Polymorphisms in genes affecting meat quality in European beef breeds

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INTRODUCTION – Many of the qualitative differences noticed in the meats of various calf species depend from environmental characteristics (different reproduction systems, butchering age, etc.). Undoubtedly, there are some genetic factors that influence said quality, such as the composition of fat acids (poly-saturated and saturated), fat distribution, muscle fibre, connective tissue make up. Comparison of genomic DNA sequences in different individuals reveals some positions at which two, or in some cases more than two, alternative bases can occur. These single nucleotide polymorphisms (SNPs) are highly abundant, the average number of SNPs in human genes has been found to be 1 out of every 185 bases (Stephens et al., 2001). Depending on where an SNP occurs, it might have different consequences at phenotypic level. There are SNPs in the coding regions of genes that alter the structure and/or function of the encoded proteins and can contribute to a particular character. However, most SNPs are located in non-coding regions of the genome, and have no direct known impact on the phenotype of an individual. SNPs are common among different genotypes of almost all species and the direct selection for important economically traits can be conducted by using SNPs in and around DNA coding regions. These applications require technology capable of genotyping thousands of SNPs from large numbers of individual DNA samples in an accurate, rapid and cost effective manner (Vignal et al., 2002). After the completion of the human genomic sequencing project, several other model species have been fully
sequenced (Band et al., 2000; Gibbs et al., 2002; Larkin et al., 2003; Schibler et al., 1998; Thomas et al., 2003; Venter et al., 2001). Currently (end of 2004), complete sequencing of livestock species, like pig and cattle, are also envisaged and ongoing with funds allocated for the full completion of the task. However, despite the great advantage of having a full sequence at hand, several studies must be further conducted to understand the role and functions of genes behind phenotypic traits that may be economically important. In accomplishing this task, model species particularly of human and rodents, are particularly useful, as they have been extensively characterised for several thousands of genes. For example, in Homo sapiens, slightly less than 35,000 mRNA and corresponding genes are annotated and for about half of them their function has been assessed (http://www.ncbi.nlm.nih.gov/). This database can be queried to search for homologous genes at least in mammalian livestock species. Therefore, it is quite straightforward to go from a gene in a model species with a function that may be relevant for an economically important trait to the same homologous gene in livestock species. Here we present some strategies used to find single nucleotide polymorphisms in candidates genes affecting meat quality in European beef breeds by exploiting knowledge gathered from model species, particularly human.

MATERIALS AND METHOD – Animals. Fifteen breeds across Europe (Asturiana de Valles, Asturiana de Mantaña, Pirenaica, Avileña, Limousine, Charolaise, Piedmontese, Marchigiana, Simmenthal, Danish Red Cattle, Holstein, Aberdeen-Angus, Jersey, Highland, South-Devon) were chosen, and 30 bulls from each breed were raised under similar condition up to the slaughtering, recording live traits, post-mortem traits and meat quality characteristic. DNA was extracted from biological materials (blood, semen, spleen or muscle).

Identification of SNPs. We have made up a list of 514 genes that may control variation in meat quality, based on knowledge of their physiological role in muscle development, composition, metabolism, or because they encode for structural components of muscle, have been studied. Only for 304 genes of the list, PCR primers were designed to amplify at least two different fragments, one preferably spanning exonic sequences and the other intronic ones. A panel of 30 animals (2 x 15 breeds) were analysed and several strategies have been used to detect polymorphisms and to identify SNPs (direct sequencing of PCR products, or sequencing after SSCP, RFLP-SSCP or DHPLC analysis).

The DHPLC technique has been described elsewhere (Oefener and Underhill, 1998), but in brief, has been developed to screen DNA variations by separating heteroduplex and homoduplex DNA fragments by ion-pair reverse-phase liquid chromatography. DHPLC uses unpurified PCR products that are subjected to a final denaturating/reannealing step to ensure adequate formation of heteroduplexes. Analysis time is 3 or 6 min per sample, depending on the configuration of the instrument, and the heteroduplex profiles are easily distinguished from homoduplexes (Cotton, 1997; Underhill et al., 1997).

Validation of SNPs – Primer extension. In this technique, an oligonucleotide is used, to prime DNA synthesis by a polymerase, as performed in a standard sequencing reaction. The 3’ end of the single base extension primer is positioned on the base just preceding the SNP to be tested. The DNA polymerase is then used to incorporate labelled ddNTPs, each of four labelled with a different fluorescent dye. The precise mass of the product is determined, depending on which ddNTP is incorporated. The CeQ 8800 software provides automated sizing and allele calling from single or multiplexed SNP products.

RESULTS AND CONCLUSION – Currently, 514 genes have been identified that potentially have an impact on meat quality; information on these genes is recorded in the project database (http://www.gemqual.org/). From the list of candidate genes, 354 have been assigned to four different partners of the project. Primers for 304 genes have been designed and about 680 SNPs were identified (table 1).

| 5’ UTR | 3’ UTR | exon | intron | total |
|-------|-------|------|-------|-------|
| 7     | 89    | 228  | 356   | 680   |

Table 1. A summary of discovered SNPs.
The analysis of the data for 680 mutations revealed these results: 644 SNPs are biallelic, of which 47 A/C, 228 A/G, 41A/T, 47 C/G, 234 C/T and 47 G/T; 34 SNPs are deletions or insertions, one shows 3 and one shows 4 alleles; 182 SNPs are transversion and 462 are transition. The transitions A/G have almost the same frequency of C/T. 98 of the 228 SNPs found in exons correspond to amino acid substitutions, while 130 are synonymous codon changes. Considering the SNP number relative to the total length of the amplified DNA fragments, the frequency found is about 2 SNPs every 1000bp and no differences were found in relation to SNPs position.

Several methods for SNPs genotyping have been tested, such as Perkin Elmer AcycloPrime™ system, primer extension or mini-sequencing (Lindblad-Tok et al., 2000; Pastinen et al., 2000), Ampliflour (Nazarenko et al., 2002) and Oligonucleotide Ligation Assay (OLA) (Tobe et al., 1996) and TaqMan (Livak et al., 1995).

An important point to consider when a laboratory chooses a technique for genotyping SNPs is the type of project envisaged, since it is quite different to perform genotypes with a limited number of SNPs on very large population samples, or a large number of SNPs on a limited number of individuals. In the GeMQual project we will have to test 680 SNPs on 973 animals and we resolve to use the primer extension or TaqMan techniques. Analysis of the meat quality data and of possible associations between the candidate genes and variations in meat quality, will require sophisticated statistical approaches. The methods used in other studies and other potentially relevant statistical techniques have been reviewed and initial tests using simulated data completed.

Further development of the methods will be carried out as real data emerges from the project.

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