Pig Genome I

An Overview of Cutting-edge Genomics with Emphasis on the Pig

Parco Tecnologico Padano

Lodi - Italy

February 20-21, 2006

Scientific Program

Abstract book

Main Lectures

Posters and Oral Communications

SCIENTIFIC COMMITTEE

COST European Network for Pig Genomics
"PigNet" (COST action 861)

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SCIENTIFIC PROGRAM

MONDAY - February 20, 2006

h. 08.00 - 09.00 Registration

h. 09.00 - 09.10 Welcome
ELISABETTA GIUFFRA *(local organizer)* and FRANÇOIS HATEY *(PigNet Chairman)*

h. 09.10 - 12.20 STRUCTURAL GENOMICS AND BIOINFORMATICS - morning session

Chairman: MARTIEN GROENEN

h. 09.10 - 09.50 *keynote speaker* PEER BORK

Comparative Metazoan genome analysis for predicting functional divergence and deducing evolutionary features

h. 09.50 - 10.30 *keynote speaker:* PIETRO LIÒ

Using microarrays for identifying regulatory motifs and analysing time series gene expression

h.10.30 - 10.50 *Coffee break*

h. 10.50 - 12.20 **WG2 and WG4 meeting - Selected presentations**

1. J. M. REECY • **NRSP-8 Pig Genome Informatics: Databases and Resources.**
2. A. CAPRERA • **A web-database platform for data mining and statistical analysis in a swine epidemiological study.**
3. J. GORODKIN • **Analysis of digital expression in the porcine genome using 98 cDNA libraries and 1,021,891 ESTs.**
4. E. TORARINSSON • **Conserved RNA structure between genomic regions of human and mouse that are unalignable in primary sequence.**
5. A-B. NYGAARD • **Alternative splicing of pig pre-mRNA.**
6. A. BONNET • **Transcriptome of pig ovarian cells: discriminant genes involved in follicular development.**

h.12.20 - 14.15 *Lunch buffet - first poster session (groups: 1,2,5,6)*

h. 14.15 - 17.05 STRUCTURAL GENOMICS AND BIOINFORMATICS - afternoon session

Chairman: ALAN ARCHIBALD

h. 14.15 - 14.55 *keynote speaker:* JANE ROGERS

Pig genome sequencing - progress and prospects

h. 14.55 - 15.35 *keynote speaker:* JONATHAN FLINT

Whole genome association reveals the genetic architecture of complex traits in mice

h. 15.35 - 17.05 **WG2 and WG4 meeting - Selected presentations**

1. M. MANCINI • **Illumina Solutions to Large Scale Genotyping and Gene Expression Analysis using BeadArray® technology.**
2. H-J. MEGENS • **Assessment of genetic diversity and extent of LD in European and Chinese pig breeds using large scale SNP genotyping.**
3. J. Demars • **High resolution physical map of porcine chromosome 7 QTL region and comparative mapping of this region among vertebrate genomes.**
4. S. BRAGLIA • **An association study of myopalladin and titin polymorphisms with meat quality and production traits in pigs.**
5. S. KUSZA • **Study of non-classical class I MHC genes in pig.**
6. A. THOMAS • **Innate immunity in pigs: study of several genes.**

h.17.05 - 17.30 *Coffee break*

h.20.30 **GALA DINNER**

TUESDAY - February 21, 2006

h. 09.00 - 12.45 **PIGS, HUMAN MEDICINE AND ETHICS** - session

Chairman: PATRICK CHARDON

h. 09.00 - 09.40 *keynote speaker:* ANNA OLSSON
Pig Genomics and the public

h. 09.40 - 10.20 *keynote speaker:* CESARE GALLI
Pig somatic cell nuclear transfer and its application in biomedical research

h. 10.20 - 10.40 *Coffee break*

h. 10.40 - 11.20 *keynote speaker:* DAVID SIMPSON
The pig eye as a model for human retinal degenerative diseases and development of stem cell therapies

h. 11.20 - 12.00 *keynote speaker:* SILVIA VINCENT NAULLEAU
The Melanoblastoma-bearing Libechov Minipigs: an accurate model to improve the knowledge of anti-tumoural self defence

h. 12.00 - 12.45 **WG1 and WG5 - Selected presentations**

1. S. GENINI • **Porcine Arthrogryposis Multiplex Congenita (AMC), a disease model for human medicine.**
2. C. KNORR • **Molecular decipherment of porcine hernia inguinalis and scrotalis.**
3. A.M. NEETESON • **Experience with dissemination of research results and dialogue with industry.**

h.12.45 - 14.15 *Lunch buffet – second poster session (groups: 3,4,7)*

h. 14.15 - 17.25 FUNCTIONAL GENOMICS AND PROTEOMICS

Chairman: MAX ROTHSCHILD

h. 14.15- 14.55 *keynote speaker:* MICHEL GEORGES

Uncovering roles for miRNAs in shaping phenotypic variability in livestock

h. 14.55 - 15.35 *keynote speaker:* ROB BEYNON

Protein turnover in muscle: strategies to understand a dynamic Proteome

h.15.35 - 15.55 *Coffee break*

h. 15.55 - 17.25 **WG3 - Selected presentations**

1. P. SCHÜSSLER • **70mer microarray probes applied in whole genome expression analysis of pig (*Sus scrofa*).**
2. S. BOTTI • **Complementarity of gene expression data obtained by SSH and Operon 13K microarrays during PRRSV infection in pigs.**
3. E. SOSTARIC • **Profiling porcine oviductal epithelial cell surface membrane proteins.**
4. T. SAYD • **Proteome analysis of the sarcoplasmic fraction of pig muscles differing by meat quality traits: colour and tenderness.**
5. M. DAMON • **Transcriptomic analysis of destructured ham.**
6. N. BUYS • **Selection for the porcine IGF2-intron3-G3072A substitution: implications for the industry.**

h. 17.30 Conference closing
FRANÇOIS HATEY

MAIN LECTURES

Comparative Metazoan genome analysis: Predicting functional divergence and deducing evolutionary features

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Functionality predicted from genome sequences comes at different scales. Individual point mutations can hint at precise enzymatic changes, homology between genes reveals more loosely diversification of molecular function, and gene context (e.g. co-occurrence of genes in genomes) implies interactions of the encoded proteins and modification at the pathways level. Depending on the phylogenetic position of the genome of interest, different methods will be more powerful than others; for example in close species such as chimp and human it is important to identify the information content of individual mutations while the focus in a comparison of fish and human is the change of the repertoire of domain families and associated functions.

I will introduce and exemplify the different concepts of function prediction and will emphasize genome quality and systematic annotation errors that need to be considered. Furthermore, I will also highlight evolutionary patterns that can be observed when multiple genomes are compared such a the correlation of different genetic mechanisms leading to

divergence such as intron loss, point mutations or genome shuffling. The quantification of these measures is important as they are tightly coupled with functional features. The faster a genome evolves the more effort is needed to identify functional features shared with its relatives.

Using microarray for identifying regulatory motifs and analysing time series gene expression

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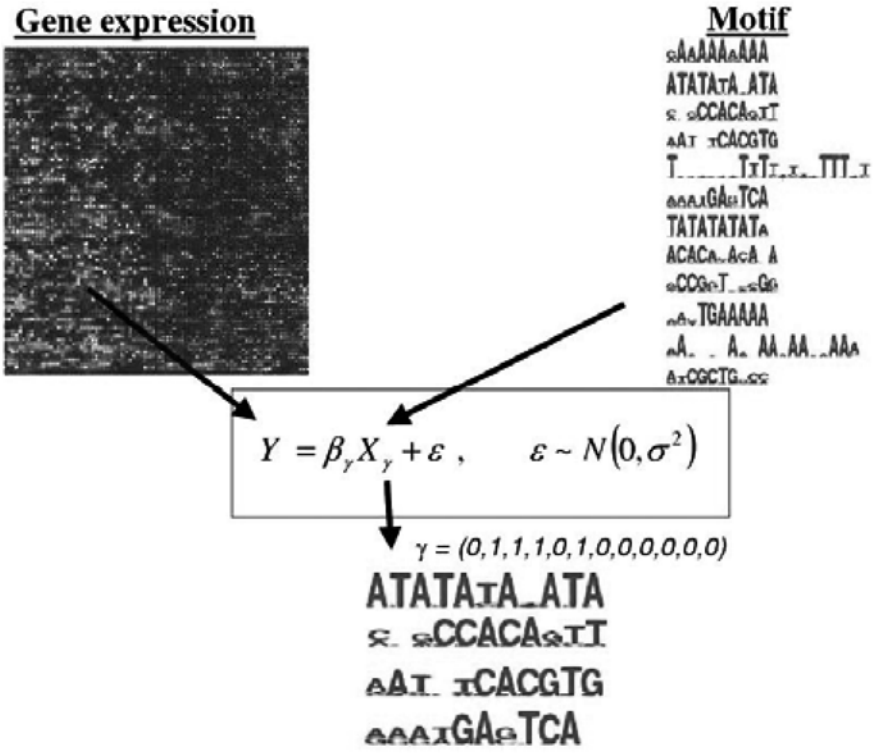
Control of gene expression is essential to the establishment and maintenance of all cell types, and is involved in pathogenesis of several diseases. Computational identification of regulatory sites is currently based mainly on three different approaches: (1) identification of conserved motifs using interspecies sequence global alignments (Pennacchio 2001); (2) motif-finding algorithms that identify conserved motifs in the promoters of co-regulated genes (Hughes et al 2000, Eskin et al 2002); (3) computational detection of known experimentally identified motifs in genes' promoters for which binding factors are unknown (Kel et al 2003). The limitations of the first approach are caused by the high mutation, deletion and insertion rates in gene promoter regions, that prevent a correct alignment of the promoter region. As experimental data is accumulating on known DNA binding elements, increasing amount of information can be used to search for similar elements in genes for which transcription factors are unknown.

DNA microarrays provide a simple and natural vehicle for exploring the regulation of thousands of genes and their interactions (Conlon et al, 2003; Tadesse et al., 2004). Genes with similar expression profiles are likely to have similar regulatory mechanisms. A close inspection of their promoter sequences may therefore reveal nucleotide patterns that are relevant to their regulation. This motivates the following strategy: (1) candidate motifs can be obtained from the upstream regions of the most induced or most repressed genes; (2) a score can be assigned to reflect how well each motif matches the upstream sequence of a particular gene; and (3) regression analysis and variable selection methods can be used to detect sets of motifs acting together to affect the expression of genes. We implement steps 1 and 2 using existing procedures and available software. For step 3, we propose the use of Bayesian variable selection methods as an alternative to stepwise selection procedures used by other investigators. Bayesian variable selection methods use a latent binary vector to index all possible sets of variables (nucleotide patterns). Stochastic search techniques are then used to explore the high-dimensional variable space and identify sets that best predict the response variable (gene expression). The method provides joint posterior probabilities of sets of patterns, as well as marginal posterior probabilities for the inclusion of single nucleotide patterns. Stepwise methods, on the other hand, perform greedy deterministic searches and can be stuck at local minima. Another limitation of the stepwise pro-

cedure is that it presumes the existence of a single 'best' subset of variables and seeks to identify it. In practice, how ever, there may be several equally good models. We first use simulated data with similar structure to the real datasets considered for analysis and show how Bayesian variable selection can outperform stepwise procedures. We then exemplify our method using eukaryotic genomes and microarray data from different stress experiments.

We demonstrate through simulated data the improved performance of the Bayesian variable selection method compared to the stepwise procedure. We then analyse and discuss the results from experiments. We identify regulatory motifs known to be related to the experimental conditions considered. Some of our selected motifs are also in agreement with recent findings by other researchers.



Graphical representation of methodology—we fit a linear regression model that relates gene-expression data to pattern scores. A Bayesian variable selection method is used to identify variables included in the model. This is accomplished through a latent binary vector Á , updated via stochastic search MCMC techniques. Motifs with high posterior probability are selected and indicate promising sets for further investigation.

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Pig Genome Sequencing – progress and prospects

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Over the past five years, a compelling case has been developed to obtain a reference sequence of the swine genome. The pig is a member of the Cetartiodactyla order, (a clade distinct from rodent and primates), that last shared a common ancestor with man 79-87 million years ago (Kumar and Hedges 1998). It is an important species for meat-based protein production and as a model organism for biomedical research into human diseases that may be modelled less well in rodents, e,g, obesity, arthritis and cardiovascular disease.

The pig is also interesting for its potential to supply organs, tissues and cells for transplant through so called Xenotransplantation, providing that issues surrounding porcine endogenous retroviruses can be resolved.

A programme to obtain a reference sequence that will be publicly available is being developed by a consortium of laboratories from ten countries. The project is being developed in two phases. The first phase has focused on the production of a physical map of the swine genome in mapped, bacterial large insert clones. The map is based principally on a BAC library generated from a single female pig (CHORI-242), which has been end-sequenced and fingerprinted iat the University of Illinois and at the Wellcome Trust Sanger Institute, but also draws in additional end-sequences and finger-prints from three other libraries (CHORI-44 (P. de Jong, Children's Hospital, Oakland CA), pigEBAC (Roslin Institute, Edinburgh, U.K.) and INRA (INRA porcine BAC library, Toulouse)). The physical map is being assembled on the basis of overlapping fingerprints, using alignment of end sequences to the human genome sequence to help orient and order contigs and markers positioned on the radiation hybrid map of the swine genome generated at the University of Illinois, to position contigs on the swine genome. Using this approach, the map has been contiguated rapidly and currently provides highly continuous coverage across the 18 pig autosomes and the X chromosome in 176 contigs. There is also localised representation of the gene rich regions on the Y chromosome.

The map can be accessed through WebFPC, at (http://www.sanger.ac.uk/Projects/S_scrofa/WebFPC/porcine/large.shtml) and represents an entry point for rapid electronic positional cloning of genes, fine mapping of QTLs, and templates for targeted functional genomics studies. The physical map will also be used to define a tilepath of minimally overlapping clones across the genome as the basis for the genome sequencing project.

In contrast to the approach taken for most draft mammalian genome sequences, the strategy that will be used to sequence the 2.7Gb swine genome is a combined whole genome sequencing and mapped clone sequencing approach. The majority of the sequence data will be derived from the single animal used to generate the CHORI-242 BAC library. Over the next two years, with funding announced recently from the USDA-CREES, a 3-fold depth draft sequence of the genome will be assembled from shotgun sequences of a minimal tilepath of BAC clones identified from the physical map. This will be supplemented by additional clone shotgun sequence and whole genome shotgun sequence data generated from DNA derived from the sow used for the CHORI-242 BAC library. A number of funding partners have been identified and the aim is to achieve a 6-7-fold draft sequence of the genome by 2008. As they are produced, sequence trace data will be deposited in the GenBank and Ensembl trace archives and clone sequence assemblies will be deposited in the HTGS divisions of EMBL/GenBank/DDBJ. Clones will be selected, largely, by chromosome, and as the sequencing of chromosome tilepaths are completed, the chromosome sequences will be assembled, and displayed with automated annotation in the Ensembl genome browser. One advantage of using a clone-based sequence strategy is that targets of particular interest, that require detailed gene annotation and/or SNP positioning, can be identified for production of high quality finished sequence and, if funding is available, forwarded to the high throughput finishing pipeline at the Sanger Institute.

Whole genome association reveals the genetic architecture of complex traits in mice

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Although over two thousand quantitative trait loci (QTLs) have been mapped in crosses between inbred strains of mice, little is known about the genetic architecture of quantitative variation. One major obstacle to progress has been the difficulty of obtaining high resolution QTL mapping.

We have tackled this problem by developing methods for mapping QTL in outbred mice of known ancestry. By exploiting of historical recombinants that have accumulated in a genetically heterogeneous stock (HS) of mice descended from eight inbred progenitor strains (A/J, AKR/J, BALBc/J, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and LP/J) we have shown that a QTL explaining 5% or less of the phenotypic variation can be mapped into an interval of under a centimorgan (cM). I will present data from a large HS QTL mapping experiment which provides the first omprehensive high resolution analysis of quantitative variation in the mouse. We included in our phenotypic battery mouse models of three common human diseases: anxiety, type II diabetes and asthma. In addition, we collected biochemistry, haematology and immunology profiles of each mouse. Our extensive phenotypic assays allow us to compare the genetic architecture of different phenotypes and to investigate the pleiotropic action of QTL.

Pig genomics and the public

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Pig genomics is an intellectual effort which not only cuts across disciplines. It also cuts across different sectors in society which traditionally have not had much contact with each other: Pig production, biomedicine and basic biological research. By crossing traditional borders new intellectual and practical opportunities arise. However, some of the borders that are being crossed also seem to have great ethical significance.

Take the border between pig production and biomedicine. From studies applications are seen as more acceptable than agricultural applications. To many people it may appear provocative that knowledge concerning pig genomics within the same research consortium is considered both as a means to increase disease resistance in order to make pig production more efficient and as a means to create pig models of serious human diseases.

Take the border between research in the public domain and research aimed at commercial goals. We know that there is a widespread concern that commercial interests are not always for the benefit of the common good. And when the researchers from the public domain start to collaborate closely with industry there may be a fear that they will be led by narrow commercial interests.

Furthermore there may be a worry regarding secrecy that important decisions are taken behind closed doors and that the public will only be involved when it is too late.

Finally across the borders there is a widespread worry that animals may be treated in ways which are not considered ethically acceptable. There may be concerns about animal welfare: that the animals may be caused to suffer more or feel extra stress within pig production, or that pigs which serve as models of serious human diseases may suffer as a consequence. However there may also be concerns about animal integrity: That animals may be manipulated in ways which display a lack of respect for nature.

One aim of the talk is to bring forward what we at present know about concerns of the public in relation to research and development regarding pig genomics.

To a wider public the notion "pig genomics" probably does not mean much.

However, as indicated above some of the applications that may result from the developments within pig genomics may give rise to concerns. This part of the talk will be based on a number of the surveys and interview studies which in recent years have been conducted regarding biotechnology and various forms of animal use.

A second and equally important aim is to discuss how the issues which may be relevant from the point of view of the public can be integrated in the work done regarding pig genomics. The underlying idea is that it is much better to deal with these issues at an early stage rather than late in the development of various applications. There are several reasons why this is so. Firstly, the research will appear much more trustworthy and less arrogant if it is seen as taking seriously the views and concerns of the surrounding society. Secondly, it may be possible to develop the applications of pig genomics in a way that does not conflict with public acceptance . In the presentation we will discuss two examples of such a development, taken from pig breeding and pig genomics research.

Pig somatic cell nuclear transfer and its application in biomedical research

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The model for mammalian development is and has always been the laboratory mouse. A wealth of cellular and molecular biology techniques have been developed in this species, facilitated by its size and reproductive cycle. In the last twenty years many of these techniques have been applied also in larger animals including humans. Molecular embryology of large mammals is now a well established discipline that allows the manipulation of gametes and early pre-implantation stage embryos capable of generating viable animals following transfer in the uterus of synchronised recipients (Galli et al, 2003a). Somatic cell nuclear transfer represents the most recent technique, developed in the last 10 years in several mammalian species including the pig (Galli et al 2003b). The success of this technique is largely dependent on conventional assisted reproduction procedures as in vitro maturation of oocytes and in vitro culture of manipulated embryos that are subsequently transferred to synchronised recipients to generate animals with the genetic make up of the somatic cell used to create the embryo. When these embryo–technologies are combined with genetic engineering the production of genetically modified animals is simplified opening the possibility of making transgenic large animals for the study of functional genomics in mammals other than the mouse.

Although, in general, the proof of principle of any new experimental hypothesis and/or any therapeutic protocol is first demonstrated in the mouse, there is the need to have an intermediate model closer to the human to fully address safety issues involved in novel therapeutic approaches. The pig represents the ideal species for a number of reasons including size, lifespan, anatomy, physiology and metabolism.

Nuclear transfer

Several techniques for pig nuclear transfer have been applied successfully (Polejaeva et al 2000, Onishi et al 2000, Betthauser et al, 2000) using both in vivo and in vitro matured oocytes. At present, all techniques rely on oocytes collected from slaughtered animals and matured in vitro being an inexpensive and unlimited supply of material required for cloning. After maturation the oocytes are enucleated (removal of their nucleus) to create a cytoplast that will receive the nucleus of a somatic cell. After nuclear transfer the cytoplast containing the somatic nucleus is given the appropriated stimuli to start embryonic development. Manipulated embryos are transferred shortly after fusion and activation to the oviducts of synchronised recipients. Recent refinements of the manipulation techniques involving the removal of the zona (Vajta et al 2002) and of the culture of pre-implantation embryos in vitro up to the blastocyst stage (Galli et al unpublished) has allowed to increase the number of embryos that can be generated in any given day by a single operator. This technological advance opens the possibility to attempt non surgical embryo transfer in the pig making the technique less invasive and costly. In our experience the surgical transfer of day 5 zona free blastocysts has resulted in 2 out of 4 sows pregnant with the delivery of a total of 14 cloned piglets.

Embryonic stem cells (ESC) and cell therapy

Degenerative diseases like Parkinson, Alzheimer, artritis, infarction, myocardiopathies result in an irreversible cellular damage that cannot be treated with conventional, drug-based therapies. Moreover, when the option of organ transplantation is available, like in the case of heart transplant, then the chronic lack of suitable organs represents a major obstacle.

Recent advances in the biology of stem cells can offer alternative routes to the complete organ transplantation. There is the prospect that cell therapies based on stem cell transplantation can rescue the organ functionality by replacing some of the damaged cells. The potential of this therapeutic approach is only partially explored and it is today one of the primary objectives of the biomedical research and of regenerative medicine in particular. In this context the derivation of pig ESC creates the opportunity to develop a pre-clinical model of stem cell transplantation and even autologous transplantation if the cells are derived from cloned embryos and are transplanted in the cell donor animal. In this instance there will be no immune rejection since the cloned embryo and the recipient animal will be genetically identical, thus extending the principle of the so called “therapeutic cloning” (Barberi et al, Nat Biotechnol. 21:1200-1207, 2003) to a pig model. It will also offer the opportunity to test the safety of such therapeutic approaches in a large animal model before they are attempted in humans.

Xenotransplantation

The possibility to genetically modify the pig genome for making pig organs immuno-compatible to humans has been explored for many years, however it is only since the development of cloning that this research has gained a renewed interest. Gene transfer techniques are well established in many mammalian cell types and are currently used on fetal or adult fibroblast. These cells, in contrast to embryonic stem cells, have a limited proliferative capacity that is sufficient to go only through one round of selection following gene transfer. Besides gene insertion it is also possible to target a specific allele (KO knock out) by homologous recombination (Capecchi, 2005) resulting in the loss of gene function in case of double KO. This strategy has been used (Lai et al, 2002) to make the GAL KO pig, harbouring the loss of function of the alfa1-galattosyl transferase, an enzyme that adds sugars to the cell membranes protein. Since this enzyme was lost during evolution in humans, the product of this enzyme is believed to be the primary target of hyperacute rejection. However, several other genetic modifications of the pig genome will be required to warrant pig organ transplantation to humans. A number of genes have already been identified that are involved in the acute and chronic rejection and are the candidates for additional genetic modifications.

Conclusion

Here we have mentioned some aspects of the new biotechnologies that can be developed in the pig for biomedical research. Many more opportunities exist to develop functional genomics in this species as new information becomes available from genome sequencing. All of them however will be based on efficient protocols to manipulate the genome, the gametes and the embryos.

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The pig eye as a model for human retinal degenerative diseases and development of stem cell therapies

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The eye comprises both optical and nervous components: The cornea and lens focus light onto the neural retina. In the retina the photons of light are detected and converted into a nervous signal, which is processed and then transmitted to the visual cortex of the brain via the optic nerve. The pig eye provides an excellent model for ophthalmic investigations because of its size and anatomical similarity to that of the human. The ability to create transgenic animals provides insights into normal physiology and allows the development of disease models. In the Centre for Vision Science at Queen's University Belfast Dr WJ Curry, T Cogliati and myself are investigating inherited retinal degenerations, in particular Retinitis Pigmentosa (RP). This disease is inherited in a Mendelian fashion and is caused by mutations in one of a large number of possible genes (see Retinal Information Network: *http://www.sph.uth.tmc.edu/Retnet/*). Although there is a range of phenotypes, RP patients generally begin to lose peripheral vision in childhood and progress to tunnel vision and ultimately complete loss of sight in later life.

There are many natural and transgenic rodent models of RP, but Prof Bob Petters (North Carolina State University, USA) was the first to create a pig model. This overexpresses a mutant form of the gene encoding the visual pigment rhodopsin ($Rho^{Pro347Leu}$), which is implicated in the most comon forms of RP. The $Rho^{Pro347Leu}$ pig undergoes retinal degeneration and remodelling akin to that observed in postmortem RP human eyes. We are currently analysing at both the RNA and protein level the changes in gene expression which accompany the degenerative process. Preliminary experiments using microarrays provided by the U.S. Pig Genome Coordination Program and comprising Qiagen pig genome oligonucleotides have demonstrated significant changes in mRNA expression. However, since proteins are the ultimate effector molecules in the cell it is important to analyse their expression directly. The small amount of tissue available from rodent eyes is extremely limiting for proteomic analysis, but use of a pig model makes this feasible. 2-dimension (2-D) gel electrophoresis was used to define the proteome of the normal porcine retina. Comparisons with the $Rho^{Pro347Leu}$ retina have revealed a range of significantly altered proteins.

The retina contains over 50 cell types which are arranged in an elegant laminar structure. This heterogeneity makes it difficult to interpret observations from whole retinal extracts and changes in a single cell type are difficult to detect. We therefore employed planar sectioning to isolate specific retinal layers and validated their composition by RT-PCR using known markers. An alternative approach is subcellular fractionation and a protocol has been developed to isolate vesicles and study their protein content.

There are currently no therapies available to prevent or reverse the retinal degeneration that occurs in RP or other retinal degenerative disorders. Implantation of neural stem cells into the eye may be a means by which to retard or even reverse degeneration of the retina. Several studies have demonstrated the ablility of stem cells to incorporate, albeit to a limited degree, into the retina. Retinal progenitor cells (RPCs) are neural stem cells specific to the retina and have been isolated from the ciliary margin in a range of adult animals, including humans. We have demonstrated that it is possible to obtain RPCs from pig eyes. They form pigmented neurospheres containing proliferating cells. Expression of Nestin and Pax6 confirmed the identity of the RPCs, which in the presence of serum-containing medium differentiate into glia and neurons, as demonstrated by expression of Glial Fibrillary Acidic Protein (GFAP) and _-tubulin respectively. RPCs from various species, from murine to human, have proved difficult to maintain in culture. The total number of cells from one isolation increases up to passage 3 and then declines until all cells are lost. We are now investigating different approaches for maintenance and expansion of porcine RPCs to allow their further characterization and assess their availability and viability for cell replacement in the diseased retina.

The pig is a good large animal model for the human eye and it is anticipated that more pig models for degenerative eye disease will become available. It is already providing novel biomedical information and is highly suited for preclinical testing of potential treatments for retinal degeneration, including gene therapy and stem cell replacement. These applications are likely to increase now that the disadvantage of limited genomic information is being removed, although the lack of eye-specific/enriched mRNAs from retinal cDNA libraries remains a limitation.

The Melanoma-bearing Libechov Minipigs: an accurate model to improve the knowledge of anti-tumoral self defence

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Cutaneous malignant melanomas stem from the neoplastic transformation of skin melanocytes. It is a public health problem due, first, to the considerable increase of its incidence over the past forty years among the Caucasian population and, secondly, to its high metastatic spreading and its poor response to radio-, chemo- and immunotherapy. The only successful treatment of melanoma is excision of the primary tumor before the start of invasion. Sun exposure is the major environmental risk factor but approximately, 10% of malignant melanomas occur in a familial setting. This cancer provides a good example of a multifactorial disease, associating genetic and environmental factors. Relevant experimental models of melanoma are rare and many of them require a combination of carcinogen and UV treatments or insertion of a known oncogene. Most of them are developed in mice where the origin of precursor melanoma cells differs from that of human cells. In swine, skin anatomy and histology are comparable to those of human. We have imported from Czech Republic the Melanoma-bearing Libechov Minipigs (MeLiM) model in order to study the genetic susceptibility of this tumor and try to understand the molecular pathway involved in the surprising spontaneous regression of this cancer in swine. This presentation will make the focus on our recent results in this swine melanoma model.

1 - Clinical and histological characterization

In the MeLiM model, melanoma occurs in utero or soon after birth. The clinical and histological characterization has been done in collaboration with anatomo-pathologists of the Gustave Roussy Institute (Villejuif, France), the Curie Institute (Paris, France) and the Leon Bérard center (Lyon, France) using human melanoma classification. The pigs developed three different types of pigmented lesions. The flat lesions are benign for most of them and correspond either to lentigo or to intraepidermal atypical melanocytic proliferation. Some of them, highly pigmented correspond to melanoma restricted to epidermis. The small elevated lesions (dome or plateau shaped lesions) correspond either to Superficial Spreading Melanoma (SSM) or Nodular Melanoma (NM), with a maximal invasion in the reticular dermis (Clark's level IV). These two kinds of lesions never give metastasis when they are alone on piglets. Finally, large exophytic lesions represent invasive melanoma in 50% of MeLiM swine, SSM or NM, reaching a Clark's level V (hypodermis invasion). They are often ulcerated and developed preferentially on pigs with black coat colour. Half of these melanomas spread in regional lymph nodes and some of them in visceral organs. Nevertheless, most of them spontaneously regress in the first year of life. Regression begins often in the second month, with reduction in pigmentation and tumour size and ends by formation of scar tissue. *(Vincent-Naulleau S.,2004)*.

2 - PET scan analysis

Regression affects primary melanomas and also metastasis as we demonstrated in a PET scan study. Serial whole-body FDG PET scans were conducted on MeLiM swine. Sensitivity was better for tumors with vertical growth than the flat lesions and reached 75% for cutaneous melanoma and 62.5% for lymph nodes metastases. The spontaneous clinical regression could be followed by a decreasing of the PET scan signal until a total extinction in the cutaneous melanoma as in the lymph node metastasis. The results obtained in the MeLiM model correlating well with those described in human melanoma imaging, support the possible use of this model to test new tracers specific for tumoral cells. *(Boisgard R., 2003)*.

3 - Genetic characterization

Two complementary approaches have been used to search for genes involved in melanoma oncogenesis: 1) a linkage analysis, both on candidate loci and on the overall genome, to detect predisposing genes, 2) a genetic analysis of the tumour to detect somatic alterations.

3 - 1 Linkage analysis:

This analysis was performed in collaboration with the Genetic Department from INRA. Due to the high

level of inbreeding in the MeLiM families, we crossed affected MeLiM with healthy Duroc pigs to produce F1 and Backcross (BC) generations, the later segregating for the melanoma loci. The clinical and histological characterization allowed us to determine the phenotypes of pigs. Melanoma segregation in families revealed that swine melanoma was inherited as an autosomal dominant trait with incomplete penetrance.

3 - 1 - a - Candidate loci:

The genes CDKN2A, CDK4, MC1R and BRAF which are involved in human melanoma were studied in swine families. These genes were mapped on the swine radiation hybrids map respectively on *Sus Scrofa* chromosomes (SSC) 1, 5, 6 and 18, and the nearest markers were used in the genome scan. The CDKN2A gene, mapped to 1q25 region (*Le Chalony C.; 2000)* was excluded as a major susceptibility gene in this MeLiM family by a haplotype analysis *(Le Chalony C., 2003)*.

3 - 1 - b - Genome scan:

A significant genome-wide scan, with 153 microsatellite markers, was conducted in 331 BC pigs deriving from 5 MeLiM animals. Interval mapping analysis, using the QTLMAP software developed at INRA, provided evidence that melanoma development was linked to five regions on SSC 1, 2, 13, 15 and 17 ; some of them were already found by a preliminary analysis conducted on 79 BC *(Geffrotin C, 2004)*. Analysis of 7 specific traits revealed additional QTLs, the more significant lying on SSC 10 (ulceration), 12 (melanoma present at birth), 13 (lesion type), 16 and 17 (number of tumors). Association was observed with a MeLiM allele for a marker close to the MC1R gene which codes for the melanocortin receptor, a regulator of melanin synthesis on SSC 6p1.5 In addition, interactions were observed between MC1R and markers located on SSC1.The markers close to CDK4 and BRAF genes segregated independently from melanoma suggesting that these 2 genes were unlikely to be melanoma susceptibility genes in this model *(Geffrotin C, 2004)*. Therefore, these results demonstrate the polygenic nature of melanoma susceptibility in the MeLiM model as also suggested in human melanoma.

3 - 2 Genetic analysis of the tumour:

This approach has been realised in collaboration with the Curie Institute (UMR 147, Paris). Comparative Genomic Hybridization (CGH), which allows to detect gains and losses of genetic material, was applied to DNA from tumoral cells isolated from 11 tumours (4 SSM and 7 NM) after laser microdissection. Consensus regions of chromosome gains were identified on both subtypes, on SSC 3p13-p17, 12q and 14q11-q21. Chromosomal loss was restricted to NM lesions on SSC 13q31-49 *(Apiou F, 2004)*.

4 - Molecular analysis of melanoma regression

Spontaneous regression of malignancy is rare; therefore, our MeLiM model seems to be very useful to study this process. As there could be various factors which can affect host resistance, we have choose to approach the mechanisms involved in this process by a global analysis of the genes differentially expressed between growing tumours and those in the first phases of regression defined by clinical and histological parameters. The study was performed both on tumour tissues and cells extracted from tumours. We applied the suppression subtractive hybridization (SSH) technique to isolate differentially expressed transcripts in the two phases of tumour evolution. Analysis of the differentially expressed genes between progressive and early regressive tumors is underway. In the future, a large-scale analysis will be done using GeneChip® Porcine Genome array (Affymetrix).We hope that these studies might lead to point out some pathways involved both in oncogenesis and regression of melanoma and to conduct research towards more specific fundamental and applied studies with a therapeutic goal. In addition, these analyses might allow to identify new tumour antigens and find some markers specific for tumour regression..

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Uncovering roles for miRNAs in shaping phenotypic variability in animals

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Our laboratory has been actively involved in the positional identification of QTN influencing agronomically important traits in livestock over the last 15 years. In two instances - both times when studying muscular development in the sheep - this has let us to the demonstration of important roles of miRNAs in regulating the inheritance of muscular hypertrophies.

The first case deals with the callipyge muscular hypertrophy, which uniqueness stems from its unusual mode of inheritance, referred to as polar overdominance, in which only heterozygous individuals having received the CLPG mutation from their father express the phenotype. We have demonstrated that +/CLPGPat individuals are hypermuscled because of the ectopic expression of the paternally expressed imprinted DLK1 gene in skeletal muscle as a result of the cis-effect of the CLPG mutation. That CLPG/CLPG animals do not express the phenotype is due to translational inhibition of DLK1 mRNAs mediated by maternally expressed imprinted non-coding RNA genes. The demonstration if RNAi mediated allelic transinteraction at the imprinted Peg11 locus strongly supports a direct role of miRNA in polar overdominance.

The second case deals with the muscular hypertrophy of Txel sheep. We have demonstrated that the MSTN allele of Texel sheep is characterized by a G to A transition in the 3'UTR that creates a target site for miRNAs which are highly expressed in skeletal muscle. This causes translational inhibition of the myostatin gene and hence contributes to the muscular hypertrophy of Texel sheep. Analysis of SNPs databases for human and mice, demonstrates that mutations creating or destroying putative miRNA target sites are abundant and might be important effectors of phenotypic variation.

Latest results will be presented.

Protein turnover in muscle: strategies to understand the dynamic proteome

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Proteomics aims to define the complete pattern of protein expression in a cell or tissue at a particular physiological or pathophysiological state. We can distinguish three types, or phases of proteomics: a) identification proteomics, which is predominantly concerned with gaining the identities of proteins that

demonstrate a response of interest, b) characterisation proteomics, which emphasises the gain of further qualitative information on proteins of interest, such as sites of phosphorylation, and c) quantitative proteomics in which the amounts of specific proteins are determined. We can further subdivide quantitative proteomics in subcategories. Relative quantification expresses the quantity of protein in one state relative to a second state, whereas absolute quantification measures the amounts of the protein without reference to a second state.

A further complication reveals itself when we consider that the level of any protein in the cell is the product of two opposing processes, namely protein synthesis and protein degradation. For example, a protein might increase in the cell through accelerated synthesis, or through diminished degradation. The outcome is the same, but the mechanism and connectivity to other cellular processes, is very different. We can anticipate that the rate of synthesis of a protein is more immediately linked to the level of the cognate transcript (and therefore, rates of transcription and translation) whereas the rate of degradation of a protein is a stochastic selection for proteolysis controlled by the environment of the protein, and thus linked more closely to the metabolome. Thus, a full quantitative description of the proteome in the cell will also seek to express the levels of protein in the cell in terms of synthesis and degradation.

We have been developing methods to allow measurement of the rates of protein turnover of individual proteins, on a proteome-wide scale. In brief, we measure the incorporation or loss of stable isotope labelled amino acids from proteins, monitored through the analysis of tryptic fragments by mass spectrometry. Simple to implement in single cell systems maintained in culture, the challenges faced in delivering similar methods in intact animals are considerable.

At the same time, we have also developed a novel approach to multiplexed absolute quantification of proteins in a proteome. The well established approach of using stable isotope labelled peptides as a surrogate for the protein in isotope dilution experiments is extended by the design and construction of totally new synthetic genes, heterologously expressed in bacteria which yield QconCAT proteins (*www.qcats.com*). After proteolysis with trypsin, a series of stoichiometrically equal peptides are generated, each of which can be used for quantification of a different protein. In my lecture, I will review our approach to proteome quantification in terms of turnover and absolute quantification.

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Posters and Oral Communications

Structural genomics and Gene mapping

1 High resolution physical map of porcine chromosome 7 QTL region and comparative mapping of this region among vertebrate genomes

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On porcine chromosome 7, the region surrounding the MHC contains QTL, influencing many traits. Previous studies highlighted evidence for the presence of a fragment of ~3.7 Mb located 23 Mb upstream of the position expected in this region, comparing to the human order and internal rearrangements were suggested. Characterization of this region and identification of all human chromosomal fragments orthologous to this porcine fragment remains to be carried out.

17 genes and 2 reference microsatellites were ordered on the high resolution IMNpRH2$_{12000rad}$ Radiation Hybrid panel. A framework 1000:1 map covering 550 cR$_{12000}$ was established. This study was accomplished by the development of a complete contig of the region, taking advantage of the fingerprinting of the INRA BAC library, and of the anchoring of BAC end sequences on the human genome. New micro rearrangements were highlighted. A bovine RH map was also developed in this region by mapping 16 genes. Comparison of the organization of this region in pig, cattle, human, mouse, dog and chicken genomes revealed that if the translocation of the whole fragment is observed in bovine and porcine genomes, internal rearrangements are specific of the porcine genome.

We estimate that rearranged fragments cover 5.2 Mb of the porcine genome. The study of this complete BAC contig showed that human chromosomal fragments homologs of this heavily rearranged QTL region are all located in the region of HSA6 that surrounds the centromere. This work allows us to define a list of all candidate genes that could explain these QTL effects.

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2 Sequencing, evolutionary characterization and mapping of the porcine mitochondrial transcription factor A gene

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Mitochondrial transcription factor A (TFAM) is an integral part of the basal mitochondrial transcription machinery in mammals. TFAM interacts with the mitochondrial D-loop region and facilitates transcription of mammalian mtDNA from the light-strand and heavy-strand promoters in the presence of mitochondrial transcription factors B1 (TFB1M), B2 (TFB2M) and mitochondrial RNA polymerase (POLRMT). In mammals, TFAM gene has already been isolated and characterized in human, mouse, rat, cattle and silvered leaf-monkey. Characterization of the *TFAM* gene in pig is of economic importance because of its possible impact on production traits, such as general energy metabolism and fat deposition. In the present study, we determined full-length cDNA and proximal promoter sequences of the porcine TFAM gene, which were then used for comparative characterization of the TFAM gene with other species. Using Radiation Hybrid panel the TFAM gene was mapped to the porcine chromosome 14 (SSC14). RT-PCR analysis revealed alternative splicing of the porcine TFAM primary transcript. A G557A substitution in intron 1 of porcine *TFAM* gene was detected and genotyped on a total of 252 animals, including 165 from seven Chinese and 87 from five Western pig breeds. The statistical analysis revealed that these two groups of pigs were well separated regarding this locus during the breed history. As there are marked differences in fatness and lean meat production between Western and Chinese pig breeds, the *TFAM* gene deserves further investigation in order to evaluate its phenotypic effect on fat deposition and carcass traits in commercial pig populations.

3

Characterization of the porcine spermadhesin gene family – an expanding gene cluster in the pig genome

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Approximately 80% - 90% of mammalian genes are conserved in a 1:1 orthologous relationship across different mammalian species. Differences in the gene content between different mammalian species are frequently observed with genes involved in immune defence or reproduction.

We sequenced a genomic region on SSC 14q28-q29 containing a cluster of five genes encoding spermadhesins. Spermadhesins are soluble proteins in the seminal plasma of boars and they constitute a major fraction of the total seminal plasma protein in the pig. The porcine spermadhesin genes share a common genomic organization and an unusual high degree of sequence identity in non-coding regions, whereas parts of the coding sequences of the spermadhesin genes have diverged considerably. The spermadhesin genes seem to have acquired a special functional significance in the pig as there are five functional porcine genes that are all strongly expressed in the male genital tract and one member is even expressed in the uterus of sows. In contrast to the pig, other mammalian species with sequenced genomes mostly have two spermadhesin gene copies. Among these species, cattle seems to be the only species where these two spermadhesin genes are functional. In human, chimpanzee, and dog deleterious mutations have inactivated the spermadhesin genes. In the rodent species mouse and rat finally, the spermadhesin locus has been deleted from the genome entirely.

4

Progress on fine mapping of meat quality QTL on porcine chromosome 4 in a commercial sire line

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QTL effects on meat quality traits have been pinpointed in a half-sib structure of a commercial finisher cross. A large area of 90 cM on the long arm of chromosome 4 surrounding the QTL region was subjected to fine mapping by genotyping more families, developing and genotyping more markers, and genotyping the purebred nucleus line itself. After genotyping the publicly available microsatellites in the region, new markers have been developed from the publicly available BAC-end sequences (BES) and the Sino-Danish data on 0,66 X coverage whole-genome shotgun sequence of the pig (Wernersson et al., 2005).

Targeting of markers was performed anchoring the porcine BES hits to the human reference sequence and using the pig-human comparative map information. From a selection of 30 markers genotyped on the population, 25 markers (83%) could be mapped to the region of interest. The development of new SNP markers from porcine ESTs and BES information proofed to be much more time consuming than the development of additional microsatellite markers. Genotyping and mapping of microsatellites from shotgun sequence data is currently underway. The results refine the comparative map of SSC4q with human chromosome 1and 8.

Molecular characterization of the porcine differentiation factor PTHLH

5

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The differentiation factor PTHLH (parathyroid hormone-like hormone) has an established role as a local modulator of epithelial-mesenchymal interactions such as those of bone, teeth, mammary gland and nipple development. Through alternative splicing, four different mRNA species are transcribed from the PTHLH gene, which translate into two protein variants differing in the N-terminal end. Downstream of its receptor, PTHLH modulates the activity of the differentiation factor IHH (Indian hedgehog homolog). PTHLH is expressed in placenta and in a variety of tissues at embryonic stages; however its role in adult tissue has not been well reported. PTHLH-knock out mice died at birth from complications of a chondrodysplastic syndrome. Mice lacking the PTHLH receptor displayed, in addition, lack of teeth, mammary glands and nipples.

Based on these observations, we are investigating the PTHLH gene as a candidate gene for nipple development in a F2 Meishan x Iberian experimental pig intercross. These breeds differ in several reproductive traits including teat number. Only one of the four PTHLH variants has been sequenced in pig. Using human, mouse and partial pig sequence information we have designed primers to amplify the four cDNA variants in pig. Our sequencing data shows that pig PTHLH exhibits a 93.72% homology with human sequences. We are currently investigating the distribution of expression for each variant of this gene in a variety of adult and embryonic tissues. In addition, we are also looking for polymorphisms by sequencing a number of Meishan x Iberian animals in order to carry out association studies with reproductive traits.

Deciphering porcine SSC17 QTL for meat quality: from genome scan to fine mapping to sequence information

6

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Numerous quantitative trait loci (QTL) for a variety of economically important traits for the swine industry were identified in the last decade. In order to fully utilize these QTL in advanced pig selection, identification of the causative mutations is needed. To fine map several meat quality QTL on SSC17, genetic markers were added to the existing map and thereby increasing the marker density in the QTL regions of interest. As new maps were obtained, additional QTL analyses have been performed. The availability of the full DNA sequence of the region, where specific QTL are located, would be an extremely helpful resource. A highly contiguous, integrated BAC physical map of the porcine genome has been completed by members of the Swine Genome Sequencing Consortium. This resource allowed the selection of a minimally overlapping tile path of BAC clones that covered the entire QTL region. The BAC tile path spanning the meat quality QTL on SSC17 was then sequenced by the Sanger Institute. With the high quality finished sequence information now becoming available, it is possible to look for polymorphisms, insertions, deletions and new gene candidates, which will ultimately lead to the identification of the mechanisms that control the SSC 17 meat quality QTL. The approach and the added value of the sequence are illustrated with this example of QTL for meat quality traits on chromosome 17.

7

The porcine *Mx1* and *Mx2* genes

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MX proteins are GTPases and some are known to confer an innate resistance against several viruses. In laboratory mouse strains, allelic polymorphisms at the Mx1 locus affect the probability of survival after experimental influenzal disease, which raises the possibility that identification of an antiviral MX isoform in pigs might allow selection programmes aimed at improving their innate resistance. Only partial-length porcine Mx1 and Mx2 cDNA clones were isolated and sequenced (Müller et al. 1992 J. Interferon Res. 12(2): 119-129). The present study was designed to isolate the porcine Mx1 and Mx2 genes and to investigate their genomic organisation.

Using specific primers and the SMART RACE® technology, we amplified cDNA fragments and cloned them within the pCRII vector. Sequence analysis of different clones revealed the complete 5'- end sequences of *Mx1* and *Mx2*. A genomic bacterial artificial chromosome (BAC) clone containing both genes was identified for the porcine library RPCI-44 (CHORI). The insert was sequenced by the shotgun technique. The comparison between the genomic and the cDNA sequences allowed the identification of the promoters, exons and introns. Each promoter contains 2 proximal typical interferon-stimulated response element (ISRE) sequences. The porcine *Mx1* gene is made up of 15 exons, as the bovine *Mx1* gene. In contrast, the porcine *Mx2* contains only 8 exons. In conclusion, we established the porcine *Mx1* and *Mx2* genomic structures and produced sufficient flanking intronic sequences to enable simple PCR amplification of the promoters and the coding portions of these genes. Promoter and expression analysis was also performed.

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Conserved RNA structure between genomic regions of human and mouse that are unalignable in primary sequence

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A main limitation in comparative genomics is that it requires a good alignment of the genomes primary sequences. The use of multiple organisms can to some extend compensate for this. Nonetheless, when searching for non-coding RNA genes, aligning the sequences by both primary sequence and secondary structure is the desired approach when comparing corresponding genomic regions with sequence conservation typically less than 50%. The Sankoff based approach, FOLDALIGN [1] has recently been updated to conduct a mutual scan for conserved structure along two such genomic regions, by simultanously aligning and folding the considered sequences. The approach has been applied on a large comparison between human and mouse including the 10 chromosomes for which transcriptional fragments have been mapped [2]. Our scan has resulted in an estiamte of approximately 1800 ncRNA candidates between the genomes. We are currently in the process analyzing the predicted candidates and have so far verified the expression of 40 mouse ncRNAs (using RT-PCR) for which the corresponding human candidates overlap with the transcriptional fragments [2].

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Porcine SPLUNC1: Molecular cloning, characterization and expression analysis

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SPLUNC1, originally named PLUNC for palate, lung and nasal epithelium clone, is a small protein which is secreted from epithelial cells of the nasal cavity and the upper respiratory tract in humans, mice, rats and cows. SPLUNC1 is structurally homologous to the two key mediators of host defence against Gram-negative bacteria, lipopolysaccharide binding protein (LBP) and bactericidal permeability increasing protein (BPI). SPLUNC1 is therefore believed to play a role in the innate immune system.

This work reports the cloning and analysis of the porcine (*Sus scrofa*) homologue of SPLUNC1. The *SPLUNC1* cDNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using oligonucleotide primers derived from *in silico* sequences. The porcine cDNA codes for a protein of 249 amino acids which shows a high similarity to bovine (74%) and to human (69%) SPLUNC1. The predicted *Sus scrofa* SPLUNC1, SsSPLUNC1, polypeptide contains a putative signal peptide of 19 residues. A similar signal sequence is also found in all other members of the PLUNC family. *In silico* search analysis with the local EST database at The Danish Instititute of Agricultural Sciences revealed presence of homologues of all members of the human PLUNC gene family. Expression analysis by RT-PCR demonstrated a very high expression level of the porcine SPLUNC1 homologue in trachea and lung tissue only. This airway-specific expression might be of particular interest in the study of airborne diseases in pig.

Illumina Solutions to Large Scale Genotyping and Gene Expression Analysis using BeadArray® technology

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Analysis of the upstream region of the *dominant white/kit* locus in pig

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The *KIT* gene located on SSC8, encodes the mast/stem cell growth factor receptor, which is essential in melanogenesis, gametogenesis and hematopoiesis. Seven *KIT* alleles with distinct phenotypic effects have so far been described: recessive wild-type i, *Patch* I^{P}, *Belt* I^{Be}, *Dominant White* I^{1}, I^{2}, I^{3}, and the I^{L} allele. We have previously reported that I^{P} is associated with a large duplication (~450 kb) including the entire *KIT* coding sequence while the *Dominant white* alleles are associated with the same duplication plus a splice mutation in intron 17. The Belt allele (I^{Be}) causing a white belt across an otherwise pigmented body is neither associated with the duplication nor the splice mutation. It has been proposed, on the basis of similarity to some mouse *KIT* mutations that I^{Be} is most likely caused by a regulatory mutation upstream of KIT. In the present study the *KIT* upstream region, corresponding to approximately 380 kb has been amplified from different *KIT* genotypes as overlapping long-range PCR fragments. The fragments have been screened for insertion/deletion polymorphisms in a first attempt to map and identify the mutation causing the Belt phenotype. Several microsatellites in the 5′ *KIT* region and SNP′s located in the *KIT* coding region have been analysed. A ~ 13 kb region including two ultraconserved elements have been partially sequenced and microsatellite polymorphisms have been determined for different *KIT* genotypes.

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MC1R gene polymorphisms in Mora Romagnola and Cinta Senese for pig breed traceability

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In Italy many different autochthonous pig breeds, as Mora Romagnola and Cinta Senese, characterised by small consistency and high quality products, are raised. The identification of molecular polymorphisms useful for breed identification could contributed to the valorisation of local genetic resources. Due to the importance of pig breeds coat colours standardisation, porcine genes involved in pigmentation are useful candidates for breed molecular traceability. In order to identify polymorphisms useful for the identification of specific markers for local breed pig products traceability, we have investigated, in Cinta Senese (n.11) and Mora Romagnola (n. 13) the polymorphisms of MelanoCortin-1 Receptor (MC1R) gene, previously known as Extension locus, one of the most important and polymorphic genes involved in porcine coat pigmentation. The Mora Romagnola is described as black and tan whereas Cinta Senese as white belted over a black coat colour. Cinta Senese shows the G124A mutation and the absence of the insertion of two nucleotides at codon 22, denoted the MC1R*3 allele also known as dominant black. On the contrary, Mora Romagnola shows the G164C and G243A mutations characterizing the MC1R*4 allele, also known as red allele. In these work we present also the results obtained in the wild boar (n.5) and in two cosmopolitan breeds, the Duroc (n.4) and the Large White (n.7) discussing them with the phenotypic descriptions of the breeds. The results confirm the usefulness of MC1R polymorphisms in some porcine breed distinction but only the study of other coat colour genes could permit a more discriminant breed analyses.

Preliminary analysis on genetic diversity of Cinta senese pigs

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Cinta senese is an autochthonous pig breed native of Tuscany. The breed currently produces fresh and cured products with high market price. One of the factors associated with this high price is the origin of the raw material but consumers require that Cinta senese pig be the only breed involved. Microsatellite markers could help in breed identification and the first step to achieve this objective is the knowledge of the genetic variability.

The purpose of the present work was to determine the genetic variability of Cinta Senese (CS) breed with ten microsatellites (SW2038, SW024, SW0017, SW153, SW1370, SW1035, SW1695, SW1873, SW1823 and SW1556) using Large White (LW) breed as reference. Genetic characterization was performed on 52 and 14 subjects for Cinta senese and Large white respectively. The following statistics were calculated: allele frequencies; mean heterozygosity values; average expected heterozygosity; coefficient of gene differentiation. The analysis of microsatellite loci revealed observed heterozygosities ranging from 0.31 to 0.92. The expected heterozygosity ranges from 0.444 to 0.892. A large part of the total genetic diversity can be explained by the variation within breeds (94.2%) and to a smaller extent by the variation among breeds although a large difference has been found among loci with Gst values ranging from 0.3% to 12.57%. Even if the mean number of alleles is similar between the two breeds (5.1), Cinta senese breed shows a almost unique pattern with many specific alleles and this is a good release point to setup a protocol for breed identification.

Identification of SNPs in the porcine *adiponectin* gene

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Adiponectin (referred too as ADN and ApM1) is a fat derived hormone involved in lipid and glucose metabolism. The pig gene, mapped on SSC 13, can be considered a putative functional and positional candidate gene for growth and for meat production and quality. The aim of this work is to search for variability in the porcine adiponectin gene and to analyse new polymorphisms in different breeds (Italian Large White, Italian Landrace, Duroc, Hampshire, Landrace Belga and Pietrain). Three primer pairs were designed in order to amplify and to sequence amplicons of approximately 1100 bp each. The three different PCR products allowed to determine the sequences corresponding to the terminal part of intron 1, the exon 2, the intron 2 and the almost complete encoding region of the 3th and last exon of the ADN gene. New SNPs were found both in exons and introns regions. In particular it was identified a G-A polymorphism within the exon 2, which determines the val-ile substitution in the collagen-like domain of the protein. To study this polymorphism a further primer pair was designed and a PCR-RFLP protocol was set up. The most frequent variant was the G in all the examined breeds. Differences in allelic frequencies between the six pig breeds were observed. The polymorphism identified will be useful to perform association studies with meat quality and production traits in pigs.

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SNP analysis of *FASN* gene in Italian pig breeds

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Fatty acid synthase gene (FASN) plays a central role in fatty acids biosyntesis catalyzing the conversion of acetyl-CoA and malonyl-CoA, in the presence of NADPH, into long-chain saturated fatty acids. In animals the enzymatic activities of FASN are organized in one large polypeptide. In pigs FASN gene has been assigned to chromosome 12p1.5 and two SNPs were described by Munoz et al. (Animal Genetics, 2003, 34:234-6). One of these polymorphism found in the fourth exon creates a polymorphic FnuHI restriction site, was used in the present research to characterise the pig breeds reared in Italy. We analysed the allele frequencies of this SNP of FASN gene in 11 pig breeds Italian Large White, Italian Landrace, Duroc, Belgian Landrace, Hampshire, Piétrain, Calabrese, Cinta senese, Casertana, Nera siciliana, Mora romagnola and the chinese breed Meishan. For each breed a sample from 11 to 57 pigs was analysed. In 8 out of 11 analysed breeds allele 2 was the most frequent and its value was higher than 0.75 in Duroc, Belgian Landrace, Piétrain, Calabrese, Cinta senese, Nera Siciliana breeds. Meishan samples were monomorphic for this allele. The same FASN SNP was used to genotype pigs with divergent EBVs for production traits backfat thickness (BFT) and lean cuts (LC) in a sample of 200 Italian Large White. Significant differences between allelic variants at FASN locus were found ($P<0.05$) between the pigs extreme for BFT. Further studies are necessary in order to verify these findings and determine if FASN gene can have effect on carcass quality in pigs.

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Study of non-classical class I MHC genes in pig

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The class I region of Major Histocompatibility Complex (MHC) comprises classical Ia and non classical Ib genes, which share the same general organization and present high sequence similarities. The class Ia genes, which are expressed at the cell surface for endogenous and viral peptide presentation to the $CD8^+$ T lymphocytes, are highly polymorphic and have no tissue specificity. These genes are referred to as HLA-A, -B and -C in man and SLA-1, -2 and -3 in pig. In contrast, the class Ib genes are less polymorphic, show a tissue specific expression and their roles are still unclear. The Ib genes are referred to as HLA-E, -F and -G in man and SLA-6, -7 and -8 in pig. Comparisons of both sequence and position within MHC region between human and pig Ib genes do not indicate any obvious orthology. Our aim is to analyze the polymorphism and tissue specificity of SLA-6, -7 and -8. We have designed gene specific primer pairs amplifying the whole exon 2 to study the polymorphism of eleven Chinese and twenty European breeds. To study their expression, we have designed primers amplifying the junction between exons 2 and 3 and are developing real-time quantitative PCR experiments using 33 distinct tissues. We will present our current results on both aspects of this work.

A novel polymorphism of the porcine prolactin (PRL) gene

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Prolactin is a protein hormone playing a role in the maintenance of pregnancy in the pig by action on *corpora lutea* cells and possibly initiating production of progesterone. The *prolactin* gene is 10 kb in size and is composed of 5 exons and 4 introns. The present work is a report of the swine PRL gene – comparative DNA sequence analysis and the SNP revealed in the promoter region. Basing on the bovine prolactin gene, three primer pairs were designed using the Primer3 *on-line* software. The overlapping fragments covered about 400 nucleotides of the promoter and 87 nucleotides of exon 1. The fragments were amplified, the two of them were sequenced and deposited in the GenBank database (AY341908 and AY905690). In all three fragments the multitemperature SSCP analysis was performed. The third fragment appeared to show a different MSSCP pattern. The samples of differing MSSCP conformers were sequenced and the C499T transition was identified in the 5'UTR region of the gene. The restriction analysis allowed to chose *HphI* restriction enzyme to recognize the novel SNP found. The alignment for homology analysis was performed between the pig, bovine (X01452) and human (NM_000948) DNA sequences available in GenBank database, using BLAST software. The comparative homology analysis results varied in dependence on the species and functional region of the gene.

Assessment of genetic diversity and extent of LD in European and Chinese pig breeds using large scale SNP genotyping

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A breed is characterized by having a different history from other breeds, implying different parent stock, rates of drift, selection pressure etc. Such differences are expected to leave signatures in the genome, for instance in levels of heterozygosity and patterns of linkage disequilibrium (LD). Our main focus is to compare patterns of single nucleotide polymorphisms (SNP) in breeds of two major areas of pig domestication, China and Europe. Eleven Chinese breeds were selected to cover maximum geographic diversity. The ten European breeds represent both commercial lines as well as traditional breeds, and one wild boar population from France was also included. SNP were derived from various sources such as earlier studies on SSC 2 and 18, and EST databases. A total of 1536 SNP were assayed using the Illumina BeadArray technology, with a particular focus on SSC 2, 3, 4, 6, 9 and 18. For a high resolution comparison of LD in various breeds around 400 SNP located on three regions of 0.7 to 4 megabase each from SSC 18 were selected, with spacings between a couple of hundred to several megabases. The rest of the SNP data was used to look for evidence of long range LD and general patterns of genetic diversity. Our presentation will provide preliminary results of this study.

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Pig and minipig liver drug metabolizing enzymes: similar to the man or not?

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The most important enzymes metabolizing compounds foreign to the organism (xenobiotics, as drugs, pollutants etc.) in animals as well as in plants are heme enzymes, cytochromes P450 (abbrev. CYP). Structural similarity of these enzymes obtained from pigs and minipigs allows for the use of minipigs as experimental animals; high degree of identity with human orthologous forms makes them also good models for human metabolism of xenobiotics. This is especially valid for the CYP3A human and pig forms as well as for the CYP2E1 form. On the other hand, there are some structural changes which probably cause that even two orthologous forms with almost identical substrate specificity (human and minipig CYP2E1) differ in their active site flexibility and hence in the ease with which they denature (the human enzyme is more prone to the denaturation). Proteomic studies aimed at the presence of other CYP enzymes orthologous to their human counterparts in the minipig liver are currently underway in our laboratories.

Financial support from the Czech Ministry of Education to the COST B 861.001 project through the 1P05OC050 grant is gratefully acknowledged.

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A Genome-wide Scan for Novel QTLs of Hereditary Cutaneous Malignant Melanoma in the MeLiM Swine Model

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Human cutaneous malignant melanoma is a complex trait affected by both multiple genetic and environmental factors, and has been yearly increasing in populations of Caucasian origin. To search for melanoma susceptibility loci and help human melanoma studies, genetic analyses of the MeLiM swine model were completed. Quantitative trait loci (QTLs) on *Sus Scrofa* (Sscr) chromosomes 1, 13, 15 and 17, were detected to be associated with melanoma synthetic traits, which combined all phenotypic information, and at the same time, some regions on Sscr 2, 6, 10, 12 and 14 contain QTLs underlying various specific traits. All data indicate that melanoma development in pigs is also a multigenic process. Furthermore, after sequencing the entire coding region of *MC1R* and then SSCP identification analyses, black pigs with allele *MC1R-2* were found to have predisposition to develop melanoma significantly, after calibration of the coat color effect. Massive radiation hybrid mapping comparatively localized orthologous genes on *Homo Sapiens* (Hsap) chromosome 9p21, which was related with Sscr 1 chromosomal regions of some putative QTL(s). Our previous work and the characteristic composition pattern of Sscr 1 putative QTL peaks compared to Hsap 9p21 region suggested that novel putative gene(s) underlying melanoma susceptibility might be in these regions, which needs further investigation.

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Radiation hybrid mapping to SSC5 of 18 positional and functional candidate genes for Arthrogryposis Multiplex Congenita (AMC), a pig disease model for human medicine

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Arthrogryposis multiplex congenita (AMC) is one of the most common congenital defects observed in piglets and other mammals. In humans it occurs in one of every 3000 births. Piglets born with this syndrome contain multiple defects to the legs and spinal column and are not viable. In this study we report the chromosomal assignment with the INRA-Minnesota radiation hybrid panel of 18 porcine homologues to human genes. These are interesting positional and/or functional candidates for this autosomal recessively inherited disease, mapped between *SW152* and *SW904* on *SSC5. C3F, NR4A1* and *Q6ZUQ4* were chosen based on the results of a study using 13K oligo microarrays. *CACNA1C, COL2A1, CPNE8, C12ORF4, DDX11, GDF11, HOXC8, KCNA1, MDS028, MGC5576, PHB2, PRICKLE1, SCN8A, TUBA8* and *USP18* were selected from pig-human comparative database analysis. *CPNE8, PRICKLE1, Q6ZUQ4, TUBA8* and *USP18* were mapped in the pig AMC interval and therefore are important candidates to screen for the causative mutation or to identify genetic markers that can help eliminate the disease. Additionally, the present findings for SSC5q12-q22 revealed the existence of one small chromosomal interval of HSA22q11.2, between the segments of HSA12p13 and HSA12q12. Totally, we identified seven breakpoint blocks between HSA12, HSA22 and SSC5 in the proximity of the AMC region. These comparative data provide an important road map for future studies in humans and other species. To conclude, the swine represents a particularly attractive animal model for studying the common genetic forms of human AMC, due to the phenotypic similarity of AMC disease in humans and pigs.

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Genome-wide linkage analysis of inguinal hernia in Norwegian Landrace pigs

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Inguinal and scrotal hernia are a problem in pig production, with frequencies varying from 1.7% to 6.7% for different breeds, causing poor animal welfare and severe economic loss for the pig producers. By definition, an inguinal hernia refers to hernial contents present in the inguinal canal and scrotal hernia refers to hernial contents present in the scrotum. In these conditions, most frequently the distal jejunum and ileum pass through the vaginal ring and enter the inguinal canal. The aim of this project was to identify genomic regions that increase the susceptibility to develop inguinal/scrotal hernia in pigs, and therefore a complete genome scan based on affected sib pairs was performed to identify genomic regions affecting inguinal hernia in pigs. The genome scan included 52 litters with altogether 127 affected and 67 unaffected piglets, and they were all genotyped using 137 microsatellite markers evenly distributed over the entire genome. The data was analysed with nonparametric methods (Non-parametric Linkage (NPL)), using the computer package ALLEGRO 1.0, and the Transmission Disequilibrium Test (TDT)). Using the non-parametric linkage analysis, the linkage score for a QTL on inguinal hernia exceeded the nominal significance level of $p<0.001$ for chromosomal regions in SSC2 and SSC15. Furthermore, the linkage score exceeded the nominal significance level of $p<0.01$ for six chromosomes (SSC4, SSC5, SSC13, SSC17, SSC18, and SSCX). All markers, except one, that reached significance level of $p<0.05$ in the TDT analysis were also significant in the linkage analysis (NPL).

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Porcine Hernia – functional/positional candidate genes

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Hernia inguinalis/scrotalis result from either an abnormal wide inguinal canal (IC) or a not obliterated processus vaginalis (PV). During testicular descent the gubernaculum serves to expand the IC, whereas an overexpansion due to an unphysiological development of the gubernaculum may lead to an open IC. Further, smooth muscle cells (SMC) derived from the gubernaculum propel the testis into the scrotum. After testicular descent, SMC should undergo programmed cell death (PCD). A perturbation of the PCD cascade inhibits the obliteration of the PV.

Thus, genes involved either in the expansion of the gubernaculum or the PCD of smooth muscle cells were chosen as functional candidates.

A recently accomplished genome wide linkage analysis and the Genomic Mismatch Scanning approach show evidence of regions on SSC 3, 6, 7, 12, and 15 involved in the inheritance of hernia in German pig breeds.

GUSB gene encoding the ß-Glucuronidase, which is involved in the degradation of hyaluronan during the involution of the gubernaculum, was mapped on SSC3p13-15. *GUSB* was sequenced and five SNPs were detected. 84 half-sib families (512 animals) were genotyped for three of the SNPs. Further a SNP described in the *SERCA1* gene that encodes an intracellular calcium pump in the endoplasmatic reticulum of SMC was genotyped in the 512 animals. So far Transmission Disequilibrium Tests (TDT) analyses revealed no significant association between one of the allelic variants and the disease outcome. Nevertheless SNP analyses contribute to the finemapping of the previously detected hernia-associated chromosomal regions. Additional SNPs within functional/positional candidate genes on SSC 3 are subject of our ongoing studies.

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Genetic variation and in vitro analysis of the porcine Toll-like receptor 4 (TLR4) promoter

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TLR4 is essential for initiating the innate response to lipopolysaccharide (LPS) from Gram-negative bacteria by acting as a signal-transducing receptor. Common mutations in *TLR4* have been associated with several human and mice disease-related phenotypes. As the pig industry faces a unique array of related pathogens, it is anticipated that the genotype of swine TLR4 could be relevant for future strategies aimed at improving genetic resistance to infectious diseases. The promoter is a crucial region of the gene that we investigated in details. Several putative binding sites described in the human and murine promoter of TLR4 genes have been identified in the 5'flanking region of *poTLR4*. Conversely, this region lacks a TATA box, consensus initiator sequences, or GC-rich regions.

We described an inventory of naturally occurring variation in a portion of 436 bp of the porcine *TLR4* promoter segregating in a panel of 245 animals belonging to commercial and autochtonous breeds. At least two of the six SNPs found fell within or in close proximity to the putative binding sites. All SNP variants are been tested in vitro for a possible role in changing the promoter's activity in response to LPS, by cloning in pGL3-prom vector and cell transfection, followed by a dual-luciferase reporter assay protocol (Promega, USA).

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Analysis of the genetic background of juvenile hairlessness in pigs

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A new pig phenotype mainly characterized by juvenile hairlessness and thin skin has been discovered in a Danish pig herd. The trait shows autosomal co-dominant inheritance with all three genotypes easily distinguishable. Since the phenotype shows great resemblance to the integrin subunit β6 -/- knockout phenotype seen in mice, *ITGB6* is considered an obvious candidate gene for this trait. Integrins are a family of cell surface transmembrane proteins each consisting of an α- and a β-subunit. Comparative mapping predicts that the porcine *ITGB6* ortholog maps to SSC15. An experimental family (n=113) showing segregation of the trait has been established. The candidate region has been confirmed by linkage analysis with four microsatellite markers in the candidate region giving LOD scores from 8.4 to12.1. Sequencing of the *ITGB6* coding sequence from affected and normal pigs has revealed extensive alternative splicing of the pre-mRNA as well as several SNP's but no evidence of a causative mutation. A preliminary quantitative PCR study has suggested an up-regulated expression of a splice variant in homozygous affected pigs. Histological examination of skin samples has demonstrated a significantly thinner dermis and fewer hair follicles in affected pigs compared to normal pigs. Ongoing work focusing on expression studies of splice variants and ITGB6 protein expression is in progress.

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Molecular and functional characterization of the lung surfactant protein C (*SP-C*) gene in pig

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Surfactant protein C (SP-C) is a component of the protein-phospholipid mixture secreted by epithelial type II pneumocytes that prevents lung collapse on expiration. We have characterized the porcine *SP-C* gene at genomic, transcriptional and protein levels. The *SP-C* is coded by a single copy gene on SSC14. Sequencing the pig *SP-C* genomic region showed five synonymous SNPs. Two *SP-C* cDNAs were found in a pig newborn lung cDNA library: a full-length clone (FL) and a clone missing the complete exon five (*SP-CD*^{exon5}). Sequence comparison between two cDNA clones and cDNAs from four production pigs revealed four synonymous and two nonsynonymous substitutions. Two of the cDNA sequences had also in frame insertions at the beginning of exon five. Comparison of the *SP-C* coding region between several mammalian species showed high levels of conservation.

Expression studies using Northern blotting and real-time RT-PCR showed specific expression of the *SP-C* gene in lung, appearing in 50 days old foetus and increasing during lung development. The FL and *SP-CD*^{exon5} variants are mainly expressed in lung with a 260 fold change between them. Additionally they showed a quite high expression in ovary. Both mRNAs were significantly down-regulated in necrotic lung of pigs infected with *Actinobacillus pleuropneumoniae* (AP). In agreement with these results, the protein levels were also decreased or absent in the necrotic tissue as shown by immunohistochemistry and Western blotting studies.

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Chromosomal Localization, mRNA Transcription Profile, and Mutational Analysis of Golgi UDP-N-Acetylglucosamine Transporter from Pig

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Understanding the basic transport mechanism of nucleotide sugar transporters requires a comprehensive picture of their structure-function relationships, which are scarcely characterized. The gene *SLC35A3* encodes a Golgi UDP-N-acetylglucosamine (UDP-GlcNAc) transporter, which is highly conserved among mammalian species. In these studies, the porcine *SLC35A3* was assigned to *Sus scrofa* chromosome 4 (SSC4) by two-point linkage analysis using CriMap and SNP genotypic data. Subsequently the chromosomal localization was verified by the use of a porcine-rodent somatic cell hybrid panel in which the regional assignment was either 4q21-23 or 4q24. Real-time RT-PCR studies indicated transcription of *SLC35A3* mRNA in all examined tissues, although with highly variable expression levels. The highest expression was observed in stomach, which was ~8 fold higher than in vastus intermedius, showing the lowest expression.

The deduced 325 amino acid (aa) sequence revealed a highly hydrophobic protein with 8-10 predicted transmembrane helices. Functionally important regions of the protein were studied by complementation cloning in a *Kluyveromyces lactis* mutant deficient in transport of UDP-GlcNAc into the Golgi apparatus. Expression of full length SLC35A3 efficiently restored UDP-GlcNAc transportation in the yeast mutant. Additionally, truncation mutagenesis showed that the N-terminal 7-aa hydrophilic tail was dispensable, whereas the 14-aa tail in the C-terminus was essential for complete transport activity. Further truncations, concerning either the first or the last predicted transmembrane helix, inactivated the transporter.

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Complementarity of gene expression data obtained by SSH and Operon pig 13K microarrays during PRRSv infection in pigs

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Porcine Respiratory and Reproductive Syndrome (PRRS) causes significant economic losses in the swine industry every year throughout the world. PRRS is caused by a RNA virus (PRRSv) member of *Arteviriade* family, which has the ability to infect macrophages.

We are investigating the molecular mechanisms of the pathogenesis of PRRSv *in vitro* to identify host factors of resistance/susceptibility to PRRSv by complementing two gene expression technologies: Suppression Subtractive Hybridization (SSH, Clontech, USA) and oligo microarray analysis (Operon 13K pig array, Qiagen).

mRNA populations of primary porcine alveolar macrophages (PPAM) challenged with European PRRSv strains *in vitro* were extracted at different time of infections (4 -8 -24 - 48 hours post infection). Cells were collected by lung lavage of three commercial hybrids pig lines (PRRS-negative). A field strain of Northern Italy (Gozio) was used to infect PPAM at 0,1 moi.

Microarray analysis identified a list of activated and down regulated genes that could be assigned to specific pathways of the immune response.

Overall, SSH allowed to enrich the results obtained by the operon 13K microarray by identifying additional genes not represented on the array and/or not detected by slide hybridization

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Pre- and postnatal hepatic expression profiles: insight into functional networks of metabolic regulation

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The liver plays a central role in the regulation of the metabolic status and expenditure of energy and nutrients. We aim to expression profile liver using different experimental designs. In order to show hepatic genes with trait associated expression eight discordant sib pairs were selected from a Duroc x Berlin Miniature pig F_2 experimental cross representing extremes for traits related to body composition in order to build up groups of four high and four low performing individuals. Using an application-specific porcine hepatic cDNA microarray covering genes shown to be preferentially liver expressed and/or regulated due to different feeding regimes revealed nine genes that are regulated by the factor ≥ 2 between the high and low performing group. The transcript levels of four liver genes (apolipoprotein H (APOH), pigment epithelium-derived factor (PEDF), organic anion transport polypeptide 2 (SLC21A8), thyroxine-binding globulin (TBG) were shown to be significantly different between the phenotype groups by quantitative real time PCR of individual samples. Currently we display the expression profile of liver at three prenatal stages (35, 63, and 91dpc) plus adult stage in breeds differing in body composition and utilization and partitioning of nutrient. Porcine embryonic and foetal liver development can be divided into three periods: period of differentiation and haematopoietic activity (18-40 dpc), period of metabolic activity (40-80 dpc) and period of glycogen accumulation (80-113 dpc). Functional networks identified using genome-wide microarrays reflect these stage-specific major hepatic activities.

Alternative splicing of pig pre-mRNA

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Alternative splicing of pre-mRNA is a major source of protein diversity in eukaryotes. The number of described alternative splice events in pig has so far been limited. The Sino-Danish Porcine Genome Project has established a total of 98 porcine cDNA libraries representing different tissues and tissue-developmental stages. From the 98 cDNA libraries approximately 700.000 ESTs have been generated. From this resource porcine genes were selected on the basis of known alternative splicing of pre-mRNA in the human orthologous genes. The porcine EST data was used for verification of the alternative splice event in pig, as well as indication of tissue-specific or developmental-specific alternative splicing.

Fifteen selected porcine splice events were experimentally verified with RT-PCR using splice-specific primers. Twenty different tissues were collected from eight 25 kg pigs. In addition 12 different tissues were collected from four different developmental stages. Total RNA was isolated, treated with DNase I, and reverse transcribed into cDNA using oligo-dT primers. Real-time quantitative PCR analysis (qPCR) was used to determine the relative expression of alternative transcripts of the selected splice events. An index with three housekeeping genes was used for normalizing the qPCR data. ANOVA was performed for identifying statistically significant alternative splicing events in various tissues.

Eight of the genes showed no tissue-specific splice pattern, however seven of the genes had tissue-specific alternative splicing. One gene indicated tissue-developmental-specific alternative splicing. The tissues mostly subjected to tissue-specific alternative splicing were brain, heart and muscle.

Genomic mismatch scanning (GMS) and a genome-wide microsatellite scan identify identical chromosome regions associated with porcine hernia inguinalis/scrotalis

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Genomic mismatch scanning is a hybridization-based technique designed to enrich regions of identity by descent (IBD) between two individuals without the need of genotyping or sequencing. We performed this kind of linkage mapping using DNA of related and unrelated pigs affected with hernia inguinalis/scrotalis. About 300 IBD fragments between 20 bp to 650 bp length were generated and sequenced. Chromosomal location by PCR-screening of hybrid panels was performed for IBD fragments larger than 100 bp. A total of 14 IBD fragments was assigned to SSC 1, 3, 4, 7, 10, 12, 14, and 15.

In parallel, linkage and association analyses were performed to identify loci affecting susceptibility to hernia by scoring microsatellites and single nucleotide polymorphisms. The genome-wide scan based on affected half-sib families and included 84 families with a total of 512 animals with 81 boars, 156 sows and 275 affected piglets among them. The pedigree was genotyped for 139 DNA-markers distributed across the 18 autosomes. Non-parametric linkage scores were calculated using the program package Allegro 1.0. Significant linkage results were obtained for regions on SSC 3, 6, 7, 12 and 15.

Comparison between the GMS results and the results of the linkage and association analyses revealed that the three SSC3 IBD fragments mapped to chromosomal regions significantly associated with the phenotype. The five IBD fragments assigned to SSC 7, 12 and 15 fit to regions with suggestive linkage. None of the further six IBD fragments was located on any chromosomal region associated with the phenotype.

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Effect of 20 years of selection on Large White pigs: design of an *in vitro* model of dendritic cell infection with the Pseudorabies virus (PrV) for comparative transcriptomic analysis of the immune response

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Pig selection has been intense for the last 20 years in the French Large White pig breed and was shown to be efficient to improve production traits such as meat quality and fat content. During this period of selection, the immune response capacity of pigs was not taken into account and may have decreased. INRA has launched a program to study the impact of the last 20 years of selection and two pig populations were produced. The non selected population (1977) corresponds to pigs obtained after *in vitro* insemination of present-day sows with frozen semen of sires born in 1976 and used in Artificial Insemination Centers in 1977. The highly selected population (1998) corresponds to present-day pigs. Our aim is to evaluate the immune response capacity of pigs from both populations with a transcriptomic approach, on a model of *in vitro* infection of dendritic cells by the Pseudorabies virus (PrV). Blood from 6 animals of each population was sampled for lymphocyte purification, monocyte selection by immunomagnetic cell sorting and differentiation into immature dendritic cells by incubation for 72h with IL4 and GMF-CSF. Cells were infected for 18h and collected for further total RNA extraction and hybridization onto DNA chips. The DNA chips comprise 1856 pig probes, including 530 sequences of the Major Histocompatibility Complex (MHC) region, 80 additional genes of the immune response, the 80 PrV genes and 1166 randomly chosen cDNAs. We will present data obtained from these DNA chip hybridization analyses.

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Analysis of digital expression in the porcine genome using 98 cDNA libraries and 1,021,891 ESTs

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A resource of 685000 porcine ESTs has been analysed together with 400000 publicly available porcine ESTs. The former set of ESTs were extracted from 98 cDNA libraries of which 97 were unnormalized. The Distiller assembly program (1) has been used to assemble the sequences. We obtained toughly 48000 clusters, 73000 singletons. Approximately 27% of the clusters (covering two thirds of the resource reads) have a good blast match to existing sequences in UniProt and approximately 70 sequences have fairly stringent match to non-coding RNAs. We have investigated correlation patterns between the EST libraries and we find that the expression of sequences correlates for libraries from related tissues. For the fraction of the ESTs which had good blast match, we systematically compared the Gene Ontology profiles tissue by tissue. Even though the profiles varied from library to library we find that several categories correlate, for example the level transcriptional regulator activity correlates with the level of translational regulatory activity and the proportion is independent od which tissue (library) is considered. Furthermore, we find that libraries from brain and testis are among the most diverse tissue. We also identify sets of genes that are highly and uniquely expressed among the libraries.

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The Italian FIRB pig project: analysis of gene expression profiles in pig to identify candidate genes useful for meat quality and production improvement

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Genetic improvement of pig meat quality is an important aim of the Italian pig breeding industry whose production is mainly oriented of high quality typical products such as Parma and S.Daniele ham. In order to overcome the antagonism between lean meat content of the carcass and meat quality it is essential to identify the genes affecting parameters that define qualitative characteristics of dry cured meat. To this aim an Italian MIUR project (The pig FIRB project) is studying the differences in gene expression patterns comparing the transcriptome of several tissues and organs 1) among local and cosmopolitan breeds divergent for several meat quality and production traits 2) among different breeds that were treated just before slaughtering with high or minimal stressing conditions that may influence meat quality traits 3) between extreme divergent animals for growth and several parameters affecting the technological characteristics of meat processing. The analysis of gene expression is carried out with different techniques: DDRT-PCR, SSH, cDNA-AFLP,SAGE and microarray hybridisation. The differentially expressed genes will be chosen to search for mutations and polymorphisms. The regulatory regions will be characterised as well using in-vitro assays. Furthermore high-throughput methods of analysis will be defined to screen pig populations in association studies with meat quality and production traits.

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An IGF2 expression study in different muscle types of piglets at four different ages

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A paternally expressed QTL affecting muscle mass, fat deposition and heart size in pigs maps to the distal end of chromosome arm SSC2p, to the IGF2 (insulin-like growth factor 2) region. The QTL is caused by a nucleotide substitution in an evolutionary conserved CpG island in intron 3 of IGF2. The QTL explains 15-30% of the phenotypic variation in muscle mass and 10-20% of the variation in back-fat thickness. Previously it was reported that the mutation abrogates in vitro interaction with a repressor, which leads to a threefold increase of IGF2 expression in post-natal muscle (*gluteus*) in those pigs that inherited the mutation from their father.

The aim of our study is to investigate differences in IGF2 expression between three muscle types (red muscle (*M. Masseter*), white muscle (*M. Longissimus*) and heart) in the two paternal genotypes, qpat and Qpat.

Muscle samples were taken from piglets of 4, 8, 16 and 26 weeks of age and expression levels were measured using a real-time quantitation assay with specific probes and primers.

It was found that the expression of IGF2 in red muscle was significantly higher than expression in white muscle or heart muscle at all ages. In addition, expression levels of Qpat were higher compared to expression levels of qpat, in piglets of all four ages in all muscle-types. Whether or not these differences in expression levels can be correlated with meat quality parameters is currently under investigation.

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Transcriptomic analysis of destructured ham

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Meat quality is a complex trait which depends on various muscle biological characteristics affected by genetic, nutritional or environmental regulation factors. In pig, two major genes, i.e. RYR1 and RN, have already been identified to explain the Pale, Soft and Exsudative (PSE) and acid meat syndroms, respectively. However, there still remains an unacceptable level of variation of meat quality. Among them, the destructured meat syndrom has been identified but is still poorly understood. The affected muscles lose their fibrous aspect and give a soft whitened muscular mass without any organised structure.

In order to identify groups of genes potentially involved in the syndrome, a pig high-density nylon microarray consisting of 3443 PCR probes was used to compare gene transcription profiles between normal (n=8) and destructured (n=9) semimembranosus muscles. Normal and destructured groups of pigs were allotted post-mortem based on normal (43.9 +/- 2.2) or high (60.5 +/- 2.2) L* values of the semimembranosus muscle, respectively. Transcriptome analysis was performed for each animal and expression was normalized according to the sum of the intensities of the microarray. Fifty four clones were selected as differentially expressed (p=0.002) by ANOVA and 90% of them were upregulated in the affected muscles. Myofibrillar proteins involved in the regulation of the interaction between actin and myosin (TNNC2 and TNNT3) or in the integrity of the sarcomere (tropomodulin, ankyrin, myomesin) were increased in the affected muscle. Interestingly, the glycolytic pathway was particularly affected since 6 genes encoding enzymes of glycolysis were upregulated in the destructured muscles.

A study of a meat quality trait, tenderness, by the analysis of the transcriptome of a pig muscle *(Longissimus dorsi)*

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The aim of our project is to characterize gene expression variability in relation to meat quality traits through the transcriptome analysis of the skeletal striated muscle.

F2 pigs were obtained by crossing two different male lines (France Hybrides). The muscle samples from *Longissimus dorsi* were taken from about one thousand animals and characterized by shear force (Warner-Bratzler method, WBSF) as a tenderness evaluation.

We selected thirty samples displaying, after cooking, extreme WBSF values (high and low). RNA isolated from these samples were hybridized on Nylon microarrays spotted with 3456 PCR products obtained by amplification of pig sequences from AGENAE multi-tissues cDNA library (INRA, France) and from a muscle specific cDNA library (Department of Genetics and Biotechnology, DIAS, Denmark).

Using a linear regression model, we sorted out 151 cDNA clones showing an expression significantly correlated with shear force variation (FDR<0.05). Main functions represented are cytoskeleton and metabolism. We also found genes for signal transduction and transcriptional regulation. Using an original powerful statistical method, the random forests, we selected, among these 151 clones, the most important for shear force. The expression analysis of four significant genes by another approach, real-time RT-PCR, gave the same results as microarrays. These results demonstrate the possibility of using transcriptome analysis to identify variation in gene expression in relation to a meat quality trait. Further analysis of our microarrays data are needed to define a combination of biomarkers for meat tenderness.

Proteome analysis of the sarcoplasmic fraction of pig muscles differing by meat quality traits: colour and tenderness

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Semimembranosus (S) and *longissimus dorsi* (LD) muscle samples were taken immediately after slaughter (T0) on 1000 pigs originated from F2 crossing between Pietrain and a synthetic line (Large White x Duroc x Hampshire). L* value of resulting meat of *S* was measured 36 h *post mortem* and shear force of LD was measured after cooking. Two groups of 12 animals (chosen by pair within 12 half sibs families) were constituted according to extreme L* values (light and dark groups), and two other groups of 12 animals were constituted, according to extreme shear force values (tough and tender groups). Sarcoplasmic proteins were extracted from the T0 samples and separated by 2-D electrophoresis using a 5-8 pH gradient in the first dimension and 12% acrylamide in the second dimension. Gels were visualized and analyzed using the two dimensional electrophoresis (2DE) image analysis software PDQuest (Bio-Rad). Peptides of proteins of interest were identified using a MALDI-TOF mass spectrometer. Signification of differential expression of proteins is discussed with regard to each meat quality traits (colour and texture).

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Transcriptome of pig ovarian cells : discriminant genes involved in follicular development

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In order to identify genes and gene networks involved in pig ovarian follicular development, we built subtractive suppressive hybridization libraries (SSH) from granulosa cells of healthy follicles at different developmental stages (small, medium or large). The RNA isolated from these cells was used to hybridize cDNA nylon micro-arrays designed from these SSH libraries and from a pig multi-tissue cDNA library.

Data analysis using a gaussian linear mixed model showed that 83% of the variability is due to the genes.

Two hundred fifty one regulated genes out of the 656 expressed genes were significantly regulated as demonstrated by a Fischer test (pvalue <0.001). They were clustered into three groups according to the follicle size. Among these genes were genes already known to be regulated such as aromatase or IGFBP2 which supported the validity of our experimental model. A random forest analysis sorted out the genes by their importance in the discrimination of the three follicle classes and put forward thus the most discriminant genes. Sixty genes were selected on the basis of VarUsed importance criterion.

Using GO nomenclature, regulated genes can be assembled into pathways such as those involved in cell modelling (Stathmin…), regulation of transcription (PPAR γ…), apoptosis during follicle growth (Cyclophilin…).

The next step will be to describe more precisely the spatio-temporal expression patterns of the genes put forward by these experiments but unidentified.

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Kinetics of infection of pig PK15 cells with the Pseudorabies virus (PrV): a transcriptomic approach

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Knowledge of the genes which are induced during virus infection is important to identify molecules involved in host resistance mechanisms and to understand the physiopathology of infection. The Pseudorabies virus (PrV), which is a well characterized pathogen in pig, is a good model to follow virus infection. Our aim is to analyse modifications of cellular transcriptome due to viral infection as a marker of physiopathological modifications. In our experimental design, porcine renal epithelial PK15 cells were mock-infected or virus-infected. Cells were collected for RNA extraction just after infection and 1, 2, 3, 4, 8 and 12 h after infection for kinetics studies. To analyse the cellular transcriptome, two DNA chips were hybridized. The first array, which was produced in the laboratory is a cDNA chip targeting the SLA (Swine Leucocyte Antigen) region. The SLA complex contains genes encoding molecules involved in the innate and adaptative immune response, such as proteins presenting antigens to T cells. This array contains 1856 probes including 530 sequences of the SLA region, 80 additional genes of the immune response, 80 PrV genes and 1166 randomly chosen cDNAs. The second array (Qiagen-NRSP8 array) is an oligo chip which contains 13297 probes. Both arrays are used to identify genes differentially expressed between infected and mock-infected cells and genes which are differentially expressed during time in parallel with viral genes expression.

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Profiling porcine oviductal epithelial cell surface membrane proteins

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Several important reproductive events take place in the oviduct leading to the establishment of pregnancy. Oviductal epithelial cell (OEC) plasma membranes (PM) are the site of interaction between gametes/embryos and female reproductive tract. We aimed this investigation towards identifying OEC PM surface proteins.

The OEC were isolated from sow oviducts and cultured for 18hrs. Surface PM proteins of viable and intact OEC were subjected to biotinylation. The cells were homogenized, solubilised and biotinylated proteins were purified using monomeric avidin columns. The purified biotinylated proteins were subjected to 2D gel electrophoresis, stained and all individual protein spots were identified. In addition biotinylated proteins were subjected to a variation of multidimensional protein identification technology (MudPIT); proteins were separated by 1D gel electrophoresis, stained and gels were cut into bands. Proteins present in each band were identified. Using 2D gel electrophoresis we were able to identify 42 proteins. However, the MudPIT approach allowed us to identify approximately 290 proteins. From those proteins identified by MudPIT approach, over 200 were predicted to have one or more than one trans-membrane domains (TMpred prediction program). Only 60% of MudPIT identified proteins sub-cellular localization was classified in Gene Ontology database. From which 37 (22%) were known membrane proteins.

In conclusion, here we report a simple technique to enrich cell surface PM proteins. This technique, in combination with mass spectrometry and especially MudPIT can be used to identify surface PM proteins with potential involvement in cell-cell interactions and communication.

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70mer microarray probes applied in whole genome expression analysis of pig (*sus scrofa*)

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DNA microarrays are commonly used in expression profiling and genetic analysis experiments. Today still many DNA arrays are based on short oligonucleotides or cDNAs. These can provide either high specificity or high sensitivity respectively, but cannot combine these qualities. Operon has designed probes of different lengths to various positions in the Open Reading Frames (ORFs) and the results clearly show that 70mers offer the optimal combination of specificity and sensitivity.

Using the Ensembl database *http://www.ensembl.org* or other databases that display genome and transcript information allows the design of common, partial common and individual transcript 70mers. This design strategy is suitable for differentiating alternative splice variants and is maximizing the number of represented transcripts. Whenever possible, oligos were designed to be fully contained in one exon (exon oligos) avoiding the location of oligos across exon borders. Therefore exon oligos are also applicable for CGH (comparative genomic hybridizations) experiments. Operon has designed Array-Ready Oligo SetsTM (AROS) consisting of optimized 70mers for expression analysis for a number of genomes, including Pig and Cattle. The Pig AROS V1 based on data from TIGR Pig Gene Index 5.0 and contain 10.665 70mer oligos representing 10.665 sus scrofa gene sequences with a hit to known human, mouse or pig genes. The Pig genome extension set contain 2632 oligos representing pig sequences that contain at least one EST (expressed sequence tag).

A new upcoming Pig AROS V2 will contain about 20.000 oligos representing sequences from the current data set of the TIGR Pig Gene Index release 11.0. To help users deal with microarray experiment data, we have linked our relational Database OMAD (*http://www.operon.com/arrays/omad.php*) to the corresponding public databases. Therefore our users can always access the latest information for target genes and transcripts. Experiments and user data will be shown and demonstrate the specificity, sensitivity and reproducibility of results obtained by applying 70mers for expression profiling arrays.

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Boar sperm surface proteome related to sperm maturation and fertility

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Mammalian sperm differentiation and maturation occurs during the transit through the epididymis where spermatozoa aquire their motility and fertility. Epididymal maturation involved the progressive loss of most of the testicular sperm surface proteins and gain of new transient or permanent proteins on the surface membrane of mature and fertile spermatozoa. The aim of this work was to identify these sperm surface modifications in boar. Surface proteins of the mature and immature gametes were labelled with sulfo-NHS-SS-biotin. Differential extractions of surface proteins were realised on intact sperm and on isolated membrane obtained by nitrogen cavitation. Peripheral proteins (low affinity membrane-bounded proteins) were released with high ionic salt buffer and integral proteins were extracted with a non-ionic detergent.

The biotinylated proteins from these different extracts were affinity purified on streptavidin column and separated by one and two dimensional gel electrophoresis (IEF/SDS-PAGE). Identification of the different proteins was done by mass spectrometry (MALDI-TOF and nano LC MS/MS).

Between mature and immature spermatozoa, several different surface proteins have been electrophoretically characterized and most of them have been identified by MS. Comparative analyses between species were carried out. This systematic identification shows that important modifications occurred both on the surface membrane and in the inner membrane of spermatozoa during epididymal maturation. Several of these proteins modifications may be associated with sperm fertility and potentially can be used as markers either in animal selection or human fertility pathology.

Gene expression for androstenone using microarray

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Boar taint is an unpleasant odour and flavour that can occur in the meat of intact male pigs, and is primarily caused by high levels of androstenone and/or skatole. Castration of male pigs avoids the problem of taint. This will, however, be prohibited in Norway from 2009, which claims for a reduction of boar taint in the meat of uncastrated pigs. Genetic differences between animals expressing high/low levels of androstenone and skatole are interesting for breeding purposes, since the heritabilities show that selection against these components should be possible. However, cautions need to be shown to avoid unfavourable selection on performance and sexual maturation. Thus, before starting such a selection one have to promote a mechanistic understanding of the complex genetic system controlling boar taint and take into account possible side effects of other traits. As one of several approaches toward understanding the molecular mechanisms of boar taint, we used a pig specific cDNA microarray to study differential expression of genes between boars with extreme high/low levels of androstenone. Samples of testicle tissue from 120 boars of the Norwegian Landrace and Duroc breeds were hybridized to the arrays using a balanced block design. A linear model was fitted to the data and our analysis identified several candidate genes for the genetics underlying androstenone. Resequencing of interesting genes obtained from microarray studies revealed several SNPs that will be further studied to clarify their functionality. Transcript profiling using real competitive PCR allowed absolute quantification to confirm differential expression of the candidate genes.

Investigation of genetic and epigenetic mechanisms underlying stage and breed specific differences in the transcription of SPP1 gene during porcine myogenesis

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The number of muscle fibers and proportion of the different fiber types are essentially determined during prenatal muscle development and have a significant impact on muscle growth potential and meat quality. To identify functional candidate genes for pork quality comparative transcriptional profiling of 7 key stages of myogenesis between Pietrain and Duroc breeds was employed (EU-funded project PorDictor – QLK5-2000-01363). Transcription of the SPP1 gene was found to be associated with myogenesis, peaking at days 35 and 77 of gestation when formation of primary and secondary fibres takes place, and showed consistently higher transcription level at all stages in Duroc. Furthermore upregulation of the SPP1 gene transcription at three stages was associated ($p<0.05$) with lowered meat quality, however only for Duroc embryos/foetuses. To elucidate genetic factors underlying the differential expression of SPP1 gene between Pietrain and Duroc breeds comparative sequencing of the SPP1 gene 5′ flanking region was performed. Two variable sites were identified that may have an impact on the transcription. In silico analysis of the 5′ region of SPP1 gene revealed presence of a 300bp long CpG island. DNA methylation in the context of CpG dinucleotides is an established epigenetic mechanism involved in the regulation of myogenesis and represent a candidate factor underlying both stage as well as breed associated variation in the SPP1 transcription.

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Combining QTL- and expression-analysis: functional and positional evidence for candidacy of genes of myogenesis for meat quality and carcass traits

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Genome scans allow detecting QTL without prior hypothesis on the physiology of a trait. However, disentangling complex traits in their constituent phenotypes may facilitate the identification of QTL. Function-oriented holistic expression profiling is a complementary approach to derive hypothesis on the genetic background of phenotypic variation. Skeletal muscle consists of three different types of muscle fibres each characterised by certain biophysical, biochemical and metabolic properties. Their number, size, and proportion are to a large extent determined during the prenatal development and are endogenous factors on meat quality traits. We identified significant QTL for fibre type distribution traits as well as for meat quality and muscularity on SSC1, 2, 4, 14, and 15 in a porcine experimental F2 population of Duroc and Berlin Miniature Pig. In these regions effects on the complex traits related to meat quality and body composition might be the result of genetic variation primarily affecting fibre type distribution traits. In order to complement the QTL approach we compared transcription profiles of fetal M. longissimus dorsi tissue at seven key stages of myogenesis in Pietrain and Duroc breeds by RT-DD-PCR. Expression profiling revealed a list of functional candidate genes for meat quality and carcass traits with trait-, breed-, and stage-associated expression of various physiological networks. Application of bioinformatic tools, use and generation of mapping information of differentially expressed genes combined with QTL information revealed segregating functional positional candidate genes. For six genes we exemplarily proofed association to meat and carcass traits in commercial crossbred populations.

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Abnormal gene expression results in the immotile short tail sperm defect in Finnish Large White

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The immotile short tail sperm defect is an autosomal recessive disease within the Finnish Yorkshire pig population. This disease specifically affects the microtubuli structure of sperm flagella, whereas cilia in other tissues appear unaffected. Recently, the disease locus was mapped to a 3 cM region on porcine chromosome 16. To facilitate selection of candidate genes we constructed a porcine-human comparative map, which anchored the disease locus to a region on human chromosome 5p13.2 containing 8 annotated genes. Sequence analysis of a candidate gene revealed the presence of an inserted porcine endogenous retrovirus within an intron. The insertion affects splicing of the gene transcript in two ways; it either causes skipping of the exon preceding the insertion, or it leads to inclusion of intronic sequence as well as part of the insertion in the transcript. Both changes alter the reading frame, leading to premature termination of translation. Further work revealed that the aberrantly spliced exon is expressed predominantly in testicular tissue, which explains the tissue-specificity of the defect. Furthermore, expression studies of various tissues revealed several mRNA isoforms and a tissue specific splicing pattern of the gene. These findings may provide insight into common cilia and flagella malformations and abnormal development in mammals.

An association study of myopalladin and titin polymorphisms with meat quality and production traits in pigs

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Genes coding for skeletal muscle proteins play key roles in muscle mass accretion and several studies have reported their relationships with meat production and quality traits in pigs. Thus, DNA markers in genes coding for such proteins may be associated with meat production traits.

In the present work, we investigated two skeletal muscle genes, myopalladin *(MYPN)* and titin *(TTN)*, located in regions where quantitative trait loci for meat quality traits were mapped on chromosome 14 and chromosome 15, respectively.

For these two genes, we have already identified single nucleotide polymorphisms in the 3' untranslated regions. Using a selective genotyping approach in Italian Large White pigs, we have identified a possible association of *MYPN* on lean content and this result was then confirmed in a large crossbred population.

Here, to further evaluate the putative effects of the two genes on carcass and meat quality traits, we genotyped 272 sib tested Italian Large White pigs. In this sample, *MYPN* allele *T* and *TTN* allele *C* showed the highest frequency (0.80 and 0.86, respectively). Association between the *MYPN* and *TTN* genotypes with meat technological parameters and estimated breeding values for carcass and meat quality traits was evaluated by a general linear model procedure of the SAS package. *MYPN* and *TTN* resulted associated to average daily gain ($P < 0.10$) and pH_u ($P < 0.10$), respectively.

Further investigations are needed to better define the obtained results.

Analysis of mutations in the porcine PGAM2 and PRKAG3 genes and associations with muscle glycolityc potential and meat quality traits in Italian Large White pigs

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Conversion of muscle to meat is determined by several biochemical processes that take place after the slaughtering of the animals and that depends, in part, by the energy supply of the muscle. This supply is expressed by the glycolytic potential (GP) that defines the glucide level of the skeletal muscle that can be converted into lactate during the *post mortem* phase. Thus GP is related to the pH_u that in turn affects meat colour, drip loss, tenderness and processing yield. Applying a candidate gene approach in pigs to identify DNA markers associated to this parameter, we recently investigated several genes involved in the regulation of the energy balance, glycogen metabolism and glycolysis of the skeletal muscle. Here we selected two of these genes (muscle phosphoglycerate mutase 2, *PGAM2*; protein kinase, AMP-activated, gamma 3 non-catalytic subunit, *PRKAG3*) to be used in an association study with meat quality parameters. Glycogen and lactate content, GP, pH_1 and pH_u of muscle *semimembranosus* were determined for 220 sib tested Italian Large White pigs. PCR-RFLP protocols were used to genotype the single nucleotide polymorphisms of the *PGAM2* and *PRKAG3* (N30T and G52S mutations) genes as already described. Association between genotypes of the analysed genes and meat technological parameters were evaluated by means of the GLM procedure of SAS. *PGAM2*, that is a gene that codes for an enzyme involved in the last steps of the glycolysis, resulted associated ($P<0.10$) with lactate content of the muscle.

50 Linkage mapping of the porcine Mucin 4 gene on SSC13 and association with the *Escherichia coli* F4ac receptor gene

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Linkage analysis was performed in order to assign the Mucin 4 *(MUC4)* and the *Escherichia (E.) coli* F4ac receptor *(F4acR)* genes to a map of nine SSC13 microsatellites. The analysis included 202 Landrace/ Large White pigs from our experimental herd (EH) and 78 pigs representing the Swiss porcine population (SPP). The most likely order of the loci was *SW207 – MUC4 – S0283 – S0075 – [SW2007 – SW1876] – SW225 – [SW1030 – SW698] – SW398*, and *F4acR* could be assigned between *SW207* and *SW1876*. A microscopic adhesion test was performed for *E. coli* F4ac in 196 EH and in 78 SPP pigs in order to test the association with *MUC4*. Comparison between F4ac adhesion and the *MUC4-Xba*I polymorphism in intron 7 revealed that 92% of the F4ac susceptible SPP pigs had the expected *C/C* or *C/G* genotypes. For five pigs, the *C/G* genotype did not correspond to susceptibility and for one pig the *G/G* genotype did not correspond to F4ac resistance. Therefore, we conclude that *MUC4* is a good marker, but not the gene controlling the *F4ac* receptor, and that the *MUC4* marker can be used to estimate F4ac susceptibility in Swiss pigs. The *MUC4 Xba*I polymorphism was determined in 193 boars from four breeds currently used in artificial insemination in Switzerland. The *C/G* and *C/C* genotypes were found in 75% of Large White, 73% of Landrace, 13% of Duroc and in 4% of Piétrain x Duroc. These results confirm the differences between the breeds in susceptibility to *E. coli* F4ac.

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51 Characterization of the polymorphism in swine *egf, rbp4,cox2 and igf2* genes and their association with reproduction traits

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Efficiency of swine production is highly influenced by reproductive success, especially litter size. One of the approaches to gather genomes information that might be used in genetic improvement of litter size is the candidate gene approach. The current investigations cover several genes, the product of which is associated with different aspects of reproduction. The effect on litter size was observed between other for Epidermal Growth Factor *(EGF)*, Retinol Binding Protein4 *(RBP4)*, Cyclooxygenaze 2 *(COX2)* genes and microsatillite sequence in *IGF2* gene. It was examined the polymorphism in these genes for 500 sows from Polish Synthetic line 990. The polymorphism of the genes was determined by the PCR/RFLP method. The relationship between candidate gene genotypes and reproductive traits will be evaluated with the Least Squares Method. The fixed effects of the season of the farrowing, year of the farrowing, number of parities and boars will include into linear model. Additional regression coefficients will be obtained for additive and dominance effects of RYR1 genotypes (HAL^A and HAL^D). The following traits were included in the study: total number born (TNB), number born alive (NBA) number of piglets at 21 days of age (NP21), number of weaned piglets (NWP), litter weight at 21 days of age (LW21), litter weight at weaning. (LWW), farrowing interval (FI). The *EGF* locus genotype was found to have a significant effect on the TNB, NBA, NP21, NWP and FI. The A/A animals showed the highest reproductive efficiency compared to those of B/B and A/B genotypes. The effect of *RBP4* genotype was significant for the traits LWW and FI. The 2/2 homozygous have also higher litter weight at weaning and shortest farrowing interval than 1/1 and 1/2 sows No significant effects of the *COX2* sows genotype were found in part due to absence of one of the homozygous genotypes at this locus observed in the tested material. The *IGF-2* locus (microsatellite sequence) was found to have a 10 different alleles. Some of the genotypes in this locus were connected with reproduction traits in the tested material.

ESR gene polymorphism and reproductive traits in polish sows

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Estrogens are known to regulate the numerous vital functions, such as reproduction, cell growth and differentiation, mammary gland development and lactogenesis, homeostasis and oncogenesis. Estrogen receptor (ESR) can regulate the synthesis of estrogen and affect the reproduction traits and is indicated as a major, or strictly linked to major gene responsible for litter size of pigs. The aim of this study was to find the frequency of the mutation of *ESR* gene identified with *PvuII* enzyme as well as a possible relation between *ESR* genotypes and reproductive characters (total number of piglets born, number of piglets born alive, number of piglets weaned, the age of sows on the day of farrowing in the 1st, 2nd, 3rd and later parities) in the herd of sows of the Polish Landrace breed. The study was carried on 296 Polish Landrace sows. DNA from pigs blood collected into K_3EDTA was prepared with Master Pure kit, according to producer's instructions. Genotypes analyses were preformed using the PCR-RFLP method. The fragment of DNA of 120 bp was amplified through starter sequences described by Short et al. (1997). Three ESR/*PvuII* genotypes were identified in the studied pedigree herd of sows: *AA, AB* and *BB* genotypes. The frequency of *AA* genotype was 0.17, that of *AB* genotype – 0.23 and that of *BB* was 0.60. The present study shows that the *ESR/PvuII* polymorphism is strongly associated with litter size in parities sows. The results obtained should be verified on greater number of animals.

Characterization of the porcine *acyl-CoA synthetase long-chain 4 (ACSL4)* gene and its association with growth and meat quality traits

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Long-chain acyl-CoA synthetase (ACSL) catalyzes the formation of long-chain acyl-CoA from fatty acid, ATP and CoA, activating fatty acids for subsequent reactions. ACSL thus plays an essential role in both the lipid biosynthesis and the fatty acid degradation. The *ACSL4* gene was evaluated as a positional candidate gene for the QTL located between SW2456 and SW1943 markers on chromosome X. We have sequenced 4906 bp of the pig *ACSL4* mRNA in twelve pigs from the Iberian, Landrace, Large White, Pietrain, Meishan and Duroc breeds. Sequence analysis allowed us to identify ten polymorphisms located in the 3'-UTR region and linked in only two ACSL4 haplotypes. Furthemore, a QTL and an association study between polymorphisms of the *ACSL4* gene and traits of interest was carried out in an Iberian x Landrace cross. We report QTL that have not been identified previously and an association of the *ACSL4* polymorphisms with growth and percentage of oleic fatty acid. Finally, we have determined the allelic frequencies of the G/A polymorphism located at position 2645 (GenBank accession number DQ14454) in 140 pigs belonging to the Iberian, Landrace, Large White, Meishan, Pietrain, Duroc, Vietnamese, Peccary and Babirusa.

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Association of Leptin Receptor (LEPR) gene variants with growth traits in pigs

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The Leptin Receptor gene is a biological and positional candidate for growth and fatness traits. A recent study has shown a significant association of *LEPR* haplotypes with different backfat measures in an Iberian x Landrace intercross. The evidences suggest that these effects could probably be due to a missense mutation located on exon 14. The physiological role of the receptor is related with the transmission of the leptin signal in the hypothalamic regulation of energy homeostasis and voluntary feed intake. Thus, a functional mutation on *LEPR* gene with an effect on fatness would be expected to have also an influence on body weight. The objective of this work was to test for association of *LEPR* exon 14 SNP with growth traits in the IBxLR resource population (377 F_2, 86 F_3 and 136 backcross individuals). Information of the *LEPR* exon 14 SNP (Leu663Phe), one SNP on MC1R gene and 13 microsatellites located on SSC6 was used for QTL detection and marker assisted association test for two live weight measures: Live weight at 150 days of age (LW1) and live weight at the end of the trial (LW2). The QTL detection results show the existence of one cryptic QTL for LW2 at position 133cM, coinciding with the previously identified QTL and the *LEPR* gene position. The marker assisted association test shows significant association results for both traits (a= 2.44±0.77, p<0.009 for LW1; a=3.17±0.72, p<0.002 for LW2). Results obtained support the hypothesis of the functional implication of *LEPR* exon 14 variants on productive traits.

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Performance traits in relation to GH gene polymorphism in large white sows

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Traits associated with reproduction are taking under consideration by selection of sows. Growth hormone (GH) plays an important role in many physiological pathways, also in reproductive processes. Besides growth and development GH is also responsible for sexual differentiation, pubertal maturation, gonadal steroidogenesis, gametogenesis and ovulation. Gene which codes GH in pigs is assigned to chromosome 12. and consist of five exons. Mutations in this gene may be associated with some phenotypic traits. The aim of this study was to determine relationship between different genetic variants of *GH* gene and following traits: total number of piglets born, number of days to weaning, number of piglets weaned, number and percent of falls. Investigations were performed on 300 Large White sows. To detect polymorphism in *GH* gene PCR-RFLP method was used. Variations in 2 *loci were* analyzed: *GH/HaeII* in exon 2 and *GH/MspI* in intron 2. The following allele frequencies were found: $GH/HaeII^+$ - 0.31, $GH/HaeII^-$ - 0.69 and $GH/MspI^+$ - 0.58, $GH/MspI^-$ - 0.42. Statistical analysis showed only significant associations ($P \leq 0.05$) between number of falls, percent of falls and individual genotypes of swine GH gene. In investigated herd affirmed significant differences between observed and theoretically counted values for *GH/HaeII* and *GH/MspI* genotypes.

Effects of the pig melatonin receptor 1a gene *(MTNR1A)* on litter size in an Iberian x Meishan F_2 population

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The melatonin receptor 1a gene (MTNR1a) has been of particular interest because of the previously described association between different genotypes of this gene and fertility in autumn lambing sheep flock. The *MTNR1a* gene is located at chromosome Ssc17 where a QTL for prolificacy has been detected in previous studies. We have sequenced a fragment of 395 bp of the pig *MTNR1A* gene in 10 individuals of diverse pig breeds. The comparative sequence analysis revealed a silent T → C polymorphism at exon 2. An association analysis between this polymorphism and sow reproductive traits (total number born and number born alive) in an Iberian x Meishan cross pigs has been performed. The dataset consist of 882 data from 289 F_2 sows. The statistical analysis was carried out using and animal model with repeated measures. The model considers order or parity as systematic effects, and random polygenic and permanent effects. First, we included in the model the T → C mutation without considering the QTL effect, and we found a significant dominance effect for total number born (p = 0.01) and number born alive (p = 0.05). When we included the Ssc17 QTL effect, the significance of the dominance effects was even higher for both total number born, (p = 0.0008) and number born alive (p = 0.007), whereas the QTL still retained a significant additive effect.

PRLR – Candidate Gene For Litter Size In Polish Pigs

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The progress in research on the porcine genome enables identification of polymorphic loci of individual genes that control reproduction characters and thus may influence reproductive performance. Examples of genes affecting reproduction is the prolactin receptor *(PRLR)* gene localized in the swine chromosome 16 and recognized as a "candidate" gene of reproductive traits. The aim of the experiment was to use the DNA mutations in the *PRLR* gene to determine associations between the genotypes and litter size in Polish Large White x Landrace crossbred sows. Reproductive traits investigated were: total number of piglets born (TNB), number of piglets born alive (NBA) and number of piglets weaned (NW). The polymorphism in *PRLR* gene was detected using the PCR-RFLP method, with specific primers and the *AluI* restriction enzyme. Two different alleles of *PRLR* gene were identified: allele *A* and *B*, whose frequencies were 62% and 38%, respectively. The relationship between the *PRLR* genotypes and TNB, NBA and NW were analyzed. The analysis showed in first parity sows statistically significant ($P \leq 0.01$) differences between sows with the *AA* genotype compared to sows with *AB* and *BB* genotypes. The present study showed that the *PRLR* gene is strongly associated with litter size in first parity sows. In later parities the *AA* genotype still had the largest litters, but the difference was statistically not significant. However, this should be verified by a larger number of animals.

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Relations between the polymorphisms in the coding and 5'-flanking regions of the porcine *MYOD1* and *MYF5* genes and productive traits in pigs

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Myogenic factors 3 (myf3) and 5 (myf5) are the products of *MYOD1 (MYF3)* and *MYF5* genes, respectively, belonging to the *MyoD* family. The *MyoD* gene family consists of four structurally related genes: *MYOD1, MYF5, MYOG (myogenin)* and *MYF6*. These genes encode transcription factors with bHLH domain, which control the processes of myogenesis and induce the expression of muscle-specific genes. We identified seven new mutations in the coding and 5' flanking regions. To evaluate the relationship between new mutations and productive traits in pigs were tested the animals of several breeds and line: Polish Large White, Polish Landrace and synthetic line L990. The analysis RealTime PCR showed that transversion *A65C* in the *MYF5* gene does not affect the level of its transcription in the *m. longissimus dorsi*. Homozygotes of the "wild" allele as regards all three mutations of the *MYOD1* taken into consideration in the present study apperead to be more profitable for traits characterizing carcass meatiness. In turn, heterozygotes for mutations identified in the *MYF5* gene proved to be most favourable as regards carcass meatiness. The effect of *MyoD* genotypes on performance traits was observed to be similar irrespective of the animals breed. This may suggest that new mutations, identified in the coding and 5' flanking regions of the both genes, could be considered as causative mutations and their usefulness for selection is better than those discovered earlier in the non-coding regions of these genes.

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NRSP-8 Pig Genome Informatics: Databases and Resources

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Bioinformatics tools and databases have been integral part of the U.S. Pig Genome and Bioinformatics Coordinators' Programs. Over the past several years, we have developed and maintained a number of public database resources and web functions to facilitate the community effort in pig genome research. The databases include the Pig EST Database, the Pig QTL Database (PigQTLdb), databases for NRSP8 shared materials and reagents such as primers, probes and microarray, the mapping references database updated by L. Crittenden, genetic analysis software database, and a mirror site for TheArkDB (pig).

The tools include a blast server for pig sequences, and a computer program, Expeditor, to design gene-specific primers using consensus sequence information. In addition, we have also created and maintained the AnGenMaP mailing list, with discussion archives and content search capabilities. We have recently made significant progress in the integration of existing databases, such as linking QTL information to the NCBI Gene database, to pig clone finger printed contig (FPC) maps, to radiation hybrid (RH) maps, and comparative maps to human genome, etc. The pig genome web site has served as a central information hub for pig genome research, such as research reports, updates, name lists, upcoming meetings, links to related online databases and web resources, and a collection of educational materials for students and general public on pig gene mapping and genome research. Opportunities for further development and growth are presented.

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PGP: the PTP Genomics Platform facility serving the Ag-Biotech community

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The Parco Tecnologico Padano, Lodi – Italy, has recently established a new facility (PTP Genomics Platform – PGP) for high-throughput genotyping and gene expression analyses of plant and animal samples. The Platform is integrated with a LIMS (Laboratory Informatics Management System) to track and store information on DNA/RNA input samples, reagents and output genotyping and gene expression results enabling the full traceability of the workflows, tasks and results. The key features of PGP are: 1) Flexibility both in services and technologies, 2) GLP compliance – ISO 9001, 3) Quality control based upon the use of LIMS and bar code systems, 4) Customized genotyping experimental design an assays covering SNPs, SSR, AFLP and TILLING technologies. PGP is collaborating in the high-throughput implementation of several projects focused on Molecular breeding, Animal Resistance to Pathogens, Pathogen Monitoring, Food Safety, Biodiversity and Traceability.

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A web-database platform for data mining and statistical analysis in a swine epidemiological study

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The main objective of the project is to create a client-server database for routine collection and analysis of production and epidemiological records of a commercial swine population. The commercial population consists of the animals present on 11 herds in Lombardy, Italy. The data recorded include all of the major events in the lives of the animals, including dates of birth, weaning, transfer from one management group to another, breeding, parturition, and exit from the farm (with reason for removal). In addition, important production traits such as measures of growth and estimation of body conformation are recorded. Finally, another set of parameters are recorded to periodically provide a picture of the health status of each hog: randomly collected blood samples are analyzed and about 15 environmental conditions recorded. Evidence of any clinical problem is recorded each time symptoms are observed by either the farmer or when a formal diagnosis is made by a veterinarian, during routine visits. When a clinical evidence occurs, all data from laboratory-based diagnoses of problems is recorded. All of the participating breeders keep routine pedigree information on all animals and practice artificial insemination with sires from the same sources. This latter fact guarantees genetic connectness among the participating herds. All of the information collected is subsequently stored in a relational database. The database has a client-server architecture, and is written in MySQL DBMS. The user interface is web-based and written in the PHP language. This interface ameliorates data entry and performs simple checks to help ensure accuracy, as well as facilitating visualization and query of the database contents. The website includes pages of documentation and controls access to the content and is hosted on an Apache server running Linux. Export of the data for analysis is automated and produces files in SAS format. Results are updated each time that new data is added, producing a set of standard charts and tables. In addition, automated analyses include simple ANOVA and logistic regression. The entire process guarantees a continuous flow of information to and from the farmer. The database includes not only the phenotypic information derived from the farm and pedigree data from the breed association and but also genomic information from our laboratory through a connection to the LIMS of the PTP. The information from these different sources is combined and analyzed together through a bioinformatic pipeline with options to compare sequences to public databases chosen by the user.

Experience with dissemination of research results and dialogue with industry

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For the future of the animal breeding industry, a good knowledge base in Europe is important. It is important for their competitiveness, and for the decisions of companies to maintain their (head) office in Europe. Next to good science, the application of science into breeding programmes is a key item.

In EADGENE, a network of excellence on animal disease genomics, the dialogue with industry and implementation of research is addressed throughout the project in several ways. Operational genomics is part of the joint research programme. Furthermore, a Club of Interested industries active in the field is formed, and working parties on knowledge management and technology transfer. In the FABRE Technology Platform both industry and research are developing a vision on animal breeding and reproduction, and will be working out a Strategic Research Agenda in 2006 with the involvement of a wide range of stakeholders.

The presentation will address the key factors of knowledge, experience and learning from knowledge, technology transfer and the challenge to use the strong points of each in industry-academia cooperation, with examples from the EADGENE project and opportunities for the next 15-20 years time set out by the FABRE Technology Platform.

Operational Genomics: bridging the gap between research and industry

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As animal disease genomics research progresses, it is becoming apparent that there is a 'gap' between research outputs and practical solutions for industry. Operational genomics aims to bridge the gap in animal disease research to enable the industry to produce safer animal products and improve animal welfare. As part of the EADGENE network we aim to implement operational genomics in disease studies by firstly evaluating and then integrating outputs from structural, functional and population genomics research and, secondly, determining the strategies and tools best able to create usable solutions for industry. This could involve disease monitoring and surveillance, intervention strategies, for example through vaccines, and breeding for increased disease resistance. An initial step in this process is to identify target diseases that are amenable to an operational genomics approach, i.e. diseases which pose the greatest risks to animal product safety and animal welfare and where, at the same time, genomic research is sufficiently advanced to allow novel and cost effective strategies using genomics to be developed. It is hoped that by using these methods we will create tangible outputs for use in production animals to improve food safety and animal welfare in Europe

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