Annotation of the immunity-related genes in the pig genome

. Immune Response Annotation Group (irag), Claire Rogel Gaillard, Christopher K. Tuggle, Jane Loveland, Jennifer Harrow, Elisabetta Giuffra, Joan Lunney, David Hume, Alan Archibald, Harry Dawson, et al.

P5053 Whole genome re-sequencing of Japanese Black cattle for SNP discovery in critical regions for *Marbling-2, CW-2* and *FMA*

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Marbling-2 and *CW-2* are QTL for marbling and carcass weight, and *FMA* is a genetic disease for Forelimb-girdle Muscular Anomaly. *Marbling-2* and *CW-2* have been mapped with progenies of Bull A, while *FMA* has been mapped with a progenies of Bull B. The critical regions for *Marbling-2, CW-2* and *FMA* were 4.6-Mb (BTA7), 0.9-Mb (BTA6) and 2.4-Mb (BTA26), respectively. To identify responsible/causative genes or SNPs from these relatively large regions, we performed whole genome re-sequencing of Bull A and B that are maternal half-sib.

A single-end library and three size-different paired-end libraries were prepared according to the manufacture's protocol, followed by sequencing with 36-bases reads using Genome Analyzer *II* (Illumina).

More than 3 x 10^9 reads were generated from Bull A and B, respectively, of which 11.5-M reads (412.3-Mb; x 52.03) and 11.7-M reads (415.3-Mb; x 58.96) were mapped to the target regions. Nearly 90% of the target regions were covered by ≥10 depth in both Bull A and B, and 21,136 (Bull A) and 19,598 SNPs (Bull B) were detected in comparison with Btau4.0 reference sequence. Based on criteria that Bull A is heterozygous for *Marbling-2* and *CW-2*, and Bull B is heterozygous for *FMA*, responsible or causative SNP will be searched among the SNPs.

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Resistance to disease and control of immune responses are complex processes that may be studied at several levels including individuals, populations, species, and phylogenetic groups. Indeed, there are mechanisms and responses shared between species and others that are species-specific, partly due to co-evolution of hosts and pathogens. Large scale sequencing of complex genomes provides unique opportunities to annotate large sets of genes and their structural variations for further comparative analyses. An automatically annotated draft assembly of the pig genome was released with Ensembl 56 in October 2009. The Immune Response Annotation Group (IRAG), comprising scientists working on resistance to disease and immunity in swine, was established to identify shared and species-specific immune responses and refine the annotation of immunity-related genes. A list of close to 1700 genes was drawn using information from gene ontology annotation (GO: 0002376 for immune system process), a core set of genes involved in host pathogen interplay (Jenner and Young, 2005), and gene sets under positive selection in humans (Barreiro and Quintana-Murci, 2010) and cattle. Manual annotation of these genes has begun using the WTSI Otterlace software. Alignments of genomic contigs with publicly available mRNAs, ESTs and proteins are analyzed to delineate exon and intron boundaries, thus providing a refined functional annotation of genes. Preliminary results have identified gene duplications and many new alternative splice variants for known genes. Our future challenge will be to identify the underlying biology specific to the pig species that can be identified from this major annotation effort.

P5055 Gene expression and pathways analysis of white blood cells from cattle orally challenged with Bovine Amyloidotic Spongiform Encephalopathy (BASE) one year post-infection

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Bovine Amyloidotic Spongiform Encephalopathy (BASE) is a recently discovered atypical form of BSE, transmissible to primates, and has been proposed as an equivalent of sporadic Creutzfeldt-Jacob Disease in man. Although transmissible, it is unknown whether BASE is acquired through infection or arises spontaneously. In the present study, the gene expression patterns of white blood cells (WBCs) from five cattle one year after oral BASE challenge were compared with negative controls using a custom microarray containing 43,768 unique gene probes.

A total of 140 genes were found to be differentially expressed between BASE and control animals with a log fold change of 1.5 or greater. Of these, 70% were up-regulated in the infected animals. Microarray data were then confirmed by qRT-PCR. The majority of the differentially expressed genes are related to immune functions, and several belong to the same pathways as revealed by KEGG analysis. In particular, BASE animals appear to have significantly modified expression of genes linked to the differentiation of subsets of immune cells, T and B cell development and activation, Natural Killer activity and inflammatory response. Samples from the same animals were examined by qRT-PCR at different time-points for the most interesting pathways to define when changes in gene expression are first observed and their kinetics.

The potential impacts of these gene expression changes will be discussed, together with their potential use as biomarkers and their contribution to better define PrP related gene functions in immune cells.