

KEYNOTE LECTURE 1:

STOCHASTICITY AND NETWORKS IN GENOMIC DATA

Professor John Quackenbush (Dana-Farber Cancer Institute and the Harvard School of Public Health, USA)

Two trends are driving innovation and discovery in biological sciences: technologies that allow holistic surveys of genes, proteins, and metabolites and a realization that biological processes are driven by complex networks of interacting biological molecules. However, there is a gap between the gene lists emerging from genome sequencing projects and the network diagrams that are essential if we are to understand the link between genotype and phenotype. 'Omic technologies were once heralded as providing a window into those networks, but so far their success has been limited. To circumvent these limitations, we developed a method that combines 'omic data with other sources of information. Here we will present an approach that uses literature networks as constraints on a Bayesian Network analysis of microarray data, we show that we are able to recover evidence for a wide range of known networks and pathways, even in experiments not explicitly designed to probe them.

With a putative gene-interaction network, the problem of producing viable models of the cell remains. While systems biology approaches that attempt to develop quantitative, predictive models of cellular processes have received great attention, it is surprising to note that the starting point for all cellular gene expression, the transcription of RNA, has not been described and measured in a population of living cells. To address this problem, we propose a simple model for transcript levels based on Poisson statistics and provide supporting experimental evidence for genes known to be expressed at high, moderate, and low levels. Not only do these data confirm our model, but this general strategy opens up a potential new approach, esoscopic Biology, that can be used to assess the natural variability of processes occurring at the cellular level in biological systems.

KEYNOTE LECTURE 2:

RECENT ADVANCES IN DECIPHERING THE MOLECULAR BASIS OF POLAR OVERDOMINANCE

Prof Michel Georges (University of Liege, Belgium)

KEYNOTE LECTURE 3:

COMPLEX GENETIC CONTROL OF SUSCEPTIBILITY TO MALARIA: LESSONS FROM THE MOUSE

Professor Philippe Gros (McGill University, Canada)

Susceptibility to infections is affected by both genetic background of the host and by environmental factors, including pathogen-associated virulence determinants. Infectious pathogens have been suggested to be the single most important selective pressure on the human genome. When studied in inbred mouse lines, the genetic component of inter-strain differences in susceptibility may be inherited as a monogenic trait amenable to positional cloning, or may behave as a complex trait with multiple genes each contributing to only a fraction of the variability. Over the past 15 years, our laboratory has studied the complex genetic control of susceptibility to malaria in mouse models of the erythroid (*Plasmodium chabaudi*) and cerebral phases of the disease (*Plasmodium berghei*). These studies have identified major gene effects that independently affect replication inside the erythrocyte (*pklr*), early immune response (*Vnn3*), and control of late stage replication (*lcsbp*). In one case, passive administration of the product (cysteamine) of the biosynthetic pathway (pantetheinase) impaired in susceptible animals was shown to restore resistance, showing promise of this molecule as a novel "host-based" anti-malarial drug. Finally, we have identified a gene in which mutations cause susceptibility to blood-stage infection but yet protect against the cerebral stage of the disease, highlighting how the same host response may be beneficial or detrimental to pathological manifestations of the same infection.

OPEN SESSION

O-1/ISAFG-P97

PROTEOME ANALYSIS OF BOVINE MYOGENESIS GIVES NEW LEADS TO OPTIMIZE MEAT QUALITY AND MUSCLE GROWTH EFFICIENCY

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Meat quality goes through a good understanding of skeletal muscle development and a good knowledge about the abundance of potential marker of meat quality through myogenic process. Histological and biochemical experiments allowed the identification of representative stages in bovine myogenesis. In order to improve our knowledge for cattle development, a proteomic analysis, based on the *in vivo* analysis of the *Semitendinosus* muscle from Charolais fetuses, at five crucial stages was conducted using two-dimensional gel electrophoresis and mass spectrometry. Stages 60, 110, 180, 210 and 260 dpc were kept for the analysis of muscular proteome dynamics. The proteomic approach revealed 496 spots common to these five times. Among these spots, Principal Component Analysis and Hierarchical Clustering analysis of proteome dynamics during the nine months of gestation revealed 245 proteins which abundance changed. Mainly, three categories of proteins were displayed: protein from contractile apparatus (many isoforms of MyHC and troponine T), proteins belonging to cytoskeletal apparatus (desmin, actin, HSP27) and essential proteins for cell cycle regulation and / or involved in the control of the balance proliferation / apoptosis of cells. This last group of about 25 proteins including PAK2, PARK7, SNX6, Desmin, Galectin 1 and others, could lead to the identification of potential marker of the total number of muscle fibres (TNF) acquired at 180dpc for *Bos taurus*. This TNF is of particular interest in meat production since it is one of the most important parameters that control muscle mass.

O-2/ISAFG-P8

COMPARATIVE BIOINFORMATIC ANALYSIS OF THE TRANSCRIPTIONAL RESPONSE TO *SALMONELLA ENTERICA* SEROVAR CHOLERAESUIS SUGGESTS NOVEL TARGETS OF NFκB ARE ACTIVATED IN THE PORCINE MESENTERIC LYMPH NODE

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We are investigating the porcine response to *Salmonella* infection through expression profiling and integration of structural/functional genomic data across species. We identified differentially expressed genes in pig mesenteric lymph nodes (MLN) responding to infection with *Salmonella enterica* serovar Choleraesuis (*S. Choleraesuis*) at 8 hours (h), 24h, 48h and 21 days post-inoculation (pi) using Affymetrix technology. Analysis of variance showed that 1,853 genes exhibited significant changes in expression (p -value <0.01 , q <0.26 and fold change >2). Gene expression and annotation clustering revealed down-regulation of translation-related genes at 8 and 24 hpi, and up-regulation of genes involved in the Th1, innate immune/inflammation response and apoptosis pathways at 8-48 hpi. Antigen presentation/dendritic cell (DC) function pathways were not affected significantly during early infection, indicating systemic infection may be caused by poor DC antigen presentation in the MLN. Quantitative-PCR of 22 genes confirmed the strong NFκB-dependent response detected by the microarray results, where 59 known NFκB target genes were induced at 8, 24 and/or 48 hpi. We identified transcripts that have both a similar expression pattern as these known NFκB targets and a computationally-identified NFκB-binding motif within the orthologous human/mouse gene promoter, and hypothesize that these are putative novel NFκB target genes. To test this hypothesis, we performed gel shift analyses using a mouse macrophage nuclear extract and showed that NFκB bound to candidate NFκB motifs at five orthologous mouse promoters. Our study provides novel transcriptional data on the porcine response to *S. Choleraesuis*, and expands the understanding of NFκB-dependent signaling in response to *Salmonella*.

O-3/ISAFG-P32

TISSUE-SPECIFIC EXPRESSION ANALYSIS OF ALTERNATIVE SPLICING IN THE PIG

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Introduction

Since at least half of the genes in mammalian genomes are subjected to alternative splicing, alternative pre-mRNA splicing plays an important contribution to the complexity of the mammalian proteomes. Expressed sequence tags (ESTs) provide evidence of a great number of possible alternative isoforms. With the EST resource for the domestic pig now containing more than one million porcine ESTs, it is possible to identify alternative splice forms of the individual transcripts in this species from the EST data with some confidence.

Methods

The pig EST data generated by the Sino-Danish Pig Genome project has been assembled with publicly available ESTs and made available in the PigEST database. Using the Distiller package more than 2,500 EST clusters with candidate alternative isoforms were identified in the EST data with high confidence. This corresponds to approximately 9% of the porcine genes in the resource having two or more isoforms. Using information about tissue and developmental stage of the libraries from which the ESTs were derived, a list of 100 genes with presumed tissue-specific alternative splice events was generated. To confirm this, 10 genes were arbitrarily selected from which 16 individual splice events were chosen for experimental verification by quantitative PCR (qPCR).

Results

Strikingly good agreement was found between *in silico* predicted and experimentally verified expression of tissue-specific alternative transcripts. Six of the experimentally verified genes were shown to have tissue specific alternatively spliced transcripts with expression patterns matching the EST data. The remaining four genes had tissue-restricted expression of alternative spliced transcripts. Five out of the 16 splice events were found to be pig specific. This study supports the notion that alternative splicing provides an important impact on species differentiation.

O-4/ISAFG-P1

INFERENCE ON HIDDEN POPULATION SUBSTRUCTURE AND GENETIC BACKGROUND OF CATTLE FROM DENSE SNP GENOTYPE DATA

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Introduction

Hidden genetic substructures, if unrecognised, will confound association studies. In many cases, information on herd of origin and pedigree may be unavailable for a group of animals. Indeed, it is not uncommon in commercial animals for there to be a degree of crossbreeding, even among mostly purebred herds. To be able to use these animals it is necessary to quantify the breed origins of an animal.

Methods

Using animals of known ancestry and breeding, we segregated animals into their property, herd of origin, and parentage groups. Genetic distances between pairs of 189 animals from seven different breeds were determined by estimating the proportion of alleles that are identical by state across ~10,000 SNPs. Unsupervised clustering was performed on those data to assign animals into groups. Additionally, for each SNP, we predicted the amount of genetic diversity and extent of selection pressure by estimating the amount of departure of observed heterozygosity from that expected under Hardy-Weinberg Equilibrium.

Results

This approach successfully re-classified all animals into their respective breeds. More interestingly, these seven sub-clusters were pre-divided into two larger clusters, corresponding to their tropical and temperate breed-types. We extended this analysis to 1,440 Brahman and composite animals, which allowed us to identify substructure among the composite animals.

Discussion

An obvious benefit of this method is in association studies where unrecognised population substructures are a known confounding factor – animals can be grouped using their genetic relationships revealed by SNP panels. A further use of this study is in its potential to identify alleles at various genomic regions that can be used to differentiate between breeds or to quantify the genetic background of any given cross-breed.

O-5/ISAFG-P73

ACCURACY OF GENOMIC BREEDING VALUE PREDICTION USING DIFFERENT MODELS

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Genomic selection is based on breeding values predicted from a large number of marker haplotype effects across the genome. Different models have been suggested, and the aim of this study was to test the effect of different haplotype definitions on the accuracy of predicted breeding values. Four models were used, where haplotypes were: 1) represented by single marker alleles (SNP1), 2) constructed from two adjacent marker alleles (SNP2), 3) constructed from two markers using the identical-by-descent (IBD) probability as a covariance between haplotypes (HAP_IBD2) and 4) as in HAP_IBD2, but now taking 10 markers into account (HAP_IBD10). IBD probabilities were calculated from combined linkage and linkage disequilibrium information. Simulations included heritabilities of 0.5 and 0.1 and five marker densities varying from 119 to 2343 SNPs on a 3M genome. Haplotype effects were estimated using a Gibbs sampler derived from BayesB, that avoids the Metropolis Hastings step. For the trait with a heritability of 0.1, the differences between models were small across marker densities. For the trait with a heritability of 0.5, the HAP_IBD10 model yielded the highest accuracies of predicted total breeding values for non-phenotyped and phenotyped animals across marker densities. HAP_IBD models had a benefit when the associations (LD) between single markers and a QTL were low, whereas SNP1 had a benefit when LD between a single marker and a QTL was high. Genomic selection was considerably more accurate than traditional selection, especially for juvenile animals and low heritability, and this effect was gained even with relatively simple models.

O-6/ISAFG-P77

GENOME-WIDE EXPRESSION CONSEQUENCES OF A DISEASE RESISTANCE QTL ARE STRONGLY INFLUENCED BY THE GENETIC BACKGROUND.

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The underlying assumption in QTL mapping and use is that genetic polymorphisms largely carry their phenotypes with them. We have combined a public murine 8 million SNP data set with Affymetrix-derived expression data under twelve conditions from inbred mice and sets of congenic mice derived from them. This has provided estimates of the proportions of genes that show *cis* and *trans* regulation. *cis* regulated genes appeared atypical in that they showed evidence of exceptionally consistent and tissue-independent expression. Most genes appeared to be regulated by *trans* acting mechanisms, but their expression levels were exquisitely sensitive to the genetic environment. Thus the introduction of a small region of the genome from one inbred line onto a background of another line has more widespread and less predictable effects on gene expression profiles, both within and outside the congenic region, than expected. Consequently, the differential utilization of pathways in inbred mice is unlikely to reflect the pathways that are differentially expressed in the mapping populations that are used to discover QTL, or the populations into which QTL are introgressed.

O-7/ISAFG-P31

GENE EXPRESSION AND IMMUNE STUDIES IN *BOS TAURUS* AND *BOS INDICUS* CATTLE INFESTED WITH *RHIPICEPHALUS MICROPLUS*

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Rhipicephalus microplus is a serious external parasite of cattle. Ticks and the diseases that they can transmit are of world-wide economic significance with costs of approximately \$US2.5 billion per annum. It is well known that *Bos indicus* cattle develop stronger tick resistance than *Bos taurus* cattle but the basis of this tick resistance is poorly understood. Six *B. taurus* and six *B. indicus* were infested with *R. microplus* larvae for five weeks and tick counts undertaken demonstrated the ability of *Bos indicus* cattle to resist tick infestation. Blood samples were collected after infestation for microarray analysis and immunological testing. Using Affymetrix bovine microarrays, significant differences in the expression of 230 genes were noted. Genes significantly upregulated in the tick resistant *B. indicus* included FOXP1, granzyme B and interleukin-2 receptor alpha (IL-2RA). Quantitative real-time PCR was used to verify differential expression of several genes and validate the results obtained via microarray. Immune assays revealed that peripheral blood of *B. indicus* contained higher percentages of CD4 and CD25 (IL-2R) cells and quantitative real-time PCR revealed higher expression of IL-2, IL-2RA, TNF-alpha and CCR-1. Furthermore, sera of susceptible *B. taurus* cattle had higher levels of tick-specific antibodies measured by ELISA and recognized more tick antigens in immunoblotting. The results suggest that the pathways of tick resistance in resistant *B. indicus* cattle involve immunological processes including activated T cells to enable the ensuing humoral response.

POSTER SESSION

ISAFG-P2

TRANSCRIPTS QUANTIFICATION IN WHITE MUSCLE OF *ONCORHYNCHUS KISUTCH* (COHO SALMON): A METHOD FOR MONITORING GROWTH

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Growth in salmonids represents a character of great importance in the commercial culture of these fishes. Currently, there are not any methods or technique to select salmonids with high growth rate, generated by the muscular increase and not by fat accumulation. In this context, in the present work it was evaluated the relation between levels of gene expression and muscle growth rate in *Oncorhynchus kisutch*. The selected gene corresponds to gene associated to growth, myosin. By application of real-time PCR technique, was quantified the transcripts presents in the white muscle of fishes under several planes of nutrition by 35 days in vitro. Furthermore, it verified relationship in vivo established between the transcripts levels of the same gene and individuals of different sizes of *Oncorhynchus kisutch*. The results obtained indicated that for organisms in conditions *in vitro*, with highly feeding and conversion rate, it was observed an increase of the transcripts number. In conditions in vivo, individuals obtained from cultives, it was observed that the specimens of big size presented a number elevated of transcripts compared whit the smalls. Of this form, the results in vitro and in vivo obtained in this work validate the relation between the levels of expression of the myosin gene and the growth rate in *Oncorhynchus kisutch*. Finally, this study suggests an interesting possibility to use genomic functional like biotechnological tool in the field of the salmoniculture.

Acknowledged

Functional Genomics in Salmon, 05CT6PPT-10

ISAFG-P3

THE EXPRESSION AND ANTIMICROBIAL ACTIVITY OF AVIAN BETA-DEFENSIN 10 IN THREE DISTINCT LINES OF CHICKEN REARED IN LOW AND HIGH HYGIENE ENVIRONMENTS

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Antimicrobial peptides (AMPs) are a group of host defence peptides 1-10kDa in size which contain spatially separated hydrophobic and cationic amino acids. To date a number of chicken (*Gallus gallus domesticus*) AMPs have been reported; these include the avian β -defensins (AvBD) or 'Gallinacins', a group of 13 AMPs encoded by genes clustered on chromosome 3. We have focused on one of these, AvBD10. Using RT-PCR this study investigated gene expression in male birds, aged 7 and 35 days, of 3 different lines reared in either a low (LH) or high hygiene (HH) environment. Gene expression was identified in nearly all tissues tested but semi-quantitative analyses suggested increased gene expression in the liver, kidney, testes and lung in most birds relative to the other tissues. Significantly a reduction in gene expression was noted in the 35 day birds living in the pedigree environment relative to the day 7 birds, and those reared in the low hygiene conditions. The AvBD10 peptide was hyper-expressed *in vitro* and shown to have antimicrobial activities against *Salmonella typhimurium phoP* and clinical isolates of *Staphylococcus aureus* and *Escherichia coli* spp. These data suggest important functional roles for this peptide in the avian innate defences and in particular in young birds. Moreover environmental parameters can potentially affect gene AMP expression levels.

ISAFG-P4

DRB3 ALLELES AFFECT IMMUNE RESPONSE TO A FMDV PEPTIDE IN CATTLE

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An ideal vaccine would consist of the minimum number of immunodominant epitopes that induce protection in most individuals. However, the role of host genetics in variation in response to vaccines with a limited spectrum of epitopes is unclear. Complex traits such as immune response involve many genes, but polymorphisms in Major Histocompatibility Complex (MHC) genes play a critical role. The MHC class II gene - *DRB3* is one of the most polymorphic genes in the cattle genome, with 104 alleles identified, although within most herds a limited range will be present. These polymorphisms can alter the conformation of the peptide binding cleft (PBC), thus affecting the binding efficacy of the vaccine peptides and consequently the acquired immune response. A safe synthetic vaccine to foot-and-mouth disease virus (FMDV) needs to include the loop region of the viral capsid (VP1) as this is the main focus of neutralising antibody. We investigated associations between *DRB3* genotypes and variation in immune response to a FMDV peptide containing this loop region in a genetically diverse Charolais-Holstein cross population of 250 individuals. Allele *DRB3*1601* conferred a low responder phenotype in terms of both IgG1 and IgG2 responses ($P < 0.05$). In contrast T cells from animals which were *DRB3*1201* positive exhibited higher proliferation in response to the FMDV peptide ($P < 0.05$). It is probable that differences in the PBC conformation in **1601* and **1201* affect the binding of the peptide and thus the outcome of the immune response. Therefore FMDV vaccine design needs to take account of MHC allele interactions.

ISAFG-P5

HOST MAF TRANSCRIPTION FACTORS ARE REGULATED BY THE PROTOZOAN PARASITE *THEILERIA ANNULATA*

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MAF transcription factor family members play an important role in haematopoietic events. In particular, c-maf and mafB are involved in monocyte and macrophage differentiation, proliferation and survival. These cellular processes are modulated during the infection of bovine macrophages with the protozoan parasite *Theileria annulata*, leading to the reversible transformation of the host cell. The parasite regulates several host cell signalling pathways, but the transformation mechanism has not been elucidated. We are currently investigating if MAF transcription factors play an important role during *Theileria annulata* infection.

Microarray analysis, using our bovine macrophage specific microarray, and quantitative RT-PCR have been used to investigate the response of bovine monocytes to *T. annulata* infection. The analyses have revealed that c-maf is up-regulated during infection, but to a significantly lower level than in monocytes cultured in medium only, suggesting that *T. annulata* suppresses the up-regulation of c-maf. Furthermore, c-maf and mafB expression is much lower in *T. annulata* infected macrophages than uninfected macrophages. To confirm that the suppression of c-maf and mafB are caused by the parasite, established *T. annulata* infected cell-lines were treated with the anti-theileriacidal drug buparvaquone. MAF transcription factor mRNA levels significantly increased after buparvaquone treatment.

This is the first report of *T. annulata* regulating host MAF transcription factor expression. Furthermore, this is the first report of a protozoan parasite modulating the expression of MAF transcription factors. We hypothesize that *T. annulata* regulation of host MAF transcription factor expression is essential for parasite-induced survival and proliferation of the infected host cell.

ISAFG-P6

A PHYLOGENETIC APPROACH TO IDENTIFY SUBSTITUTIONS OF FUNCTIONAL RELEVANCE IN BOVINE TOLL-LIKE RECEPTOR 2

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Due to their central role for host defence, polymorphism within TLR genes is associated with the predisposition of several diseases in human and mouse. Growing evidence suggests that polymorphism in TLR genes might also be an underlying reason for observed variation in disease resistance traits in livestock.

Here we analyse the bovine TLR2 gene in several important cattle breeds for polymorphic variants and use a phylogeny based approach to predict their functional relevance. Sequencing of pools containing amplified TLR2 coding regions of approximately 100 individuals belonging to the most predominant ten cattle breeds revealed as yet unreported polymorphic amino acid sites. Many of them are located at or near sites of functional relevance in human or mouse.

Further mammalian species were either sequenced at TLR2 or the sequences extracted from databases and used to analyse their molecular evolution. A Phylogenetic Analysis using Maximum Likelihood (PAML) detected sites shaped by either positive or negative selection in a complete mammalian set of sequences and in an alternative set limited to ruminant sequences. Selective pressures on ruminant TLR2 differed significantly from those of the complete mammalian set, suggesting a different role of selection in the phylogeny of ruminant TLR2. Sites shaped by ruminant-specific positive or negative selection or neutral evolution were identified.

This approach enabled an identification of polymorphic sites shaped by positive selection in ruminants which are likely to exert functional consequences.

ISAFG-P7

THE ROLE OF MHC CLASS II GENES IN NEMATODE INFECTION OF LAMBS.

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Nematode infection is one of the most important diseases faced by sheep. Current methods of treatment are threatened by the evolution of drug resistance in parasite populations. Selective breeding of nematode-resistant animals represents an attractive method of nematode control. In the UK, essentially all grazing sheep are exposed to a mixed, predominantly *Trichostrongylus axei* infection. Faecal nematode egg counts are widely used to classify lambs but the use of genetic markers would simplify the identification of superior animals. One genetic marker is the *DRB1* locus in the class II region of the major histocompatibility complex. The G2 allele has been found to be associated with resistance in both Scottish Blackface and Suffolk lambs. Understanding the mechanisms underlying this association would assist in identifying the causal mutation and improve the recognition of resistant animals. The major mechanisms underlying resistance appear to be the IgA-mediated suppression of nematode growth and fecundity as well as the IgE-mediated prevention of nematode establishment and possibly survival. Suppression of nematode growth and fecundity accounts for the majority of the genetic variation in faecal egg count. However, the *DRB1* locus is associated with differences in IgE activity and in the number of *T. circumcincta*. Targets of the IgE response have been identified by 2-dimensional gel electrophoresis followed by tandem mass spectrometry. The targets include paramyosin and tropomyosin both of which are allergenic in a wide variety of host-parasite systems.

ISAFG-P10

EXPRESSION PATTERN OF TIR8/SIGIRR IN DOMESTIC ANIMALS

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Toll-like/IL-1R (TIR) superfamily belonging receptors have a relevant role in the innate immunity and during inflammation. Their activation can lead to exaggerate inflammatory responses and potentially devastating local and systemic reactions, if not tightly regulated. TIR8/SIGIRR receptor belongs to the TIR superfamily; it negatively modulates the inflammatory responses in the gastrointestinal tract in mice. Tir8 acts as a decoy, trapping key components of TIR signaling. Since chronic intestinal diseases are becoming more relevant for the economy and the management of livestock and small animals, it is important to elucidate some aspects of these processes in other species than human and murine. The aim of this study was to investigate Tir8 conservation, expression and eventually its role in inflammatory processes in few domestic animals. The expression of Tir8 was evaluated in a wide panel of organs by Northern Blot analysis. Although with specie-specific traits of expression, TIR8 was mainly detected in kidney and gastro-intestinal tract, as in human and in mouse. Through alignment analysis, Tir8 sequence in those species revealed higher homology to the human sequence than to the murine one. Conserved nucleotide sequences and conserved expression in evolution among these species may suggest a similar function in inflammation and immunity.

ISAFG-P11

EXTRAHEPATIC ACUTE PHASE PROTEIN RESPONSE IN PIGS WITH PLEUROPNEUMONIA CAUSED BY EXPERIMENTAL *ACTINOBACILLUS PLEUROPNEUMONIAE* INFECTION

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The acute phase response is an important part of innate immunity and includes a dramatically altered hepatic synthesis of acute phase proteins. Extrahepatic expression of acute phase proteins has been sporadically demonstrated, however little is known about extrahepatic production of acute phase proteins in pigs.

Ten 9-week-old pigs were inoculated with *A. pleuropneumoniae*. Five non-inoculated pigs were used as controls. Animals were sacrificed 14-18h after inoculation. Blood- and tissue samples (liver, tracheobronchial lymph nodes, spleen, tonsils) were taken immediately after sacrifice. Quantitative real-time RT-PCR was performed to determine differential expression of genes between infected and non-infected animals in blood- and tissue samples. Extrahepatic expression and regulation of several acute-phase proteins as C-reactive protein, Haptoglobin, and Serum amyloid A2 as well as IL-6 were found 14-18h after experimental infection with *A. pleuropneumoniae*.

The current study provides one of the first examples of porcine extrahepatic expression and regulation of acute phase proteins and proposes that many different cell-types in the organism can be involved in the production of these proteins. The results further suggest that extrahepatic acute phase protein expression is tightly regulated during an acute phase response indicating that it might be an important part of the innate defence system.

ISAFG-P12

GENE EXPRESSION PROFILING OF THE EARLY RESPONSE IN LUNGS OF PIGS EXPERIMENTALLY INFECTED WITH *ACTINOBACILLUS PLEUROPNEUMONIAE*

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A frequent finding in lungs of pigs infected with the gram-negative bacterium *Actinobacillus pleuropneumoniae* is very well demarcated areas of necrotic tissue whereas the rest of the lung is seemingly unaffected by visual inspection. The focus of this study is to evaluate the gene expression profile in samples taken from three different sites in lungs of infected pigs; 1- Necrotic areas, 2-Margins of necrotic areas, 3-Visually unaffected areas.

Ten 9-week-old pigs were infected with *A. pleuropneumoniae* while five non-infected pigs were used as controls. Animals were sacrificed 14-18h post infection and lung samples were taken. Samples were analyzed using pig cDNA microarrays (DJF_PIG_55K4_v1, GEO accession: GPL6173) containing 2 x 27.744 spots representing approximately 20.000 porcine genes. It was found that 4702 (FDR<0.05) genes were differentially expressed in necrotic areas compared to control tissue from uninfected pigs whereas only 289 genes were differentially expressed in samples taken from the margin of the necrotic areas compared to uninfected controls. In samples taken from the visually unaffected areas, in the challenged pigs, it was found that 65 genes were differentially expressed compared to tissue from uninfected pigs. Although the majority of differentially expressed genes are found in necrotic areas, these genes are mostly related to GO-terms as metabolism, catabolism and biosynthesis whereas the GO-terms for the two other tissue areas investigated are clearly related to immune responses.

In this study different local tissue responses have been demonstrated in lungs of infected pigs, using broad gene expression analysis of unaffected, marginal tissue, and necrotic tissue.

ISAFG-P13

GENE EXPRESSION PROFILES IN LYMPH NODE TISSUE OF TICK-RESISTANT AND TICK-SUCEPTIBLE BEEF CATTLE.

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This study was carried out to identify gene expression patterns in lymph node tissues collected from tick-susceptible (*Bos taurus*) and tick-resistant (*Bos indicus*) beef cattle. Tick-naïve Aberdeen Angus (n=5) and Nellore (n=5) calves were subjected to a single artificial infestation with approximately 20,000 *Rhipicephalus (Boophilus) microplus* larvae. Inguinal lymph nodes were biopsied from calves at 10 days before and 21 days after tick infestation. Additionally, a group of 10 uninfested calves (5 Nellore and 5 Angus) were used as experimental controls. RNA from lymph nodes were hybridized to 20 Bovine Long Oligo Arrays using a reference design where samples collected prior to infestation were used as references against samples collected post infestation for each animal. Hierarchical clustering of differentially expressed genes ($p < 0.05$) revealed a unique gene expression pattern in the infested Angus animals. Clusters containing genes particularly involved in immune response, cell communication and protein modification were found to be induced in the Angus infested group. Furthermore, clusters harboring genes related to development, signal transduction, and transport were induced exclusively in the Angus control group. In contrast, infested Nellore calves did not show a clearly defined pattern of expression. For instance, clusters containing genes with functions related to protein metabolism, development, immunoglobulins, response to stimulus, and cell communication showed altered expression not only in infested but also in uninfested Nellore animals. Our results highlight tick-resistance characteristics in Nellore breed, provide new insights into host-parasite interaction, and indicate potential targets to be used in genetic improvement of beef cattle.

ISAFG-P14

DEVELOPMENT AND APPLICATION OF A SNP ASSAY TO ASSESS MHC DIVERSITY AT CLASS I LOCI IN HOLSTEIN CATTLE

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The highly polymorphic major histocompatibility complex (MHC) genes encode molecules integral to adaptive immune responses to infectious disease. Populations with high MHC diversity may be better equipped to fight infection. It is hypothesised that intensive selective breeding of cattle for production and disease resistance traits is leading to a decrease in genetic variation of cattle MHC genes. Reference strand mediated conformation analysis (RSCA) of DNA from 75 premium Canadian Holstein bulls revealed a propensity towards two MHC haplotypes, A14 and A15, which are identical at 2 out of 3 class I loci. A number of single nucleotide polymorphisms (SNPs) were identified to demonstrate presence or absence of the A14 and A15 haplotypes and determine zygosity, and these formed the basis of a custom-designed assay based upon the SNP-extension technique. The assay has been used to analyse samples from 6 dairy herds to assess whether these two haplotypes were as common in the wider population as in the Canadian bull sample. Preliminary results indicate that the bias towards the A14 and A15 haplotypes is also evident in the UK herd. We aim to expand this novel technique to detect a number of other common MHC haplotypes. The ability to rapidly MHC- type cattle from a DNA sample will enable stockmen to devise breeding programmes that promote MHC diversity in the future UK dairy herd and may improve many aspects of herd health.

ISAFG-P15

GENETIC AND NON-GENETIC FACTORS AFFECT THE VARIABILITY IN IMMUNE RESPONSE TO A FOOT-AND-MOUTH PEPTIDE IN A CATTLE CROSS POPULATION.

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Genetic and non-genetic factors determining immune responsiveness were investigated in a Charolais-Holstein cattle cross population, immunised with a foot-and-mouth disease virus (FMDV) peptide. The 40-mer peptide comprised two virus derived VP1 capsid sequences joined by a Pro-Pro-Ser (PPS) spacer, representing the major neutralising antibody epitopes. Although this peptide protects a proportion of cattle against viral challenge, considerable variation is seen. Protection does not always correlate with high titres of neutralising antibody. Previous studies have highlighted a potential role for the MHC locus, but it is clear that other unknown factors are important. Phenotypic measurements, IgG1, IgG2 and T-cell proliferation were taken across a 10 week period. Considerable variation was seen in all traits. REML and ANOVA analysis was used to investigate relationships between this variation and the factors: age, sire, dam, cohort and breed. Cohort had a significant effect ($P < 0.05$ IgG1 and IgG2), sire was significant ($P < 0.01$) for T-cell proliferation and IgG2, whereas dam was significant ($P < 0.01$) for IgG1 and T-cell proliferation. Age and breed, however, had no effect. These results suggest that genetics contribute significantly to the observed variability in immune response. The eventual aim of this project is to locate candidate genes controlling immune responsiveness via QTL analysis. The outcomes of this project may lead to the development of more effective vaccines.

ISAFG-P16
PIG PERIPHERAL BLOOD CD11R1⁺ MONONUCLEAR LEUKOCYTES –
CHARACTERIZATION, HERITABILITY AND CORRELATIONS WITH PERFORMANCE.

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Introduction

Genetic markers for increased resistance to a wide range of infectious diseases would facilitate genetic improvement of health and performance under health conditions seen on commercial farms. Previous work has shown that CD11R1⁺ cells, from pig peripheral blood mononuclear leucocytes (PBML), were heritable and negatively correlated with performance, but we hypothesize that these effects will depend upon farm health status. Also, although pig natural killer (NK) cells express CD11R1⁺ it is not clear if both populations belong to the same sub-set.

Methods and Results

a. 1598 pigs from three specific-pathogen free (SPF) farms and 1029 pigs from four non-SPF farms were monitored for average daily gain. PBML CD11R1⁺ cell proportions were measured in a sub-group of 537 SPF pigs and 604 non-SPF pigs. CD11R1⁺ cell proportions were heritable regardless of farm health status ($h^2 = 0.42(0.09)$). Negative genetic correlations between CD11R1⁺ cell proportions and daily gain were only detectable amongst the non-SPF pigs.

b. Pig PBML CD11R1⁺ cells were characterized by FACS analysis (n = 8). Most CD11R1⁺ cells were CD3⁻CD16⁺ and CD8 α ⁺, a phenotype suggestive of NK cells. However, one third of CD11R1⁺ cells were CD3⁺CD16⁺ and these cells may be NK T cells.

Discussion

This data show that CD11R1⁺ cells are phenotypically heterogeneous. Also, the negative association between CD11R1⁺ cell proportions and performance under non-SPF conditions may reflect a response to infection which is under genetic control. Having demonstrated these genetic effects, we are now completing a whole genome scan to detect genetic markers associated with innate immunity.

ISAFG-P17

GENOMICS OF HOST RESISTANCE TO BOVINE TUBERCULOSIS

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Bovine Tuberculosis (TB) is a severe, chronic infectious disease of the respiratory tract. It has severe implications for animal welfare, food safety and human health. The incidence of bovine TB has increased in the last two decades in the UK, particularly in Northern Ireland. TB is caused by infection with *Mycobacterium bovis*. Other possible hosts of *M. bovis* are wild life animals and humans. The current control strategies are based on the cattle 'test and slaughter' scheme, to control spread of the disease, and on the control of wild life transmission (badgers). Research into vaccines is still in progress.

The purpose of this project is to quantify the host genetic differences in TB resistance and to identify the causes of the genetic differences between resistant and susceptible animals. A statistical analysis of TB incidence and the genetic influence on incidence will be undertaken on information collected on herds in Northern Ireland and a genome wide scan will be conducted on 1000 animals using a case-control strategy, with the recently released 50K SNP Chip. In addition, genetic studies on the different strains of the bacteria will be undertaken, and risks associated with strain differences will be assessed. The project outcomes will enable us to quantify the genetic influence of disease resistance/susceptibility, identify sires that are highly resistant to TB and identify genetic markers that can be used to select for increased TB resistance.

ISAFG-P18

MICROARRAY-BASED GENE EXPRESSION ANALYSIS OF MEDIASTINIC LYMPH NODE IN RESPONSE TO NATURAL PORCINE CIRCOVIRUS TYPE 2 (PCV2) INFECTION IN PIGS

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Porcine circovirus type 2 (PCV2) is recognized as the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS). Under field conditions, a high percentage of pigs becomes infected by PCV2, but only a limited proportion of animals suffer from PMWS. The mechanism underlying the development of PMWS has not yet been identified and, among others, a genetic predisposition has been pointed out. This work aims at characterizing the transcriptional profile of PMWS naturally affected pigs. A total of 25 pigs (13 healthy and 12 PMWS cases) from 3 different farms were included in the analysis. PMWS diagnosis was made based on the international accepted criteria, which demands presence of clinical wasting, histopathological lesions in lymphoid tissues (moderate to severe lymphocyte depletion with histiocytic infiltration) and moderate to high amount of PCV2 within lesions. Total RNA from mediastinic lymph node was hybridized to Affymetrix Porcine GeneChip[®]. The Bioconductor software was used for array normalization (RMA) and statistical analyses (moderated t-test). In total, 386 genes were found to be significantly differentially expressed (DE) between healthy and PMWS-affected pigs (FDR<0.1, M>1). Of them, 219 and 167 genes were up-regulated and down-regulated, respectively, in the PMWS-affected group. Remarkably, genes related to the immune system (immune response, leukocyte homeostasis, and antigen processing and presentation) and catalytic activity (apoptosis, proteolysis) were mostly up-regulated; whereas the expression of genes related to transcription processes appeared to be down-regulated. This work provides insight on the molecular mechanisms involved in the pathogenesis of PCV2 infection. Further validation by quantitative PCR of most relevant genes will be performed.

ISAFG-P19

DIFFERENTIAL EXPRESSION OF CELL SURFACE RECEPTOR RNA IN CHICKEN IMMUNE DEFENSE AGAINST *SALMONELLA ENTERITIDIS* INFECTION

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Activation of the host immune response by pathogens includes direct cellular communication between antigen-presenting cells and T cells. The objective of this study was to characterize effects of *Salmonella enteritidis* infection in young chickens on RNA expression levels of immune cell surface receptors (CD3 ξ , CD28, MHC II α and MHC II β). Chicks from the F8 generation of two advanced intercross lines (AIL; broiler X Fayoumi, broiler X Leghorn) were orally inoculated with *S. enteritidis* at 1 day of age. At 1 week of age the spleens and ceca were harvested to measure RNA expression levels by SYBR green quantitative RT-PCR. Cecal content and spleen tissue of all infected birds were *Salmonella enteritidis*-positive. Cecal RNA expression was significantly higher ($p < 0.05$) in *S. enteritidis* infected than uninfected chicks for all four cell receptors (range, 1.5 to 14-fold differences). No significant differences in splenic RNA expression levels between infected and uninfected chicks were detected. There were significant differences between the two AIL for splenic CD3 ξ and CD28 RNA expression in combined data of both infection states, where the broiler X Fayoumi had greater RNA expression levels than the broiler X Leghorn AIL. The results suggest that *S. enteritidis* infection initiates a localized supramolecular activation complex in the cecum, including antigen-presenting cells (MHC II) and T cells (CD3 ξ and CD28). The data also reveal genetic line differences in expression of genes (CD3 ξ and CD28) that may result in specific elevation of splenic T cell activation via the NF- κ B pathway.

ISAFG-P20

**A NEW TOOL FOR STUDYING EXPRESSION OF WELL-ANNOTATED GENES IN CATTLE:
THE CAFG BOVINE LONG OLIGO MICROARRAY**

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Past bovine microarray resources from the Center for Animal Functional Genomics (CAFG) at Michigan State University have been extensively used to probe complex questions in animal and veterinary sciences. Our mission to improve specificity, slide reproducibility, spot morphology, background and ease of use prompted us to begin work in 2004 on a bovine long oligo microarray (BLO) resource representing 7,449 unique and well-annotated genes. In 2007 a set of 1,865 bovine 70-mer oligos was designed from the highly published BOTL-5 cDNA microarray to replace it and to expand the BLO microarray. The new BLOPlus microarray now includes many genes important in studies of bovine immunobiology.

Validating the BLOPlus was accomplished using a twelve-array common reference design experiment. An investigation was conducted using a bovine macrophage cell line (BoMac) to observe the effect of CD40L on Mycobacterium avium subsp paratuberculosis (MAP)-infected macrophages. BoMac cells at passage 4 were incubated with MAP for 2 hours to allow for phagocytosis and then washed. After 22 additional hours the MAP-infected cultures were treated for 6 hours with CD40L. The experimental design consisted of comparing MAP-infected cells, CD40L-treated cells, MAP-infected cells treated with CD40L, and Nil cultures to a common reference sample.

Assuring the sensitivity and specificity of the 70-mer oligos on the BLOPlus microarray is of utmost importance. Microarray parameters examined included features found, hybridization efficiency, background levels, and false-positive rate. Data demonstrating that the new BLOPlus microarray is a robust tool for investigating gene expression in cattle will be presented and discussed.

ISAFG-P21

TRANSCRIPTOME MODIFYING EFFECTS OF PARTURITION ON BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS

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Cattle are particularly sensitive to parturition-induced immunosuppression. The capacity for mitogen-induced proliferation of peripheral blood mononuclear cells (PBMCs) is substantially diminished in the first weeks following birth, potentially influencing immune responsiveness to vaccines and infectious pathogens. This might be caused by dramatic fluctuations that occur in circulating steroids and cytokines that have potent transcriptional activating and suppressing effects on PBMCs. However, little is known about the molecular basis of parturition-induced changes in PBMCs because, until recently, genome-level exploration of potentially affected genes has not been possible. We conducted a study of parturition-induced reprogramming of PBMCs aimed at explaining dairy cows' disease susceptibility and highlighting therapy targets. RNA from purified (> 98%) PBMCs collected from three cows on days -8, 0.5, 2, 7, and 14 relative to parturition were used to interrogate a series of 1200-member BOTL-3 arrays in a loop design consisting of one loop per cow and 5 arrays (one per sample day) per loop. We identified that parturition induces a "blossoming" in the activity of 147 bovine PBMCs genes from 24 different ontological clusters, with 35 of these genes being up or down regulated ($P < 0.05$) up to 14 days postpartum. Functions such as transcription, chromatin and extracellular matrix remodelling were substantially altered. Our study highlights the effects of bovine parturition on genes that regulate immunological functions of PBMCs, possibly explaining why female dairy cattle succumb to opportunistic infections of the mammary glands, reproductive and respiratory tracts in the first month following birth.

ISAFG-P22

IN DEPTH GLOBAL ANALYSIS OF GENE EXPRESSION LEVELS IN PORCINE ALVEOLAR MACROPHAGES FOLLOWING INFECTION WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine worldwide. Infection of the preferential target cells, porcine alveolar macrophages (PAMs), by PRRSV causes significant changes in their function by mechanisms that are not understood. Serial Analysis of Gene Expression (SAGE) libraries were constructed from in vitro mock-infected and PRRSV strain VR-2332-infected PAMs at 0, 6, 12, 16 and 24 hours post-infection. Each SAGE library was sequenced to obtain >95,000 tags per time point. The sequences were processed to account for sequencing error before generating tag:count databases of relative abundance. Tags were mapped to transcripts and genes by exact regular expression matching to sequences in available databases. More than 590 unique tags with significantly altered expression levels were identified ($p < 0.01$ with Bonferroni correction). Validity and kinetics of expression of SAGE identified genes were confirmed using real-time RT-PCR. Expression of the identified innate immune response genes (including both cytokine and chemokine encoding transcripts known to be altered by other pathogens) showed no or very little change post-infection. Expression of arginase showed a significant, short-lived increase in expression at 6 hours post-infection indicating possible inhibition or lack of a pro-inflammatory response by inhibition of inducible nitric oxide synthase (iNOS) activity. This study represents the first comprehensive evaluation of gene expression in PRRSV-infected PAM, including identification of potential proteins and pathways that may be utilized for the control of PRRSV infection.

ISAFG-P23

SALMONELLA TYPHIMURIUM MODULATES THE EXPRESSION OF NODS IN BOVINE MACROPHAGES AND DENDRITIC CELLS.

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Salmonella enterica serovar Typhimurium is the main *Salmonella* species that infects human and cattle. It is zoonotic and causes similar symptoms in humans and cattle, mainly gut pathology and diarrhoea. Macrophages (MΦ) and dendritic cells (DC) are antigen-presenting cells (APC) and the direct involvement of both cell types in the immune response to *Salmonella* in human and bovine hosts has been previously identified. MΦ and DC bridge innate and acquired immune responses by performing a variety of functions including phagocytosis, secretion of cytokines and antigen presentation. The aim of this project is to investigate how MΦ and DC interact in the initial stages of infection in cattle with *Salmonella*. It is planned to use a functional genomics approach by comparing samples from non-infected MΦ and DC with MΦ and DC infected with heat-inactivated and live bacteria using Affymetrix microarrays. Secondly the role of Nod like receptors (NLRs) is being investigated. Because of their recent discovery, NLRs are not fully represented on the Affymetrix microarray. NLRs are a group of pattern recognition receptors (PRRs) including NOD1, NOD2, NOD3 and IPAF which are known to be associated with the host response to intracellular invasion by bacteria. Preliminary studies show differences in expression of NLRs in response to stimulation with dead bacteria and bacterial endotoxins as well as differences in NLR expression between cattle breeds. Knowledge gathered from these studies will provide new insights into immune response and pathogenesis of Salmonellosis in cattle.

ISAFG-P24

TRANSCRIPTOMIC ANALYSIS OF ATLANTIC SALMON (*SALMO SALAR*) HEAD KIDNEY CELLS INFECTED WITH INFECTIOUS PANCREATIC NECROSIS VIRUS

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Infectious pancreatic necrosis (IPN) is the most serious viral disease affecting the UK salmon farming industry. IPN is caused by infectious pancreatic necrosis virus (IPNV), a member of the Aquabirnavirus genus within the family Birnaviridae. The mechanism by which pathology is induced in IPNV infection is unknown, which compromises breeding programmes aimed at improving disease resistance in Atlantic salmon, and the rational development of vaccines to counter IPN. To characterize the host-pathogen relationship in IPNV, we have analysed transcriptional profiles in IPNV-infected salmon head kidney (SHK-1) cells. The cell line SHK-1 was developed from Atlantic salmon head kidney leucocytes, and exhibits macrophage-like enzyme activity. RNA was extracted from IPNV-infected SHK-1 cells using the Trizol method and subsequently amplified using the amino allyl MessageAmp procedure. Amplified RNA was Cy3/Cy5 labelled then hybridized to the TRAITS / SGP 17000 feature Atlantic salmon cDNA microarray enriched for immune-related genes. At three days post-infection, 340 genes were differentially expressed in IPNV-infected SHK cells. These included genes encoding proteins involved in cell signalling, transcription, innate and adaptive immunity, cellular metabolism, apoptosis, and glycolysis. Differentially expressed immune-related genes included: NF-kappaB inhibitor, ubiquitin specific protease 18, C-type lectin receptor A, complement factor-H precursor, histamine N-methyltransferase, proteasome subunit beta type 5, and endothelial leukocyte adhesion molecule (ELAM-1). This work forms part of a larger study which aims to characterize transcriptional expression in vivo and which will allow comparisons between in vitro and in vivo expression.

ISAFG-P25

STREPTOCOCCUS DYSGALACTIAE ELICITS A FAST BUT PROLONGED INNATE IMMUNE RESPONSE IN THE BOVINE MAMMARY GLAND

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Mastitis is one of the most economically significant dairy production diseases. On Irish dairy farms, the Gram-positive *Streptococcus dysgalactiae* is a major mastitis-causing pathogen. Mechanisms aimed at boosting the bovine immune response to intracellular pathogens, such as *S. dysgalactiae*, may help in mastitis control. The objective of this study was to profile local and systemic bovine immune gene expression (IL-1 β , IL-8, IL-12, TNF- α , NF- κ B, TLR2 and TLR4) in response to a *S. dysgalactiae* challenge in 5 disease-free Holstein Friesian cows. One quarter from each animal was infused with 1500 CFU of *S. dysgalactiae*. Milk samples were taken from the infused quarter and an adjacent quarter which served as an internal animal control. SCC was recorded and RNA was isolated from milk somatic cells at pre-infusion, 7, 24, 48, 72hrs, 1 wk and 2 wks post-infusion. RNA extracted from blood, taken at the same time points, monitored the systemic response. Real-time PCR was performed using Lightcycler and SyBr Green technology with gene specific primer pairs. Gene copy number was determined by absolute quantification. In all animals, a substantial localised upregulation of the pro-inflammatory cytokines occurred between 1 and 7 days post infusion. For example, an increase of almost 500,000 gene copies of TNF- α by 48 hrs post-infusion was observed. The prolonged nature of the infection and the elevated SCC of up to 2 weeks post-infusion were of interest. The results in this study differ from work with Gram-negative pathogens, where a shorter infection has been observed.

ISAFG-P26

DIRECTED ALTERATION OF A NOVEL BOVINE ANTIMICROBIAL PEPTIDE IMPROVES EFFICACY AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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Antibiotic resistance is of growing concern in medical and veterinary circles and a major health issue for the future. Methicillin-resistant *Staphylococcus aureus* (MRSA), a zoonotic bacterium resistant to standard antibiotic therapies, has become increasingly prevalent and have recently been isolated from livestock that have been fed with growth-promoting antibiotics thereby acting as a possible reservoir of human infections. Consequently the search for novel antimicrobial agents is critical.

Innate immune defence mechanisms activated against invading pathogens involve activation of signalling pathways that stimulate expression of antimicrobial peptides (AMPs). AMPs are short, structurally diverse cationic proteins that kill microbes via a range of effector mechanisms including the disruption of pathogen cell membrane integrity. We analysed a panel of recently discovered bovine AMPs to identify sites subject to positive selection (PS) over the course of evolution that may be functionally important against antibiotic resistant bacteria.

Amino acid modifications were introduced to increase charge at PS sites; to make charge neutral changes at PS sites; to increase hydrophobicity and to confer a hydrophilic C-terminal. While all four peptide modifications increased antimicrobial activity against MRSA when compared with the native form of bovine β -defensin 123 (BBD123; $P = 0.02$); conferring the hydrophilic tail caused the most significant increase, with an LD₅₀ of 3.91 μ g/ml. The peptide with increased charge at PS sites showed the most significant increase in antimicrobial activity against a non-resistant strain of *S. aureus* ($P = 0.02$). Therefore, informed modifications of amino acid sequence can significantly affect the specificity and antimicrobial activity of a peptide, opening a route to improved therapeutics.

ISAFG-P27

GENOMIC INVESTIGATION OF THE HOST-PARASITE INTERACTION IN SHEEP SCAB WITH A VIEW TO IDENTIFYING AND DEVELOPING POTENTIAL VACCINE CANDIDATES FOR THE CONTROL OF THE DISEASE

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Sheep scab is a highly contagious ectoparasitic disease of sheep caused by infestation with the mite *Psoroptes ovis*. It is an important welfare issue due to the disease symptoms which include intense pruritis and severe exudative dermatitis. The host response to infestation is directed against mite secretory/ excretory products and is typical of an immediate hypersensitivity reaction. Sheep scab is traditionally controlled by chemical intervention, however there have been concerns over chemical residues in meat and also on the effect of these chemicals on human health and the environment. In addition there is an emerging problem of resistance to the organophosphates and synthetic pyrethroid compounds used in treatment. Vaccine candidates have been identified by sequentially fractionating mite proteins, however vaccine efficacy has been relatively low (despite further fractionation) and isolation of the proteins involved has proved difficult. In order to further improve the development of a vaccine against sheep scab we must gain a better understanding of the host-parasite relationship. Sheep scab provides us with an excellent model with which to achieve this as we have access to both host and parasite material. In order to interrogate the host parasite interaction we are utilising a genomics approach, combining an in-house *P.ovis* cDNA microarray with an ovine skin cDNA microarray developed by Belinda Norris and colleagues at CSIRO, Australia. It is hoped that these resources will provide us with a greater understanding of the underlying biology of sheep scab infection and will also lead to the identification of potential vaccine candidates.

ISAFG-P28

CORRELATING CLINICAL AND MOLECULAR CHANGES POST-PARTUM TOWARD AN IMPROVED UNDERSTANDING OF BOVINE UTERINE INNATE IMMUNITY

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Calving results in uterine exposure to several bacterial species and activation of inflammatory processes. Unsuccessful resolution of inflammation leads to metritis and endometritis. Although uterine infection is the most common cause of reproductive disorder in the cow little is known regarding the role of the uterine innate immune response in the resolution of post-partum inflammation in dairy cows.

This study was designed to investigate the correlation between clinical, cytological and histopathological findings with molecular gene expression changes in uterine tissue and peripheral blood of Holstein-friesian dairy cows 14 days post-partum. 10 dairy cows that had calved 13 to 16 days previously were presented for veterinary examination. Clinical examination, ultrasonography, bacterial culture and uterine cytology were performed. Blood samples and uterine biopsies were obtained, RNA extracted and cDNA synthesised. Gene specific primers were designed for genes commonly involved in pathogen detection and innate immune response to bacterial infections, including Toll-Like Receptors (TLRs), cytokines and AMPs analysed by quantitative real-time PCR (qRT-PCR).

All 10 cows presented with a vulval discharge, while 4 cows had intra-uterine *E-coli* infection. Neutrophils and macrophages were evident on cytology. Preliminary results show that *LAP* and *TAP* genes are increased in expression in mRNA from peripheral leukocytes from cows with metritis. *BNBD5* gene expression is decreased in expression.

Further work will analyse the expression of a wider panel of immune genes and correlate them with clinical changes. Altered expression of innate immune gene expression in bovine uteri and PBL may be a useful prognostic indicator of specific uterine infection.

ISAFG-P29

DIFFERENTIAL GENE EXPRESSION PROFILING SHEDS LIGHT ON THE INNATE IMMUNE RESPONSE DIFFERENCES IN CHICKEN INTESTINE BETWEEN COMMENSAL (CAMPYLOBACTER) AND PATHOGENIC (SALMONELLA) BACTERIA

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Although *Campylobacter jejuni* is the major cause of infectious food poisoning worldwide, very little is known regarding its relationship with the immune system of its primary host – the chicken. Commonly regarded as a commensal, even high experimental doses of *Campylobacter* in chickens does not cause pathology. It is unknown whether the intestinal immune response has a role in establishing the commensal state of *Campylobacter*.

A comparative *C. jejuni* and *Salmonella enterica* infection model was carried out to investigate the innate immune responses of 4 week-old broiler chickens. Challenged and control birds were sacrificed 6 hours post-challenge and tissue samples taken for RNA extraction. Selected genes involved in pathogen detection and innate immune response were profiled by quantitative real-time PCR.

In the caecum, a significant increase in the expression of *TLR4*, *TLR7* and *TLR21* was observed after challenge with both types of bacteria ($P < 0.05$) compared to control birds. In contrast, *TLR5* expression was increased 2-fold after *S. enterica* challenge only ($P = 0.05$). A significant increase in *IL6* was only seen after pathogenic challenge. *IL8* mRNA expression was decreased 4-fold in response to *C. jejuni* but increased 5-fold to *S. enterica* challenge ($P = 0.00$). Preliminary studies in the caecums of birds 20 hours after *C. jejuni* challenge has revealed a 24-fold increase in the expression of *IL8* ($P = 0$).

Results indicate that colonisation by *Campylobacter* in the chicken intestine actively induces expression of innate immune genes.

ISAFG-P30

DEVELOPMENTAL REGULATION OF THE CHICKEN INNATE IMMUNE ARSENAL *IN OVO*.

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Avian β -defensins (AvBDs) and cathelicidins are the two major sub-classes of Antimicrobial Peptides (AMPs) known to play a fundamental role in both the innate and adaptive immune response to bacterial, viral, fungal and parasitic pathogens. The adaptive immune system is not developed when chickens hatch so the innate immune system has evolved a range of mechanisms to deal with early pathogenic assault. In this study, we profile the expression of a range of innate immune genes including pathogen receptors (Toll-like receptors, TLRs), cytokines and effector molecules (AMPs) during the first 12 days of chicken embryogenesis. We demonstrate that chicken embryos express specific patterns of TLRs (*TLR2* and *TLR15*), the complete repertoire of 14 known AvBDs and selected cytokines (*IL1 β* , *IL8*, and *IL10*) at different stages during development. This expression is developmentally regulated and we further identify differential patterns of expression across the developmental timecourse using quantitative real-time PCR. Functional analyses of two antimicrobial peptides were shown to have significant pathogen-specific antimicrobial activity when tested against a panel of pathogens (*E. coli*, *Salmonella typhimurium* and *Staphylococcus aureus*, and *listeria monocytogenes*) *in vitro*. This is the first study to examine the expression of the chicken antimicrobial peptides and demonstrates the innate preparedness of developing embryos for pathogenic assault *in ovo*, or post-hatching.

ISAFG-P33

AN *INSILICO* APPROACH TO STUDY THE MECHANISM OF *TFS1P* –AN INHIBITOR OF MAPK SIGNAL TRANSDUCTION DOWN REGULATOR IN SACCHAROMYCES CEREVISIAE AND ITS USE IN PREVENTING CANCER

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Ras genes encoding a family of protein are very important molecular switches for a wide variety of signal pathways that control the process of cell proliferation, cell adhesion, apoptosis and cell migration. Mutation of *Ras* family of proto-oncogenes are very common. This paper aims at molecular modeling with a view to arrive at a similar structured molecule which could reintroduce regulation of *Ras* system or kill cells during uncontrolled pathways. The TBD and *Ira2* *Ras* GAP structures were predicted and their interaction with *Tfs1p* were studied. Where *Tsf1p* is a PEBP family of protein which can interact with *Ras* thereby down regulate signal transduction pathway. The docking study revealed that the amino acids involved in the interaction could be helpful in drug designing against the catalytic sites of *Tsf1p*. So the interactions could be blocked and in turn inhibition of *Ras* GAP activity could be prevented. Since *Ira2* resembles human NF1 (neurofibromatosis type –1) and the 3-D structure of TBD is similar to human *Ras* GAP. The interactions between TBD, *Ras* GAP and *Tfs1p* were done using Hex software. There is no 3-D structure available for the TBD. The structural similarity of yeast GAP with the human *Ras* GAP will pave its way for further exploration and thus will be useful for treating Human and Animal Cancer.

ISAFG-P34

INVESTIGATION OF ARTIFICIAL NEURAL NETWORK (ANN) ABILITY IN PREDICTION OF SEMEN PRODUCTION OF IRANIAN MARKHOUS BREED BY TESTIS PHENOTYPIC INFORMATION

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The aim of this investigation was to study the ability and accuracy of ANN (Artificial Neural network) in estimate and predicting total volume of semen from phenotypic data, for selection high producing he-goat as prospective and parent of next generation, in addition reduces financial costs.

The data consisted of 20 he-goat information from Iranian Markhouz breed which collected and measured in summer and fall season. In order to train ANN, tow variables (testis volume and testis circumference) as input variable and one variable (total semen volume in 100cc) as output variable were introduced to system (MATLAB, 2007), through 150 records, 134 were used for ANN training and 16 records were selected randomly for simulated system (ANN). The constructed network was a back propagation Artificial Neural Network with four layers of input, hidden (include 2 layers) and output. The layers had 2, 20, 20 and 1 neuron, respectively. The Result demonstrated there was no difference between predicted and observed data ($p>0.05$), the high correlation ($r_p=0.92$) and proper root mean square error (RMSE=28.19) showed that the predicted average for semen concentration ($r_p=0.92$) were close to the observed value.

The major use of any predictive process is to support accurate decisions which are dependent on prior knowledge of the possible outcome. The result showed, phenotypic trait information could be widely used in prediction of semen volume with highly correlation of observed data. Also, ANNs are reliable as decision support system that helps breeders to choose a ram to be left or culled from herd.

ISAFG-P35

SYNTHESISING TECHNOLOGIES FOR MICROARRAY QUALITY OPTIMISATION

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Microarrays are widely recognised as a useful tool in genomics, proteomics and drug discovery, providing huge quantities of data which, effectively analysed, add to our growing body of knowledge about how humans and other animals function when they are well and malfunction when they are ill. Quality data should in theory lead to quality conclusions but microarray technology can be complex. A successful microarray experiment requires the marriage of ultra low volume fluidics with a good quality library to generate your microarray; effective hybridisation and scanning technology; and finally a data analysis tool capable of weeding out the 'noise' in an experiment without corrupting genuine informational data. Failure or mediocrity in one or more component of the system leads to inconclusive or misleading results. This paper describes the synthesis of technology from 3 separate groups to generate better quality microarray results. A novel inkjet print head is used to produce DNA microarrays using a proprietary library. The microarrays are in turn hybridised via a continuously hydrating hybridisation mixer system.

Optimisation of your microarray technology, from array production to data output, can produce dividends. An optimised system delivers better quality data reducing the likelihood of data analysis leading to inconclusive or misleading results.

ISAFG-P36

EFFECT OF DIETARY N-3 POLYUNSATURATED FATTY ACIDS ON GENE EXPRESSION IN THE BOVINE UTERUS USING MICROARRAY TECHNOLOGY

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Modern high-yielding dairy cows display poor reproductive performance. Nutrition is known to play a key role in reproduction and there is emerging evidence that dietary long chain n-3 polyunsaturated fatty acids may act as specific potent regulators of some reproductive events, however their role as mediators of uterine function has not been well studied. Microarray technology has provided the opportunity to examine the simultaneous expression of thousands of genes within a cell type of interest. The objective of this study was to use a 23K oligonucleotide bovine microarray to examine the effects of dietary supplementation of n-3 PUFA on bovine uterine endometrial gene expression and to relate changes in gene expression to key biochemical pathways using novel bioinformatic tools. Reproductively normal crossbred beef heifers were fed a straw and barley/beet pulp based concentrate and supplemented with a rumen protected source of either saturated fatty acid or high n-3 PUFA for 45 days. Uterine endometrial tissue was harvested from all animals and total RNA was extracted using TRIzol reagent, from endometrial tissue from seven animals on High and seven on Low PUFA. Gene profiling was carried out and microarray data was normalized and statistical analysis of gene expression was carried out using the Puma method. Data were analyzed using Ingenuity Pathway analysis. A total of 418 genes were significantly differentially expressed ($P < 0.05$). Animals supplemented with a high PUFA diet showed differential endometrial expression of genes involved in regulating key biological processes that could potentially influence bovine fertility.

ISAFG-P37

IDENTIFICATION OF MARKER GENES FOR *ACTINOBACILLUS PLEUROPNEUMONIAE* (APP) INFECTION IN PIG

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Introduction

Bacterial respiratory tract infections cause severe losses to the pig industry in the European Union and worldwide. Genetic traits associated with host resistance have not been identified to date. Therefore, the aim of this project is the identification of porcine genetic markers, putatively associated with resistance to infections caused by bacterial respiratory tract pathogens.

Methods

For identification of candidate genes a well established aerosol infection model with *APP* was used. Pigs of different breeds were infected, clinically monitored during the stages of infection and probed on day 4 (acute phase) or 21 (chronic phase) post infection. All animals were subjected to pathology, a lung lesion score was determined, and samples were taken from lung tissue without and with heavy pathological changes, respectively. These samples as were used for transcriptome analysis using the Affymetrix Gene Chip Porcine Genome Array enabling a genome wide analysis of porcine gene expression. Several statistical methods have been applied in order to identify differentially expressed genes and associated pathway and functional information.

Results

In order to recover potential marker genes we used three approaches, namely (i) correlation analysis of expression profiles and clinical scores, (ii) statistical tests for differential expression, and (iii) ANOVA (analysis of variance). The genes identified as being strongly regulated on day 4 post infection show important differences in the pig strains pointing to strain-dependent responses to APP lung infection.

ISAFG-P38

MEAN SPOT INTENSITY OR MEDIAN SPOT INTENSITY: DOES IT REALLY MATTER?

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Some open systems for microarray data acquisition (microarray scanners) offer its users possibility to gain data of mean spot intensity or median spot intensity. Aim of the present study was to compare different methods of microarray data analysis using results of mean or median spot intensity. Samples of total RNA were obtained from control mice and mice with streptozotocin-induced diabetes and processed with SuperScript™ Plus Indirect cDNA Labeling System with Alexa Fluor® Dyes (Invitrogen) and hybridized on commercially available microarray slides containing 35852 oligonucleotide probes in automatic hybridization station. Values of spot intensities minus background were automatically normalized with LOESS method by microarray scanner software. The following methods of ranking genes were used: Wilcoxon statistic, fold change and signal to noise. Wilcoxon statistic revealed 106 probes with $P < 0.05$ when mean spot intensity values were analyzed and 157 probes with $P < 0.05$ when median spot intensity values were analyzed. These two groups of results shared 72 common probes. In the group of 106 "means" only 27 differ in fold change analyses by more than 1.6. In the group of 157 "medians" number of probes differing by more than 1.6 was 60. As far as signal-to-noise is concerned in the group of "means" value > 0.5 observed in 11 probes and in the group of "medians" in 30 probes. Our results suggest that using for statistical analyses values of median spot intensity gives larger number of probes (genes) differing statistically.

ISAFG-P39

PRODUCING A MEANINGFUL PICTURE OF MICROARRAY DATA THROUGH STATISTICS

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The main purpose of statistical processing and analysis is to eliminate all possible noise factors that can lead to misinterpretation of microarray data and finally concentrate on a list of genes that appear to be biological significant. This process requires modelling of technical, dye artefacts and finally at the analysis stage, modelling of gene variance.

Technical artefacts are removed using spatial bias correction. This is carried out separately for each print-tip by subtracting corresponding row and column means of log-ratios from each data spot.

Dye artefacts are gene dependent and the reason is that some genes are systematically badly labelled by Cy5 or Cy3 respectively. Due to the fact that the data cloud is neither constant nor linear there is a need for non-linear normalization. The most widely used method to dispose of the dye effect is by loess normalization, either for every slide or for every print-tip.

The normalized log ratios on each microarray slide are analyzed for each gene by ANOVA. t-tests are constructed and then modified by the Limma eBayes correction, giving the so-called "moderated t-statistics". The Limma eBayes correction, shrinks the residual standard errors towards the approximate median value, and so avoids declaring genes significant which have small differential expressions together with very small standard errors. Genes with false discovery rate (FDR) ≤ 0.05 are usually considered significantly different.

This procedure of data processing and analysis can be followed through an already analyzed experiment which involves the comparison of tRNA from two groups of sheep kept at different day lengths/photoperiod, either long day or short day, and sampled at 4 hours, 12 hours and 20 hours after lights-on. The aim of the experiment is to identify differential gene expression between long-day and short-day sheep at each time point.

ISAFG-P40

ASSESSING THE THRESHOLDS FOR DIFFERENTIAL GENE EXPRESSION BETWEEN BOVINE MUSCLES IN A MICROARRAY EXPERIMENT

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Introduction

The reliable detection of differentially expressed (DE) genes remains a main challenge in the analysis of microarray data. A microarray experiment was performed to detect the transcriptional profile between two types of skeletal muscle in bovine. A Bayesian approach for data normalization, analysis and model-based clustering was applied for the detection of DE genes. Real time RT-PCR confirmed the existence of false negatives. Thus, the need to explore other statistical approaches arose. The aim of the present communication was to use several statistical procedures to ascertain the optimal thresholds for differential expression.

Methods

Model-based clustering methods, for three expression contrasts, and a BH multiple comparison were applied to detect DE genes. Techniques were evaluated in terms of the estimates of FDR, FNDR and Risk. The correlation among the expression of DE genes was also evaluated based on a bovine gene co-expression network.

Results

While the number of DE clones changed dramatically across the various approaches, the number of biological functions involved remained alike. Furthermore, three of the four approaches detected as DE the previously confirmed false negatives.

Discussion

When using microarray expression profiling to identify sets of candidate genes for further research, the comparison of different statistical techniques is recommended in order to distinguish DE from artifacts. Additionally, independent techniques, such as real time RT-PCR, could be used to set the biological thresholds for differential expression.

ISAFG-P41

PREFERENTIAL TRANSCRIPT EXPRESSION IN PORCINE LIVER CONDITIONAL ON SEX AND FEEDING LEVEL

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To identify hepatic gene expression patterns in male and female pigs fed with high and low feeding levels we have used expression microarrays. Ten Iberian pigs (five females and five males) were allocated to each one of the two feeding levels. Liver samples were collected from pigs slaughtered at 211 days of age. RNA samples from two pigs of each feeding level by sex combination were hybridized with 24,123 probe sets of the Affymetrix porcine GeneChipTM. Expression data quality evaluation and normalization was carried out using affyPLM package and RMA function in Bioconductor. Data analysis was performed using mixed model methodology, and a false discovery rate of 1% was assumed. A large number of significant expression differences have been found between sexes, 124 transcripts appear overexpressed in males and 114 in females. Out of them, 16 transcripts (five male-biased and 11 female-biased) showed more than ten-fold change in gene expression. There was not overrepresentation of any chromosome except for Y chromosome. Differences in expression levels between sexes of several *Cyp450* family genes, that codify enzymes responsible for metabolizing drugs and xenometabolites, have been detected. This *Cyp450* sex dependent expression has been shown to be determined by growth hormone in human and rodents. Restriction of feeding level leads to overexpression of 16 transcripts and underexpression of 56 transcripts. These transcripts included relevant genes related with lipid metabolism as *delta-9 desaturase*, *fatty acid binding protein 3* and *apolipoprotein A-I*. Validation of some relevant results has been done by Real Time PCR.

ISAFG-P42

FUNCTIONAL GENOMICS APPLIED TO THE IDENTIFICATION OF MARKERS OF BEEF TENDERNESS

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The objective of this study, included in the MUGENE French program, was to identify new markers of beef tenderness by functional genomics approaches. The expression level of a large number of genes and proteins as well as biochemical characteristics were analyzed in the *Longissimus thoracis* (LT) muscle from Charolais young bulls slaughtered at 15 or 19 months of age. The muscular transcriptome and proteome were compared on the basis of tenderness estimated by sensorial analysis or shear force measurement of 55°C grilled meat. Among the identified markers, some were related with a better tenderness (citrate synthase, apoBEC, apolipoprotein, slow isoform of the myosin heavy chain, ubiquinol-cytochrome-c reductase and ATP synthase beta chain) of meat. A major result is the identification of a negative relationship between the expression of the DNAJA1 gene and tenderness of beef after 14 days of aging in the studied young bulls which was confirmed in the LT of Charolais steers. This gene codes for a chaperonne protein of the "heat shock" proteins family (hsp40). The expression level of other proteins of stress (especially HSPB1 which encodes Hsp27) was positively correlated with shear force at the mRNA or protein levels. These proteins have anti-apoptotic activity and could thus slow down the process of cell death and consequently the maturation of the meat, favourable to the tenderization of muscle after the slaughter of the animal. The validation of the role of these proteins in meat tenderness is in progress in other breeds and muscles.

ISAFG-P43

GENETIC DIVERSITY IN INDIAN LIVESTOCK BREEDS FOR EXPLOITATION AS GLOBAL GENE POOL USING GENOMICS FOR FUTURE FOOD SECURITY TO MANKIND

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India is one of the hot spot of mega biodiversity centres in the world, as reflected in the form of over 140 well defined breeds of livestock and poultry in addition to 37 lesser known breeds characterized with both phenotypically and molecular markers. These breeds are mainly kept by individual farmers, normally in small herds/flocks raised under low input system, hardly suffer from any infectious diseases. The high heat and draught tolerance of these breeds often exposed to these inhospitable conditions invariably high. In cattle 33 breeds and 7-8 lesser known populations differ in body size, conformation and production levels, weighing as high as 450-500 and milk production 25-30 kg per day. In 22 goats breeds, there are large diversity for milk, meat, prolificacy and cashmere. 42 sheep breeds varying in wool yield and quality, body size and fecundity having Fec+B allele. 18 indigenous poultry breeds like close to red jungle fowl, game breed Aseel and Kadaknath black meat breed is an important germplasm of the country. Some never suffered bird flu kept at almost zero input under backyard system. Still other valuable species Yak and mithun also exist in India. Our SNP data shows the number of SNPs and resulting alleles are much higher than any other studies in the western world. This magnitude of genetic diversity calls for a greater global attention to these rich genetic resources for the identification of alleles related production, heat and drought tolerance and disease resistance. This would help in developing global strategies for improvement of animal genetic resources to meet the ever growing animal protein requirement and global food security.

ISAFG-P44

IDENTIFICATION AND ANALYSIS OF AGE TISSUE AND BREED SPECIFIC TRANSCRIPT-DERIVED FRAGMENTS (TDFS) USING *CDNA-AFLP* TECHNIQUE IN A PANEL OF FOUR SELECTED CATTLE BREEDS.

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A complete gene expression profiling study has been performed using cDNA-AFLP technique in a panel of four selected breeds. The age specific gene expression profile was generated from the young bulls at the age of 6, 9 and 12 months. While, the breed specific gene expression profile was generated from the Polish HF, Polish Red (milch breeds), Limousin and Hereford (beef breeds). Two tissues, namely, pituitary and thyroid were selected to produce the tissue specific gene expression profile. This cDNA-AFLP experiment was aimed to identify the putative differential, identical and single displayed (DD, iDD and sDD) transcript derived fragments (TDFs) bands in context to muscle growth and body composition trait in cattle. The investigation was carried using a total of 64 different combinations of selective primer pair *TaqI* / *MseI* restriction enzymes, to create the tissue, breeds and age specific gene expression profile. Results revealed mass identification of iDD, DD and sDD TDFs bands in age, tissue and breeds specific gene expression profiling cDNA-AFLP experiment. The investigated results based on both pituitary and thyroid tissues profiles, revealed the higher frequencies of DD TDFs bands in comparison to iDD band. However, varying frequencies of sDD TDFs bands were observed among the young bulls at different ages in the investigated panel of four selected breeds. This performed cDNA-AFLP experiment has built up a platform for next stage of experiment i.e., band excising, isolation and cloning of putative TDFs fragments and eventually the sequencing, to produce the putative expression sequence tags (ESTs) in context to muscle growth and body composition traits in cattle.

ISAFG-P46

EFFECT OF HOLSTEIN FRIESIAN GENOTYPE ON HEPATIC EXPRESSION OF GENES INVOLVED IN THE GH-IGF AXIS DURING EARLY AND MID-LACTATION.

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The New Zealand Holstein Friesian (NZHF) has lower milk production but superior fertility compared to the North American Holstein Friesian (NAHF) strain. Energy demands of early lactation exceed energy intake resulting in a state of negative energy balance (NEB). Differences in fertility between the two strains may be due to greater NEB during early lactation, and a longer period of preferential partitioning of nutrients to milk production for the NA strain. The objective of this study was to examine the effect of cow genotype and stage of lactation on the expression of genes in the GH-IGF axis. Ten mature NAHF cows and 10 mature NZHF cows were selected as representative of their respective strains. Liver tissue was collected from all cows at 35 and 150 days postpartum. Real-Time RT-PCR analysis was performed on genes involved in the IGF system. IGF-1 was the only gene that was affected by cow genotype with mRNA abundance greater in the NZ breed. Acid labile subunit (critical to IGF-1 stability) also tended to be greater in NZ cows compared to NA cows. Across genotypes, mRNA abundance of IGFBP-1, IGFBP-2 and GHR1A decreased from day 35 to 150, whereas increased IGF-I and ALS mRNA were observed during this period. Consistent with greater IGF-I mRNA abundance, the NZ strain also exhibited greater circulating IGF-I concentrations, which may play explain their superior reproductive performance. However there was no genotype by day interaction for any of the genes measured.

ISAFG-P47

GENE NETWORK ANALYSIS ON MUSCLE EXPRESSION DATA FROM DUROC PIGS WITH EXTREME VALUES FOR CHOLESTEROL AND FAT DEPOSITION TRAITS

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We have used *GeneChip Porcine Genome*® arrays (*Affymetrix*) to study differences in gene expression between extreme animals for cholesterol and fat parameters in a commercial Duroc pig population. A total of 70 *Gluteus medius* (GM) muscle samples were processed, belonging to animals with the most extreme levels (HIGH and LOW groups) for traits such as plasma lipoprotein and triglycerides concentrations, percentage of intramuscular fat and fatty acid composition in muscle. After normalizing data with the GCRMA algorithm, class comparison between groups was performed with a t-test obtaining 1007 genes whose expression levels differed significantly ($p\text{-value} < 10^{-7}$) between the HIGH and LOW groups. We used the bioinformatic software *Ingenuity Pathways Analysis* (IPA) to set up a potential network to identify the molecular differences between these two groups of animals. This software generates signalling networks based on the known interaction/relations among target genes, determining those involved in well documented canonical signal transduction or metabolic pathways. Gene expression fold changes were over-laid on the 17 primary pathways represented in the results. There was a consistent overrepresentation of pathways leading to regulation of circulating thyroxine (LEP, NPY, TRH, TSHR, FGF1, NCOA1). Many known signalling pathways were confirmed to be differentially regulated such as those related to myocyte size and proliferation, muscle thickness and skeletal muscle cell death, adipocyte proliferation, storage of fat and inhibition of lipid deposition. Three genes, NPY, LEP and TRH, represented a link between several endocrine pathways (glucose, lipid and thyroxine plasma levels) and an inhibitory role over cellular growth and proliferation pathways.

ISAFG-P48

ASSOCIATION OF SNP IN THE EXON2 OF LEPTIN GENE WITH MILK PRODUCTION TRAITS IN SARABI CATTLE BREED OF IRAN

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Leptin plays a role in the regulation of appetite, energy partition, feed intake and body composition of mammals. The leptin gene influences economic traits in cattle such as meat production, milk performance, reproduction traits, and carcass quality. The objective of this study was to investigate the genetic variations in the exon2 region of the leptin gene for the Sarabi cattle breed of Iran. The measured traits included milk yield (305-days), fat yield, fat percentage, protein yield, and protein percentage. Blood samples were randomly collected from 274 Sarabi cows for genotyping of the leptin gene. The C and the T allelic frequencies were 0.67 and 0.33, respectively. Three genotypes (CC, CT and TT) were detected in the population with frequencies of 0.394, 0.555 and 0.051, respectively. The Average Heterozygosity was 0.436 in the population. The chi-square test confirmed Hardy-Weinberg equilibrium in the population. A univariate linear model was used to evaluate the association of each genotype with the traits. The CC and TT genotypes had significant effects on milk and protein yield ($P < 0.05$). Cows with the TT genotype gave higher performance for milk yield, fat yield and protein yield. The association analysis showed a significant effect of the leptin SNP on protein yield and 305-day milk yield ($p < 0.05$).

Key Words

Leptin, Polymorphism, PCR-RFLP, Sarabi cattle breed

ISAFG-P49

CLONING AND CHARACTERIZATION OF THE 5' REGULATORY REGIONS OF FATTY ACYL Δ 6 DESATURASE GENES IN ATLANTIC SALMON AND COD

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Fatty acyl desaturases (DES) are critical enzymes in the pathway for the biosynthesis of highly unsaturated fatty acids (HUFA) in fish. Δ 6 DES cDNAs from Atlantic salmon and cod have been isolated and functionally characterised. Here we report the characterisation of the promoters of these DES genes. Atlantic salmon and cod genomic DNA libraries were probed with full-length salmon and cod Δ 6 DES cDNAs. The 5' sequences upstream of the transcription start site obtained were 4163bp and 6222bp for salmon and cod Δ 6 DES, respectively. The DNA fragments of the 5'flanking region generated by PCR cloning were fused to a promoterless luciferase reporter gene and transfected to salmon (AS) cells. Luciferase assays were performed at 48h post-transfection. Progressive 3' deletions in the putative promoter regions were examined for their effects on reporter activity. Significant promoter activity was observed in the region between nts -321 to -546, and -40 to -163 upstream of the transcription start site for salmon and cod DES, respectively. Further *in silico* analysis of the sequences of this region revealed the presence of several potential transcription factor binding sites. Site-directed mutagenesis of these binding sites identified a CCAAT box as the important cis-element. Mutagenesis of the potential Sp1, C/EBPalpha and GATA-1 binding sites also significantly decreased cod DES promoter activity. We also found that treatment of cells with EPA (20:5n-3) caused a significant reduction in transcriptional activity for both salmon and cod DES. These results suggest complex regulation of Δ 6 DES gene expression in salmon and cod.

ISAFG-P50

EFFECT OF LEVEL OF EICOSAPENTAENOIC ACID (EPA) ON THE TRANSCRIPTIONAL REGULATION OF Δ -9 DESATURASE IN AN *IN VITRO* BOVINE INTRAMUSCULAR ADIPOCYTE CELL CULTURE MODEL

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Conjugated linoleic acid (CLA; *cis*-9, *trans*-11) is reputed for its human health benefits. CLA is formed by tissue desaturation of vaccenic acid (VA) by Δ -9 desaturase. Bovine muscle VA concentrations may be enhanced by supplementing cattle diets with n-3 polyunsaturated fatty acids (PUFA) and linoleic acid however increases in VA are not paralleled with equivalent augmentation of tissue CLA concentrations. It has been reported that this may be a consequence of n-3 PUFA, particularly eicosapentaenoic acid (EPA), mediated inhibition of mRNA expression for Δ -9 desaturase. In this study, a bovine intramuscular adipocyte cell line was developed and employed to examine the effect of EPA on the expression of Δ -9 desaturase and its putative transcription factor, SREBP-1c in an *in vitro* intramuscular adipocyte cell culture model. Adipocytes were incubated for 24h in medium containing 0 μ M, 50 μ M EPA or 100 μ M EPA. RNA was extracted and mRNA expression of Δ -9 desaturase and SREBP-1 was measured by quantitative real time RT-PCR. Expression of Δ -9 desaturase mRNA was decreased 5 and 7 fold, respectively following 50 μ M and 100 μ M EPA incubation. Gene expression of SREBP-1c was decreased 6 and 18 fold in cells supplemented with 50 and 100 μ M EPA, respectively. Regression analysis showed a significant negative relationship between EPA concentration and gene expression of both Δ -9 desaturase and SREBP-1c, while there was a significant positive relationship observed between Δ -9 desaturase and SREBP-1c gene expression. This is first report demonstrating that EPA decreased gene expression of Δ -9 desaturase and SREBP-1c in bovine intramuscular adipocytes.

ISAFG-P51

MICROARRAY ANALYSIS OF THE HYPOTHALAMO-PITUITARY GONADAL AXIS AFTER REMOVAL OF FOOD RESTRICTION

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Broiler breeder hens develop polyfollicular ovaries if fed ad-libitum. Feed restriction prevents this and is essential to sustain fertility. There is no clear hypothesis on the cause of polyfollicular ovaries. This study aimed to establish the effects of lifting feed restriction on genes in the hypothalamo-pituitary gonadal axis using micro-arrays to develop new hypothesis on polyfollicular ovaries.

Breeder chicks (n=16) were reared on restricted rations, at 29 weeks of age half the birds were fed *ab-libitum* and all were killed 2 weeks later. Ovarian stroma, F1, 5-6 and 6-7 mm follicles, pituitary and hypothalamus tissues were taken to probe a chicken 17K oligo micro-array. Confirmatory real-time quantitative PCRs (QPCR) were run using SYBR green. Release from food restriction significantly increased body weight, the number of yellow yolky follicles (>8mm), ovarian stroma and pituitary weight. The largest number of genes found to be differentially expressed from the micro-array and QPCR was in the pituitary and the F1 follicle (P>0.05). Genes differentially expressed in the pituitary included myosin light chain kinase, TSH β , Ras-related associated with diabetes (RRAD), Dachhund, HSPB1 and in the F1 follicle, C-type lectin and MHC class II. All genes were up regulated in the ad-libitum group except RRAD.

Dachhund is involved in the differentiation of pituitary cells and may maintain the hypertrophy after food restriction. The observed increase in TSH β suggests involvement of thyrotrophs. We are now in a position to test hypothesis on the role of these factors on ovarian follicle recruitment in broiler breeders.

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ISAFG-P52

GENE NETWORK DYNAMICS FOR SKIN AND WOOL DEVELOPMENT UNDER NORMAL AND PERTURBED CONDITIONS

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The skin is a specialist epithelial tissue that forms the first point of contact with the external environment. We are interested in identifying gene networks that define skin homeostasis so that we might better understand the changes occurring during developmental stages or in response to other perturbations such as disease.

We have focused on Merino sheep skin tissue and defined the homeostatic state using samples originating from ten healthy adults. The perturbed state is represented by ten samples originating from various embryonic stages and disease challenged animals. Samples were analyzed across three microarray experiments, spanning 61 hybridizations. We used a mixed model to normalize the fluorescent intensity levels and a combination of an information theoretic approach with partial correlation coefficients to identify significant co-expression between genes separately for the two states. A total of 1,887 genes were included in both networks. We identified 286 genes that are differentially expressed and/or differentially connected between the normal and perturbed states (FDR < 1%). Over-represented pathways include ECM-receptor interaction, focal adhesion, cell cycling and TGF-beta signaling. A cluster of keratins expression and connectivity were significantly decreased from normal to perturbed conditions. Further, this cluster of keratins was negatively correlated with a cluster of collagens and other genes encoding for extracellular matrix proteins. In comparing and contrasting gene expression networks representing normal and perturbed states we have identified various common and specialist profiles that reflect the adaptive response of skin. This has expanded understanding of the general mechanisms of skin development, growth and maintenance.

ISAFG-P53

GENE EXPRESSION PROFILES OF IMMUNE-RELATED GENES IN MYCOBACTERIUM BOVIS INFECTED AND HEALTHY CONTROL CATTLE

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Cases of bovine tuberculosis (BTB) caused by the facultative intracellular pathogen *Mycobacterium bovis* are a major economic burden to farmers. Early and accurate diagnosis of disease is important as infections could become chronic and also be rarely transmitted to humans. The expression profiles of key immune genes (including cytokines, chemokines and transcription factors) between *M. bovis* infected and healthy cattle were compared to identify candidate biomarkers of infection that could form the basis of diagnostic assays for BTB. Peripheral blood mononuclear cells (PBMC) were isolated from the blood of seven healthy and seven BTB-infected cattle. Total RNA was extracted, quantified and reverse transcribed into cDNA. The expression of eight putative stable reference genes was determined by real time quantitative reverse transcription PCR (qRT-PCR) and the geNorm program was used to analyse results and identify the most stable reference gene among all the control and BTB-infected animals (*H3F3A* – the H3 histone family 3A gene). Additional real time qRT-PCR reactions were carried out to quantify the expression levels of 14 immune related genes and results were analysed using the Delta-delta Ct method. Fold changes relative to the healthy animals were determined for the BTB-infected animals and results were statistically analysed. Expression of *CCL2*, *IL2* and *NFKB1* was decreased in BTB-infected PBMC, while *TGFB1* was found to be increased. The remaining 10 genes were not found to be significantly differentially expressed. Future work will confirm the above results in an additional set of healthy and BTB infected animals.

ISAFG-P54

RESPONSE OF BOVINE MONOCYTE-DERIVED MACROPHAGE (MDM) TO PURIFIED PROTEIN DERIVATIVE (PPD) FROM MYCOBACTERIUM BOVIS AND MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS

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The causative agents of bovine tuberculosis (*Mycobacterium bovis*) and Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*) have the ability to cause debilitating chronic infections in cattle. Following initial infection, these bacteria can survive and grow in the host by residing in different cell types—particularly macrophages—by successfully avoiding their bactericidal activity and by suppressing the immune response generated by these cells. To investigate the host response to these two economically important pathogens, an *in vitro* cell culture model of monocyte derived macrophages (MDM) was established. Following the isolation of peripheral blood mononuclear cells by density centrifugation from fresh blood, monocytes were purified using an anti-CD14 monocyte specific antibody. Monocytes were cultured for 7-8 days to mature to MDM and their identity was confirmed by flow-cytometry and phagocytic activity by incubation with fluorescently labelled latex beads. MDM cultures were challenged with purified protein derivate (PPD) from *M. bovis* and *M. avium* subspecies *paratuberculosis* at two different concentrations. As a positive control MDM cultures were also challenged with lipopolysaccharide and untreated control MDM cultures were included in all experiments. Following 24 hours incubation with the different treatments total RNA was extracted. The MDM response to the different treatments was investigated by measuring the expression levels of key inflammatory genes by real time quantitative reverse transcription PCR (qRT-PCR). Statistical analysis was based on MDM isolated from 8 different cattle and differences in the response of cells to the PPD from *M. bovis* and *M. avium* subspecies *paratuberculosis* were determined.

ISAFG-P55

COMPARISON OF THE GENE EXPRESSION PROFILE IN NORMAL AND INVERTED TEATS IN LACTATING SOWS

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The inverted teat defect is the most common disorder of the teat in pigs. The number of genes involved in the development of this disorder is unknown. The aim of this study was to investigate the genome-wide gene expression profile in porcine teats at the lactating stage, and to evaluate the differences between normal and defect teats in lactating sows. Samples of normal and defect teats were collected from 2 lactating sows. RNA was extracted and further used for microarray analysis performed using genome-wide porcine Affymetrix arrays. Analysis of correlations and differential expressions were performed using the program SAS (version 9.1). Further, real-time PCR was performed to validate the expression of selected genes. In total 1,253 transcripts were found being differentially expressed between normal and inverted teats, of which 695 transcripts were found being higher and 558 transcripts lower expressed in normal compared to inverted teats. Validation of five selected genes using real-time PCR revealed a significantly higher expression of CTGF (connective tissue growth factor) and IGF-II (Insulin-like growth factor 2) in inverted teats, whereas GDF8 (growth differentiation factor 8) was highly expressed in normal teats. For both EGF (Epidermal growth factor) and EGFR (Epidermal growth factor receptor) no differentially expression could be verified. In conclusion this study promotes the functional candidate genes CTGF, IGF-II, and GDF8 as candidates for the inverted teat defect in pigs.

ISAFG-P56

A TIME COURSE MICROARRAY ANALYSIS OF *POST MORTEM* SKELETAL MUSCLE TRANSCRIPTIONAL PROFILE IN PIGS

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Transcriptional profile of *post mortem* skeletal muscle tissue and the changes in time course are unexplored topics in molecular biology applied to meat quality studies. This issue is of particular interest considering the fact that the availability of *post mortem* muscles at commercial abattoirs is usually associated with substantial delays. On the other hand, the possibility of using *post mortem* samples for gene expression could represent a new approach for the transcriptome analysis with potential applications for product authentication, quality assessment and forensics. The recovery of high quality RNA has been considered a prerequisite for gene expression investigation. However, it is commonly asserted that RNA is subjected to irreversible damages starting from the death of the animals. Therefore, it is usually considered that RNA cannot be efficiently used for expression analysis of tissues at different *post mortem* stages, even if several experiments with human and laboratory animal specimens have demonstrated that, depending on the tissue and *pre mortem* and *post mortem* conditions, RNA can remain stable for a long period after death. In this study, for the first time, we investigated the porcine skeletal muscle transcriptional profile in a *post mortem* time course. RNA extracted from porcine *Semimembranosus* muscles sampled at 20 minutes, 2, 6 and 24 hours *post mortem* was assessed by agarose gel and microfluidic capillary electrophoresis. Similar RIN values were obtained for all samples indicating their suitability for downstream gene expression evaluations. Then, Affymetrix GeneChip porcine genome microarrays were hybridised with RNA extracted at all *post mortem* time points. The results indicated that most of the genes did not change their level, confirming that RNA degradation might not play a major role during the first 24 hours *post mortem*. However, two groups of genes showing different trends of expression during the *post mortem* stages were identified. These results open new perspectives for the analysis of hypoxic/anoxic skeletal muscle gene expression and the possibility to identify meat quality predictors.

ISAFG-P57

BOS TAURUS AND BOS INDICUS CATTLE UNDERGOING INFESTATION WITH RHIPICEPHALUS MICROPLUS HAVE DIFFERENT PATTERNS OF GENE EXPRESSION AND IMMUNE REACTIONS

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Rhipicephalus microplus is a serious external parasite of cattle. Ticks and the diseases that they can transmit are of world-wide economic significance with costs of approximately \$US2.5 billion per annum. It is well known that *Bos indicus* cattle develop stronger tick resistance than *Bos taurus* cattle but the basis of this tick resistance is poorly understood. Six *B. taurus* and six *B. indicus* were infested with *R. microplus* larvae for five weeks and tick counts undertaken demonstrated the ability of *Bos indicus* cattle to resist tick infestation. Blood samples were collected after infestation for microarray analysis and immunological testing. Using Affymetrix bovine microarrays, significant differences in the expression of 230 genes were noted. Genes significantly upregulated in the tick resistant *B. indicus* included FOXP1, granzyme B and interleukin-2 receptor alpha (IL-2RA). Quantitative real-time PCR was used to verify differential expression of several genes and validate the results obtained via microarray. Immune assays revealed that peripheral blood of *B. indicus* contained higher percentages of CD4 and CD25 (IL-2R) cells and quantitative real-time PCR revealed higher expression of IL-2, IL-2RA, TNF-alpha and CCR-1. Furthermore, sera of susceptible *B. taurus* cattle had higher levels of tick-specific antibodies measured by ELISA and recognized more tick antigens in immunoblotting. The results suggest that the pathways of tick resistance in resistant *B. indicus* cattle involve immunological processes including activated T cells to enable the ensuing humoral response.

ISAFG-P58

TRANSCRIPTIONAL ASPECTS OF THE BOVINE GHRELIN RECEPTOR GENE IN ARCUATE NUCLEUS

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The ghrelin receptor (GRLN-R)(growth hormone secretagogue receptor type 1a (GHS-R 1a)) is a typical G-protein-coupled receptor (GPCR). It is widely distributed and mediates many of the biological actions of ghrelin and synthetic GHS. GHS-R1b, a splice variant of the GHS-R1a, is even more widely distributed in the central and peripheral tissues but its function is still unknown. The transcriptional aspects of the GRLN-R gene in the arcuate nucleus have not been revealed in cattle thus far. Therefore, we have sequenced the 5'-untranslated region (UTR) and 3'-UTR of transcripts of the GRLN-R gene expressed in the arcuate nucleus of the brain of a 2.5-month-old Holstein-Friesian bull calf using RT-PCR, 3'-RACE, 5'-RACE, nested-PCR and sub-cloning analysis. The sequences of the 5'-UTR region of the transcripts were identical to the 5'-sequence of the genomic GRLN-R gene (NW_001493715). 2 major types of 5'-UTR were observed: (1) splicing type (1a; 4 subtypes: a major transcription start site:-991) and (2) non-splicing type (1b; 3 subtypes:-649). The splicing type (1a) did not include the sequence from position -390 to position -135. A branch point sequence (YNCURAY) was not observed in this splice-out region. Three types of 3'-UTRs were observed in the 3'-UTR sequence of the transcripts:(1) type A(489 bp from the terminal signal), (2) type B(388 bp) and (3)type C(21 bp: the major type). Type A and B have the putative polyadenylation signal AATAAA and an embryo deadenylation element sequences.

ISAFG-P59

VARIATION IN THE HSP90AA1 PROMOTER AMONG SHEEP BREEDS FROM DIFFERENT GEOGRAPHIC LOCATIONS

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The physiological mechanisms that underline an organism's ability to cope rapidly with changing environmental conditions remain an area of strong scientific interest. In this regard, molecular chaperones, known as heat shock proteins (Hsp), have long been understood to be preferentially transcribed in response to multiple perturbations of cellular homeostasis.

In this study, the polymorphism of the gene encoding the inducible form of the cytoplasmic Hsp90 (HSP90AA1) was addressed in 23 sheep breeds habiting in different geographic regions of Europe and Asia.

As a result, significant differences in the genotypic frequencies for a C/G SNP located at position -660 in the HSP90AA1 5'flanking region were found among sheep adapted to different environments.

It has been previously described that this polymorphism may affect a possible regulatory element of the ovine HSP90AA1 gene. Thus, maintenance of the different genotypes could be due to selective advantage manifested in response to the different environmental conditions. Nevertheless, further analysis should be performed in order to see if there are any differences in HSP90AA1 expression that could be explained by the polymorphism identify in the present work.

ISAFG-P60

SEX AND FEEDING LEVEL INFLUENCE ON MICROARRAY GENE EXPRESSION OF PORCINE MUSCLE AND ADIPOSE TISSUES

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Microarrays have been used to study the effects of sex and feeding level on the gene expression patterns of diaphragm, *psoas major* muscle and adipose tissue in Iberian pigs. Two groups (five males and five females each) were fed at two different feeding levels. Tissue samples were collected at slaughter (211 days of age). RNA samples of the three tissues from two pigs of each feeding level by sex combination were hybridized with Affymetrix porcine GeneChipsTM. Statistical analysis of differentially expressed (DE) genes was carried out with the GEAMM software, applying a false discovery rate of 5%. Regarding the sex effect on gene expression, we detect 29, 53 and 23 DE genes on diaphragm, *psoas* and fat, respectively. One gene (*CLOCK*, upregulated in females) is common to the three tissues, and other six are common to both muscles. The DE transcripts show overrepresentation of Y chromosome on both muscles but not in adipose tissue. The feeding level induces differential expression of 10, 24 and 27 genes on diaphragm, *psoas* and fat, respectively. None of them is coincident among tissues. The DE transcripts include a number of genes related with protein catabolism and lipid metabolism. High feeding level leads to downregulation of transcripts related with proteolysis and upregulation of lipid catabolism genes in both muscles. In fat, high feeding level increases proteolysis and lipid synthesis genes' expression. Also, in *psoas* muscle some genes related with myogenesis and muscle cell differentiation are upregulated on the high feeding level group.

ISAFG-P61

THE EFFECTS OF DIETARY LIPID COMPOSITION AND DEVELOPMENTAL PHASE ON GENE EXPRESSION IN THE LIVER OF ATLANTIC SALMON (*SALMO SALAR*)

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The expression of genes involved in highly unsaturated fatty acid (HUFA) synthesis in Atlantic salmon liver is influenced by nutritional and environmental factors. For example, delta-6 and delta-5 fatty acyl desaturase expression is up-regulated in fish fed vegetable oil (VO) compared to fish oil (FO) in both freshwater and seawater phases of the salmon life-cycle. We report here on global liver gene expression in salmon fed FO and VO at four time points during the two-year growth cycle.

Atlantic salmon were fed for two years from first feeding on diets containing either FO or a VO blend. Liver samples were collected from fish at four time-points (36, 50, 53 & 86 weeks) and derived cDNAs were subjected to microarray interrogation (17K Atlantic salmon TRAITS / SGP microarray; 96 separate hybridisations).

While > 4000 features (22%) showed differential gene expression over the time course (11 months), only 15 showed differences between diets. Homology searches revealed putative gene identities for 10 of these 15 differentially expressed features, all of which appeared to be up regulated c. 1.5 – 2.0 fold in fish fed VO. All identified genes were involved in either HUFA or cholesterol biosynthetic pathways and their differential expression confirmed by Q-PCR. The VO diet has not substantially affected gene expression levels. Key genes in the HUFA biosynthetic pathway were confirmed as being differentially expressed, even though fold differences were relatively low. The effect of VO feeding on the cholesterol biosynthesis pathway is a novel finding.

ISAFG-P62

VALIDATION OF FOUR SELECTED HOUSEKEEPING GENES FOR RELATIVE QUANTIFICATION OF GENE EXPRESSION IN PIGS

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As the stability of expression varies greatly between genes, tissues and organisms, this study is focused on determination of the set of reliable housekeeping (reference) genes for normalization of relative quantification of gene expression. We tested stability of expression of *EEF1A1*, *GAPDH*, *HPRT1* and *TOP2B* genes in different tissues in pigs - *musculus longissimus dorsi*, heart, diaphragm, liver, spleen, kidney and lung.

mRNA was isolated from given tissues of ten hybrid pigs. Analysis was done using real-time reverse transcription polymerase chain reaction. We used specific primers designed on the bases of mRNA sequences (GeneBank). Specificity of PCR products was verified by construction of a melting curve and direct sequencing. Real-time PCR was performed in 7500 Real Time PCR System and with SYBR Green chemistry. For data evaluation, we used geNorm software application.

We determined most stable gene in every tissue separately and suggested that *EEF1A1* is good reference gene for given set of tissues in pigs. *GAPDH* was the least stable of these genes.

ISAFG-P63

EQUINE CDNA MICROARRAYS AND THE TRANSCRIPTOMIC RESPONSE OF THOROUGHBRED SKELETAL MUSCLE TO EXERCISE.

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With the recent advances in the sequencing of the equine genome it is important to utilize this information in the laboratory in conjunction with *in silico* studies. We are using equine cDNA microarrays to identify variations in gene expression in equine skeletal muscle in response to exercise. This will lead to a greater understanding of the molecular networks that control cellular function relating to muscle physiology in the horse. Eight untrained four year old thoroughbred geldings were exercised to maximal heart rate or fatigue on an equine high-speed treadmill. Skeletal muscle biopsies were taken from the middle gluteal muscle before, immediately after and four hours after exercise. Individual comparisons between time points will enable an understanding of each individual's response to exercise. This reduces the statistical importance of inter-individual variability in mRNA level which is high in untrained human subjects. mRNA abundance is being analysed using Linear Models for Microarray Data (LIMMA), a software package for the analysis of gene expression microarray data to determine statistically significant expression changes and to identify gene pathways relevant to exercise. Results will be confirmed using quantitative realtime RT-PCR. This study will be the first to characterize global mRNA expression profiles in equine skeletal muscle using an equine-specific microarray platform and will provide valuable information regarding the response to intense exercise and mRNA expression during recovery from exercise.

ISAFG-P64

GENE EXPRESSION DIFFERENCES BETWEEN BOVINE EMBRYO BIOPSIES DERIVED FROM BLASTOCYSTS RESULTED IN DIFFERENT PREGNANCY OUTCOMES AFTER TRANSFER TO RECIPIENTS

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Bovine embryos which differ in their developmental potential are known to differ in relative abundance of developmentally important genes. However, the direct connection between transcript abundance and further developmental capacity has been a challenge for the last decade. Here we aimed to establish this connection through gene expression analysis of biopsies derived from blastocysts prior to transfer. For this in vivo derived bovine embryos were subjected to biopsy and reexpanded embryos were transferred to recipients and based on the pregnancy outcome, biopsies were pooled in three groups: those resulted in no pregnancy, those resulted in resorption and those resulted in calf delivery. Triplicate pools (each with 3 biopsies) representing the three groups were used for gene expression analysis using BlueChip (with ~2000 clones) cDNA array. Microarray data analysis revealed a total of 50 and 52 genes were differentially regulated among biopsies derived from blastocysts resulted in no pregnancy vs. calf delivery and resorption vs. calf delivery respectively. Biopsies from calf delivery group were found to be enriched with genes regulating nucleosome assembly (H2FAZ), translation (RPLP0), cell cycle (RGS2), and metabolic process (ELOVL1). Biopsies from no pregnancy and resorption group are enriched with transcripts involved in mitochondrial electron transport (FL405), response to stress (HSPD1), and cell cycle arrest (PA2G4 and 7030402D04Rik). The transcript abundance of six differentially regulated genes was confirmed by quantitative RT-PCR in single independent biopsies from the three groups. Further functional analysis of these candidates in bovine embryogenesis will supplement the results of the present study.

ISAFG-P65

GENE EXPRESSION PROFILING OF BOVINE SKELETAL MUSCLE TISSUE OF ANIMALS WITH EXTREMES OF HIGH AND LOW WARNER-BRATZLER SHEAR FORCE VALUES

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While tenderness is an important factor determining the palatability of beef, unacceptable levels of variability in this quality trait still remain. The objective of this project was to identify differential gene expression profiles of bovine *longissimus thoracis et lumborum* muscles with different technological values of meat tenderness. High quality RNA (RIN ≥ 7) was extracted from slaughter-weight commercial cattle representing high (n = 7) and low (n = 7) levels of Warner-Bratzler shear force (WBSF). RNA was further processed for microarray analysis and hybridised to custom made muscle-specific cDNA microarrays created from SSH library clones. The arrays consisted of 597 sequences printed in triplicate representing a total of 408 genes, 69 expressed sequence tags (ESTs) and 99 candidate genes for meat quality. Twenty three genes were identified as significantly differentially expressed ($P < 0.1$) between animals representing high and low levels of WBSF following GeneSpring microarray data analysis. To validate the array analysis, the expression patterns of 10 selected sequences were further analysed using quantitative real-time reverse transcription PCR (QRT-PCR) which confirmed the differential expression of the selected sequences. Annotation of the 23 differentially expressed sequences identified some interesting putative genes and pathways. These genes are involved in a number of biological functions and pathways such as protein modification, proteolysis, energy metabolism, nucleotide metabolism and signal transduction and are targets for further research on genes influencing tenderness in meat.

ISAFG-P66

TRANSCRIPTOMIC PATTERN OF SKELETAL MUSCLES OF BULLS WITH MYOSTATIN GENE POLYMORPHISM

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The aim of the present study was to compare polymorphism-dependant *mstn* expression as well as transcriptomic patterns of skeletal muscle (*m. semitendinosus*) in bulls with different myostatin genotypes. Polymorphism concerned was described earlier substitution in the 5'flanking region of the myostatin gene at position -7828 (relative to ATG). The experiments were performed using cDNA microarray (*the NBFGC EST collection*) with 18.263 cDNA probes representing different bovine tissues in different physiological states (*Michigan State University, USA*). Statistical analyses of microarray data were performed using Wilcoxon statistic and fold change analysis. *mstn* gene expression in animals with different genotypes we measured with Real-Time PCR technique. Gene expression measured with Real-time PCR showed that CC genotype had the lowest and GG and GC genotypes statistically higher expression of *mstn* gene.

The studies using DNA microarrays revealed 109 genes with different expression between CC-GC genotypes, 60 genes with different expression between CC-GG genotypes and only 15 genes with different expression between GG-GC genotypes. The results obtained indicate that the diversity in gene expression profile in skeletal muscle of bulls with different genotypes were correlated with differences in *mstn* gene expression. The largest differences in *mstn* expression between CC and GC genotypes corresponded with largest diversity in transcriptomic pattern (109 genes). The lowest differences in *mstn* expression between GG and GC genotypes corresponded with lowest diversity in transcriptomic pattern (15 genes). It could be hypothesized that *mstn* expression is important determinant of transcriptome in bulls skeletal muscle.

ISAFG-P67

PLEITROPIC EFFECTS OF SEVERE NEGATIVE ENERGY BALANCE IN THE POSTPARTUM DAIRY COW ON GLOBAL GENE EXPRESSION IN THE SPLEEN: CONSEQUENCES FOR IMMUNE FUNCTION.

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Negative energy balance (NEB) is a severe metabolic disease affecting high yielding dairy cows in the postpartum period. Increased mobilisation of body fats to make up the energy deficit cause by increased milk production results in an increased systemic concentrations of metabolites such as non-esterified fatty acids (NEFA), beta hydroxyl butyrate (BHB), the onset of oxidative stress and disruption of normal metabolism and physiology. The immune system is also depressed in early lactation and dairy cows are therefore more vulnerable to bacterial infections causing mastitis or endometritis at this time. The objective of this study was to determine the effects of NEB on immune function in the postpartum cow using a model of severe NEB (SNEB). Spleen tissue was removed post mortem from five SNEB and five medium NEB (MNEB) cows 15 days post-partum. A bovine Affymetrix oligonucleotide array and bioinformatic analysis was used to determine differential gene expression and to explore significant gene networks. SNEB balance resulted in increased systemic concentrations of NEFAs ($P < 0.001$) and BHB ($P < 0.001$) and in a reduction in circulating lymphocyte numbers ($P < 0.05$). These effects were in turn associated with pleiotropic effects on splenic gene expression including signalling pathways and networks associated with oxidative stress, down-regulation of IL-15, BCL-2 and IFN γ ; up-regulation of BAX and CHOP and increased apoptosis with a potential negative impact on immune function. This study shows for the first time to our knowledge likely functional links between SNEB, splenic IL-15 expression and suppression of immune function in dairy cows.

ISAFG-P68

**EFFECT OF SUPPRESSION AND INHIBITION OF DNA (CYTOSINE 5)-
METHYLTRANSFERASE 1 (DNMT1) GENE IN BOVINE EMBRYOS USING RNA
INTERFERENCE AND 5-AZACYTIDINE**

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DNA (cytosine 5)-methyltransferase 1 (DNMT1) is believed to be involved in DNA methylation which is the well-characterized epigenetic modulator that has been shown to have essential functions in germ line and embryo genomic imprinting. Here we aimed to investigate the consequences of suppression and inhibition of bovine DNMT1 transcript using sequence specific siRNA and 5-azacytidine, respectively. For this *Smart pool*® siRNA designed to target the bovine Dnmt1 and 5-azacytidine were used for microinjection at zygotes stage of bovine development. A total 1107 in vitro fertilized bovine zygotes were categorized into four groups namely; those injected with siRNA (n=211), those injected with 5-azacytidine (n=266), nuclease-free water (n=318) and uninjected controls (n=312). Consequences of microinjection of siRNA and 5-azacytidine on development, mRNA and protein expression levels were determined. Microinjection of siRNA and 5-azacytidine at the zygote stage has significantly reduced the proportion of 8-cell stage 44.3% and 50.8%, respectively, compared to those injected with water (59.7%) and uninjected control (65.0%) 48 hrs after microinjection, with out significant difference in cleavage rate 24 hrs post injection. Microinjection of siRNA targeting DNMT1 has resulted in suppression of DNMT1 mRNA at 8-cell stage by 74% compared to the uninjected controls. Subsequently, suppression of Dnmt1 mRNA had significant effect in protein expression as observed in western blotting. The expression of other isoforms (DNMT3a and DNMT3b) was not affected due to the suppression of DNMT1, showing the specificity of the suppression experiment. Microinjection of 5-azacytidine had no effect on both the mRNA and protein expression.

ISAFG-P69

BIRC6/APOLLON IS ESSENTIAL DURING BOVINE PREIMPLANTATION EMBRYO DEVELOPMENT

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In activation of Birc6/Apollon/Bruce is known to activate apoptosis and resulted in embryonic lethality in mouse. However, its involvement in bovine preimplantation embryo development is not yet known. Here we have investigated the role of Birc6 in bovine preimplantative embryos by targeted suppression of the Birc6 mRNA using gene-specific long double-stranded RNA (dsRNAi) and short hairpin RNA (shRNA). For this, in vitro fertilized zygotes were categorized into four groups: those injected with long dsRNA (n= 302), shRNA (n = 352), water injected (n = 399) and uninjected controls (n = 377). Following culture in vitro total blastocyst rate at 8 and 9 dpi was significantly ($P < 0.05$) lower in shRNA and dsRNA injected zygote groups compared to uninjected and water injected control. Furthermore, the total cell number was significantly ($P < 0.05$) lower in dsRNA and shRNA groups compared to the control groups. The mRNA level of Birc6 was reduced by 40% in the blastocysts derived from RNA injected groups. In embryos that did not reach to the blastocyst stage at 8 dpi, the mRNA level of Birc6 was reduced by 83% in the dsRNA and 45% in shRNA injected zygote groups. On the other hand, Bax mRNA was increased by 50% and 100% in blastocysts derived from zygotes injected with dsRNA and ShRNA, respectively compared to the control groups. Therefore, inefficient developmental competence accompanied by reduction in mRNA level of Birc6 in the RNA injected group may indicate the involvement of Birc6 mRNA in bovine preimplantation embryo production.

ISAFG-P70
EST CLONES FOR FARM ANIMALS

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ARK-Genomics is a BBSRC funded facility designed to provide the UK scientific community with access to high quality and specialised research facilities and resources to study the livestock genome. The facility collects, stores and distributes cDNA/genomic libraries and clones from all major farm animal species including the large sequenced chicken EST set from Nottingham, Manchester, Dundee, Roslin and Incyte and the chicken RNAi vectors collection. These clones are sequence verified upon request and prior to dispatch to collaborators. Gridded array filters and microarrays are custom prepared from any of the clones in the libraries held. The extensive cDNA collections are used to create both custom designed and large generic microarrays for each of the major farm animal species. Extensive liquid handling and bacterial curatorial services are available for high throughput tasks such as colony picking, clone cherry picking, plasmid/template preparation, PCR product purification and quality control.

The EST clones are accessible to the scientific community by registering with ARK-Genomics. The procedures for registration are available on our web site www.ark-genomics.org. or email at info@ark-genomics.org

Acknowledgement

In addition to BBSRC we acknowledge financial support for infrastructure and staff from ERDF and SEEL

ISAFG-P71
PRODUCTION OF SHEEP cDNA MICROARRAY

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Introduction

The aim for this project was to create a sheep cDNA microarray, to permit screening for gene expression changes correlated to parasitic infection.

Resources

The resources used were from two sheep libraries. A sheep gut normalised cDNA library, prepared from RNA isolated from abomasal mucosa, gastric lymph node, efferent gastric lymph and Peyer's patch of sheep infected or uninfected with the parasite *Teladorsagia* and ovine rectum and large colon challenged or unchallenged with *E.coli*. Cloned inserts were tagged to allow identification of its source tissue. Ten thousand clones were sequenced from both 5' and 3' ends. A sheep spleen/brain library was prepared from mixed spleen and brain RNA from scrapie infected sheep. Clones were selected by subtractive hybridisation with normal sheep spleen and liver to remove common genes. Five thousand clones were 5' sequenced and clustered. A single representative clone from each cluster was amplified for inclusion onto the array.

Method

15,000 clones were PCR amplified, analysed on 1% agarose gels and checked for quality. Purified products were quantified using a Picogreen assay. 11,942 PCR products passed the QC and were normalised in microarray printing buffer and printed onto glass slides using an Arrayjet printer. A GAL file was produced and annotated with respect to bovine sequence homology using Unigene, DfCI Gene Index, Ensembl cDNA library and the International Protein Index.

Results

The resulting sheep cDNA microarray represents a significant novel resource for investigation into gene expression changes following parasitic infection and has successfully identified differentially expressed genes.

ISAFG-P72
PRODUCTION OF FARM ANIMAL MICROARRAYS

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Microarrays are now used for a range of techniques for the analysis of the transcriptome and the genome. ARK-Genomics, a BBSRC funded facility, provides the scientific community with access to high quality and specialised research facilities and resources to study the livestock genome. The laboratory is designed for the automated analysis of gene expression and genome mapping using state of the art robots and microarray scanners. The centre is open for scientific visits to use the equipment and resources or the centre can supply arrays for use in your own facility. Staff can provide training on the range of techniques used within ARK Genomics and the SOPs for each procedure. Both cDNA and oligonucleotide microarrays for pigs, cattle, sheep, salmon and chickens are produced by the centre. The centre also provides access to Affymetrix and Agilent Gene Expression arrays, CGH and SNP arrays. If required, analysis of your data can be carried out by our team of bioinformaticians. The facilities and its resources are accessible to the scientific community by registering with ARK-genomics. The procedures for registration and details of material available on our web site www.ark-genomics.org. or email at info@ark-genomics.org

Acknowledgement

In addition to BBSRC we acknowledge financial support for infrastructure and staff from ERDF and SEEL.

ISAFG-P74
PORCINE MATERNAL INFANTICIDE

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Introduction

The aim of our study was to identify quantitative trait loci (QTL) associated with maternal (infanticide) sow aggression, which is defined by sows attacking and killing their own newborn offspring, within 24 hours of birth.

Methods

An affected sib pair whole genome linkage analysis using 121 sib pairs was carried out with 80 microsatellite markers covering the 18 porcine autosomes and the X chromosome. This should obtain 100% genome coverage at 80% power and detect QTL within 10cM. Analysis was completed using the non-parametric linkage test of Whittemore and Halpern, as implemented in the Merlin software.

Results

4 QTL mapping on *Sus scrofa* chromosomes 2 (SSC2), 10 (SSC10) and two on X (SSCX) were identified. The peak regions of these QTL are syntenic to HSA 5q14.3-15, 1q32, Xpter-Xp2.1 and Xq2.4-Xqter respectively.

Conclusions

Identification and understanding of the maternal infanticide phenotype is of utmost importance to the agricultural community with the ultimate aim of putting in place measures to reduce maternal aggression in the sow. Several potential candidate genes were found to lie in the 4 QTL in addition to relevant abnormal behavioural QTL, found in humans and rodents. We are currently developing a SNP chip covering these chromosomes for the purpose of refining our QTL and developing a predictive test. This will have both an economic impact for the pig industry as well as being important in terms of animal welfare. We have also proposed that this could be an animal model for human postnatal psychiatric illness.

ISAFG-P76

GENOMICS APPROACHES TO STUDY OF THE BIOLOGY UNDERLYING RESISTANCE TO TRYPANOSOMIASIS - SOME UNEXPECTED LESSONS.

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An international multidisciplinary consortium is conducting a programme of research into the host response to trypanosome infection. This builds upon QTL mapping which identified genome regions influencing susceptibility to pathology following *T. congolense* infection in both cattle and mouse. The approach uses large scale expression analysis to examine the response of both susceptible and resistant strains and a series of novel informatics tools to identify pathways which are activated as a result of challenge, and those which are differentially used by resistant and susceptible strains. Of particular interest are those pathways which are significantly differentially activated and which contains genes within QTL regions. However, it is important to stress that we do not require those genes to be differentially expressed themselves.

Comparison of murine and bovine systems has proved highly informative. In particular, the use of congenic mouse lines in which fragments of resistant genome surrounding the QTL have been placed on a susceptible background has proved to be a powerful means of studying the action of QTL – and several trypanotolerance QTL co-localise to QTL involved in a range of other traits. We see a number of cases in which genes of susceptible origin show an altered pattern of expression as a result of the presence of a distant gene of resistant origin. For example, a structural polymorphism within Daxx within the QTL was correlated with altered expression of p53 with which Daxx interacts on a different chromosome.

In all cases, we find a gene network rather than a gene-by-gene approach to be highly informative and we see strong indications that survival or death is a result of differential use of number of very generic pathways. This is perhaps surprising and is turning out to have implications beyond trypanosomiasis into areas such as human survival following sepsis and under many other stresses.

ISAFG-P78

DESIGN NEW ALLELIC SPECIFIC PCR METHOD FOR DETECTION COMPLEX VERTEBRAL MALFORMATION IN HOLSTAIN-FRIESIAN

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Introduction

Complex vertebral malformation (CVM) is a recessively inherited disorder with onset during fetal development, leading to frequent abortion of fetuses or prenatal death, and vertebral anomalies. The CVM is caused by a point mutation from G to T at nucleotide position 559 in exon 4 of the bovine solute carrier family 35 member 3 (SLC35A3) gene. The aim of this study was to design new allelic specific PCR (AS-PCR) for detect CVM in Holstein breed cattle.

Methods

For this purpose we design new primers (F₁B: 5' CTCTCCCCACAGTCAGTTTCCTTAG 3' and R₁B: 5' GAAAAAGGAACCAAAGGGATGTG 3'; F₂: 5' TCACAATTTGTAGGTCTCATGGCAG 3' and R₂: 5' CACTGGAAAAACATGCTGTGAGAAA 3') with primer-primer 5 (software) for separated wild and mutant gene. These primers generated tow or three fragments, in order that independent to G or T nucleotide place. Amplification of species fragments was carried out in a final volume of 25 µl containing 10 pM from each primer and 200 ng of DNA template.

Results

The result demonstrated wild-type was represented by fragments of 1104 and 284 bp, so the mutant-type was represented by fragments of 1104 and 869 bp, whereas the carrier-type was represented by fragments of 1104, 284 and 869 bp.

Discussion

We present new allele-specific polymerase chain reaction (AS-PCR) and it's a useful method for extensive screening and diagnosis of CVM allele, the AS-PCR requires selected DNA polymerases and strictly controlled reaction conditions to obtain reliable results. This method could be wildly used in diagnostic laboratories

Key words

Complex vertebral malformation; Holstein; Allelic specific PCR.

ISAFG-P79

A TRANSGENIC APPROACH TO QTL ANALYSIS IN A TRYPANOTOLERANT MOUSE MODEL.

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We are using a mouse model to investigate the genetic basis of trypanotolerance in African cattle. We have used a transgenic approach in order to determine candidate genes underlying a Quantitative Trait Locus (QTL) for trypanotolerance in mice. We have created three transgenic mouse lines that contain BAC clones from the trypanotolerant C57BL/6 covering the Tir1 QTL for response to infection with *Trypanosoma congolense* in a susceptible A/J background. Three overlapping BAC clones from the RP23 and RP24 C57BL/6 BAC libraries have been introduced into A/J x Balb/c donor oocytes by microinjection. Founder mice for two lines were backcrossed to A/J for four generations and then subjected to trypanosome challenge. One transgenic line showed no variation in survival time compared to the susceptible non-transgenic control, which may rule out the genes carried by this BAC as candidates. One line showed a slight increase in survival time. It is not yet known if this is due to the transgene or any remaining heterozygosity. This will be investigated by locating the insertion point of the transgene and genotyping the surrounding area. The third transgenic line will be backcrossed into the A/J background and challenged with trypanosome infection.

ISAFG-P80

POLYMORPHISM OF THE PIG ACETYL COENZYME A CARBOXYLASE ALPHA (ACACA) GENE IS ASSOCIATED WITH FATTY ACID COMPOSITION IN A DUROC COMMERCIAL LINE

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Acetyl-coenzyme A carboxylase α (ACACA) is an enzyme deeply involved in the biosynthesis of long-chain fatty acids by playing a key role in the conversion of acetyl-CoA to malonyl-CoA. Interestingly, the location of the pig ACACA gene coincides with a SSC12 QTL for fatty acid composition. The main objective of our experiment was to identify polymorphisms in the ACACA coding region and to investigate their association with diverse lipid metabolism traits. RNA was extracted from liver samples corresponding to 13 Duroc pigs and eight overlapping fragments, encompassing 7.4 kb, were amplified and sequenced. Twenty three polymorphic sites were identified although any of them involved an amino acid substitution. Ten of these SNP were genotyped in a commercial Duroc population (N = 370) by pyrosequencing and primer-extension. Statistical analysis revealed a strong association between ACACA genotype and fatty acid composition at the longissimus dorsi. These associations were particularly important for SNP1633 and SNP1732, with significant effects on the abundance of both saturated and polyunsaturated fatty acids. Further analysis to identify the causal mutation is currently underway.

ISAFG-P81

DETERMINATION OF ASSOCIATION OF LITTER SIZE IN PIGS AND GENOTYPES BASED ON MICROARRAY METHOD

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Introduction

The five genes (*IL10*, *CXCL12*, *SLA-PD14*, *EHMT2*, *MX1*) have been suggested as candidate genes for pig reproductive traits, because of their essential roles in the physiological mechanisms related to functions in reproduction and immunity. The objectives of this study were to determine the genotype of 6 SNPs from *IL10*, *CXCL12*, *SLA-PD14*, *EHMT2* and *MX1* and test if these mutations are associated with litter size in sows.

Methods

The genotype and allelic frequencies of 6 SNPs from five genes were determined by genotyping 282 pig samples using a high through put Microarray-based methodology. The potential associations of polymorphisms with litter size and teat number were analysed using SPSS for windows (version 11.5) and SAS 8.0.

Results

The results showed that 1) the polymorphisms of the loci *SLA-PD14*-SNP3 and *IL10*-SNP1 were significantly associated with litter size; 2) Number born alive of first parity (NBAF) of sows with CC genotype were significantly higher than that of sows with the AC or AA genotype at the *SLA-PD14*-SNP3 locus; 3) compared to the TG genotype at the *IL10*-SNP1 locus, sows with GG had more ($p < 0.05$) total number born at first parity (TNBF), NBAF, total number born at later parities (TNBL) and number born alive at later parities (NBAL).

Conclusions

The two SNPs of *SLA-PD14*-SNP3 and *IL10*-SNP1 may be an effective potential tool used in conjunction with traditional selection methods.

Key words

litter size, SNP, *IL10*, and *SLA-PD14*.

ISAFG-P82

ASSOCIATION ANALYSIS BETWEEN GENOTYPES AND PSEUDORABIES ANTIBODY IN PIG

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Introduction

Pseudorabies has become endemic and represents a widespread problem for pig production in the world, causing great economic losses associated with reproductive failure and neonatal mortality. Mutations (SNPs) from the coding regions of the mediators of pro-inflammatory responses or other candidate genes in pigs could indicate potential involvement in susceptibility or resistance to PrV (Pseudorabies Virus) infection. There have been no previous association studies with candidate host genes that may influence PrV phenotypic traits.

Methods

In order to perform association studies to identify genes contributing to PrV phenotypes, the genotypes of 5 SNPs from three genes (*IL10*, *CXCL12*, *BAT2*) were determined for 178 sow samples using Microarray-based methodology. PrV antibodies were tested by enzyme-linked immunosorbent assay (ELISA) to determine whether there was an association between antibody levels and particular genotypes. The association between SNP genotypes and the PrV antibody levels were analyzed using the Duncan method of one-way ANOVA procedure using SAS software package.

Results

The results showed that the gE-ELISA antibody of pigs with genotypes 11(AA) and 12(AG) was significantly higher than in pigs with genotype 22(GG)($p < 0.05$) of SNP in the gene *BAT2*-SNP1.

Conclusions

The SNP1 of *BAT2*, which is HLA-B associated transcript 2, localized in the vicinity of the genes for TNF alpha and TNF beta and within the human major histocompatibility complex class III region, may be an effective potential tool to identify susceptible and resistant animals when used in conjunction with traditional selection methods.

Key words

PrV, SNP, *BAT2*, ELISA, genotyping of Microarray, antibody

ISAFG-P83

EFFECT OF DGAT1 K232A POLYMORPHISM ON MILK PRODUCTION TRAITS IN HOLSTEIN CATTLE

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The DGAT1 gene is located within to a QTL on BTA14 for milk fat content. Extensive sequencing of the gene led to the identification of a nonconservative AA to GC dinucleotide substitution in exon 8, which results in a lysine to alanine substitution at amino acid 232. Our objective was to determine the relationship between the K232A polymorphism with milk performance traits in the Iranian Holstein cattle. A 411 bp product including the K232A polymorphism was amplified and digested with the restriction enzyme *CfrI* to determine the genotypes for 206 Holstein cows. Allele substitution effects of the lysine allele for all traits in the first and second lactations were estimated separately as described by Falconer and Mackay (1996). The estimated allele frequencies were 0.66 and 0.34 for the alanine and lysine alleles, respectively. The relatively low frequency of the lysine variant in the Iranian Holstein population may be due to selection for milk yield in recent years. The association analyses showed positive effects of the lysine allele for fat and protein content traits, as well as for the fat yield in both lactations. In contrast, negative effects were found for milk and protein yield. It is important to note that recent studies have shown that the K232A polymorphism is not the only polymorphism underlying the QTL for milk production traits at the proximal end of BTA14 (Bennewitz et al., 2004) and therefore other markers in the region should be investigated.

ISAFG-P84

COMPREHENSIVE SNP ANALYSIS IN THE REGION RESPONSIBLE FOR ETECF4AB/AC RESISTANCE IN PIGS

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Approximately 170.000 pigs in Denmark succumb each year to infection by enterotoxigenic *Escherichia coli* (ETEC F4ab/ac). An effort to reduce the prevalence of this infection will thus have a huge impact on pig welfare and greatly diminish the use of antibiotic treatments in pig production. SNPs segregating with susceptibility/resistance identified by our group in intronic regions of MUC4 are presently used in selection programmes by the pig breeding industry. To elucidate the molecular mechanisms involved in ETECF4ab/ac infection in pigs, we have established a detailed haplotype map of the porcine candidate region 13q41. This region covers approximately 2.6 Mb as determined by comparative mapping to human chromosome 3. The family material used for the study has been established by crossing two wild boars and eight large whites resulting in 26 offspring in the F1 population, and F1 intercrosses producing 200 F2 animals. All animals were phenotyped using an adhesion assay. The 10 founders contained 18 informative chromosomes (12 susceptible and 6 resistances).

By a targeted approach, the 13q41 region was subjected to SNP screening mainly focusing on intronic sequences. A total of 22 genes were partially sequenced and SNPs were identified in GP5, CENTB2, APOD, MUC20, MUC4, ACK1, ZDDHC19, OST α , and PCYT1A.

Overall, 132 SNPs were discovered in the parental generation. This very detailed haplotype map of the SNPs serves as valuable resources for further fine mapping of the locus involved in ETECF4ab/ac adhesion and ultimately for the identification of responsible mutation.

ISAFG-P85

RELATIONSHIP BETWEEN EYE SIZE AND BODY SIZE IN 3-WEEK OLD CHICKENS FROM AN ADVANCED INTERCROSS LINE (AIL)

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Purpose

In humans, axial elongation is correlated with myopia progression, and eye size is related to height. Thus, identifying factors which influence ocular dimensions may provide insights into myopia aetiology. This study examined the relationship between eye size and body size in chickens in an F₁₀ generation from a cross between Layer (small body and eye size) and Broiler (large body and eye size) lines.

Methods

Chickens (n=509; age 3-weeks) from the AIL were assessed using in vivo high-resolution A-scan ultrasonography. Eye diameter and eye weight were measured after enucleation. Variations in eye size parameters that could be explained by body length (BL), body weight (BW) and head width (HW) were examined using multiple stepwise linear regression.

Results

Together, BL, BW and HW explained 44%, 33%, 51%, 51% respectively of the variation in axial length, vitreous chamber depth, eye diameter and eye weight. For axial length, BL, HW and BW were all significant independent explanatory variables ($P < 0.05$). One phenotypic standard deviation change in BL, HW and BW was associated with a 97 μ m, 44 μ m and 30 μ m change in axial length, respectively. BL showed strongest association with vitreous chamber depth and eye diameter, whereas BW was most strongly associated with eye weight.

Conclusion

Eye size is associated with Body size in these AIL chickens, suggesting that genes controlling body size also influence eye size. Thus, mapping the Quantitative Trait Loci that determine body size may improve understanding the genetic determination of eye size. The results also indicate that other factors are involved.

ISAFG-P86

IDENTIFICATION, VALIDATION AND GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) WITHIN BOVINE IMPRINTED GENES

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Genomic imprinting, a feature of at least 70 mammalian genes, results in the monoallelic expression from one of the two parental chromosomes. Many of these genes have been shown to play a significant role in foetal growth and development. In addition, it has been shown that many complex production traits in livestock are influenced by imprinted genes.

To screen for polymorphisms that may influence economically-relevant traits in cattle, we have: (1) assembled a panel of candidate bovine imprinted genes using the human and murine imprinted gene knowledgebase, and (2) used the bovine genome sequence to identify and validate putative DNA sequence variants within these genes. These polymorphisms are being genotyped in DNA samples collected from phenotypically-defined animals from beef cattle populations ($n \geq 270$) using custom-designed TaqMan[®] SNP assays. Statistical analyses using appropriate animal models will identify SNPs in imprinted genes that are associated with production traits and that can be formatted for high-throughput assays suitable for marker-assisted selection (MAS).

ISAFG-P87

GENETIC VARIABILITY OF THE *PRNP* GENE IN GOAT BREEDS FROM NORTHERN AND SOUTHERN ITALY

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Introduction

Scrapie is a fatal, neurodegenerative disease that occurs in sheep and goats. It is characterized by the accumulation in the central nervous system of an abnormal isoform (PrP^{Sc}) of a host-encoded cellular prion protein (PrP^C). In sheep, scrapie appears to be entirely an infectious disease in which genetic susceptibility, related to prion protein gene (*PRNP*) polymorphisms, plays an important role. Little is known about caprine *PRNP* variability and its involvement in goat susceptibility to scrapie; this study, determining the polymorphisms of the *PRNP* in goats from Northern and Southern Italy, contributes to fill this lack of information.

Methods

Genomic DNA, from 478 goats, was polymerase chain reaction (PCR)-amplified for the coding region of the *PRNP* gene and then sequenced.

Results

In total, 13 polymorphic sites were identified: G37V, T110P, G127S, M137I, I142M, I142T, H143R, R154H, P168Q, T194P, R211Q, Q222K and S240P (substitutions I142T and T194P are novel) giving rise to 14 haplotypes. Clear frequency differences between Northern and Southern breeds were found and confirmed by genetic distance analysis.

Discussion

Differences in allele distribution were found between Northern and Southern goats, in particular regarding the M142 and K222 alleles, possibly associated to scrapie resistance in goats; phylogeographical analysis supported the idea that Northern and Southern breeds may be considered as separate clusters. Moreover the finding of significant differences among allele distributions in Northern and Southern goats, especially if involved in modulating resistance/susceptibility, need to be carefully considered for the feasibility of selection plans for resistance to scrapie.

ISAFG-P88

AMBP AND TNC GENES EXPRESSION ANALYSIS AND THEIR QUANTITATIVE TRAIT LOCI (EQTL) RELATED TO WATER HOLDING CAPACITY TRAITS IN PORCINE MEAT

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A whole-genome scan was carried out to detect expression quantitative trait loci (eQTL) affecting drip loss and pH traits in porcine meat. A previous microarray study, based on phenotypic (high vs low drip loss) and genotypic (pQTL on SSC 5 and SSC 18) revealed 104 significant genes that were differentially expressed. Alpha-1-macroglobulin/bikunin protein precursor (AMBP) and Tenascin C (TNC) were selected for the expression abundance using quantitative real-time PCR. Transcript abundances were normalized by 3 reference genes (GAPDH, RPL32, and TBP) with different normalization approaches applied to the data. A linear regression analysis based on 122 markers on pig autosomes revealed 25 eQTL at the 5% chromosome-wide significance level (15 for AMBP) on SSC1, 2, 5, 8, 14, 16, 18, and (10 for TNC) on SSC1, 2, 3, 4, 5, 7, 13, 14, respectively. Three of which eQTL were found highly significant for AMBP ($p < 0.05$) and four eQTL were also found significant for TNC. The most significant eQTL was found located on SSC3 (with the best likely position at 45 cM) for AMBP and on SSC13 (with the best likely position at 117 cM) for TNC, respectively (F -values > 7). According to the positions of AMBP (SSC1q) and TNC (SSC1q 21.1-.3), the results showed eQTL for AMBP (SSC1 at 105-111 cM) and TNC (SSC1 at 176-192 cM) likely to be cis-regulated genes, whereas 23 eQTL shows trans-regulation. The detected eQTL in this study promotes AMBP and TNC genes as a direct candidate gene in meat quality traits.

ISAFG-P89

PERFORMANCE OF A GENOME-WIDE 7K PORCINE SNP CHIP

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Studies of the genetic control of complex traits by whole genome association studies require large numbers of informative genetic markers providing good genome coverage and a technology platform for high throughput genotyping. The emerging wealth of genome and transcript sequence data for the pig has provided a source and template for the discovery of single nucleotide polymorphisms (SNPs). The Illumina Infinium® BeadChip system provides a technology platform for high throughput genotyping.

We present an analysis of a custom 7K porcine Illumina Custom Infinium® BeadChip for SNP genotyping. The SNP content of this chip was derived from three sources: 1) in silico mining of porcine Expressed Sequence Tag (EST) data; 2) targeted re-sequencing of specific cDNAs; and 3) targeted re-sequencing of Sequence Tag Sites (STSs) corresponding to BAC end sequences (BES) selected from the pig physical map.

BeadChips were created for 92.6% of the sequences originally forwarded to Illumina giving a total of 6523 different beads.

A configuration template of genotype clustering was created using a dataset containing different breeds, replicate samples and nuclear family structures. Analysis showed that 85.6% of the SNPs could be genotyped successfully. Of these about 83% worked perfectly, 13% did not segregate in the material and 4% were only partly genotyped due to software limitations, low intensity values or presence of extra alleles. The remaining 940 SNPs were classified as “not working” but up to 25% of these could at least be partly genotyped if manual genotyping was possible.

The apparent large number of “not working” SNPs might mainly be explained by the method of SNP detection. While 69.7% of the predicted SNPs could be genotyped, 360 did not segregate in the data material. As this, however, is a rapid method for developing candidate SNPs for species currently lacking a complete reference genome sequence, the result fulfils the expectation and is therefore satisfactory.

ISAFG-P90

SCRAPIE GENE IS ASSOCIATED WITH COAT COLOUR OF SHETLAND SHEEP

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Susceptibility to scrapie is controlled by the *PrP* gene. Field observations in some sheep breeds suggest that some *PrP* genotypes are more common in sheep with certain morphological characteristics. The objective of this study was to investigate the relationship between *PrP* gene and coat colour and presence and number of horns in four UK native breeds. Records from 2529 Shetland (coat colour and hornedness), 103 Soay (coat colour), 855 Hebridean (hornedness), and 314 Manx Loghtan (horns) sheep were collected using postal surveys. Data were analyzed using a generalised logistic model assuming a logit link function using maximum likelihood method with SAS software. The model adjusted for effect of sex and age of the animal. There was a high significant association of *PrP* gene with coat colour in Shetland sheep. Grey katmoget animals had higher odds (1.8 to 5.3) of having ARR/ARR genotype than any other genotype. In contrast, animals with a gulmoget coat had significantly lower odds of having ARR/ARR genotype compared with other genotypes. Using the limited Soay data, no significant association of *PrP* gene with coat colour was revealed. Similarly, there was no significant association of *PrP* gene with presence or number of horns in any of the breeds studied. The association found between *PrP* gene and coat colour in Shetland sheep might be due to linkage with some *agouti* loci located on the same chromosome. This association could be utilized to select for scrapie resistance though selection intensity would be less compared with direct *PrP* alleles selection.

ISAFG-P91

CHARACTERIZATION OF *ITIH1*, *ITIH3* AND *ITIH4* AS CANDIDATE GENES FOR REPRODUCTIVE TRAITS IN PIGS

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An F₂ intercross was created from 3 Iberian boars and 18 Meishan sows with the aim to identify genes related to reproductive traits in pigs. The *inter-alpha-trypsin inhibitor heavy chains* (*ITIH1*, *ITIH3* and *ITIH4*) genes were selected as physiological candidate genes due to their participation in the stabilization of the *cumulus* extracellular matrix and maintenance of uterine surface glycocalyx during placental attachment. cDNA sequencing and quantitative PCR were performed to search for polymorphisms or variable gene expression that could explain differences in prolificacy of the F₂ sows. Sows were classified into 3 groups according to the number of embryos (NE) at the sacrifice day (30-32 days of gestation): low (NE<9, n=12), medium (10<NE>13, n=14), and high (NE>14, n=14). For each sow, two samples of uterus were analyzed: apical (proximal to the ovary) and basal (close to cervix). Data analysis determined significant differences in the expression of *ITIH1* and *ITIH3* genes between both uterus samples. In apical uterus, differential expression regarding prolificacy was only identified for *ITIH3*, where high and medium prolificacy sows had 2 times more *ITIH3* expression than low prolificacy sows. Several polymorphisms were identified in *ITIH1*, *ITIH3* and *ITIH4* genes. Four SNPs of the *ITIH4* genotyped in the Iberian x Meishan population were used for an association study with prolificacy traits. Results obtained showed a significant effect of these polymorphisms that determined an increment of 0.43 piglets born alive favourable to the haplotype of *Meishan* origin.

ISAFG-P92

NOVEL QTL ON THE SEX CHROMOSOMES OF PIGS INCLUDING THE PSEUDOAUTOSOMAL REGION

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A QTL analysis of pig chromosome X (SSCX) was carried out using a methodology which accounts for the unique features of SSCX and its pseudoautosomal region with the Y chromosome. A three-generation full-sib design was developed (Pietrain x commercial dam line). 386 animals from 49 families were genotyped for 8 markers covering SSCX. Phenotypic data of 315 F₂ animals were available for carcass characteristics measured at slaughter weight (140 kg) and chemical body composition, growth and feed intake throughout growth (30-140 kg). In the pseudoautosomal region (0 to 12 cM), QTL were identified for entire loin weight and lean meat weight of the loin. This region thought to be important for meiotic pairing and male fertility is therefore also involved in the genomic regulation of loin tissue growth. A suggestive QTL for daily feed intake (DFI) was identified, for which Pietrain alleles (cryptic) are associated with higher feed intake, unexpected for a breed known for its low feed intake capacity. At the telomeric end of the q arm, in a region where no QTL for fat tissue were identified, QTL for jowl weight and lipid accretion rate (LAR) 90-120 kg were detected. At this QTL, Pietrain alleles are associated with higher LAR. Suggestive QTL for chemical body composition at 30 kg body weight were found indicating that protein and lipid content are influenced by the sex chromosome. Information about the genomic regulation of growth and body composition can be used to improve carcass quality and feed efficiency using marker-assisted selection.

ISAFG-P93

WHOLE GENOME ASSOCIATION STUDY OF JOHNE'S DISEASE IN CATTLE

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Bovine Paratuberculosis, commonly referred to as Johne's disease, is a contagious bacterial disease estimated to be present in over 65% of US dairy herds and results in annual losses in the hundreds of millions of US dollars. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the bacteria responsible for Johne's disease. The purpose of this study was to identify novel bovine loci associated with Johne's disease. Cases were defined as Holsteins with tissue samples positive for MAP, whereas control samples were tissue negative for MAP. Genotyping was conducted on 111 cases and 102 control cows from four US dairy herds with the Illumina BovineSNP50 BeadChip, which contains > 50,000 single-nucleotide-polymorphisms (SNPs). Nine animals were removed from the analysis because of a no call rate of more than 10%, leaving 107 cases and 97 control cows. 46,966 SNPs were polymorphic and had a call rate of greater than 80%. Whole genome association analysis was conducted using the R statistical environment and the R package SNPAssoc. The study identified multiple SNPs associated with Johne's disease after stringent multiple testing correction (adjusted p-value < 0.05). The SNPs identified as being associated with Johne's disease included previously unreported regions near the centromere of chromosome one and the pseudoautosomal region of the X chromosome. To our knowledge, this is the first whole genome association study of Johne's disease in cattle and the first use of the recently released BovineSNP50 BeadChip for disease association.

ISAFG-P94

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN CANDIDATE GENES WITH MEAT QUALITY IN CROSSBRED CATTLE

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Tenderness, intramuscular fat (IMF) level and waterholding capacity are beef quality traits with moderate heritability, but which are difficult to improve by conventional selection. We aimed to test published SNP in candidate genes for association with these traits in crossbred cattle. Four published SNP in candidate genes for quality including protein kinase adenosine monophosphate activated gamma3-subunit (PRKAG3), growth hormone receptor gene (GHR), stearoyl-coa desaturase (SCD) and calpain I (CAPN1) were genotyped in 190 crossbred cattle using PCR-RFLP. Association analysis of genotype frequencies with phenotypic traits revealed a significant relationship between alleles at the PRKAG3 SNP and cook loss in *M. longissimus dorsi* ($p < 0.01$) and *M. semimembranosus* ($p < 0.05$). A SNP in the GHR gene was influential on IMF % in *M. semimembranosus* ($p < 0.05$) and sensory flavour ($p < 0.05$). A SNP in the SCD gene was associated with colour in *M. longissimus dorsi* ($p < 0.05$) and *M. semimembranosus* ($p < 0.005$) and the CAPN1 SNP was associated with sensory quality of the *M. longissimus dorsi* ($p < 0.05$). Previous research has detected an association of SNP variants in the PRKAG3 gene with pork quality but not to date in beef. These results provide evidence of the potential of SNP variants in candidate genes to influence diverse palatability traits in crossbred cattle populations. These markers have the potential to be incorporated into marker-assisted breeding programmes or meat management systems.

ISAFG-P95

BAYESIAN ANALYSIS OF EPISTATIC AND SEX-SPECIFIC QTL FOR BODY COMPOSITION IN CHICKENS

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Body composition is influenced by multiple genes, the interactive effects of these genes and the environment. The detection of these effects using traditional statistical methods is limiting thus Bayesian models have been used as a more effective alternative. Our initial studies on chickens focused on QTL with main effects on body composition but excluded epistatic effects. The goal of our current study is to investigate the presence of gene-gene and gene-sex interactions for body composition traits in chickens. We used the Bayesian model selection approach to map marginal, epistatic and sex-specific QTL for carcass composition and abdominal fat traits in a large (~695) F₂ population of chickens divergently selected for high and low growth. Strong epistatic effects (BF > 3) were detected on chromosomes 2, 5 and 7 for breast meat yield. Furthermore, strong QTL x sex interactions were identified on several chromosomes for fat weight. Pectoralis major was associated with significant main, epistatic and sex-specific QTL on chromosomes 2, 5 and 7. Chromosome 2 had significant main effects (BF > 2) on most traits. Some novel additive and epistatic QTL were identified in this study. Detecting gene-gene and gene-sex interactions has favourable implications for health and agriculture as it may improve the search for genes associated with obesity-related traits.

ISAFG-P96
POLYMORPHISM DETECTION STRATEGIES

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ARK-Genomics is a BBSRC funded facility designed to provide the UK scientific community with access to high quality and specialised research facilities, resources and experience to study the livestock genome. Genome mapping has been an integral part of this facility providing high through put microsatellite and SNP genotyping.

There are extensive microsatellite resources available for genetic studies of chicken, turkey, cattle and pigs giving full genome coverage. In addition resources for horse, sheep and duck are under development.

SNP typing is presently done at a low to medium through put level mostly using the ABI SNPlex kit where up to 48 SNPs can be typed in a single assay. Also in the near future the Illumina Golden Gate assay will be available for high through put SNP typing.

To demonstrate how microsatellite and SNP typing have been used we describe the following projects carried out by ARK-Genomics. An efficient SNP assay to detect the mutation K232A in the DGAT 1 gene of Guernsey Cattle. Using microsatellite genome scans to detect the loci of the mutations Talpid3, RDD and RGE.

Acknowledgement

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ISAFG-P98

STUDY OF VINCULIN EXPRESSION AND DISTRIBUTION IN OVARY OF SOWS

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The objective of this experiment is to study genes and proteins affecting reproductive traits in swine. We have generated an F2 cross between Iberian and Meishan pigs. These two breeds differ in their phenotypic performance for reproductive traits. RNA from ovary from sows on heat, 15 and 45 days of pregnancy have been hybridised in porcine oligonucleotide microchips to characterize changes in gene expression profile between different reproductive stages. Data analysis was performed with a home-development experimental software using Bayesian statistics. Significant differences in 281 genes ($p < 10^{-11}$) have been found between expression at different stages in ovary. One of these genes, vinculin, showed 100 times more expression on heat than at 45 days of pregnancy, so we chose that gene for immunohistochemistry and western blot analysis. For immunohistochemistry, we analyzed samples of sows on heat and 30 days of pregnancy, and we found that theca and granulosa cells showed stronger vinculin staining in all follicular development stages than ovary stroma, as well as epithelium. This major quantity of vinculin was higher in ovaries of sows on heat than at 30 days of pregnancy. These results are in agreement with those found in microarray data. For western blot, we used samples of ovary on heat, 15, 30 and 45 days of pregnancy. Significant differences appeared only between heat and 45 days of pregnancy, while pregnant sows showed no differences between band intensities. However, low sample number produced high standard errors in the pooled analysis.

ISAFG-P99

UNCOVERING THE PROTEOMIC AND GENOMIC BASIS FOR VARIATION IN PORK QUALITY

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Many important meat quality traits are at least moderately heritable and several genes influencing pork quality have been identified. Our aim was to examine the variability that exists in meat quality and produce proteomic profiles for a Large White x Landrace/Large White population. Thirty-one gilts were randomly selected and slaughtered under controlled conditions. Technological quality of *Longissimus dorsi* was measured post-slaughter (pH and temperature decline, conductivity, colour, drip loss, Warner-Bratzler shear force). Individuals divergent in important pork quality phenotypes (water holding capacity and intramuscular fat) have been identified. The important pathological conditions pale soft and exudative (driploss >6%, pH at 45 minutes <6.2) or dark firm and dry meat (driploss <3%, ultimate pH >5.8) were present in 7 individuals. Eight individuals diverged in intramuscular fat content (>1.2% versus <0.3%). Centrifugal drip samples were characterized for all 31 samples using 10% PAGE 1-D electrophoresis. One sample lane was dissected into 24 bands and in-gel digested. Peptides were extracted, purified and separated on a RP column interfaced to a nano-ESI linear ion trap mass spectrometer. Several proteins were identified, the vast majority of which are muscular isoforms of enzymes involved in glucose metabolism. Porcine *longissimus dorsi* centrifugal drip proteins will form the target for a search for novel biomarkers predictive of meat quality traits and associations with quality will be further characterized using 2-D electrophoresis. In parallel, muscle RNA samples from individuals divergent in these important quality phenotypes will also be characterised by microarray analysis and Real-time PCR.

ISAFG-P100

A SYSTEMATIC, DATA-DRIVEN APPROACH TO THE COMBINED ANALYSIS OF MICROARRAY AND QTL DATA.

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High throughput technologies inevitably produce vast quantities of data. This presents challenges in terms of developing effective analysis methods, particularly where the analysis involves combining data derived from different experimental technologies.

In this investigation, we applied a systematic approach to combine microarray gene expression data, QTL data and pathway analysis resources in order to identify functional candidate genes underlying tolerance of *Trypanosoma congolense* infection in cattle (see abstract by Agaba *et al*). We automated much of the analysis using Taverna workflows previously developed for the study of trypanotolerance in the mouse model.

We identified pathways represented by genes within the QTL regions, and subsequently ranked this list according to which pathways were over-represented in the set of genes that were differentially expressed (over time or between tolerant N'dama and susceptible Boran breeds) at various timepoints after *T. congolense* infection. The genes within the QTL that played a role in the highest-ranked pathways were flagged as strong candidates for experimental confirmation.

ISAFG-P101

DESIGN, PRODUCTION AND USAGE OF A 25K PORCINE LONG-OLIGO DNA MICROARRAY

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A porcine long-oligonucleotide DNA microarray has been designed, produced and used for global gene expression studies. The array consists of 27.648 features - 25.210 unique oligonucleotides representing approximately 19.000 porcine transcripts and 2.438 control features consisting of positive and blank spots, oligonucleotides for Arabidopsis genes, random sequence oligonucleotides and the Lucidea Universal ScoreCard.

The primary sequence source used for designing the oligonucleotides was generated in the Sino-Danish Pig Genome Sequencing Project including more than 0.8 million EST sequences and 3.8 million whole-genome shotgun sequences. Additional porcine sequences were retrieved from public domains yielding a total of approximately 6.2 million porcine sequences.

A pipeline was set up to generate input sequences for oligonucleotide design. For human gene transcripts, 15.700 sequences were generated and used as input for oligonucleotide design with SEPON. In addition, 5.500 transcript sequences for other mammals and 5.100 sequences for uncharacterized genes were generated and used as input to OligoArray. The oligonucleotide design programs produced a total of 25.210 oligonucleotides representing approximately 19.000 genes.

The 70-mer oligonucleotides were synthesised by Isogen Life Science, DNA Technology and TIB MOLBIOL, transferred to 384 well spotting plate, dried and resuspended in spotting solution followed by spotting on UltraGAPS slides using a ChipWriter Pro MicroArrayer with 48 x 946 MP2.5 pins. Slides were dried, UV cross-linked and stored in a vacuum desiccator until use. The 25K porcine oligonucleotide DNA microarray has so far been successfully used for global expression profiling of porcine liver tissues and several different muscle types.

COMPANY PRESENTATIONS

Company Name: APPLIED BIOSYSTEMS

FAST AND RELIABLE HIGH THROUGHPUT DNA ANALYSIS USING TAQMAN® SNP GENOTYPING ASSAYS AND THE BIOTROVE™ OPEN ARRAY SYSTEM

Phoebe White (Applied Biosystems, USA)

Single Nucleotide Polymorphisms (SNPs) are the most common type of genetic variation in the human, animal and plant genomes investigated so far, making them a rich source of markers for mapping and genomics projects. Although microsatellite markers are still widely used in plant and animal genetics, development and utilization of SNPs is increasingly becoming an attractive research focus because they are highly abundant and well suited for automated high throughput genotyping (Gupta et al, 2002). An efficient SNP genotyping technology is critical for typing, validating and screening SNPs on a routine basis. TaqMan® SNP Genotyping Assays from Applied Biosystems are the technology of choice for plant and animal SNP Genotyping. Custom TaqMan SNP Genotyping Assays are available for any possible SNP in any organism, and also for Multi Nucleotide Polymorphisms, and for insertions/deletions of up to six bases. Applied Biosystems is collaborating with BioTrove, Inc. to commercialize the OpenArray™ analysis platform for high-throughput genotyping applications. Applied Biosystems will develop and market custom-built arrays of TaqMan® SNP Genotyping Assays pre-loaded on BioTrove's OpenArray™ platform. This platform will integrate the ease-of-use, accuracy, and reproducibility of Applied Biosystems' TaqMan SNP Genotyping Assays with BioTrove's flexible high-density assay format, enabling researchers to rapidly perform high-throughput genotyping studies at a lower total cost compared to alternative commercially available methods.

Company Name: AFFYMETRIX

FUTURE POTENTIAL PRODUCTS FOR NON-HUMAN WHOLE GENOME ASSOCIATION STUDIES

Fiona Brew (Affymetrix, UK)

Company Name: EUROFINS MWG-OPERON

ULTRA-HIGHTHROUGHPUT SEQUENCING WITH THE ROCHE GENOME SEQUENCER FLX –TECHNOLOGY, APPLICATIONS AND SOFTWARE SOLUTIONS

Dr. Georg Gradl (Eurofins-MWG-Operon, Ebersberg, Germany)

End of 2006 we have established the GS 20 technology in our sequencing facility. Meanwhile we have upgraded to the next generation GS FLX. The GS FLX can deliver 400,000 sequence reads and 100 Mb of sequence data in a single run. The average read length is 240 bp. The next version with a read length of over 400 bp and over 500 Mb of sequence data per run is already announced for the second half of 2008. Sequencing with the GS FLX has many advantages over Sanger sequencing. The sequencing capacity is extremely high and the cost is significantly lower. Because there is no need for cloning, a very even distribution of the DNA shotgun fragments is achieved. Cloning gaps in AT rich regions of a genome can be avoided. In addition it is possible to sequence difficult GC rich areas without the problem of full stops. Scaffolding of assembled contigs is possible by a paired end sequencing strategy. This allows effective gap closing and finishing of genomes. Most important with highthroughput sequencing are powerful solutions for data management and analysis. Roche offers software for de novo assembly, paired end assembly, assembly with a reference sequence and mutation detection. We further developed proprietary software tools for strain comparison, mutation detection and several other applications. Our software is also optimised for handling difficult sequences with repeat regions. Another feature of our software tools allows using sequence barcodes for pooling of different experiments which increases the flexibility of our system considerably. With this strategy it is possible to sequence a large number of BACs in parallel or handle large pools of PCR fragments. The presentation will explain the technology and will give examples of such projects, like de novo and re-sequencing of genomes, cDNA sequencing and amplicon sequencing.

Company Name: SEQUENOM

ANIMAL FUNCTIONAL GENOMICS USING THE MassARRAY®System

Dr Robin Everts (Sequenom, USA)