IDENTIFICATION OF MICROSATELLITES IN EXPRESSED MUSCLE GENES AND THE EVALUATION OF A POLYMORPHIC CT-REPEAT WITHIN THE PORCINE DESMIN GENE AS A MARKER FOR PIG MEAT QUALITY

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INTRODUCTION

The use of DNA markers to define animal genotypes and to select animals for particular production traits, commonly known as marker-assisted selection, is a powerful molecular tool for animal breeding (Visscher and Haley, 1995). As the final qualitative and quantitative determinants of meat are the genes that are expressed in muscle ante-mortem, it follows that muscle genes are potential candidate genes for marker assisted selection of meat quality and quantity. Microsatellites remain the most widely used polymorphic markers in association studies. Due to their relative abundance in the genome, they could even be found in cDNA sequences (Grosse *et al*., 1995 ; Ruyter-Spira *et al*., 1996 ; Jørgensen, *et al*., 1996). In this study, we report on the novel use of a porcine skeletal muscle cDNA library to isolate clones harbouring dinucleotide repeats.

MATERIALS AND METHODS

Microsatellite screening of a porcine skeletal muscle cDNA library. Phagemid excision was performed on a porcine skeletal muscle λ-ZAP-Express cDNA library, derived from a 50-day-old foetus. This was a directionally cloned library using a *Xho*I-anchored oligo(dT) primer for reverse transcription. Subsequently, plasmid clones were generated for colony hybridisation using four different dinucleotide-repeat ($[AT]_{13}$, $[CG]_{13}$, $[CA]_{13}$, and $[CT]_{13}$) oligonucleotide probes, 5'-end labelled with $\gamma\ ^{32}P$ ATP. Microsatellite-positive plasmid DNA was prepared using the Qiaprep plasmid isolation kit (Qiagen). DNA sequencing reactions were performed using a thermo-sequenase fluorescent primer cycle sequencing kit (Amersham) with a T7 primer, and analysed in a MWG Li-Cor sequencer. Homology searches on the EMBL database were performed using the FASTA programme (Genetics Computer Group).

Meat quality data.The 44 pigs used for association studies were of a Large White × F1 genotype, where the F1 genotype was derived from a cross of two synthetic lines containing 25% Duroc genes and 75% of either Large White or Landrace. All pigs were slaughtered at 105 kg (s.d. 4.3 kg) at the Department of Food Animal Science, Bristol University, UK and a range of meat quality parameters including intramuscular fat content, drip loss, pH at 45 minutes and 24 hours post slaughter, shear force and colour were measured. Colour was analysed using 'CIELAB' colour space. This instrument measures L, a, b, hue and saturation which are measures of colour (L: dark to light; a: red to green; b: yellow to blue). Hue is colour (red, purple etc) and is calculated from the angle whose tangent is b^{+}/a^{+}. Saturation is the intensity of colour and is calculated as the square root of $(b^{+2} + a^{+2})$. All pigs were homozygous

(NN) for the wild-type halothane genotype, and homozygous (rn^+) for the Rendement Napole (RN) gene (Fujii *et al*., 1991 ; Milan *et al*., 2000).

Polymerase chain reaction and product analysis. Primers (sense: 5'-GAGGGAATCC CCGAAGGTGC C-3', antisense: 5'-GGAGGTGCGC ACCCCTGCC-3') were designed on either side of the porcine desmin CT-repeat region, to amplify a 134 bp region in the plasmid clone. The forward primer was end-labelled with FAM phosphoramidite (Applied Biosystems). About 50ng of genomic DNA were used in each 50µl PCR, containing 2.5mM $MgCl_2$, 200µmol dNTPs, 20pmol of each primer and 1 unit of Taq polymerase (Promega). The thermal cycling profile was once at 94°C (5 min), followed by 35 cycles of 94°C (30 s), 60°C (45 s) and 72°C (45 s), and once at 72°C (5 min). PCR products were purified on Qiaquick spin PCR purification columns (Qiagen) and eluted in 30 µl TE buffer. Two µl PCR products were run in presence of 10 µl formamide in an ABI PRISM™ 310 genetic analyser (Applied Biosystem) and analysed with GenScan analysis software.

Statistical analysis. Analysis of variance including a covariate (ANCOVA) was performed for all traits studied with cold carcass weight as a covariate.

RESULTS AND DISCUSSION

Detection of microsatellites in a porcine skeletal muscle cDNA library. Colony hybridisation on 50,000 plaque clones from the muscle cDNA library was separately performed with four dinucleotide-repeat probes. The estimated number of positive clones with CT-, TG-, AT-, and GC-repeats were 1.48%, 1.23%, 0.48% and 0.70%, respectively. In total, microsatellites were found in nearly 4% of all muscle cDNA clones. This relatively high incidence of CT- and TG-repeats in the cDNA library is similar to the findings in the human and rat genomes (Beckman and Weber, 1992). We therefore found that a cDNA library is a rich source of transcribed microsatellite markers.

After phagemid excision to generate plasmid clones, 78 CT-repeat clones were selected at random and sequenced from both ends. A variety of cDNA clones were found. Clear flanking sequences were found in eight potential candidate genes, 6 were muscle-specific genes. Primer pairs were eventually developed for three clones, namely desmin, IGF2 and ankyrin1. The latter two were polymorphic but did not show significant association with any of the meat or carcass traits examined (data not shown). The entire 1.7kb desmin cDNA clone, chosen for meat quality association studies, was completely sequenced to characterise the location of the CT-microsatellite within the desmin gene. It was virtually identical to a previously reported porcine desmin cDNA sequence, mapped to chromosome 15 (Tuggle *et al*., 1999). Compared with the previously reported sequence, our desmin clone was incomplete at its most 5'-end, but, notably, it possessed an extended 672bp 3'-end region. The CT-repeat microsatellite was located within this extended region, 655bp downstream from the stop codon. Appropriate primers flanking the CT-repeat region were generated for PCR genotyping. Six allelic length polymorphisms were found in the 44 Large White cross pigs, of which 8 different genotype combinations were present (Table 1). Genotypes 131bp/134bp and 128bp/135bp were the most common, comprising 66% of the population used in the study.

Association studies. There was no significant effect on average daily growth rate or eye muscle area (p = 0.735 and 0.337 respectively). Meat quality parameters of intramuscular fat content (p=0.751), drip loss (p = 0.130), shear force (p = 0.256) and pH loss, defined as the pH difference between 24 hours and 45min post-slaughter (p = 0.078) were not statistically different. However, statistical differences were found between genotypes for colour characteristics of meat (Table 1) such that animals with genotypes 131/131 or 131/135, which constituted 13.6% of the population tested, consistently produced paler meat (higher L value) and higher values for hue and b. This indicates a susceptibility of these genotypes to have pale soft exudative (PSE) meat

It should be noted that microsatellite polymorphisms do not necessarily indicate the presence of different desmin protein isoforms. Their association with meat colour may be an indirect one. It might be that one or more genes located in the vicinity of this microsatellite, are involved with meat colour. These genes could co-segregate with the desmin microsatellite if the recombination distance is sufficiently small between them. The desmin gene is in relatively close proximity to the *RN* marker. However in this study, it seems unlikely that the *RN* marker is connected to the association between the desmin microsatellite and meat colour as all pigs used in the study were rn^+ (recessive wild type). Whatever the causal relationship between the desmin microsatellite and post-slaughter muscle colour may be, the results indicate that the desmin microsatellite is a promising candidate for marker development.

Table 1. Association results of eight desmin genotypes with meat colour (n=44)

Genotype	Frequency / %	Mean L colour (± s.e.)	Mean b colour (± s.e.)	Mean hue colour (± s.e.)
131/134	22 / 50	53.1 (± 0.61)	5.6 (± 0.20)	36.0 (± 0.57)
128/135	7 / 16	53.3 (± 0.97)	6.1 (± 0.45)	38.7 (± 1.09)
131/131	4 / 9.1	59.3 (± 1.81)	7.5 (± 0.67)	41.0 (± 0.72)
128/134	2 / 4.5	53.7 (± 0.02)	4.7 (± 0.25)	35.0 (± 2.29)
131/135	4 / 9.1	59.0 (± 0.42)	5.1 (± 0.45)	34.9 (± 2.53)
131/136	2 / 4.5	51.6 (± 0.97)	4.5 (± 0.97)	23.1(± 2.34)
135/135	2 / 4.5	55.9 (± 1.69)	4.5 (± 0.64)	29.3 (± 1.65)
135/138	1 / 2.3	53.0 (± N/A)	5.4 (± N/A)	33.0 (± N/A)

CONCLUSIONS

The results demonstrate that dinucleotide repeats are widespread in a tissue-specific cDNA population. Not surprisingly, based on sequencing results (data not shown), these repeats are usually sited outside coding regions, such as in the 5'- and 3'-untranslated regions, and probably, in incompletely spliced introns. Limited association study on the desmin microsatellite suggests the potential usefulness of such markers in marker-assisted selection for improving meat quality. As for the future development of the desmin marker, more animals of different genetic background will have to be analysed to fully ascertain its general usefulness in predicting meat colour.

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