Strategic plan for use of molecular information for the swine industry

Canadian Centre for Swine Improvement

1. Main conclusion from the first three tasks

1.1 Available genes and markers

During the last decade, a large number of functional mutations, candidate genes and QTLs have been identified and mapped. We evaluated these mapped genes and QTLs in terms of statistical significance levels, size of effects and traits they affect and how well different studies mutually confirmed from each other, as well as their potential economic benefits from using the genes and QTLs. We adopted a stepwise-priority strategy and focused first on the most promising genes and QTLs. Some of the most promising genes and their potential usefulness will be described in this section.

1.1.2 Promising candidate genes and functional mutations

Swine gene mapping studies are characterized by the large number of identified candidate genes and functional mutations in addition to the QTLs mapped from interval mapping studies. In some other species such as dairy cattle, the gene mapping studies and their applications in breeding programs mainly focused on QTLs detected since few candidate genes and functional mutations have been mapped. The candidate genes can be functional candidate genes and positional candidate genes. Most of candidate genes in swine detected so far are functional candidate genes, found in association studies according to their functions in physiological process. Candidate genes usually have much larger effects than an overage quantitative gene. For example, Insulin-like growth factor 2 (IGF2) gene determines about 25% of lean yield variation of carcass. Also, the positions of candidate gene usually are quite precisely mapped on the genome. Their physical locations on chromosome and polymorphic status are often known exactly. Therefore, the information of candidate genes is very valuable in swine.

Candidate genes have been first applied in swine selection programs. Halothane gene and RN gene are among those typical examples of genes applied in animal breeding. Clear improvement of meat quality has been achieved worldwide by selection of Halothane gene and RN gene. Improving meat quality is usually difficult by conventional selection methods based on phenotypes because most meat quality traits can only be measured after slaughter. Therefore, only phenotypes of relatives can be used to estimate breeding values of breeding animals, which limits accuracy of selection. Also, some other factors can complicate the meat quality evaluation and improvement. Meat quality measurements may vary with different ways of hog raising and handling, as well as meat processing and cooling after slaughter. It is difficult to measure the genetic potential of meat quality traits objectively. For example, PH values and water-holding capacity of pork are usually influenced by pre-slaughter handling and after-slaughter processing. Marbling scores can be affected by fat-type and meat colour. More saturated fat may results in higher marbling score at given intramuscular fat content. Dark muscle may leads to higher marbling score at the same intramuscular fat. Therefore, meat quality has been identified as a trait category especially suitable for marker-assisted selection (Muir 2002; Dekkers et al. 2001). Fortunately, a number of candidate genes, functional mutations and QTLs have been identified and can be used as assistance in selection (Table 1 and Table 2).

With the increase of pork leanness, intramuscular fat (IMF) content is attracting increasingly more attention in swine breeding because it is positively correlated with the taste and eating attributes of pork, such as juiciness, tenderness and flavour. North American markets as well as the Japanese market are increasingly demanding more IMF content in the marketed fresh pork. The survey results have indicated that there is a significant proportion of pork loin which is under consumers' acceptance threshold (CDPQ 2003). Several candidate genes have been reported to be associated with intramuscular fat content, such as LEPR (Leptin receptor) and HFABP genes. HFABP gene is especially useful for improvement of intramuscular fat because it offers the opportunity to increase IMF content without affecting backfat thickness, therefore having fast growing leaner market hogs with desirable level of IMF content. HFABP gene is well mapped and determines up to 0.4% IMF, which is about 1/5 to 1/7 of average IMF of market hogs. The more details about this gene are given in the report entitled "Heart Fatty Acid Binding Protein Gene for Improving Meat Quality".

Calpastatin (CAST) gene and Calpain gene are associated with meat tenderness of pork, very promising for meat quality improvement. Calpastatin (CAST) is an endogenous inhibitor of Calpain, which is a calcium-dependent protease and plays an important role in post-mortem tenderization of skeletal muscle due to the degradation of key myofibres and related proteins. Significant association has been detected between *hpy1881* polymorphic locus in CAST gene and meat tenderness ($p<0.05$) (Ciobanu et al. 2002). CAST gene has also found to be associated with juiciness ($p<0.05$), firmness ($p<0.0005$) and average instron force ($p<0.05$) (Ciobanu et al. 2002). Calpain gene was reported by Parr et al. (1999) to be associated with pork tenderness as well. This gene produces an enzyme which weakens muscle fibres, thus increasing tenderness during the post-mortem aging process. Tenderness is an important quality attribute that pork consumers desire. However, currently there are few direct selection tools that can be utilized to improve this important trait of eating quality in swine. Calpastatin and Calpain genes can be applied to the selection of these eating attributes of pork.

Gene tests for CAST gene and Calpain gene have been widely used in beef cattle improvement. The beef CAST gene test, patented by the Australia-based company Genetic Solutions Pty Ltd. as trade mark *GeneSTAR*® *Tenderness*, has been commercially used for beef genetic improvement. The beef *m*-Calpain gene has been patented by US Meat Animal Research Centre as trade mark *Tender*GENE™, which has even larger effect on tenderness than CAST gene. The most extreme genotypes on CAST locus differ by about 1.8 lb of WBSF (Warner-Bratzler shear force).

IGF2 gene is a very promising gene and of large economic importance. This gene determined around 25 % of variation of lean yield percentage of pork (Nezer et al. 1999; Jeon et al. 1999). A recent study of Laere et al. (2003) mapped a G-A single nucleotide mutation that can add 3-4% more lean meat on hogs. IGF2 gene is an imprinting gene. The genes from boars should show the full effect on progeny, regardless of the sows' genotypes. Use of homozygous terminal sires in producing hogs should be able to increase the lean-yield uniformity of hogs because a sire, especially AI sire, can produce a large number of progeny and the Dams' IGF2 gene will not cause any phenotypic variation in progeny. Uniformity of the hogs' leanness is of large economic importance. The application of imprinted loci opens new perspectives for crossbreeding, which is common practice in commercial herds. Imprinted genes could further accommodate differentiation between sow lines, which are required to have optimal body composition to support their reproductive performance, and boar lines, which ensure high lean yield pork.

The use of IGF2 gene to increase lean yield and the uniformity of pork carcasses was confirmed by the field trial of Gentec, a Belgium-based company. Gentec selected a group of homozygous terminal sires with high lean allele at IGF2 gene. Commercial hogs from the selected sires were compared with those from unselected sires. The results confirmed that hogs from selected boars were leaner and mores uniform compared to those from unselected boars. Backfat thickness was reduced by 0.23 cm (0.09 inches). Average lean yield percentage was increased by 1.98%. Lean percentages in ham, loin and belly were increased by 0.31%, 0.43% and 0.92%, respectively. The variations of lean yield percentage, measured as the coefficient of variation, were reduced by 25% on average. IGF2 gene has no effects on birth weight, abdominal fat percentage and daily gain. The investigation also showed that IGF2 gene can be used for increasing the uniformity and carcass leanness in market hogs without influencing meat quality.

The selection for leaner carcass demanded by packing industry and consumers may conflict with the sow longevity and lead to increase the replacement costs of sows in swine production. IGF2 gene provides a possibility to solve the conflictive problem. The imprinting mechanism of this major gene could be used for producing commercial hogs with required leanness from fatter dams since only the favourable alleles from homogenous sires at IGF2 are expressed in progeny.

There are some other mapped functional mutations and candidate genes that are also very promising (e.g. Table 1). The opportunities are being investigated for employ these genes for swine genetic improvement or commercial application.

Table 1 Candidate genes and functional mutations for meat quality

Gene	Chromosome	Effect	Source
RYR1	6	Lean growth and porcine stress syndrome (PSS) and pale soft exudative pork(PSE)	Fuji et al, 1991
RN	15	Increased muscle glycogen leading to higher cooking loss.	Milan et al. 2000
HFABP	6	Intramuscular fat content	Gerbens et al. 1997
CAST	2	Meat tenderness	Ernst et al. 1998
FOS	7	Percentage of white fibre and fibre diameters	Reiner et al. 2000, 2002
AFABP	4	Intramuscular fat content	Gerbens 1998; Meadus 2000
LPTR	6	Intramuscular fat content	Ovilo 2002
MC5R	6	Meat colour, tenderness and intramuscular fat	Kim et al. 1999
PPAR*g*	13	fatty acid compositions in fat	Emnett et al. 2000
Calpain	2	pork tenderness	Parr et al. 1999
SLC2A4/ GLUT4	12	drip loss, colour and loin marbling	Grendflek et al. 2002

1.1.2 QTLs for traits of economic importance

A number of QTL mapping experiments have been conducted. These studies cover a large range of traits with varied chromosome regions. Following are some example of the QTLs for traits that can be useful in swine improvement.

Table 2. Identified QTLs for the traits of economic importance

Trait	Variance explained (%)	Chromosome location	Source
Meat color	10.1	12	Marlek et al. 2001
	4	17	Ovilo et al. 12002
	10.8	4	
Meat PH value	5	5	Marlek et al 2001
	4-6	15	
Intra-muscular fat	18.4	6	Olivo et al. 2002
Juiciness	8.3	17	Malek et al. 2001
Growth	15.6	1	Paszek et al. 1999
	16.1	4	Paszek et al. 1999
Backfat thickness and Abdominal fat	2-20	2 and 4	Andersson et al. 1994
			Nezer et al. 1999
			Knott et al. 1998
			Jeon et al. 1999
	26%	6	Ovilo et al. 2000
Litter size	3	11	Cassady et al. 2001
Ovulation rate	3-4	3, 4, 7, 8 and 9	Bidanel et al. 2001

1.1.3 Single nucleotide polymorphism

Single nucleotide polymorphism (SNP) is a single base substitution in a DNA sequence. A SNP variation comes about when a single nucleotide is replaced by one of the other three nucleotides. A SNP can be a functional mutation that results in phenotypic variations, and can serve as biological marker for pinpointing Mendelian genes such as disease genes or quantitative trait genes of economic importance. It represents a very promising technology for gene mapping researches and therefore, will be very useful for improving the accuracy of QTL mapping and breeding value estimation of livestock animals.

SNPs are abundant and stable compared to other types of DNA markers. SNP frequency ranged from one in 200 to one in 400 base pairs in swine genome. A high- density SNP map allows mapping QTL positions and estimating effects more exactly since SNPs can be seen as a type of markers that are much closer linked to QTLs and genes. Furthermore, SNPs can also be targeted within porcine genes and QTLs. The gene segregation can, therefore, be traced by SNPs directly. SNP markers are suitable for use in high throughput genotyping systems. Because of the use of Microarray technology, rapid screening of thousands of SNPs becomes feasible. Some laboratory can genotype over 70,000 SNPs a day (Siler 2003). The targeted price can be as low as $0.10 per SNP score. Therefore, application of SNPs is very efficient and economical way for gene mapping and marker-assisted selection in comparison with current microsatllite markers or RFLP markers.

1.2. Methods to use genomic information

1.2.1 Single gene selection

In principle, the information of a gene should be evaluated together with phenotypic performances and other gene genotypes under optimal rules. In reality, some genes, such as Halothane gene and RN gene, have been selected by the way of independent culling. IGF2 gene based on single gene genotypes can be used to increase leanness and uniformity of hogs. We believe that single gene selection is needed sometimes and does not always conflict with the principle of economic optimization. For some genes with adverse influences, such as Halothane gene and RN gene, single gene selection leads to a quick elimination of these genes, and therefore, can increases the reputation of Canadian swine. The use of IGF2 gene can lead a quick increase of leanness and uniformity of hogs. The reputation and uniformity may have significant economic value, which usually have not been considered in estimating economic weights.

1.2.2 Marker-assisted selection

The current method for the marker-assisted genetic evaluation is the gametic model via mixed model equations proposed by Fernando and Grossman (1989), which is based on Henderson's mixed model equations. A problem of the model is the large number of genetic effects to be estimated. Furthermore, this method has not considered the evaluation of candidate genes. A simplified equivalent statistical method has been developed. The method includes candidate genes and reduces the number of genetic effects to be estimated. The information on candidate genes and QTLs can be incorporated along with phenotypic information. Candidate gene effects are assumed as fixed while the polygenic effect is considered as random.

The breeding objective is to maximize the cumulative genetic response for a given period, e.g. t generations to avoid long-term disadvantage of marker-assisted selection (Dekkers and Van Arendonk. 1998). The estimation procedure of breeding values is simplified by including gametic effects at QTLs into polygenic effects. In this way, the number of equations for the polygenic effect and QTLs is equal to the number of animals to be evaluated. In the current genetic evaluation system, the computer programs do not need to be change considerably.

1.2.3 Marker-assisted introgression

A very promising application of marker information in swine breeding is marker-assisted introgression. It can be very useful in developing synthetic lines of pigs. Introgression is to introduce one or more desired genes or QTLs from a breed (donor) with some superior characters but inferior performance into a high performance breed (recipient). This is usually done by an initial F1 cross followed by successive backcrosses to the recipient breed. Marker information can be used to track the target genes in foreground selection. In background selection, marker information can also be useful to speed up the recovery of background genome by estimating the proportion of the recipient genome present in an individual. Individuals with the highest proportion are selected.

2. Recommendations for current use

- The areas where DNA information is especially useful are the traits not measurable on living animals, such as meat quality traits; traits of low heritability, such as sow reproduction; sex-limited traits; disease resistance and early stage selection. For the Canadian swine industry, it seems more useful to consider DNA information of meat quality and reproduction traits first in the index combining BLUP EBV. Methods and computing program for this are to be developed.
- DNA tests for IGF2 gene are suggested to increase the pork leanness and carcass uniformity. Benefits from the field application of IGF2 gene include (1) Increase lean yield percentage of hogs (by about 2%). This is going to move the hog lean yield % to the range of better payment grids, (2) Increase the uniformity of pork leanness (by 25% phenotypic variation). This allows much more hogs falling into the better-paid grids since there are no more homozygous hogs with low-lean genotypes (GG) in the commercial population.

- Another promising gene is the heart fatty acid binding protein (HFABP) gene**.** HFABP was identified to be a major gene for marbling or intramuscular fat (IMF), but not for Backfat, explaining 1/4-1/8 of mean values of IMF, and being polymorphic in all breeds tested so far. This gene offers the possibility of increasing marbling while continuing selection for lean yield. DNA tests for this gene are recommended (1) to produce pork with desirable IMF by choosing boars with known HFABP genotypes or (2) to increase IMF, during continuous selection for high lean yield.
- The negative effects of Halothane gene, such as higher incidence rate of stress death, higher incidence rate of PSE meat, lower growth rates, lower feed intakes and smaller litters are well documented. During recent years, the Hal-gene has been screened and eliminated systematically among sires in nuclear populations. There is a need to monitor the frequency of this gene in commercial and nucleus populations.
- A mutation of c-Kit receptor gene, known as the dominant white allele, disrupts normal melanocyte development in the embryo. This gene is therefore useful in detection of undesirable colour transmission in white breeds. Some countries, such as China, consider the colour uniformity seriously, as an indicator of breed purity. DNA tests for this gene can be especially useful for promoting Canadian genetics in international markets. Testing for this gene can be organized with AAFC Lacombe Research Centre (Dr. Jon Meadus) and with University of Guelph (Dr. Su Chen).
- Pork eating quality such as tenderness, juiciness is important meat attribute. However there is very little effective tool to improve those traits traditionally. Recently, some genes associated with these attribute have been mapped, such as CAST gene, Calpain gene and FOS gene. These genes could be used to improve the pork eating quality. DNA tests for these genes have been suggested to check gene frequencies and gene-trait associations in Canada swine populations.

3. Development of the infra-structure - DNA bank

Setting up a national DNA Bank as infra-structure is efficient way to catch the opportunity provided by genomic researches and, to test and utilize promising candidate genes, functional mutations, QTLs and especially functional SNPs and SNP markers in current stage and in the future. It allows making up missed opportunities and taking the advantage of the future discovery in the area of genomic researches. DNA bank can avoid repeated sampling for DNA tests. For example, all AI sires were sampled for Halothane gene tests during the past years. If we kept the leftover DNA samples in DNA bank, we would not have to collect the DNA sample again for the follow-up tests such as IGF2 gene and HFABP gene tests. A DNA bank for swine was established quite early in some countries, such as Japan (since 1994) and France (since 1999). There is a need to set up a similar national DNA bank in Canada. DNA bank as resources has a number of applications for swine industries. For example,

(1) Gene mapping and DNA tests for specific useful genes
(2) Genetic selection for swine improvement
(3) Paternity tests
(4) Traceability for retrieving parental or herd origins of animal or a cut of meat
(5) Purity tests, which would be useful to promote Canadian swine genetics in international market, especially in the developing countries.

4. Future perspectives

There is a rapid progress of swine gene mapping studies during recent years. A large number of promising candidate genes, functional mutations and QTLs have been identified for different economically important traits. Marker-assisted selection (MAS) programs of swine have been adopted by many companies in different countries. Canada is the largest pork exporting country in the world (USDA Foreign Agricultural Service 2003). Domestically, the pork industry is also recognized as a major industry sector in agriculture. To keep the competitiveness of Canadian swine industry, it is important to take steps now to employ the opportunity provided by the advancement and innovation of the genomics technology. For this, we suggest to test and evaluate the mapped genes, functional mutations under Canadian conditions and to explore the their application in Canadian swine improvement program, beginning with the most promising ones such as IGF2, HFABP, CAST, Calpain, FOS etc..

A technique that can be used for marker-assisted selection is SNP genotyping. The utilization of population wide linkage disequilibrium appears to be considerable advantage. Meuwissen et al (2001) showed that with a BLUP model based on the marker genotypes, it is possible to predict the breeding value of an individual based only on genome wide markers. This result is considered the most promising future application of MAS by Dekkers and Hospital (2001) because the same genome wide marker assessment can be applied to all traits regardless of heritability, QTL's identified or not, with or without phenotypic information, and can accommodate new QTL's as a result of mutations. This procedure is referred to by Muir (2004) as genome-wide marker-assisted selection (GMAS). GMAS provides a new way to incorporate genetic variations of different sources into genetic evaluation of livestock, and opens up a number of opportunities including gradual increase in emphasis in selection on genome wide markers and reduction in the number of phenotypic records.

5. References

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