Molecular approaches in pig breeding to improve meat quality

Roberta Davoli and Silvia Braglia

Advance Access publication date 21 January 2008

Abstract

This article reviews the advances in molecular genetics that have led to the identification of genes and markers associated with meat quality in pig. The development of a considerable number of annotated livestock genome sequences represents an incredibly rich source of information that can be used to identify candidate genes responsible for complex traits and quantitative trait loci effects. In pig, the huge amount of information emerging from the study of the genome has helped in the acquisition of new knowledge concerning biological systems and it is opening new opportunities for the genetic selection of this specie. Among the new fields of genomics recently developed, functional genomics and proteomics that allow considering many genes and proteins at the same time are very useful tools for a better understanding of the function and regulation of genes, and how these participate in complex networks controlling the phenotypic characteristics of a trait. In particular, global gene expression profiling at the mRNA and protein level can provide a better understanding of gene regulation that underlies biological functions and physiology related to the delivery of a better pig meat quality. Moreover, the possibility to realize an integrated approach of genomics and proteomics with bioinformatics tools is essential to obtain a complete exploitation of the available molecular genetics information. The development of this knowledge will benefit scientists, industry and breeders considering that the efficiency and accuracy of the traditional pig selection schemes will be improved by the implementation of molecular data into breeding programs.

Keywords: *pig; meat quality; QTL; candidate genes; genomic technologies; genetic improvement*

INTRODUCTION

During the last decade, the genetics of pig meat quality has became subject of increased research and the improvement of this trait is at present an essential element to support meat consumption and to satisfy the consumer's demands for excellent eating, healthy and nutritional quality. The increasing consumer's awareness of food quality and the development of the field of genomics have driven pig breeding companies to consider with more attention meat quality, and to include quality traits as an integral part of selection programmes, where DNA markers can be incorporated.

Meat quality is a composite concept as pointed out by Cameron [1] and therefore it is difficult to measure meat quality in a simple and unique manner. Meat quality traits are complex characteristics of considerable importance to the producers, consumers and processing industry, and the measurement of these traits usually include measurements assessing backfat, intramuscular fat, marbling, loin eye area, water-holding capacity, pH, glycolytic potential, colour, tenderness, juiciness and flavour.

Meat quality is influenced by a large number of factors including muscle characteristics (fibre size and type, fat and connective tissue), production and environmental conditions [growth rate, nutrition, age, (pre-) slaughter conditions and post-mortem ageing] and the genetics of the animal (breed, genotype). Genetic effects play a crucial role in 'designing' pig carcass composition and quality, although the heritability of quality traits is quite low [2]. Generally, between 10% and 30% of the variation in meat quality traits and meat products (ham), such as

Corresponding author. Roberta Davoli, DIPROVAL – Sezione di Allevamenti Zootecnici, Faculty of Agriculture, University of Bologna, Reggio Emilia, Italy. Tel: +39-0522290507; Fax: +39-0522290523; E-mail: roberta.davoli@unibo.it

Roberta Davoli is a professor of Genetic improvement of farm animals at Bologna University and is involved in functional genomic research projects aimed to identify porcine genes influencing meat quality.

Silvia Braglia, PhD, is a biologist working on animal genetics, with a major interest on porcine association studies between markers and phenotypic traits and gene expression profiles and data analysis.

ultimate pH, colour, water-holding capacity, drip loss, tenderness, marbling, etc. is determined by the genetic background of the animal [2].

The traits determining meat quality are difficult to improve by traditional selection, because the heritability is quite low and the measure for the quality traits is difficult, expensive and only possible after slaughter. Moreover, the lack of knowledge on the number of genes for the single qualitative characteristics of meat, on the quantitative effects of the single genes and on their interactions represents a limit for the complete exploitation of the opportunities of a selection plan. Molecular genetics can overcome these limits, offering new opportunities to the improvement of meat quality, as it supplies tools to analyse the quantitative genetic variability directly at DNA level with the possibility of detecting the individual genes influencing the qualitative characteristics.

Meat quality traits are controlled by an unknown number of genes mapping in quantitative trait loci (QTL) regions and some of these individual genes may have large effects on a specific trait. The main goal of genome research in farm animals in general is to map and to characterize trait loci [3, 4].

A lot of work has been carried out in this field to find potential genes or chromosome regions responsible for (or associated with) meat quality and processed meat products, but to date the knowledge of genes and their interactions that are involved in meat properties are very limited. As a consequence, the understanding of meat quality on a genetic basis is scanty. At the present time, the main task of genetics is to identify factors in the molecular or biological components of meat quality that will be useful for marker-assisted selection in breeding, giving the opportunity to 'design the meat' by genetic and molecular methods [5, 6].

Aim

This review aims to describe the current status of genomic meat science research in pigs and the recent developments in functional genomics to get a better understanding of molecular determinants of phenotypic variation of meat quality traits.

APPROACHES TO IDENTIFY THE LOCI OF MEAT QUALITY TRAITS

Genome research in pigs progressed rapidly in recent years, and our understanding of the pig genome has rapidly evolved from the localization of genes on specific chromosomes to high density marker maps [7].

Table 1: Current status of genes, ESTs and SNPs in pig (Source: http://www.animalgenome.org/pigs/dbEST release 101907)

	Number	Updated
Gene	3095	Oct 2007
ESTs	1471876	Oct 19 2007
SNPs	6625	March 2007

With the development of molecular markers, porcine genomic maps have been largely enriched in the last few years. The pig genome database includes more than 3000 genes and more than 6600 markers (http://www.animalgenome.org/pig/) (Table 1).

In the near future, the pig whole genome will be completely sequenced and this result represents a key milestone to exploit the pig genome and to detect the molecular basis of meat traits. The whole pig genome sequencing began in 2005 [8] and its first draft is expected to be finished in early 2009 (target of 3X genome coverage sequencing). Porcine single nucleotide polymorphism (SNP) discovery for exploitation in breeding is ongoing and several large projects have been completed; for example, the 'Sino-Danish Pig Genome Project' has published the pig genome sequence with <1X coverage [9] as well as EST [10] and SNP data [11], and other relevant projects are currently being initiated by INRA-Genescope initiative [12, 13].

The availability of an increasing number of nearly complete genome sequences is a source for a very high number of microsatellites and SNP markers.

All these genomic informations coming out from a number of projects on porcine genome, will allow the identification of gene markers for meat quality, providing significant insights into the molecular basis of phenotypic variation of production traits and assisting breeders in pig selection [5, 14].

Genome scan based on linkage mapping DNA markers and candidate gene used in association tests are two main strategies to identify, to map and to characterize trait loci influencing meat quality [4, 14, 15].

Genome scan approach

The ultimate goals of the genome scan approach are identifying the genes that underlie polygenic traits

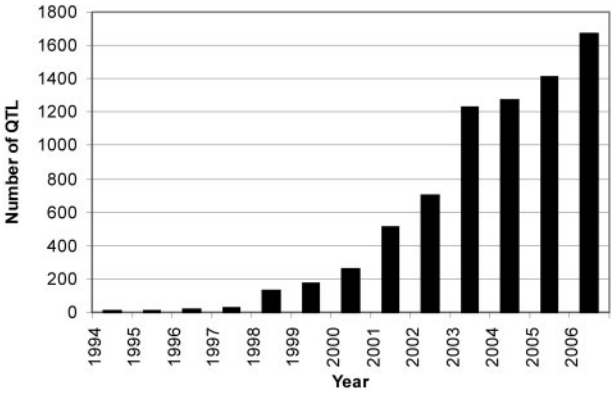

Figure 1: Identification of porcine QTLs from 1994 to 2006 (Source: PigQTLdb http://www.animalgenome.org/QTLdb/pig.html, updated January 2007).

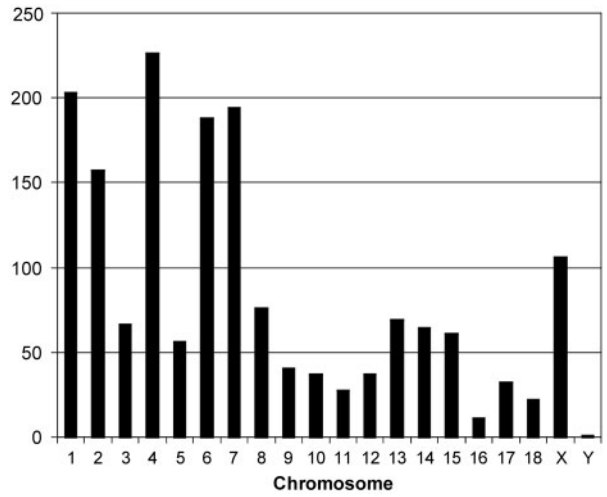

Figure 2: Number of porcine QTLs by chromosome. (Source: adapted from PigQTLdb http://www.animalgenome.org/QTLdb/pig.html, updated January 2007).

and a better understanding of their physiological and biochemical functions. In fact, a QTL region often spans 5–30 cM, and it is too large to find the target genes, so a fine mapping analysis needs to be performed. The fine mapping of a specific QTL is the goal of genome scan and it began in the relevant region by adding genetic markers and increasing the marker density to the linkage map [4, 16, 17].

In pig, the first QTL study was carried out in 1994 [18]. The first such discovered QTL was a major locus for fat deposition on chromosome 4. Since then, quantitative trait loci have been identified for a large number of traits segregating within numerous breeds. Recently, a specific porcine QTL database—PigQTLdb—became available (www.animgenome.org/QTLdb/) [19]. Over the past 15 years, QTL results from more than 100 papers have identified more than 1500 QTL (Figures 1 and 2). An extensive summary can be found at the new database PigQTLdb that combines all the published QTL information and allows the user to search by, chromosome, trait or key words from the publications [19, 20].

The main goal of setting up a QTL database was to summarize the most important QTL mapping results in pigs, in order to be able to select regions of interest for future researches with regard to different traits of interest. Traits were grouped into main classes as growth, fatness, carcass, reproduction and meat quality.

Some traits have extensive numbers of QTL (i.e. fatness), while others (i.e. health, disease resistance) have had few being discovered.

Several groups have worked on the identification of QTLs controlling meat quality in pigs. QTLs with significant influences on meat quality were located on almost every porcine chromosome. In PigQTLdb, more than 1000 QTLs are reported for meat quality [7, 20] and nine chromosomes were identified as being of most interest with regard to meat quality traits: SSC2, SSC4, SSC5, SSC6, SSC7, SC11, SSC14, SSC15 and SSC17.

It is interesting to note that only a limited number of the found QTLs have been further investigated to the point that a known causative mutation has been implicated. These include *IGF2* for muscling [21–23] and *PRKAG3* for meat quality on chromosome 15 [24, 25]. Moreover, another QTL mapped on chromosome 2 and with effect on tenderness is associated with *CAST* gene, even if the causative mutation responsible for the QTL is not yet reported [26, 27].

As a consequence of the fact that only a few QTLs have been characterized at the gene level, the implementation of marker assisted selection (MAS) in commercial breeding is limited [16]. There may be several reasons for this. For example, most of the QTL detection experiments were conducted on experimental crosses. It is not clear to what extend the detected QTLs are polymorphic within commercial populations and therefore it is necessary to perform QTL mapping within commercial populations [16]. Another drawback is the low map resolution of most of the experiments. However, even if the average distance between

markers is about 2–3 cM, some large gaps exist in the pig genetic linkage map (http://www.marc.usda.gov/genome).

Scientific advances in DNA marker technology resulting from moderate resolution linkage maps and advances witnessed in plant breeding raised high expectations for MAS in livestock. Unfortunately, these expectations of the rate of inclusion of MAS into animal production have not been fully realized. Failures include the use of genome scans that result in wide QTL regions exceeding 50 cM, thus containing hundreds of potential candidate genes. This outcome complicates the application of specific markers into outbred populations because marker–QTL relationships must be established on a within-family basis. Another rate-limiting step is that the size of the observed effect for a given QTL may be too small to validate because of the costs and time constraints, thus affecting the application of experimental findings to commercial populations [9, 16].

Critically, we may conclude that QTL mapping, up to date, is not very successful in the identification of genes underlying complex traits, like meat quality. The usually applied linkage analysis approach for detecting QTL in genome scans has limited power and QTL are positioned inaccurately. However, in view of the time-span since it first became possible to map QTL in pigs, a serious advance has been made and several developments, which may contribute to future success, have been published. These include genomic resources, animal resources, techniques and improved statistical methods. Moreover, whole genome scans for markers in population-wide linkage disequilibrium with QTL become feasible with the availability of dense marker maps combined with new analysis approaches [16].

IMPORTANT GENES AFFECTING MEAT QUALITY TRAITS IN PIGS

Researchers are currently looking for genes influencing genetic potential for meat quality traits to select animals. This is a promising approach because genotyping is now increasingly easy and less expensive to perform. The pig genome database include more than 3000 genes and 1 400 000 markers (Table 1) and the proliferation of genomic information is rapidly expanding and in constant progression [17, 19]. The extensive research, carried out by many different groups, in an effort to expand the basic knowledge regarding genes for quantitative traits, is reflected in the huge increase in the number of genes from January 2004 to October 2007 (Figure 3).

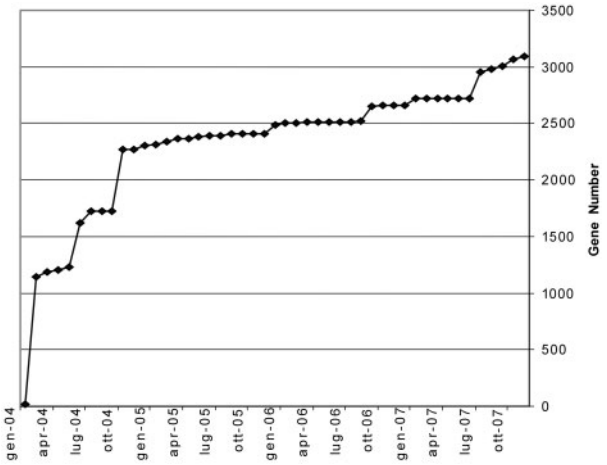

Figure 3: Pig genes discovery from January 2004 to October 2007 according to Entrez Gene database (updated 29 October 2007).

Up to now, several genes influencing body composition and meat quality have been identified using both candidate gene and genome scan approaches.

Two major genes affecting pig meat quality are known: Ryanodine receptor (*HAL* o *RYR1* gene) that regulates Ca^{++} transport across muscle cell membranes [28] and Rendment Napole (*RN*) gene that affects glycogen content of muscle [25].

Halothane sensitivity or Halothane gene is also referred as porcine stress syndrome (PSS) gene has been extensively studied. The PSE defect of meat, characterized by pale, soft and exudative muscles, has the same inheritance as PSS or malignant hyperthermia syndrome (MHS). The economic consequences of PSE defect are clear through its negative effect on the most important qualitative characteristics of meat: colour, texture and drip loss [1, 2, 28]. These adverse effects result in meat appearance (unacceptable for the consumers), reduce the production yield of cooked ham and increase the seasoning loss of dry-cured hams [29–31]. PSE meat is caused by an extensive protein denaturation due to a higher rate of glycolysis and low pH values early post-mortem in combination with the simultaneously high temperatures [29]. Halothane sensitivity also affects carcass traits and it is well documented that halothane positive pigs give heavier, shorter and leaner carcasses than halothane negative pigs [29].

Table 2: Main markers associated with meat and carcass quality in pigs applied in the industry [13]

DNA marker/Gene	Developer	Trait	First application	Reference
HALI843 (*CRC1*)	Guelph/Toronto	Stress susceptibility; MQ; Yield/FC	1991	[28]
MC4R	ISU/PIC	DG/FC/Lean	1998	[35]
RN-/rn+ (*PRKAG3*)	INRA/Uppsala/Kiel; ISU/PIC	MQ	1997/1999/2000	[24, 25]
IGF2	Liege/Uppsala	Lean	2002	[21–23]
MQ (several genes)	PIC and ISU/PIC	MQ	2001	[33]
CAST	ISU/PIC	MQ	2003	[26, 27]
RL, DA	PIC	RL, DA	2003	[5]

MQ, meat quality; FC, feed conversion; DG, daily gain; RL, reproductive longevity; ISU, Iowa State University; INRA, Institut National de la Recherche Agronomique, France.

On the whole, the gene definitely improves carcass lean content, but has a detrimental effect on pH, colour, drip loss, intramuscular fat and it reduces meat tenderness and juiciness. The molecular mechanism responsible for the effect of the *HAL* gene on carcass leanness and muscular hypertrophy remains to be elucidated. PSS phenotype is caused by an R614C missense mutation in *RYR1* gene (Ryanodine receptor 1 is an ion channel that regulates the release of Ca^{++} in skeletal muscle). A recessive mutation of the gene causes susceptibility to malignant hyperthermia, which can be triggered by stress or exposure to the anaesthetic gas halothane. Individual carrying the halothane gene are highly susceptible to stress accompanying pre-slaughter treatment, even with careful handling. This can result in a 90–95% incidence of PSE, production of carcasses that are 3–4% leaner with less backfat [29]. The *HAL (RYR1)* locus was mapped to porcine chromosome 6 [30]. A DNA test for the defective allele (HAL 1843^{TM}) is patented and used widely throughout the world [28]. World wide, the frequency of this defective allele has decreased to nearly zero, though some lines maintain the gene, in order to capture the increased lean meat produced from heterozygous pigs.

The Rendment Napole (*RN*) gene has been object of several studies over the last years as this is a single major gene affecting the meat quality traits in pigs. The *RN* gene identified in the Hampshire breed has two alleles, a dominant mutant allele RN^- and a recessive normal RN^+ allele. The mutation in the *RN* gene is associated with reduced Napole yield and leaner carcasses, and it results in poor meat quality having a lower pH because of post-mortem degradation of glycogen, described as 'acid meat' [32]. The *RN* gene was mapped to chromosome 15 in 1996 and after several years Ciobanu *et al.* [24] reported the identification of the causative mutation, which encodes a muscle-specific isoform of one of the regulatory subunits of adenosine monophosphate (AMP) activated protein kinase complex (named *PRKAG3*). Additional mutations were found in the *PRKAG3* gene associated with meat quality of pork loin [24, 26, 27]. The results of the studies clearly indicate that *PRKAG3* plays a key role in the regulation of energy metabolism in skeletal muscle. The RN^- phenotype, associated with elevated glycogen content in muscle, has no effect on early post-mortem pH values but results in a pH24h value which is associated with worsened water-holding capacity (WHC) and higher reflectance [32]. This phenotype occurs at high frequency only in the Hampshire breed. The test for the RN^- mutation is being used to remove the defect from primarily Hampshire-based lines and this genetic test represents another important additional tool to be used by pig breeders to improve meat quality.

Pig breeding companies are now paying more attention to meat quality and they are including quality traits as an integral part of selection programs to obtain simultaneous improvement in both quality and production traits. In Table 2 genes and markers being used in the swine industry are reported.

These and other genes/markers will be utilized at all levels of the meat pork industry/pork chain to improve carcass composition and meat quality.

The development of the pig gene identifications has stimulated interest in breeding for meat quality and this is particularly interesting where the trait cannot be measured on the selection candidate, but it needs to be measured on its relative's post-mortem with high costs [5, 6, 13, 33]. The advantage of incorporating markers into selection programs can

provide a very useful resource and tool. Some recent new markers for meat and carcass quality with relevant effect include polymorphism in the genes coding for calpastatin (*CAST*) [26, 27], insulin-like growth factor 2 (*IGF2*) [21, 22, 34], melanocortin receptor 4 (*MC4R*) [35]. The molecular tools that are now available make possible to use a relatively large number of candidate genes and this facilitates the development of additional markers for each trait.

RECENT ADVANCES IN FUNCTIONAL GENOMICS AND THEIR APPLICATION TO PORK MEAT QUALITY: NOVEL INTEGRATIVE GENOMICS STRATEGIES TO IDENTIFY GENES FOR COMPLEX TRAITS

Recent technological advances have created new opportunities to study the complex field of meat quality traits in pigs considering a more holistic view of the biological system under study with respect to meat traits. Instead of focusing only on the discovery of a single gene or DNA markers that co-segregate with a qualitative characteristics of pig meat, in recent times the researchers focused their interests on elucidating complex traits by the detection of the large-scale molecular gene expression profiles, gene clusters and networks that are characteristics of a biological process or of a specific phenotype [35]. Thanks to the development of high-throughput techniques such as DNA arrays and proteomic approaches allowing a global view of gene expression, it is now possible to add functional genomics to the range of approaches available for understanding the molecular basis of pork meat quality [36–39].

The application of these new genomic tools has the advantage of generating information in parallel on multiple genes and gene products, which in turn provides the opportunity to identify pathways and interacting genes [4, 38, 40]. In this way, the approach is providing insight into epistatic effects of genes that can further improve the understanding of the genetic component of meat quality.

In recent years, no other methodological approach has transformed molecular genetics more than the use of microarray. This technology has led the way from studies of the individual biological functions of a few related genes, proteins or, at best, pathways towards more global investigations of cellular activity [41].

The development of this technology immediately yielded new and interesting information and has produced more data that can be currently dealt with. It has also helped us to realize that a simultaneous molecular and structural genomic analysis is a prerequisite for the elucidation of the complex and interrelated processes affecting the phenotypic variability of a quantitative trait [42].

However, the application of the microarray technology has many issues to be dealt with. A main issue relates to the analysis of results from microarray experiments [43, 44]. Limitations to data analysis exist, although basic standards have been established and there is much to be improved with regard to data interpretation [45–47]. Although the image acquisition is well advanced, there are no standards at the level of data filtering, which is done according to the researcher's experience, leading to discrepancies at this early stage and preventing a high degree of reproducibility [46, 48]. As consequence, data comparison is difficult. Normalization, a process that adjusts microarray data for the effects that arise from variation in the technology rather than from biological differences, is another important early step in the analysis process. Fortunately, continuous progress in normalization issues is being made. Only with the establishment of commonly accepted protocols and routines will a better cross-evaluation become feasible [37, 48, 49]. The Microarray Gene Expression Data (MGED) Society is pushing towards common protocols for transcription analysis. Apart from quality issues, data interpretation is currently the main bottleneck in microarray analysis. In particular, the automated integration of complementary information in analysis algorithms is not very well established. In part, this is because of both lacking common nomenclature and data not stored in an easily queried format. The Gene Ontology Consortium and similar initiatives have taken on the crucial task of providing such a common framework [47–49].

The current challenge for genetic research on meat quality traits is to integrate structural and functional genomics [38, 41] and also to associate data from the different 'omic' sciences with phenotypic data. This process is facilitated by rapid developments in bioinformatics, which has followed the rapid expansion in genomic research. More generally, bioinformatics is becoming crucial for the

analysis of expression data with the ultimate objective to extract biologically meaningful information from the list of differentially expressed genes. A variety of bioinformatics tools are available for data-mining depending on the question being asked [49, 50]. Many experts indicated that bioinformatics and computational biology approaches will be major areas of emphasis in the next few years in both research and practical usage even for pigs [17, 51].

THE APPLICATIONS OF FUNCTIONAL GENOMICS TO THE DETECTION OF GENES AFFECTING MEAT QUALITY

The high-throughput, recently developed 'omics' techniques are capable of uncovering associations between previously unknown molecules (DNA, RNA, proteins and metabolites) or previously uncharacterized DNA/protein sequences and physiological traits of interest. Genomics in general and transcriptomics in particular describe a new scientific field midway between genetics and physiology, and are thus capable of generating new biological hypotheses that can then be further studied by more focused approaches [4]. This will impact mainly on the characterization of complex traits, which are governed by interactions between many genes with small effects. An example of such a complex trait is pork tenderness, which depends on connective tissue characteristics, lipid content, fibre composition, level of proteolysis in muscle and all complex biological aspects in themselves [52].

The field of transcriptional profiling is relatively new, especially for work in the livestock species and then also in pigs. On the whole, available pig transcriptomic data for microarray projects are somewhat fragmented and sparse. Many different platforms are being used and up to now, even if the most known commercial platforms available for the pig are the Qiagen-Operon-NRSP8 13K oligonucleotide array, the 20k porcine genechip from NRSP8 and the Affymetrix 23K Porcine GeneChip [49]. From the end of September 2007, an additional Pig oligo set platform (25K Pig microarrays, http://www-crb.jouy.inra.fr/BRC/form-e.htm) is available.

Much of the data and publications are in the very early stages of understanding RNA expression profiles and expression studies on meat quality in pig using microarray have not yet developed. In pig, gene expression profiling approach has been used up to now by some groups to better understand the changes in gene expression during porcine muscle growth and development using samples from different pig breeds [53–57].

The identification of differentially expressed genes for muscle growth and development may be of high importance also for both genetic and physiological studies related to pig meat quality. The first aim when looking at gene expression in muscle is to get a better understanding of biochemical characteristics of the tissue (muscle type), which influence meat quality traits.

Papers have been published recently on embryonic and reproductive tissues [58–62] on porcine brain [63] liver and adipose tissue [64] and one study combined microarray analysis, SNP detection within expression candidates, and association and physical mapping analyses to find liver genes affecting carcass traits [65].

The functional genomics approach will provide the opportunity to investigate global changes in known or unknown genes expression in muscle and to associate them with phenotypic characteristics, and these new approaches will generate new candidate genes to be tested for marker-assisted selection to improve meat quality in pig.

The development of genomic application in animal science may allow the discovery of gene networks and classes of genes that affect and are key drivers of a specific physiological state or a specific phenotype of a quantitative trait.

CONCLUSION

Future research in pig genetics and meat quality will be the availability of the sequenced genome and large-scale DNA arrays or SNP chips to perform low cost genome scan. It is foreseeable that the emerging functional genomics technologies will allow the identification and mapping of functional allelic variants affecting meat quality and animal performance in commercial populations. The increasing value of genomics and the potential of genomics to increase the control both of qualitative characteristics of meat and of many economically important physiological functions are expected to further contribute to improve meat and carcass quality in pig.

Key Points

- The knowledge on genes involved in pig meat qualitative characteristics and on their interactions is very poor. The understanding of meat quality on a genetic basis can give benefits to overcome limits of the traditional selection methods improving the efficiency of selection for these traits.
- To date, advances in molecular genetics have led to the identification of genes or markers associated with genes that affect the meat quality trait. Many exciting discoveries have been made in the genomics fields, in relation to meat quality which are of relevance to the meat industry. It is now foreseeable that as costs of genomic analysis are reduced and technology improves, DNA information will be increasingly used by pork industry and by pig breeders associations within the programmes of genetic improvement for meat quality.
- The availability in the near future of the pig genome fully sequenced and the emerging high-throughput technologies will allow the development of high-density SNP genotyping platforms useful to identify and map the allelic variants affecting animal performance and molecular basis of meat quality.
- Genes and proteins influencing meat quality do not function independently; they participate in complex networks that ultimately give rise to biological functions pertinent in the delivery of consistent meat quality. There is now the need to adopt a more holistic approach (systems biology) to understand how cellular processes interact within an organism to delivery consistent meat quality.
- Microarray and bioinformatics are now invaluable exploratory tools to provide information on networks of differentially expressed genes and to enhance our understanding of the biological pathways underlying the delivery of consistent meat quality. The significance of array technology lies in the potential to tie specific changes in gene expression to a phenotype of interest.

Acknowledgements
This work was supported by MIUR (PRIN 2006).

References

1. Cameron ND. Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. *Livestock Prod Sci* 1990;**26**:119–35.
2. Sellier P. Genetics of meat and carcass traits. In: Rotschild MF, Ruvinsky A (eds). *The Genetics of the Pig*. Wallingford, UK: CAB International, 1998:463–510.
3. Andersson L. Genetic dissection of phenotypic diversity in farm animal. *Nat Rev Genet* 2001;**2**:130–8.
4. Andersson L, Georges M. Domestic-animal genomics: deciphering the genetics of complex traits. *Nat Rev Genet* 2004;**5**:202–12.
5. Plastow G, Sasaki S, Yu TP, *et al*. Practical application of DNA markers for genetic improvement. *Record of Proceedings National Swine Improvement Federation Conference and Annual Meeting 2003*. Available: www.nsif.com/Conferences/pdf/PracticalDNAMarkers.pdf. (24 July 2004, date last accessed).
6. Mullen AM, Stapleton PC, Corcoran D, *et al*. Understanding meat quality through the application of genomic and proteomic approaches. *Meat Sci* 2006;**74**:3–16.
7. Gao Y, Zhang R, Hu X, *et al*. Application of genomic technologies to the improvement of meat quality of farm animals. *Meat Sci* 2007;**77**:36–45.
8. Schook LB, Beever JE, Rogers J, *et al*. Swine genome sequencing consortium (SGSC): a strategic roadmap for sequencing the pig genome. *Comp Funct Genomics* 2005;**6**:251–5.
9. Wernersson R, Schierup MH, Jorgensen FG, *et al*. Pigs in sequence space. A 0.66X coverage pig genome survey based on shotgun sequencing. *BMC Genomics* 2005;**6**:70.
10. Gorodkin J, Cirera S, Hedegaard J, *et al*. Porcine transcriptome analysis based on 97 non-normalised cDNA libraries and assembly of 1,021,891 expressed sequence tags. *Genome Biol* 2007;**8**:R45.
11. Panitz F, Stengaard H, Hornshøj H, *et al*. SNP mining porcine ESTs with MAVIANT, a novel tool for SNP evaluation and annotation. *Bioinformatics* 2007;**23**:i387–91.
12. Chen K, Baxter T, Muir WM, *et al*. Genetic resources, genome mapping and evolutionary genomics of the pig (Sus scrofa). *Int J Biol Sci* 2007;**3**:153–65.
13. Rotschild MF, Hu Z, Jiang Z. Advances in QTL mapping in pigs. *Int J Biol Sci* 2007;**3**:192–7.
14. Plastow GS, Carrion D, Gil M, *et al*. Quality pork genes and meat production. *Meat Sci* 2005;**70**:409–21.
15. Van der Steen HAM, Prall GFW, Plastow GS. Application of genomics to the pork industry. *J Animal Sci* 2005;**83**:E1–8.
16. Dekkers J. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. *J Animal Sci* 2004;**82**:E313–28.
17. Schook L. Mapping the return on investment (ROI) QTL: a progress report on animal genomics. *CAB Rev Perspect Agri, Vet Sci, Nutri Nat Res* 2007;005:12.
18. Andersson L, Haley CS, Ellegren H, *et al*. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* 1994;**263**:1771–4.
19. Hu ZL, Fritz ER, Reecy JM. AnimalQTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. *Nucleic Acid Res* 2007;**35**:D604–9.
20. Hu ZL, Dracheva S, Jang W, *et al*. A QTL resource and comparison tool for pigs: PgQTLdb. *Mamm Genome* 2005; **16**:792–800.
21. Jeon JT, Carlborg Ö, Törnsten A, *et al*. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. *Nat Genet* 1999;**21**:157–8.
22. Nezer C, Moreau L, Brouwers B, *et al*. An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. *Nat Genet* 1999;**21**: 155–6.
23. Van Laere AS, Nguyen M, Braunschweig M, *et al*. A regulatory mutation in *IGF2* causes a major QTL effect on muscle growth in the pig. *Nature* 2003;**425**:832–6.
24. Ciobanu D, Bastiaansen J, Malek M, *et al*. Evidence for new alleles in the protein kinase adenosine monophosphate-activated gamma(3)-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics* 2001;**59**:1151–62.
25. Milan D, Jeon JT, Looft C, *et al*. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* 2000;**288**:1248–51.
26. Ciobanu DC, Bastiaansen JWM, Lonergan SM, *et al*. New alleles in calpastatin gene are associated with meat quality traits in pigs. *J Animal Sci* 2004;**82**:2829–39.
27. Meyers SN, Rodriguez-Zas SL, Beever JE. Fine-mapping of a QTL influencing pork tenderness on porcine chromosome 2. *BMC Genetics* 2007;**8**:69.

28. Fujii J, Otsu K, Zorzato F, *et al*. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 1991;**253**:448–51.
29. Klont RE, Lambooy E, Van Logtestijn JG. Effect of dantrolene treatment on muscle metabolism and meat quality of anesthetized pigs of different halothane genotypes. *J Animal Sci* 1994;**72**:2008–16.
30. Davies W, Harbitz I, Fries R, *et al*. Porcine malignant hyperthermia carrier detection and chromosomal assignment using a linked probe. *Animal Genet* 1998;**19**:203–12.
31. Russo V, Nanni Costa L. Suitability of pig meat for salting and the production of quality products. *Pig News Inform* 1995;**16**:17.
32. Le Roy P, Naveau J, Elsen JM, *et al*. Evidence for a new major gene influencing meat quality in pigs. *Genet Res* 1990; **55**:33–40.
33. Knap PW, Sosnicki AA, Klont RE, *et al*. Simultaneous improvement of meat quality and growth and carcass traits in pigs. In: *Proceedings of the 7th World Congress of Genetics Applied to Livestock Production Prod. INRA, Castanet-Tolosan, France*, 2002, 339–46.
34. Carrodeguas JA, Burgos C, Moreno C, *et al*. Incidence in diverse pig populations of an IGF2 mutation with potential influence on meat quality and quantity: an assay. *Meat Sci* 2005;**71**:577–82.
35. Kim KS, Larsen NJ, Rothschild MF. Rapid communication: linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene. *J Animal Sci* 2000; **78**:791–2.
36. Schena M, Shalon D, Davis RW, *et al*. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995;**270**:467–70.
37. Bendixen C, Hedegaard J, Horn P. Functional genomics in farm animals - Microarray analysis. *Meat Sci* 2005;**71**: 128–37.
38. Tuggle CK, Dekkers JC, Reecy JM. Integration of structural and functional genomics. *Animal Genet* 2006; **37**(Suppl 1):1–6.
39. Weis BK. Standardizing global gene expression analysis between laboratories and across platforms. *Nat Methods* 2005;**2**:351–6.
40. Hocquette JF. Where are we in genomics? *J Physiol Pharmacol* 2005;**56**(Suppl 3):37–70.
41. Schadt EE, Lamb J, Yang X, *et al*. An integrative genomics approach to infer causal associations between gene expression and disease. *Nat Genet* 2005;**37**:710–7.
42. Cox B, Kislinger T. Integrating gene and protein expression data. Pattern analysis and profile mining. *Methods* 2005;**35**: 303–14.
43. Lee MT, Whitmore GA. Power and sample size for DNA microarray studies. *Stat Med* 2002;**21**:3543–70.
44. Kerr MK. Design consideration for efficient and effective microarray studies. *Biometrics* 2003;**59**:822–8.
45. Quackenbush J. Extracting meaning from functional genomic experiments. *Toxicol Appl Pharmacol* 2005; **207**(Suppl 2):195–9.
46. Yang YH, Speed T. Design issues for cDNA microarray experiments. *Nat Rev Genet* 2002;**3**:579–88.
47. Muller P, Parmigiani G, Robert C, *et al*. Optimal sample size for multiple testing: the case of gene expression microarray. *J Am Stat Assoc* 2004;**99**:990–1001.
48. Larkin JE, Frank BC, Gavras H, *et al*. Independence and reproducibility across microarray platforms. *Nat Methods* 2005;**2**:337–44.
49. Tuggle CK, Wang Y, Couture O. Advances in swine transcriptomics. *Int J Biol Sci* 2007;**3**:132–52.
50. Hanai T, Hamada H, Okamoto M. Application of bioinformatics for DNA microarray data to bioscience, bioengineering and medical fields. *J Biosci Bioengineer* 2006; **101**:377–84.
51. Fadiel A, Anidi I, Eichenbaum KD. Farm animal genomics and informatics: an update. *Nucleic Acids Res* 2005;**33**: 6308–18.
52. Ouali A. Meat tenderization: possible causes and mechanisms. A review. *J Muscle Foods* 1990;**50**:129–65.
53. Zhao SH, Recknor J, Lunney JK, *et al*. Validation of a first-generation long-oligonucleotide microarray for transcriptional profiling in the pig. *Genomics* 2005;**86**: 618–25.
54. Reecy JM, Spurlock DM, Stahl CH. Gene expression profiling: insights into skeletal muscle growth and development. *J Animal Sci* 2006;**84**:E150–4.
55. Bai Q, McGillivray C, Da Costa N, *et al*. Development of a porcine skeletal muscle cDNA microarray: analysis of differential transcript expression in phenotypically distinct muscles. *BMC Genomics* 2003;**4**:E1–13.
56. Cagnazzo M, Te Pas MF, Priem J, *et al*. Comparison of prenatal muscle tissue expression profiles of two pig breeds differing in muscle characteristics. *J Animal Sci* 2006;**84**:1–10.
57. Te Pas MF, de Witt AA, Priem J, *et al*. Transcriptome expression profiles in prenatal pigs in relation to myogenesis. *J Muscle Res Cell Motil* 2005;**26**:157–65.
58. Zhao SH, Nettleton D, Liu W, *et al*. Complementary DNA macroarray analyses of differential gene expression in porcine fetal and postnatal muscle. *J Animal Sci* 2003;**81**: 2179–9.
59. Green JA, Kim JG, Whitworth KM, *et al*. The use of microarray to define functionally-related genes that are differentially expressed in the cycling pig uterus. *Soc Reprod Fert Suppl* 2006;**62**:163–76.
60. Blomberg LA, Garret WM, Guillomot M, *et al*. Transcriptome profiling of the tubular porcine conceptus identifies the differential regulation of growth and developmentally associated genes. *Mol Reprod Dev* 2006;**73**: 1491–502.
61. Caetano AR, Johnson RK, Ford JJ, *et al*. Microarray profiling for differential gene expression ovaries and ovarian follicles of pigs selected for increased ovulation rate. *Genetics* 2004;**168**:1529–37.
62. Agca C, Ries JE, Kolath SJ, *et al*. Luteinization of porcine preovulation follicles leads to systematic changes in follicular gene expression. *Reproduction* 2006;**132**:133–45.
63. Nobis W, Ren X, Suchyta SP, *et al*. Development of a porcine brain cDNA library, EST database, and microarray resource. *Physiol Genomics* 2003;**16**:153–9.
64. Hausman GJ, Poulos SP, Richardson RL, *et al*. Secreted proteins and genes in fetal and neonatal pig adipose tissue and stromal-vascular cells. *J Animal Sci* 2006; **84**:1666–81.
65. Ponsuksili S, Murani E, Schellander K, *et al*. Identification of functional candidate genes for body composition by expression analyses and evidencing impact by association analysis and mapping. *Biochim Biophys Acta* 2005;**1730**:31–40.