What’s New in Swine Molecular Biology

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INTRODUCTION

Techniques from the field of molecular biology allow swine geneticists to monitor their breeding programs at the DNA level. Genes that affect important economic traits can now be identified in DNA isolated from a few plucked hair root cells. There are 2 basic approaches when using marker assisted selection (MAS) to generate superior genetic stock. MAS can be based on ‘identified’ genetic mutations or on linked Quality Trait Loci (QTL). Linked QTLs require pedigree analysis to correlate inheritance patterns with the physiological data since the exact genetic cause of the physiological variation is unknown. Tests based on ‘identified’ genetic mutations allow swine breeders to follow a genetic trait independent of family history. Early detection saves time and housing costs and also identifies animals that carry hidden genes that can later affect offspring or meat quality traits. Great progress has been achieved lately in the identification of genetic mutations that have a major influence on commercial pig production and quality traits. These tests are especially useful in that they identify mutations that can noticeably change the ultimate physiological qualities of the animal, regardless of their environmental or pedigree background.

Since most physiological traits such as litter size and growth rates are influenced by the additive effect of many separate genes and the environment, breeders cannot rely solely on a few genetic tests to accurately predict the ultimate adult physiological qualities of individual pigs at birth. The best scenario would add information from these tests to the existing traditional breeding programs. The genetic tests mentioned in this article (Table 1) only refer to a few of the almost 2000 genes mapped in the porcine genome. For a more in-depth review, please refer to the book by Rothschild and Ruvinsky called Genetics of the Pig (CAB International press), or the pig genome web sites at http://www.ri.bbsrc.ac.uk or http://www.genome.iastate.edu/

The RN gene.

Research conducted at the Agriculture Canada- Lacombe Research Center has focused on improving pork quality and now we are attempting to assign genetic tests to help swine breeders improve the quality of Canadian pork. Meat quality traditionally has been a difficult trait for breeders to follow since it is measured post mortem. Recently we checked if retail pork cops could be used to measure the frequency of the Rendement Napole (RN) mutation in the commercial pig population. The RN mutation causes an ~70% increase of muscle glycogen, which causes a paler color, poor ham quality, and higher drip loss. Our research findings showed that >25% of fresh pork products on retail shelves in western Canada had excessively high glycogen (Meadus & MacInnis, 2000). Using a genetic test to identify the dominant white allele of the c-kit receptor gene
(Moller et al. 1996), we also found that ~80% of the high glycogen pork products were from the commercial white pig population and not the purebred Hampshire pig population as previously assumed. The frequency of the RN gene mutation probably arose in the pig population because it can increase the loin eye muscle yield. Work is now progressing to test if the newly discovered PRKAG3 mutation (Milan et al. 2000) can be used as an accurate genetic test on pigs suspected of carrying the RN mutation. The PRKAG3 mutation inhibits a muscle specific adenosine monophosphate-activated protein kinase from breaking down storage glycogen. This inhibition is caused by a single DNA, G → A base mutation in exon 3 of the PRKAG3 gene.

The marbling fat HFABP and AFABP genes.
Another important issue affecting pork quality is the amount of marbling fat intra-dispersed in the lean meat. Blind taste panel work has determined that pork loins must have at least 2% fat in the lean meat or else the cooked product will be too dry and tasteless. A separate survey performed at Lacombe found that more than 85% of retail pork loins had less than 2% marbling fat. We have recently checked 2 new DNA tests reported to help identify genes that improve marbling fat content without increasing backfat. The promoter mutation in the Heart Fatty Acid Binding Protein (HFABP) gene and the hypervariable microsatellite in intron 2 of the Adipocyte Fatty Acid Binding protein (AFABP) gene were reported to increase marbling fat approximately 1% in Duroc and Meishan breeds, respectively. Our experiments showed that the HFABP and AFABP gene tests only explained 0.3% of the variation in marbling fat content and that new genetic markers are needed. Currently we are investigating the combined effect of dietary repartitioning agent conjugated linoleic acid (CLA) (Dugan et al. 1999) and the PPAR gene family on controlling marbling fat content.

The Halothane or RYR1 Mutation.
One of the first widely used genetic tests used by the pork industry was for the Ryanodine Receptor mutation (RYR1), also known as the Halothane gene (Hal) or Malignant hyperthermia gene (MH). The Ryanodine receptors are calcium channels which can be activated by a wide variety of ligands including, Ca2+, caffeine, calmodulin, ryanodine, and the anaesthetic Halothane (Xu et al., 1998). The HAL mutation causes the receptor to become hyper-sensitive causing the muscle to twitch rapidly, increasing carcass muscle mass and reducing fat content. The mutation became widespread in the commercial pig population because of an aggressive selection program for reduced backfat and increased lean yield. However, the HAL mutation was also found to increase the frequency of both porcine stress syndrome (PSS) and pale soft exudative pork (PSE). PSS describes animals that would lose consciousness or die from transport or environmental stress. PSE type pork, described as pale and watery is associated with a very rapid drop in pH post-mortem and extreme muscle post rigor contraction. The HAL-1843 genetic test is marketed by Innovation Foundations Inc of the University of Toronto and can detect heterozygous animals carrying one copy of the Hal mutation. Although carriers of the Hal mutation are very susceptible to producing PSE pork, the genetic mutation accounts for only 10% of the total PSE meat produced in Western Canada (Murray & Johnson, 1998).
The IGF2 gene.
Growth hormone (GH) also known as somatotrophin (ST) has long been known to have a significant beneficial effect on carcass quality in the swine industry. The level of GH can be artificially increased by injection of recombinant porcine somatotrophin (pST) which was made using molecular biology techniques to clone GH cDNA and express it in a bacteria to generate a cheap source of synthetic hormone. To promote growth, GH does not act directly on muscle cells but is instead an intermediate in a series of hormonal signaling events. This includes the PIT1 (pituitary specific transcription factor), growth hormone releasing hormone (GHRH), GH, the insulin like growth factor-1 (IGF-1), and eventually feedback inhibition by somatostatin. Altering any one of these endocrine genes or their respective receptor genes can modify growth. In a recent elegant experiment, researchers (Draghia-Akli et al. 1999) were able to increase growth rate 37%, by a simple intermuscular- injection of recombinant GHRH DNA. An attached muscle specific actin promoter controlled the injected GHRH DNA. The injected muscle cells produced recombinant GHRH protein, which was then secreted into the blood stream to stimulate GH production by the pituitary gland.

One of the intermediates in the GH endocrine pathway is IGF-2. A G→A mutation in exon 2 of IGF2 has been recently found to increase lean yield by ~2.7% in the Peitrain breed of pig which is recognized for its muscularity and leaness (Nezer et al.1999). The IGF2 mutation’s beneficial effect on the carcass is only observed if the gene is paternally inherited (from the sire). Normally, a very detailed pedigree with extensive offspring data would be needed to identify and follow carriers of the beneficial but complicated mutation. However, with genetic testing, boars of unknown heritage can now be rapidly identified and saved from castration for future breeding programs. It should be noted that the RYR1 mutation should be also tested when checking for the IGF2 in Peitrain boars, due to the high frequency of the RYR1 mutation in this purebred population. The combined effect of the RYR1 and the IGF2 mutations are believed to account for 50% of the Peitrain versus Large White breed difference in muscularity and leaness (Nezer et al. 1999).

Meat quality versus lean yield.
Meat quality is often compromised by an overly simplified selection for lean yield. In swine, this has unwittingly lead to co-selection for detrimental genes such as the RYR1 and RN. This is a common problem to breeding programs in meat producing animals as shown in Table 2. Animals such as the Callipyge lambs or the RYR1 swine might initially get a better return on carcass value at the packing plants but the overall industry will suffer once the consumers stop buying the product.

To address consumer concerns, pork producers will need to develop animals that produce more than just a cheap source of protein. The latest area of research at Lacombe is to improve the nutritional quality of pork products by selection for animals with higher levels of vitamin C and E and more unsaturated fat levels. It is expected that many of these nutritional improvements will improve the storage life, flavor and consumer perceptions of pork products as a healthy food source.
The field of molecular biology assists the pork industry in many other areas besides selective breeding. Using recombinant DNA techniques, researchers have developed new safe growth promotants, benign vaccines for disease resistance, cheaper more efficient feed enzymes, and even more environmentally friendly animals such as the transgenic Phytase pig developed at the University of Guelph. Swine molecular research has also an enormous potential in human medicine, possibly one day leading to a line of pigs designed for emergency donor organ transplants into humans.

A molecular biology refresher

- Pigs have 19 pairs of chromosomes (18 autosomes and 2 sex chromosomes XX or XY).
- There are estimated >100,000 genes in the pig genome spread out over 3.3 billion bases of DNA.
- A gene’s location (loci) on a chromosome has 2 alleles (1 paternal and 1 maternal).
- Multiple versions of same gene in a population are called allelic variants.
- Each trait can be controlled by more than 1 gene and each gene can control more than 1 trait.
- DNA is transcribed into RNA and RNA is translated into a protein.
- Gene tests identify a mutation.
- Genetic markers need to be only linked to a mutation
**Table 1. Breakthroughs in swine genetic tests.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Trait</th>
<th>Predominant Breed</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>RYR1 (ryanodine receptor, Halothane)</td>
<td>Porcine stress syndrome (PSS), pale soft exudative (PSE) pork</td>
<td>Large White, Pietrain</td>
<td>Fujii et al. 1981.</td>
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<tr>
<td>c-Kit Receptor</td>
<td>Dominant white gene blocks coat color to stop black hair follicles from staining cuts of bacon.</td>
<td>Large White</td>
<td>Moller et al. 1996.</td>
</tr>
<tr>
<td>Estrogen Receptor</td>
<td>Linked to increased litter size</td>
<td>Meishan, Landrace</td>
<td>Rothschild et al. 1996.</td>
</tr>
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<td>HFABP</td>
<td>Marbling fat content</td>
<td>Duroc</td>
<td>Gerbens et al. 1999.</td>
</tr>
<tr>
<td>IGF2 (insulin like growth factor 2)</td>
<td>Increased Lean Yield ~2.7%</td>
<td>Peitrain</td>
<td>Nezer et al. 1999.</td>
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**Table 2. Meat quality verses lean yield.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Effect on Lean Yield (one allele)</th>
<th>Effect on Meat quality</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Myostatin gene in Belgian blue and Piedmontese Cattle.</td>
<td>Increased muscle mass ~20%</td>
<td>Marbling reduced 60% and problems with calving.</td>
<td>McPherron et al. 1997.</td>
</tr>
<tr>
<td>Callipyge gene in sheep.</td>
<td>Increased muscle mass ~30%</td>
<td>Toughness increased 40% to 140%.</td>
<td>Cockett et al. 1996.</td>
</tr>
<tr>
<td>Halothane gene in pigs</td>
<td>Increased lean yield ~2%</td>
<td>Pale soft exudative (PSE) pork increased 200%</td>
<td>Fujii et al. 1991.</td>
</tr>
<tr>
<td>RN gene in pigs</td>
<td>Increased lean yield ~3%</td>
<td>Cooking loss increased 200%</td>
<td>Milan et al. 2000.</td>
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</tbody>
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References.


Rothschild MF, Ruvinsky A (Eds.) (1998) The genetics of the Pig. CAB International.
