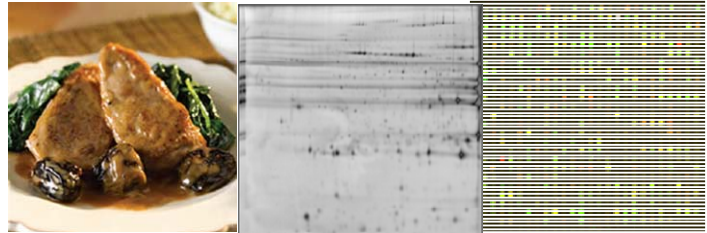


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Identification and molecular characterisation of genes influencing Irish pork meat quality



Key external stakeholders:

Pig producers, pigmeat processors, diagnostics companies

Practical implications for stakeholders:

Tools to provide early prediction of the ultimate quality of meat, i.e. shortly after slaughter, would help facilitate logistical decisions of pork processors in relation to meat management. However measurements that are currently applied by industry, e.g. carcass pH measured at 45 minutes *post mortem*, are not considered satisfactory as accurate predictors of ultimate quality.

- Molecular approaches applied in this project have led to the identification of biological markers which are associated with quality parameters. These have considerable potential as tools to predict quality in meat management systems and/or to provide a basis for the inclusion of meat quality in selection goals.
- Gene expression profiles and novel DNA markers in these gene regions were shown to be linked with meat quality in large pig populations representing Large White, Duroc and Pietrain breeds. Interestingly, several of the associations were breed-specific. Results have applications in meat management systems and breeding.
- Exudate from muscle was shown to be a readily accessible biological resource and a rich source of potential protein biomarkers of quality. More than two-hundred proteins/fragments were altered through *post mortem* ageing and are thus linked to quality. 20 proteins were linked to drip loss. The findings provide a means for the future development of high-throughput protein diagnostics for diverse aspects of meat quality in an industrial setting.

Main results:

- A detailed meat quality database (tenderness, fat content, water-holding capacity etc.) for three breeds (Large White, Pietrain, Duroc) was established.
- More than 600 candidate genes were identified whose expression levels were associated with tenderness, intramuscular fat content, drip loss or PSE-like / DFD-like meat.
- Many novel associations have been identified between 190 novel markers (SNPs) discovered in the most promising candidate genes and meat quality measurements in four cohorts (total 724 animals).
- 2D proteomics resulted in the identification of protein spots significantly associated with drip loss at the day 1 time point. These have potential to serve as early biomarkers of water-holding capacity.

Opportunity / Benefit:

A panel of proteomic markers associated with a highly relevant pork quality trait for pigmeat processors, i.e. drip loss, was identified in the course of this project. The results could be further developed into rapid tests for drip loss in a commercial context. This approach is could also be highly relevant for palatability traits, such as tenderness, juiciness and flavour. Expressions of interest in further developing this research are welcome.

Collaborating Institutions:

NUIG, UCD

Teagasc project team:	Dr. Ruth Hamill Dr. Anne Maria Mullen Dr. Jean McBryan Dr. Alessio Di Luca Dr. Ozlem Aslan Mr. Eugene Vesey Dr. Peadar Lawlor
External collaborators:	Dr. Michael Cairns, NUIG (PI) Dr. Grace Davey, NUIG Prof. Torres Sweeney, UCD Prof. Giuliano Elia, UCD

1. Project background:

Pork quality comprises a set of fresh meat qualities and processing characteristics that are important for the profitability and competitiveness of the swine industry. Genetic improvement of pigs over the past 30 years has focused primarily on selective breeding for production characteristics such as growth rate and carcass leanness and the success of such programmes is well accepted. However, strong selection for increased growth rate and reduced fat cover in pigs has had a negative effect on fresh meat quality. It is thus desirable to develop methods to improve meat quality to complement the gains in production traits. However this presents challenges, in that most aspects of pork quality can only be measured on the carcass or by consumer panels, and thus are difficult and expensive to improve by conventional means. Great opportunities, therefore, exist for the use of molecular approaches to improve the understanding of quality and to assist the industry to progress towards reliable prediction of pork meat quality by development of biological markers. This project offered an opportunity to develop an animal resource, representative of the variability in the Irish pig herd, which was comprehensively characterised for important meat quality traits and through analysis of naturally divergent animals from this resource to identify novel genetic and proteomic markers of pork quality. While this is fundamental research, such biomarkers have numerous potential applications in meat management systems to predict quality or to select animals with improved meat quality characteristics.

2. Questions addressed by the project:

- Can functional genomics approaches be applied to identify and characterise in detail, the genes responsible for variation in Irish pork meat quality?
- Can we find muscle proteins which correlate with quality and meat ageing using 2D proteomics and mass spectrometry in muscle exudate?
- Can we move a step closer to the selection of candidate gene markers in marker-assisted selection strategies for meat quality and to the identification of biomarkers which can be routinely applied in an industry context?

3. The experimental studies:

- The project involved sampling of pigs representing commonly used Irish commercial sire breeds. In total, 95 finished pigs from three genetic backgrounds (Duroc, Pietrain and Large White sires crossed to common Large White/Landrace background dams) were slaughtered at a pilot abattoir in AFRC.
- Centrifugal drip samples were collected for proteomic analysis across three time points *post mortem*.
- Detailed meat quality measurements were performed on each animal (pH, colour, drip loss, tenderness, shear force, fatty acid profile etc) at appropriate time points *post mortem*.
- Samples divergent for three major quality traits, tenderness (shear force), intramuscular fat (IMF) content, drip loss %, and PSE-like and DFD-like samples were identified.
- 1D and 2D proteomic studies were carried out on these centrifugal drip samples to determine if biological markers of drip loss and meat ageing could be identified
- Centrifugal drip samples from muscle displaying divergent phenotypes for drip loss [high (HDrip), intermediate (IP) and low drip loss (LDrip)], but excluding PSE and DFD, from day 1, 3 and 7 post mortem were compared using 2D Difference Gel Electrophoresis (2D DIGE). Findings were validated using western blot analysis of selected proteins.
- A novel muscle-specific microarray of 5400 gene probes was developed by collaborators in NUIG and microarray hybridisation experiments were carried out for four quality traits.
- The most interesting genes were sequenced to discover new genetic variation which may be influencing

quality.

- The most promising of these newly identified DNA polymorphisms (SNPs) were tested for association with meat quality in larger populations of purebred Large White, Duroc and Pietrain animals, 724 animals in total.

4. Main results:

- A panel of divergent pork samples for each of the following traits was identified; tenderness, intramuscular fat, drip loss and PSE-like and DFD-like for each breed.
- Microarray experiments identified 653 gene transcripts that were differentially expressed in relation to quality. These comprise 77 genes differentially expressed in PSE-like versus DFD-like muscle; 153 genes differentially expressed in high versus low drip loss % muscle; 253 genes differentially expressed in high versus low intramuscular fat content muscle; and 151 genes differentially expressed in muscle that was tender versus muscle that was tough on day 1 post mortem.
- These gene lists overlap with previous studies; both microarray-based and studies looking at potential candidate genes mapped on porcine chromosomal regions containing meat quantitative trait loci (QTL).
- Bioinformatic analysis of the functions of the differentially expressed genes revealed that a wide variety of functions were affected in relation to quality. For example, protein and peptide degradation processes were significantly up-regulated in pork that was tender early *post mortem*. As expected, fatty acid turnover was suppressed and fat deposition processes increased, in muscle with higher fat content, but an overall repression of carbohydrate metabolism was also observed in muscle with higher fat content. Carbohydrate metabolism was also suppressed in muscle with low drip (exudate) losses.
- From these four genelists, a shortlist of the twenty most interesting genes was created and 190 novel single nucleotide polymorphisms (SNPs) were identified in candidate gene regions in Irish pig herd.
- Many novel associations have been identified between candidate gene SNPs and a variety of meat quality measurements in 4 cohorts of a total of 724 samples.
- One dimensional proteomics and mass spectrometry (MS) indicated that the centrifugal drip fraction was reproducible and it was not dominated by a small number of highly abundant proteins. Moreover, the heat shock family was highlighted for its potential in the early prediction of WHC.
- More advanced proteomic tools (2D-DIGE) revealed three hundred and seventy six distinct protein spots in the centrifugal drip proteome and following mass spectrometric analysis the first 2D reference protein map (88 proteins/ peptides) of porcine centrifugal drip was generated.
- Major patterns in protein abundance were dominated by the post mortem time point and in likelihood the meat ageing process.
- Twenty spots significantly changed in abundance across the three phenotypes at the day 1 time point. These have potential to serve as early biomarkers of drip loss.
- Statistical models with the ability to discriminate between animals with different levels of drip loss were derived. In all comparisons the general classes of discriminatory proteins identified are metabolic enzymes, stress response proteins, transport and some structural proteins.

5. Opportunity/Benefit:

Novel SNP associations have potential utility in breeding programmes or for processors in meat management systems. A panel of proteomic markers associated with drip loss was identified which are also relevant to meat management systems. Some of the markers will be tested in collaborative projects with industry partners. Further information on potential exploitation of the project outputs can be provided by contacting the researchers.

6. Dissemination:

Dissemination has taken the form of peer-reviewed publications, technical publications, conference presentations and posters, RELAY workshop presentations, popular publications and industry information days. RELAY workshop presentations may be accessed on the RELAY website <http://www.relayresearch.ie/Public/WorkshopList.aspx>.

Main publications:

Aslan, O., Hamill, R.M., Davey, G., McBryan, J., Mullen, A.M., Gispert, M., and Sweeney, T. (2012). 'Variation in the IGF2 gene promoter region is associated with intramuscular fat content in porcine skeletal muscle'. *Molecular Biology Reports*, 39 (4): 4101-4110.

Di Luca, A., Mullen, A.M., Elia, G., Davey, G. and Hamill, R.M. (2011). 'Centrifugal drip is an accessible

source for protein indicators of pork ageing and water-holding capacity.' *Meat Science* 88 (2): 261-270.

McBryan, J., Hamill, R.M., Davey, G., Lawlor, P.G. and Mullen, A.M. (2010). 'Identification of suitable reference genes for gene expression analysis of pork meat.' *Meat Science* 86 (2): 436-439.

Popular publications:

Hamill, R.M. (2008). Genes, proteins and the future of Irish pigmeat. *The Ashtown Food Innovator*, Summer 2008, Issue 2, p.2.

Mullen, A.M., Corcoran, D., Hughes, L. and Hamill, R.M. (2007) Genomic and proteomic approaches to understanding and enhancing meat quality. *FeedInfo.com*. Available www.feedinfo.com

Dermody, J (2011) Researchers identify genetic traits in pigs to help deliver tastiest meat. *Irish Examiner* 21st October 2011.

7. Compiled by: Dr. Ruth Hamill
