calpastatin är ett protein som inhiberar calpain 1 och dess aktivitet minskar mörningsprocessen. Leptin är ett aptitreglerande enzym som har visat sig påverka slaktkroppens mängd av fett. Enzymet DGAT1 katalyserar det slutliga steget i triglyceridsyntesen medan SCD1 är ett enzym som omättar fettsyror genom att införa en dubbelbindning, vilket anses göra fettet nyttigare. DGAT1 och SCD1 har visat sig påverka mängden marmorering i nötkött. Syftet med detta arbete var att undersöka kandidatgeners inverkan på kvaliteten hos kött från ungtjurar av köttras uppfödda i Sverige. I den första studien undersöktes samband mellan djurens olika varianter av CAPN1, CAST och UASMS2 och mörhet. I den andra studien undersöktes sambandet mellan CAPN1, CAST, UASMS2, DGAT1 och SCD1 och pH, marmorering, färgstabilitet och vattenhållande förmåga.

Material och metoder
Muskelprover samlades in från 243 renrasiga ungtjurar av angus, charolais, hereford, limousin och simmental uppfödda i svenska besättningar under 2008-2010. Ungtjurar valdes eftersom de utgör en viktig kategori (40 % av alla slaktade nötkreatur förutom kalvar) i Sverige. Dessutom vet man att kött från tjurar är mindre mört än kött från kvigor och stutar. Djuren slaktades vid en ungefärlig ålder av 14 månader och den genomsnittliga slaktvikten för de olika raserna varierade från 314 till 380 kg. En 15 cm lång bit ur första delen av ryggbiffen *M. longissimus thoracis (LT)*, nära revben11-12, togs från ena sidan av slaktkroppen (valet av sida skedde slumpmässigt). Köttet vakuumförpackades och transporterades vid en temperatur på 4° C till institutionen för livsmedelvetenskap på SLU. På den sjunde dagen efter slakt mättes pH och köttet skars upp efter ett noggrant schema. En 2 cm bred, köttskiva fotograferades på båda sidor med en digitalkamera, för bestämning av marmorering. En 7 cm lång bit av köttet vakuumpackades och lagrades vid -20° C för senare mätning av mörhet och vattenhållande förmåga. För DNA-extraktion, togs en liten bit kött från den inre delen av muskeln (för att undvika kontamination), köttet hackades och packades i sterila mikrocentrifugrör.

Vid mörhetsmätningarna tinades och kokades köttet. Ur det kokta köttet skars tolv 3-cm långa stavar med en 10 x 10 mm tvärsnittsyta. Stavarna skars ut på ett sådant sätt att muskelfibrernas riktning blev parallell med stavarna. Köttets mörhet mättes på ett standardiserat sätt som Warner Bratzler shear force (WBSF) och registrerades som maximal kraft (den kraft som behövs för att skära genom ett standardiserat köttprov, N), fasthet (måttet mellan kurvans högsta topp till origo, N/mm), och total energi (arean under kurvan, Nmm). Även kompressionsprov gjordes och köttets hårdhet (N) och den energi som går åt för att trycka ihop köttet (Nmm) registrerades. Köttets vattenhållande förmåga beräknades som andel av köttets viktförlust vid tining och tillagning.

Marmorering bedömdes från bilderna både genom ett bildanalysprogram för skattning av intramuskulärt fett (%) och genom en subjektiv visuell bedömning där två personer individuellt rangordnade bilderna genom att poängsätta från den magraste biffen (1) till den mest marmorerade biffen (5). Färgmätningar utfördes på dag 7 till 14 med användning av en spektrofotometer. Den relativa halten av deoxymyoglobin (den form av myoglobin som bildas vid avsaknad av syre och ger köttet en lila färg), oxymyoglobin (som bildas vid god syretillgång under minst en timma och ger köttet en körsbärsröd färg) och metmyoglobin (som bildas vid god syretillgång under en längre period och ger brunt missfärgat kött) beräknades. Genotypning för att bestämma djurens DNA i de fem kandidatgenerna utfördes med modern metodik kallad Real-Time PCR.

Statistisk analys
För att testa effekterna av kandidatgenerna på mörhet, pH, färgstabilitet, marmorering och vattenhållande förmåga användes linjära modeller som inkluderade de fixa effekterna av genvariant och ras, en slumpmässig effekt av slakteri inom ras, och ålder inom ras som kovariat. För att korrigera för ojämn storlek på grupper mellan raserna, användes Satterthwaite's approximationsmetod. Bonferronijustering gjordes för jämförelserna av de olika genotypernas effekt på köttets mörhet.

Resultat och slutsatser
En variant av CAPN1 visade på ett gynnsamt samband med mörhet, marmorering och färgstabilitet och en variant av CAST visade på ett gynnsamt samband med mörhet. Vår studie visade att denna variant av CAST är användbar för att förutsäga mörhet hos kött från svenska ungtjurar. Köttets marmorering påverkades av DGAT1 medan CAPN1, UASMS2 och SCD1 visade sig ha betydelse för färgstabilitet hos köttet från de svenska ungtjurarna. Inga samband observerades mellan de testade kandidatgenerna och vattenhållande förmåga eller pH i köttet.

Studien visade att kvaliteten hos kött från ungtjurar påverkas av djurets gener. Det innebär att det är möjligt att förbättra det svenska nötköttets kvalitet genom att inkludera kvalitetsegenskaper i avelsprogrammen för köttraserna. Även om studien utfördes på ett begränsat material, överensstämmer resultaten med studier gjorda i andra nötkreaturspopulationer av samma raser som i denna studie.

Nyckelord: Nötköttskvalitet, kandidatgen, marmorering, färgstabilitet, vattenhållande förmåga