From *Jay Lush* to Genomics: Visions For Animal Breeding And Genetics

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15. Detection and Assay of Polymorphisms in Reproductive Gene Loci in a Commercial Broiler Breeder Population for Use in Association Studies

M.N. ROMANOV*, Y. MIAO**, P.W. WILSON[1], A. MORRIS[2], P.J. SHARP[1] & I.C. DUNN[1]

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A candidate gene approach has been adopted to determine if variance in reproductive traits in commercial broiler breeder stock can be associated with alleles of genes involved in reproduction. Alleles with favourable associations may be useful for marker assisted selection for improved reproductive performance.

Initial research focused on genes encoding for growth hormone receptor (GHR), gonadotrophin releasing hormone (GnRH) and its receptor (GnRHR), and neuropeptide Y (NPY), using published cDNA sequence information. Primer pairs designed for the genomic DNA sequence were used for Base Excision Sequence Scanning (BESS T-Scan™, Epicentre Technologies) analysis or bulk scanning with panels of restriction enzymes.

In the GHR gene, two linked polymorphic sites (A and B) were detected in intron 5. In a sample of 40 males from a pedigree broiler dam line, alleles T^+ and T^- at site A were estimated to have frequencies 0.67 and 0.33, whereas frequencies of alleles T^+ and T^- at site B were 0.33 and 0.67. A diagnostic test based on the presence or absence of *Eco*72I or *Nsp*I restriction enzyme sites was devised to distinguish the two alleles at site A in a PCR-generated DNA fragment from intron 5. This confirmed the genotyping carried out using the BESS T-Scan method.

A polymorphic region that contained a 4 base insertion was discovered at -746 bp in the NPY gene promoter. It was characterised by presence or absence of a *Dra*I restriction site, the allele frequencies being 0.22 and 0.78, respectively. About 65% (4 kb) of the GnRH gene sequence has been scanned in a broiler dam line, but no polymorphism has been found so far.

The BESS T-Scan methodology facilitates the process of identifying single nucleotide polymorphisms in the candidate genes. The alleles revealed are being used to establish possible associations with reproductive traits in a broiler dam line.

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16. Positional Candidate Cloning of a Gene for Familial Hyper-Cholesterolemia in Swine: an Animal Model for Informative Genomics of Cardiovascular Disease

J. HASLER-RAPACZ[1], H. ELLEGREN[2], M. FORNEY-PRESCOTT[3], J. VON-LINDEN-REED[3], A-K. FRIEDOLFSSON[2], B. KIRKPATRICK[1], S. KIRK[1], L. ANDERSSON[2] & J. RAPACZ[1]

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Elevated blood plasma cholesterol (hypercholesterolemia) is one of several risk factors for cardiovascular disease (CVD) in man. Experimental breeding of pigs expressing uncommon lipoprotein genotypes, derived from 37 breeds and/or groups of pigs, resulted in a gene pool of animals with plasma cholesterol from 130 to 600 mg/dL. Genetic dissection of quantitatively diverse dyslipidemia and hypercholesterolemia phenotypes led to the isolation of a monogenic cholesterol phenotype (FH-r), that is inherited in the recessive (r) manner.

A genome scan mapped the FH-r locus close to the centromere of chromosome 2. Comparative mapping showed that this region of pig chromosome 2 shares homology with a part of human chromosome 19 that harbours the low density lipoprotein receptor (LDLR) locus and therefore suggested LDLR as the prime candidate gene for FH-r.

Cloning and sequencing of hepatic LDLR cDNA from two FH-r/r and two normal (N/N) animals revealed a mis-sense mutation at codon 84 (cgc to tgc), that caused a non-conservative amino acid substitution (Arg to Cys). All hypercholesterolemic animals (r/r) were found to be homozygous T/T, whereas all normolipidemics (N/r) were heterozygous C/T. The C84 mutation co-segregates invariantly with hypercholesterolemia, which strongly suggests that this mutation is responsible for the observed hyperlipidemia. The coronary lesions from pigs expressing the FH-r phenotype show extensive lipid-rich necrotic cores and fibrosis, although they are not stenotic.

The FH pigs represent a key animal model for the study of familial hypercholesterolemia associated with atherosclerosis, and may be the ideal model to continue the search for genes that predispose humans to cardiovascular disease.