

Advances in pig molecular genetics, gene mapping and genomics

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Summary

Advances in the fields of molecular genetics and genomics have been considerable and these have led to the development of very useful linkage and physical maps of the pig genome. The genetic linkage map now has over 1,900 loci including approximately 300 genes. The physical genetic map has over 1,000 loci including a growing number of known genes. The development of new maps including those incorporating large numbers of AFLP markers map are also well underway and will add 2,000 additional markers, bringing the total to nearly 4,000 genes and markers. Several recent quantitative trait loci scans and candidate gene analyses have identified important chromosomal regions and individual genes associated with traits of economic interest in the pig. These include QTL for growth and backfat, meat quality traits, and reproduction. The causative mutations for porcine stress syndrome (HAL or CRC1) and the RN disorder, candidate genes for litter size (ESR, PRLR, RBP4), growth (MC4R), meat quality (hFABP, aFABP), disease resistance (FUT1, SLA, NRAMP), and coat color (KIT, MC1R) have also been identified. The commercial pig industry is actively using this information and in-house research to improve pig production by marker assisted selection (MAS). Furthermore, research to study the co expression of hundreds of genes is now beginning. This research will aid in our understanding of genetic systems and how to alter their relationships to improve pig production.

Introduction

Molecular genomic analysis has revolutionized how animal geneticists examine the genetic differences that exist within commercial and exotic pigs. In the past few years, efforts have been directed toward the development of genomic maps consisting of anonymous genetic markers and known genes. In addition, comparative genome maps have aided greatly in our search for interesting and potentially useful genes in the pig. The coverage on these genetic maps is now sufficient to allow researchers to conduct quantitative trait loci (QTL) linkage analyses. These QTL linkage analyses involve employing a genomic scan where generally F2 or backcross families are used and genotypes are obtained for many (>100) markers evenly spaced across the genome. Several such experiments are underway or recently completed and are beginning to produce interesting and useful results. Candidate gene and comparative mapping approaches have also been successful in identifying major genes affecting several traits. Candidate gene analyses is undertaken when a gene is chosen based on the physiology of the trait. The candidate gene is assumed to affect trait performance. This is supplemented by comparative gene analysis that allows researchers to find "positional candidate genes" in the regions associated with possible QTL. To date, several major genes have been found with the candidate gene approach. Present and future genetic improvements will result from the more detailed genetic maps and our growing understanding of the function and structure of the individual genes and gene families that are responsible for the economically important traits in pigs. The purpose of this paper is to review the recent discoveries of gene mapping and the status of QTL and candidate gene analyses in the pig and to forecast future developments and their applications in industry.

Results

Results will be presented for status of the pig genome maps and QTL and candidate gene analyses in terms of growth and performance, meat quality, disease resistance and reproduction traits. Many of the results presented earlier (Rothschild and Plastow 1999) are now updated. The author apologizes for data or reports inadvertently left out.

Status of the genome maps

The effort required to generate mapping information is very significant. Scientists and administrators realized that a cooperative effort for gene mapping in livestock would be the most productive approach. The PiGMAP project (Archibald et al., 1995) was initiated in Europe and was funded by the European Economic Community. PiGMAP involved 18 European labs and a total of 7 other labs from the U.S., Japan and Australia. In the U. S., the USDA launched two efforts. The USDA-ARS directs a sizeable gene mapping project at the Meat Animal Research Center in Clay Center, Nebraska. Secondly, the National Animal Genome Research Program was developed under the direction of USDA-CSRS in 1993. This program was designed to provide a structure which would stimulate coordination and collaboration of gene mapping in all species, including pigs. Scientists from state and private universities and federal labs cooperatively created a Swine Genome Technical Committee. The U.S. Pig Genome Coordinator activities, in concert with activities of the USDA-ARS and other international gene mapping projects have allowed the status of the pig gene map to evolve more quickly in the last several years. In 1989, only 50 genes and markers were mapped in the pig, and many of these were isozyme or blood group markers. At present, nearly 1,900 markers and genes have been mapped in pigs (Archibald et al. 1994 and personal communication; Marklund et al. 1996a; Rohrer et al. 1996), though not all have been published. There are two primary maps. The most extensive pig map has about 1,100 loci (Rohrer et al., 1996), consisting mostly of anonymous markers. A second PiGMAP publication is under consideration. In addition, the new PiGMAP genetic linkage map will have over 700 genes and markers on its map, including about close to 300 genes. Coverage for the different linkage maps varies, and while the average distance between markers is approximately 3-5 cM, some large gaps still remain. Efforts are underway to produce an integrated map including AFLP (Amplified Fragment Length Polymorphism) markers which is expected to have eventually 2000 AFLP markers (Archibald and colleagues, personal communication). Physical maps for all the species used to lag behind the genetic linkage maps but are catching up quickly due to some excellent resources such as somatic cell hybrids for physical mapping and a radiation hybrid map developed in France and being used by several groups (Hawkins et al. 1999). The physical genetic map in the pig currently consists of over 600 genes and markers, while the radiation hybrid panel map has over 1,000 markers. Despite these concerted efforts, the pig genome map still pales in comparison to the human and mouse maps. However these gene rich species provide real help to our efforts in the pig. As more genes are placed on the pig comparative map, the human genome map will have increased utility in the search for individual genes responsible for traits of economic interest .

Growth and Performance Traits

The first successful large QTL analysis was conducted using a Wild Boar and Large White three generation family and revealed major QTL accounting for 20% of the phenotypic variance for average backfat and abdominal fat on chromosome 4 (Andersson et al. 1994). A QTL for growth was also found on chromosome 13 accounting for 7% to 12% of the phenotypic variation. Additional confirmation of the chromosome 4 results have been seen in subsequent generations (Marklund et al. 1996b) and by other experiments using Chinese pig crosses (Wang et al. 1998; Milan et al., 1998; Moser and Cepica, personal communication). Using a candidate gene analysis PIT1 was identified to be associated with backfat and birth weight (Yu et al. 1995) and it maps in the center of the chromosome 13 QTL found by Andersson et al. (1994). Recent results have demonstrated that in the Iowa State University Meishan x American F2 families a QTL for early growth is centered above PIT1 and a QTL for backfat is 20 cM from PIT1 (Yu et al., 1999). Results from another specialized meat quality family confirms such a backfat QTL in this region (Malek et al. 2000; Rothschild, unpublished results).

The pig major histocompatibility complex (MHC) spans the centromere of chromosome 7. Associations between MHC haplotypes and several traits have been reported over the years. These have been confirmed, in part, using MHC class I DNA probes (Jung et al. 1989). More recently, QTL scans have identified QTL for growth and backfat traits in Chinese crosses on chromosome 7 (Rothschild et al. 1995; Bidanel et al. 1996; Chevalet et al. 1996; Moser et al. 1998; Rohrer and Keele 1998a; Wang et al. 1998). The backfat and birth weight QTL centered near the region of TNFA and S0102. A candidate gene analysis involving AMPEPN, which maps to the same region, also revealed an association with growth rate (Nielsen et al. 1996). The overall results to date suggest that at least one growth and backfat QTL exists in this region. Other results have included a growth trait QTL on chromosome 6, but it seems to be associated with the known effect caused by the RYR1 (CRC1) gene causing malignant hyperthermia (Geldermann et al. 1996) or other unknown genes in the immediate vicinity of RYR1. Some additional associations have been reported for chromosome 1 (Rohrer and Keele 1998a, Pazek et al. 1999), chromosome 3 (Casas-Carrillo et al. 1997b), chromosomes 6 and 8 (Wilkie et al. 1996), chromosome 14 (Bidanel et al. 1996) and the X chromosome (Rohrer and Keele 1998a). New data that is soon to be published shows significant QTL for growth on additional chromosomes (Rothschild et al, in preparation).

A combination of QTL scans and candidate gene analyses using GH have been performed for chromosome 12 for several performance measures (Nielsen et al. 1995; Casas-Carrillo et al. 1997a; Knorr et al. 1997) but results were not in general agreement. Analysis of the chromosome 5 region near IGF-1 revealed significant effects on average daily gain (Casas-Carrillo et al. 1997a). Additionally, Gerbens et al. (1997) and Tepas et al. (1996) reported associations of the heart fatty acid-binding protein (H-FABP) gene with intramuscular fat and the myogenin gene with average daily gain. Other candidate genes including CCK and CCKAR (Clutter et al. 1996, leptin (reference to be added) and the leptin receptor (Vincent et al. 1997) have been mapped and may prove to be associated with growth, fatness and appetite traits.

Perhaps the most interesting new results are those dealing with melanocortin 4 receptor (MC4R) and its association with feed intake, growth and backfat (Kim et al. 2000). Using information from a mouse knockout experiment done by Millennium Pharmaceuticals, the Iowa State University group in collaboration with PIC found a naturally occurring missense mutation that causes the pig to eat more (about 10%), grow faster (6-8%), and grow fatter (6-10%). This mutation can be

used to improve feed intake in certain sow lines where fat is already at the desired level and in sire lines to reduce the level of fat. This new genetic test is patented and being used in the industry.

Meat Quality Traits

The earliest QTL research in meats was by Andersson and colleagues (Andersson-Eklund et al. 1996). They have conducted one of the most complete QTL scans for meat quality using 234 markers on 191 F2 animals. QTL for several meat quality traits (pH, water holding capacity and pigmentation) were found to be on chromosome 2 and chromosome 12. Our earliest work at Iowa State University demonstrated some association of meat color and firmness scores with regions on chromosomes 4 and 7 (Rothschild et al. 1995, Wang et al. 1998). Additional associations with meat quality traits have been reported for chromosome 7 (Moser et al. 1998) and for number of muscle fibers on chromosome 3 (Milan et al., 1998). Two other interesting associations related to meat quality have been noted for chromosome 7. The activity of Malic enzyme, a lipogenic enzyme in muscle has been shown to be associated with the SLA complex on chromosome 7 (Renard et al. 1996). Furthermore, a directed QTL scan revealed that there was a major QTL for androstenone level which is associated with boar taint in the region of the SLA complex (Bidanel et al. 1996; Milan et al., 1998). Additional QTL results have also been reported for carcass composition and wholesale product yield traits. Rohrer and Keele (1998b) reported QTL for loin eye area (chromosome 1), weight of trimmed ham, loin, picnic and Boston butt (chromosomes 1 and X) and carcass length (chromosome 7).

Most recently, Rothschild and colleagues conducted a major meat quality QTL scan using two commercial breeds, Berkshire and Yorkshire. The F2 population consisted of 525 animals that were genotyped for 125 markers and measured for 40 traits for growth, carcass, meat and sensory qualities. Rothschild and colleagues (Malek et al. 2000) found over 100 significant QTL at the 5% chromosome significance level and nearly 20 at the 5% genome significance level.

Evidence for a major gene for intramuscular fat (IMF) was obtained using segregation analysis (Janss et al, 1994, 1997), and this group went on to try to identify the gene using QTL mapping. Although they recently reported evidence for suggestive linkages with markers on chromosome 1 and a marker on chromosome 3, they were not able to find identify the major gene suggested by the segregation analysis.

It has been known for some time that pale soft and exudative (PSE) pork is associated with variation in the RYR1 gene on chromosome 6. This has been well demonstrated in a QTL scan for several meat quality traits related to PSE in an F2 population originating from a Pietrain background (Geldermann et al. 1996). Focus has also centered on a gene originating in Hampshires called the RN gene that is associated with lower pH and increased glycogen content in the meat. The RN gene was first mapped to chromosome 15 (Milan et al. 1996; Mariani et al. 1996a; Reinsch et al. 1997) and surrounded by flanking markers. The actual gene had alluded researchers for some years, but DNA tests using markers for the RN gene were utilised by researchers (De Vries et al. 1997). Early this year a consortium led by Leif Andersson, Denis Milan and Christian Looft reported the identification of the causative mutation. The gene involved is a new member of a gene family coding AMP-activated protein kinases (named PRKA). Interestingly, the same gene might explain certain forms of diabetes in humans and the consortium is looking at the opportunities for their research to benefit human health. This result represented a tremendous effort by this group of labs to move from the chromosomal position to the gene itself.

This new gene discovery result represents another important addition to be used by pig breeders to improve meat quality where the Hampshire breed is used.

Among candidate genes investigated for muscle quality is the H-FABP gene that may be associated with intramuscular fat (Gerbens et al. 1997). These workers identified polymorphisms in this gene and found them to be associated with variation in IMF in the Duroc (Gerbens, 1998). The H-FABP gene maps to pig chromosome 6 in an area where some recent reports suggest a QTL in the region. A comparison of the homozygous haploid classes found that they differed by about 15% of the mean value. Interestingly, the difference in IMF content is only partially explained by backfat content. This should permit selection for increased IMF based on H-FABP genotype as long as increased backfat is countered by ongoing phenotypic backfat selection. It will be interesting to see how effective and practical this test will be in the near future. This test is patented and is available through license arrangements (Merks, personal. comm.).

Other markers which have been generated for meat quality based on the candidate gene approach include myogenin (increased fibre number, which may impact overall pork quality) (Soumillion et al. 1997) and calpastatin (Ernst et al. 1997). Coat color, though not directly associated with meat quality is of interest to the packing industry. White pigs are preferred at slaughter and the cost of a "colored carcass" at some locations may be over \$1/pig. Andersson and colleagues (Johansson-Moller et al. 1996) have now identified the KIT gene as that responsible for white coat color and a DNA test is patented and being used in MAS programs (Andersson and Plastow, personal communication). The MC1R gene has also been identified to control red and black color in the pig (Mariani et al. 1996b) and the gene test is likely to be made public in the U.S. this year.

While the normal inheritance of genetic factors is expected sometimes imprinting exists. The clearest example in pigs is the effect of the IGF2 region of chromosome 2 on muscling in pigs of different backgrounds (Jeon et al 1999, Nezer et al 1999). The ability to predict the genotype at such loci is a clear benefit of MAS.

Reproduction Traits

Given the necessity of larger resource families and the difficulty and time required to obtain information on reproduction traits, it is not surprising that results of QTL scans for these traits are limited. Initial scans have revealed promising results on chromosome 8. Wilkie et al. (1996) reported possible QTL for uterine length and ovulation rate, though in different chromosomal positions. Rathje et al. (1997) reported a sizable QTL for ovulation rate (+3.07 ova) on chromosome 8 also but some distance from the ovulation QTL observed by Wilkie and colleagues. Later work in the same lab did not confirm this finding (Pomp and Johnson, personal communication). In the French QTL experiment (Milan et al., 1998) a QTL for increased litter size of one piglet was found in the same location on chromosome 8 as Rathje. The large ovulation rate/litter size QTL on chromosome 8 is of interest as it mapped to the region which is syntenic to the Booroola fecundity gene in sheep. Interestingly, using a single microsatellite marker (OPN) in the same chromosome 8 region, (Short et al. 1997b; van der Steen et al. 1997) also found significant effects for litter size in commercial lines. Further evidence exists for at least one QTL for litter size components at the top of chromosome 8 (Rohrer et al. 1999) and perhaps another nearer the centromere (Plastow, Personal communication). Limited chromosome QTL analyses have further suggested other reproductive QTL on chromosomes 4 and 6 (Wilkie et al. 1996), on chromosome 7 (Wilkie et al., 1996; Milan et al., 1998) and chromosomes 4, 13, 15 (Rathje et al. 1997).

Candidate gene analysis for reproduction has shown considerable merit. Results have clearly demonstrated that the estrogen receptor (ESR) is significantly associated with litter size (Rothschild et al. 1996; Short et al. 1997a). Estimates of allelic effects vary from 1.15 pig/litter in Meishan synthetics to .42 pigs/litter in Large White lines. These results have not been confirmed by QTL scans using divergent crosses involving Meishan and Large White pigs. This may have resulted from small sample sizes or the fact that the ESR gene allele was not segregating in the populations involved in the QTL scans. The ESR marker was incorporated successfully into the PIC selection indices for Large White based dam lines, resulting in an increase in the rate of genetic response in its nucleus herds (Short, Wilson, McLaren and Plastow, unpublished results). Furthermore, the increase in average litter size is observed in crossbred products derived from these lines.

Other effects have been reported for retinoic acid receptor gamma (RARG), melatonin receptor 1A (MTNRIA) (Messer et al. 1996; Ollivier et al. 1997) and follicle stimulating hormone beta (FSHB) genes (Li et al. 1998). Researchers at Iowa State University, working with PIC have demonstrated that the prolactin receptor (PRLR) locus is significantly associated with litter size (Vincent et al. 1998). This has been confirmed in two smaller studies (Ohio State Univ. and WAU in the Netherlands) now being submitted for publication. More recently, retinol binding protein 4 (RBP4) has been shown in a study involving nearly 2,500 litters to be associated with an increase of about 0.25 pigs per litter (Rothschild et al. 2000). Most of the candidate gene analyses have involved considerably more sows and litters than the QTL analyses and this might explain the lack of QTL scan confirmation of the regions in which the candidate gene effects have been reported to date.

Disease Resistance and Immune Response Traits

To date, QTL scans for disease resistance or immune response QTL have been limited. An exception is work underway by Andersson and colleagues (Edfors-Lilja et al. 1998) to study some immune response parameters. Some immune capacity QTL have been identified. Also, a QTL for base cortisol level which may be related to stress and perhaps immune response, has been mapped to the end of chromosome 7 (Milan et al., 1998). Analyses of associations of candidate genes have been more substantial. The general location of the K88 E. coli receptor has been known for some time and fine mapping and candidate gene analysis of the region is underway in many labs. Two alpha (1,2) fucosyltransferase genes (FUT1, FUT2) on porcine chromosome 6q11 have been identified and are closely linked to the blood group inhibitor (S) and Escherichia coli F18 receptor (ECF18R) loci (Meijerink et al. 1997). This work has now confirmed that a polymorphism in these genes is closely linked to ECF18R in Large White, Landrace, Hampshire, Duroc and Pietrain pigs and it could be a good marker for marker assisted selection of E. coli F18 adhesion resistant animals in these breeds. Clearly now the FUT1 or FUT2 gene products are involved in the synthesis of carbohydrate structures responsible for bacterial adhesion remains to be determined. The SLA complex on chromosome 7 has recently been associated with resistance to primary infections with *Trichinella spiralis* but not to resistance to toxoplasmosis (Lunney, personal communication). The gene for Natural Resistance Associated Macrophage Protein 1 (NRAMP1), associated with resistance to *Salmonella* challenge in mice, has been recently mapped to pig chromosome 15 (Sun et al. 1998). Several other candidate genes are being investigated. Genes associated with human disorders, which have been identified and mapped in the pig, include clotting factor IX (Signer et al. 1996) and the familial hypercholesterolaemia gene (Hasler-Rapacz et al. 1996).

Genetic diversity

A final area for the use of molecular markers and genes is in the area of genetic diversity. This has traditionally been accomplished by examining at first enzyme polymorphisms and more recently using microsatellites. A final example of this approach is the European biodiversity project, coordinated by L. Ollivier from France (Laval et al. 2000). Some arguments have been placed that such approaches with anonymous markers are not sufficient to determine functional diversity and that polymorphisms within real genes should be used (Ciobanu et al., 1999; Rothschild 1999). This approach was used by Ciobanu and colleagues (Ciobanu et al., 1999) and demonstrates differences in functional genes between two native pig breeds of Romania.

Potential of DNA marker assisted selection in the pig industry

Information at DNA level can help industry breeders and geneticists to fix a specific major mutation, such as the normal Halothane allele. It can also be used to assist in the selection of quantitative traits including those that can be selected by traditional means (See Table 1). Molecular information can increase the accuracy of selection and therefore the selection response. The size of the extra response obtained through Marker Assisted Selection (MAS) schemes has been considered by many workers from a theoretical point of view. Gibson (1994) and others have shown that there is a short term benefit in using MAS, but that in some cases this can lead to a long term penalty. However, this is over a relatively long time frame. Meuwissen and Goddard (1996) considered a different set of assumptions and in particular looked at the impact for traits such as reproduction and meat quality that are difficult to progress using traditional methods. Dekkers and van Arendonk (1998) have shown that these results can be further improved by using control theory to optimise response. Altogether these results are extremely encouraging. Importantly, these responses can be sustained if new markers are continually identified. For example, new markers can be added to the selection index as old markers begin to reach fixation.

In the meantime we anticipate that significant progress will be made by utilizing candidate genes and searching for population-wide linkage disequilibrium, using tools such as AFLP. It is anticipated that AFLP analysis will be an extremely powerful tool as it generates large numbers of markers rapidly in interesting populations. AFLP can be used with bulk segregant analysis (BSA) in a form of map-less QTL analysis. For example, markers were identified close to the dominant white locus (Plastow et al. 1998). Another tool will be the use of large numbers of SNPs (single nucleotide polymorphisms) and also making use of for population-wide linkage disequilibrium.

Discussion and future developments

Additional developments in the genome maps are expected but the generation of hundreds of new random microsatellite markers is not likely. The addition of an AFLP map is likely to aid greatly in finding QTL. In addition to more extensive scans of existing populations, many new QTL experiments are underway for performance, reproduction and meat quality traits (Moran, personal communication; Nezer et al. 1996; van Oers et al. 1996). In the short time it has taken to build a considerable genetic map, many QTL and candidate analyses have yielded interesting results. The QTL scans have identified several chromosomes that are now targets for further confirmation of the chromosomal region, advanced fine mapping of the QTL, and positional comparative candidate gene analysis. This will be aided by the ever improving comparative gene maps. Additional experiments will either confirm the regions and lead to the eventual isolation of the gene or genes of interest or will produce conflicting results. Such conflicting results may be the results of

haplotype effects, epistasis or background genotype effects, or sampling. Given that we wish to detect not only large but also moderate gene effects, experimental size will need to be increased or several experimental results will need to be pooled. Recently, several PiGMaP participants pooled information on chromosome 4 to obtain additional power in the analysis (Walling et al., 2000). Additional plans are underway to use this approach to join other QTL experiments. The relevance of results found in crosses involving the Wild Boar and the Chinese breeds will also have to be evaluated in commercial lines. This is the objective of a EC funded project on the utilisation of QTL (Andersson and Plastow, personal communication) and the work by Rothschild and colleagues (Malek et al. 2000)

New technical develops continue to be provided which may yield exciting results. Several EST (expressed sequence tags) projects are underway in the pig. These projects attempt to identify bits of new genes from specified tissues. Many of these will be mapped and the comparative map of the pig will advance rapidly. The use of DNA chips or arrays to examine gene expression will offer new glimpses into the complex traits of economic importance in the pig. These and other yet to be developed methods offer great promise for the future of swine improvement.

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Table 1. Molecular Genetic Tests Used by the Swine Industry.

Parentage tests	non exclusive use
HAL	meat quality - non exclusive use
ESR	litter size - exclusive use (PIC)
PRLR	litter size - exclusive use (PIC)
RBP4	litter size - exclusive use (PIC)
KIT	white color - exclusive use (PIC)
MC1R	red/black color - exclusive use (PIC)
MC4R	growth and fatness – exclusive use (PIC)
FUT1	edema disease E. coli F18 – exclusive use (PIC/ITH Switzerland)
RN	meat quality - non exclusive tests (soon) (Uppsala, INRA, Kiel).
AFABP, HFABP	intramuscular fat - non exclusive (IPG)
IGF2	carcass composition – exclusive use (Seghers)
Trade secret tests	several traits – many companies
