ANALYSIS OF BOVINE MUSCLE DNA POLYMORPHISMS IN RELATION TO MEAT QUALITY, IN PARTICULAR INTRAMUSCULAR FAT

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Introduction

Intramuscular fat (IMF) deposition, also subjectively assessed as marbling, can be defined as the percentage of lipid content of muscle. It is a continuously varying trait in cattle exhibiting heritabilities of up to 0.65 (Marshall, 1999) and it is hypothesed to contribute to the juiciness and flavour of cooked meat (Schmutz, 2003). The identification of genes that contribute to continuous variation of meat quality, including IMF levels, is a challenge for molecular genetics and advances in molecular genetics have led to the identification of loci and DNA variations (polymorphisms) that affect traits of interest in livestock (Anderson, 2001). Some of these DNA polymorphisms may have the potential to serve as markers of meat quality. These genetic markers could help to improve the quality of beef, by marker-assisted selection (MAS) (Dekkers, 2004) by selecting these animals with a high genetic potential for a certain trait and by so producing better tasting, more valuable and more consistent beef. Three candidate genes were chosen for this study and were hypothesed to be one of the many genetic factors influencing IMF. Known single nucleotide polymorphisms (SNPs) in these genes have been determined. The leptin gene, also called the obese gene, has been assigned to bovine chromosome 4. Its product, a 16 kDa hormone called leptin, which functions in the regulation of adiposity, may be involved in controlling IMF levels. The thyroglobulin (TG) gene is located on chromosome 14 and codes for a protein TG, which is a precursor molecule of the thyroid hormones, which affect lipid metabolism. Finally the diacylglycerol-O-acyltransferase1 (DGAT1) gene, also located on chromosome 14, codes for DGAT1, a microsomal enzyme that catalyses triglyceride synthesis. Two known SNPs in the leptin gene in exon 2 (Buchanan *et al.*, 2002) and exon 3 (Haegeman *et al.*, 2000), one SNP in the TG gene in the 5' untranslated region (Barendse, W.J., 1999) and two SNPs in the DGAT1 gene in exon 8 (Winter *et al.*, 2002) have been determined. The objectives of this study were to investigate the frequency of the SNPs genotypes in the Irish cattle population and to determine if an association exists between these SNPs and IMF values. Only a few studies have been performed to show a significant effect of the SNPs on IMF values in cattle.

Materials and Methods

Blood samples were collected from pedigree bulls from a number of artificial insemination (AI) stations in Ireland. This sample set was used to investigate the frequency of the genotypes of the four polymorphisms in different breeds. To date, breeds tested include Aberdeen Angus, Charolais, Friesian, Limousin and Simmental. Secondly, bovine *M. longissimus dorsi* (LD) (249) were collected from slaughter-weight commercial cattle. Heifers were slaughtered under controlled conditions from a number of commercial abattoirs in Ireland. Meat quality measurements were analysed and the fat content for every sample was determined using the CEM analysis system, which is a hot solvent extraction (Bostian *et al.*, 1985). Genomic DNA was isolated from blood and muscle samples using the QIAamp® DNA mini kit. Diagnoses of the SNPs of leptin, TG and DGAT1 gene were detected by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism analysis (PCR-RFLP). PCR was carried out, using primers specific for the chosen candidate gene and the amplified fragments were a 545bp fragment for TG gene (Barendse, W.J., 1999), 411bp for DGAT (Winter *et al.*, 2002), and a 94bp and a 331bp fragment for two SNPs of the leptin gene (Haegeman *et al.*, 2000; Buchanan *et al.*, 2002). RFLP analysis was carried out by using specific restriction enzymes. Digests were separated and visualised on 3% agarose gels using gel electrophoresis and genotypes were assigned to each sample depending on its restriction pattern. For the DGAT1 gene, the two SNPs are positioned adjacent to each other, therefore, samples were cloned into a pCR®4-TOPO® vector and sequenced to distinguish between the different alleles. The genotype frequencies were performed using the FREQ procedure of SAS allowing differences between breeds and genotype to be discerned. Associations between the genotypes of the SNPs and IMF values were assessed using the GLM (one-way ANOVA) procedure of SAS.

Results and Discussion

Three genotypes CC, CT and TT, were detected for the leptin and TG genes and AA, KA and KK for the DGAT1 gene as previously reported (Haegeman *et al.*, 2000; Buchanan *et al.*, 2002; Barendse, W.J., 1999; Winter *et al.*, 2002). For the frequency study, some genotypes were present at a low frequency for certain breeds and not every genotype occurred in every breed (Table 1). Breed differences and geological aspects could partly influence the frequency values. For the two loci in the leptin gene, both the CC and CT genotypes are equally distributed while the TT genotype and also the KK genotype for DGAT1 occurs at a very low frequency or is even not present. The CC genotype of the TG

gene is more frequent then the CT or TT, and the TT genotype does not occur in 76 Friesians and 40 Limousins. Genotyping of a larger number of animals from each breed would establish genotype frequencies in Irish breeds, allowing breeders to increase the frequency if favourable genotypes have been associated with meat quality traits.

Table 1: Frequency (%) study of the SNPs genotypes in the Irish cattle population. 56 Friesians were used for the DGAT1 study.

Gene	TG gene			leptin exon 3			leptin exon 2			DGAT1		
Breed (n)	CC	CT	TT	CC	CT	TT	CC	CT	TT	AA	KA	KK
Friesian (76)	73.7	26.3	-	47.4	51.3	1.32	31.6	61.8	6.58	76.8	17.9	5.36 (56)
Limousin (40)	77.5	22.5	-	70.0	25.0	5.50	60.0	30.0	10.0	80.0	20.0	-
Charolais (34)	61.8	29.4	8.80	47.1	50.0	2.94	44.1	52.9	2.94	67.6	29.4	2.94
A. Angus (16)	6.30	56.3	37.5	87.5	12.5	-	12.5	75.0	12.5	62.5	37.5	-
Simmental (15)	46.7	46.7	6.70	46.7	53.3	-	60.0	40.0	-	93.3	6.67	-

Results to date show a non-significant ($P>0.05$) association between genotypes and IMF values in Irish beef for the chosen candidate genes (data not shown). Thaller *et al.* 2003 identified an associated with higher levels of IMF for T/T homozygous German Holstein cattle in *M. longissimus dorsi* for the SNP of the TG gene and in *M. semitendinusos dorsi* for the DGAT1 gene. In our study genotype TT for TG gene, is only present for 4 animals. This number of TT homozygous animals may have been too small for association detection. Buchanan *et al.*, (2002) identified the C allele of the SNP in exon 2 of the leptin gene, as the allele to be associated with fatter carcasses. While no association was found in this study, it might be that other SNPs in the candidate genes are correlated to IMF and it is also important to note that there are many other genes that are thought to contribute towards the quality trait of marbling (IMF) or to effect fat metabolism. Marbling is quite a complex trait that is affected by many biological pathways of different genes. It is important to also consider other unmarked genes, the interaction of genes and the production environment, which may all, have an effect on fat metabolism.

Conclusion

The frequency study doesn't allow us to make a final conclusion due to the small number of animals for some breeds. Although association were observed in other breeds in other studies, no association was detected so far in the Irish cattle population for the chosen candidate genes.

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