# Uncovering the genomic and proteomic basis for variation in Irish pork quality

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## Introduction

Reducing variability in the eating quality of pork is a major challenge facing the swine industry and requires knowledge of factors ranging from genetics through to post mortem handling. Meat scientists have performed substantial research on non-genetic factors such as farm, transport, slaughter and processing conditions and this has lead to considerable quality and compositional improvements. Many important meat quality traits are at least moderately heritable and several genes which influence pork quality have been identified. The aim of the current study was to examine the variability that exists in meat quality and produce molecular profiles from an Irish Large White x Landrace/Large white population in order to provide insight into the contribution of both gene and gene product (protein) to the complex processes involved in the conversion of muscle to meat.

## Materials and methods

*Animal sampling and meat quality measurements*

Thirty one Large White x Landrace/Large White gilts were randomly selected and slaughtered under controlled conditions in a pilot abattoir at the Ashtown Food Research Centre, Dublin. Samples were taken within 10 minutes post mortem from *Longissimus thoracis et lumborum*. Samples for RNA analysis were minced and placed in RNAlater stabilisation solution. Samples for proteomics were snap frozen in liquid nitrogen and stored at -80$^{o}$C. Protein samples were also collected from the same muscle at $T_{1hr}$, $T_{6hr}$, $T_{24hr}$, $T_{3day}$ and $T_{7day}$.

A variety of technological quality measurements were performed on the left side of the carcass over a 7 day period post slaughter. pH, temperature and conductivity were measured at hourly intervals for 6 hours. Colour of the *M. semimembranosus* was measured (Mini-scan XE) at 45 mins, 3 hrs, 6hrs and 24 hours. Warner Bratzler shear force measurements were made on the Instron 5543 on day 1, day 3, day 7 (Wheeler et al., 1996). Drip loss was measured using a bag method (Honikel 1998). Moisture and intramuscular fat were analyzed using the CEM SMART Trac moisture/fat analyzer. Animals were identified as divergent for phenotypes as described in Table 1. Analysis of variance indicated that all groups were significantly different to each other ($p < 0.001$).

*cDNA microarray analysis and realtime PCR*

Total RNA was prepared from *Longissimus thoracis* muscle tissue using an RNeasy maxi kit (Qiagen UK) and assessed for quality and quantity, using spectrophotometry and an Agilent 2100 bioanalyser (Agilent technologies). Samples were indirectly labelled with either Cy3 or Cy5 dyes (GE Healthcare) and purified using an Illustra Cyscribe GFX purification kit (GE Healthcare).

Approximately 5,400 SSH cDNA clones generated from a previous study (Plastow, *et al* 2005) were printed in duplicate on Schott E slides along with dye and cDNA controls. The microarray experiment was a reference design with a common reference pool of RNA from all 31 animals, incorporating dye swap. In total 60 hybridisations were performed. Microarray slides were scanned in two channels (543 and 633nm) at 5 µm resolution using a confocal laser scanner (Agilent Technologies). Using Genepix Pro 6 software, intensities for feature and background were quantified. Data were analysed using Genespring GX 7.3. Following Lowess normalisation, $\log_{10}$ ratios of signal (experimental) to control (reference) were calculated. Data were further filtered and a Welch t-test was performed to select potentially differentially expressed genes ($p < 0.05$ and $p < 0.01$). Two step quantitative Real-time PCR analysis of microarray candidates were performed in triplicate using Superscript III reverse transcriptase (Invitrogen and the Quantitech SYBR green PCR kit (Qiagen UK) on an MX3000P real-time PCR system (Stratagene, CA, USA). Data was analysed using the DDCt method (Livak &

Schmittgen, 2001) using ribosomal protein L4 (RPL4) and Tata Box binding protein (TBP) as housekeeping genes.

*Proteome analysis*

Centrifugal drip samples from all 31 animals (at days 1, 3 and 7 *post mortem*) were subjected to electrophoresis using 10 % 1-D PAGE gels and staining with Coomassie Blue R-250 (Laemmli, 1970). A single sample gel lane (Figure 1) was dissected into 24 strips and proteins were in-gel digested with trypsin. Peptides were extracted, purified and separated on a reverse phase column interfaced to a nano-ESI linear ion trap mass spectrometer (Thermo Scientific), followed by data analysis using Xcalibur™ (Thermo Scientific) and identification by comparison of theoretical and actual MS/MS spectra via TurboSEQUEST™ software.

The first dimension of 2-D gel electrophoresis was optimised by testing two types of IPG strips (pH 3-10 linear and pH 4-7 linear). The second dimension was run using 12 % SDS gels followed by silver staining, according to a modified protocol of Ramagli et al. (1985).

**Results and discussion**

*Identification of individuals divergent in meat quality attributes*

Individuals divergent in water holding capacity, intramuscular fat and tenderness were identified from the 31 Large White x Landrace/ Large White animals sampled in this study (Table 1). For traits relating to water holding capacity, individuals were segregated into four phenotypic categories according to percentage drip loss, $pH_{45}$, ultimate pH and Hunter L* colour values (PSE and DFD categories). In relation to percentage intramuscular fat (IMF), eight animals were found to be divergent (<0.35 versus >1.2%) and a further eight animals were divergent for Warner Bratzler shear force on day 1 ('tough v tender', Table 1). These animals formed the basis of cDNA microarray experiments for analysis of gene and protein expression between the different phenotypes.

| Comparison | Selection Criteria | N |
|---|---|---|
| Pale, soft and exudative (PSE) versus dark, firm and dry meat (DFD) | Drip loss > 6% , $pH_{45}$ < 6.2 versus drip loss < 3%, pHμ > 5.8 | 4 / 3 |
| High versus low drip loss, not PSE, DFD | Drip loss > 5%, drip loss < 2.3% | 3 / 4 |
| High IMF versus low IMF | < 0.3% versus >1.2% | 4/ 4 |
| Tough versus Tender | WBSF > 50N versus < 38N | 4/ 4 |

**Table 1. Criteria for selection of phenotypically divergent individuals**

*Transcriptomic profiling of divergent groups by cDNA microarray*

Following statistical analysis, 77 genes were found to be differentially regulated between PSE and DFD phenotypes, 153 genes were found to be differentially regulated between extremes of drip loss and 272 genes were found to be differentially regulated in IMF content. A further 151 genes were found to be differentially regulated between 'tender' versus 'tough' animals (Table 2). Furthermore it was possible to identify 45 genes that were potentially differentially expressed at the p<0.01 level with a fold change of 1.2 fold or greater.

| Experiment | Number of genes | Number of genes with 1.2 fold filter |
|---|---|---|
| | P<0.05 (P<0.01) | P<0.05 (P<0.01) |
| PSE versus DFD | 77 (8) | 11 (1) |
| High versus low drip loss | 153 (18) | 57 (10) |
| High versus low IMF | 272 (37) | 148 (24) |
| Tender versus tough | 151 (28) | 39 (10) |

**Table 2. Numbers of potentially differentially expressed genes (p <0.05 and p< 0.01) identified between divergent phenotypic groups.**

Real-time PCR confirmation has been carried out for a small number of microarray and literature candidates. It was possible to identify genes in both cases significantly differently expressed ($p < 0.05$) between divergent phenotypes (results not shown).

*Proteomic analysis*

1-D SDS PAGE shows evident changes over the *post mortem* ageing period (day 1, 3 and 7) in porcine exudates (Fig. 1: arrows). Differences were also observed between individuals divergent in important pork quality phenotypes (e.g. Fig. 1: S.1, S.2: normal quality phenotypes; S.3: low drip loss/ not DFD) There were differences in profiles between individuals that were normal and those with pathological conditions (PSE and DFD, results not shown). Approximately 106 proteins / fragments were detected by mass spectrometry analysis and bioinformatic analysis. The vast majority of these proteins were muscular isoforms of enzymes involved in glucose metabolism, but the fraction was not predominated by a small number of highly abundant proteins. A linear pH gradient of 4-7 was found to provide the optimal resolving power for 2-DE analysis of porcine centrifugal drip, when 100 µg proteins was loaded onto the gel (results not shown).

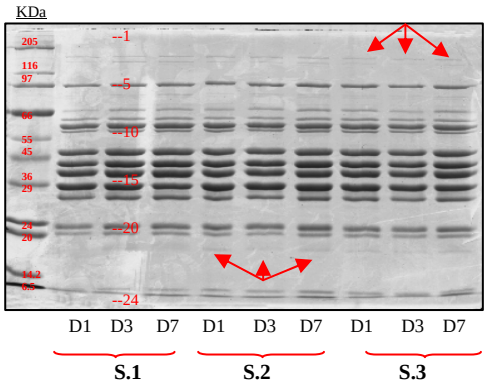


**Figure 1: 10% PAGE 1-D SDS-PAGE, stained with Coomassie Blue R-250, comparing three animals at different days *post mortem* (porcine centrifugal drip samples). Samples 1 & 2 (S.1 & S.2) are from animals with a normal quality phenotype; Sample 3 (S.3) is from an animal with low drip loss. Differences in band intensity are also evident across the *post mortem* ageing period (arrows). Numbers shown in the third lane refer to gel slices utilised for mass spectrometric**

## Conclusions

Microarray analysis of individuals divergent in important pork quality phenotypes (water holding capacity, IMF and tenderness) has provided lists of potential candidate genes up/down regulated in the traits of interest. A preliminary study of centrifugal drip proteins by 1-D electrophoresis and mass spectrometry has indicated that they are a promising source of novel biomarkers potentially predictive of meat quality traits.

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