

Genetic improvement of porcine health

At the recent IPVS Conference in Copenhagen, Merete Fredholm from the Danish Royal Veterinary and Agricultural University gave a keynote address on the genetic improvement of porcine health. In this article we will focus on the main points of her paper.

In the period from 1945 to around 1970 the primary breeding goal in the Danish breeding system was meat content. The development of more sophisticated computational and statistical methods had a great impact on the efficiency of the selection programs used in animal breeding.

In modern breeding programs a number of breeding goals, separately assigned with an economic weight, are combined for selection in the individual breeds. The most important selection objectives are production traits such as growth rate, food conversion, lean weight and litter size. The growing understanding of the genome and accompanying technological innovations has opened up possibilities for direct identification and selection of animals carrying the best genes.

Most traits of agricultural importance in livestock are influenced by variation of several or many different genes. The effects of these genes combine to produce continuous (or quantitative) variation between animals in traits such as growth rate, litter size and susceptibility to disease.

For traits that are difficult or costly to measure, such as disease resistance, it is difficult to identify which animals carry the best genes simply from their performance. In such cases, the ability to directly identify animals carrying valuable genes using DNA based tests will provide a much improved means of selecting superior animals.

Stress susceptibility

Historically the selection for aspects of lean growth has been associated with a high frequency of stress susceptibility. Stress susceptibility, as defined by halothane sensitivity caused by the HAL mutation (n), is a very interesting example of a gene with both positive and negative effects that also has an impact on both a complex or quantitative and a simple or qualitative trait.

Pigs that are homozygous for the mutation have a high frequency of death caused by stress, produce meat of low quality but they are leaner. Thus, using traditional phenotypic selection on its own, it was not possible to

eliminate this deleterious gene. In 1991 researchers showed that the gene responsible for stress susceptibility was the ryanodine receptor gene and they identified the point mutation.

This discovery allowed a direct DNA test to be developed to discriminate accurately between all three genotypes (nn, Nn, and NN). This has subsequently been widely adopted by pig breeding companies and it has been estimated that the removal of the HALn allele from a breeding line reduces the death through porcine stress syndrome from between 4-16 per 1,000 pigs to zero. At the same time this is accompanied by a substantial improvement in meat quality.

Whole genome scan

The general identification of genes underlying quantitative traits requires a lot of effort and is much more time consuming and costly compared to identification of genes underlying simple traits. In both cases, however, the general approach is to perform a whole genome scan aiming at scanning the entire genome for regions which are associated with variation in the traits of interest. This approach relies on the phenomenon of genetic linkage that allows individual markers to be used to follow the inheritance of a segment of a chromosome.

The segregation of markers spaced at intervals through the genome is studied together with the segregation of the traits of interest. Such studies result in the identification of quantitative trait loci (QTL) which are regions of the genome in which genes influencing the trait(s) of interest is located.

The results from all genome scans performed in the pig have been collected in the Pig Quantitative Trait Loci Database (PigQTLdb). This database reports on 1,263 QTL representing 236 different traits. Only six of these are listed under the trait description of 'disease resistance' and specific knowledge leading to the establishment of DNA tests has only been obtained with respect to two disease traits.

To illustrate how genome scans are con-

ducted, Merete highlighted two ongoing projects. Within the EU project 'Control of Porcine Circovirus Diseases (PCVDs): Towards Improved Food Quality and Safety' one of the work packages is focusing on molecular genetic characterisation of the host response to PCVD/PMWS infection and aims to identify the gene(s) responsible for susceptibility/resistance in the pig.

The evidence for genetic aetiology of the disease is circumstantial as both field studies and experimental infection studies have shown that in a pen or among a litter only some individual pigs exhibit clinical signs of PCVD/PMWS and certain boar lines have been reported to produce either a higher or lower number of susceptible offspring.

A total genome scan is conducted in families in which susceptibility is segregated in order to identify regions of the genome harbouring genes that influence susceptibility/resistance towards PCVD. Following identification of candidate regions expression studies of the genes will be conducted and fine mapping using additional markers will be used to identify potential allelic differences between susceptible and resistant animals.

Worm study

Merete and her colleagues are also involved in a study on *Ascaris suum*. Infestations by this parasite are often present with high prevalence in the pig population. An extremely disparate distribution of *Ascaris* with a few pigs carrying the large majority of the adult worm population is characteristic for both natural and experimental infections indicating that host genetics is important with regards to worm burden.

In order to examine the association between host genotype and resistance to parasite infections, 195 piglets have been produced after artificial insemination of 19 Landrace/Yorkshire sows with semen, from 13 Duroc boars. The resulting piglets were reared outdoors and they received *A. suum* eggs for 14 weeks.

Faecal samples were taken at regular intervals and examined for worm eggs. The results of these egg counts indicated that there are clear differences in parasite load, between litters, which most likely are related to host genotype. Linkage analyses using

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genetic markers are now underway.

These analyses will focus on candidate regions identified in the human genome in a study of *Ascaris* infected humans in Nepal. Genome scan has also been performed in order to shed light on the genetic background for a variety of different inherited defects such as anal atresia, artrogryposis multiplex congenital and hernia.

As mentioned, DNA based tests have been established for two disease traits. These traits are susceptibility/resistance towards *E. coli* F18 and *E. coli* F4. Both traits are controlled by a single gene.

Adhesion of F18 and F4 fimbriated *E. coli* to small intestinal brush border membranes causes oedema disease/post-weaning and

neonatal diarrhoea in piglets respectively. These diseases are of considerable economic significance in the pig breeding sector, thus, it is desirable to eliminate genetically susceptible pigs from breeding herds.

The gene potentially controlling expression of the *E. coli* F18 receptor ($\alpha(1,2)$ fucosyltransferase, FUT1) has now been characterised. A polymorphism resulting in the amino acid substitution at position 286 has been shown to segregate together with susceptibility/resistance towards *E. coli* F18.

The DNA test established for discriminating between resistant and susceptible animals has been patented by PIC. No information on the outcome of the selection against the disease has yet been published.

The gene that potentially controls the expression of the *E. coli* F4 receptor (mucin 4, MUC4) has also been characterised and a DNA test has been established. The test is available through commercial genotyping laboratories. In 2003 the Danish breeding company DanAvl started breeding efforts in order to breed resistant finishers.

At the start of the program the frequency of resistant animals in the Landrace and Yorkshire population was 1% and 20% respectively and comparable numbers were 88% in Duroc and 100% in Hampshire.

After approximately two years of selection, the frequency of resistant animals in Landrace has increased to approximately 11%.

Preliminary data indicates that the breeding program influences the breeding index in a positive manner.

During recent years a lot of new resources of importance for genetic research have been generated. Sequencing of the porcine genome has been initiated, thus the amount of sequence information is growing rapidly.

Through the Sino-Danish pig genome sequencing project approximately 3.84 million shotgun sequences (0.66X coverage) of the pig genome have been generated together with approximately one million sequences from coding regions.

Furthermore, 15 high throughput technologies are under development for genotyping and expression studies.

Thus, it is becoming easier to get from the knowledge of the phenotype of a trait from the characterisation of the genotype behind it. Knowledge of the genetic and phenotypic relationships between traits will enable evaluation of more efficient selection strategies.

Given that it is difficult and costly to measure disease traits it would, of course, be of great advantage if it was possible to identify a genotype that was superior with respect to disease resistance in general.

The challenge for breeding is to select superior animals and reduce the genetic variation on one side and to conserve genetic variation at loci that are involved in immunity.

Studies both of the innate and cellular immune responses indicate that it is of great importance to maintain a high degree of polymorphism in many genes involved in immunity rather than breed for a specific genotype.

More studies of host pathogen interaction together with more information about the individual components of the immune system will be of benefit to sustainable animals breeding.

In addition, specific knowledge of host pathogen interaction will be of immense importance in respect to development of more efficient vaccines. Although collection of good performance information will remain crucial for the foreseeable future, high density marker information will certainly complement or even to some extent replace pedigree recording.

The future of animal breeding is genomic breeding where all specific information concerning the genetic and phenotypic information is included together with marker information covering the whole genome. ■