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Review article

Structural and functional genomics in domestic animals: the way to understand the phenotype

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Abstract. Molecular approaches to genome analysis in livestock are reviewed by discussing the contribution of molecular genome analysis to the identification of the genetic variation underlying phenotypic variation (structural genome analysis) and to the definition of the trait-associated and environment-affected gene expression (functional genome analysis) as an important prerequisite to understanding the formation of a phenotype. Aspects of using mapped 'quantitative trait loci' (QTL) or gene variants as well as the identified trait-associated and environment-affected gene expression profile in livestock production are expounded.

Key words: candidate genes, gene expression, genome analysis, livestock, QTL.

Introduction

For many years efforts of animal breeders have been directed towards an improvement of productive performance-traits, such as growth, milk yield and feeding efficiency. During the last 5 decades, genetic improvements have tremendously increased the economic efficiency of many domestic animals, mainly due to the consequent application of the principles and means of quantitative genetics and statistics. Genetic research related to animal breeding has embarked on description and delineation of the particular genetic values of elite animals for spe-

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cific traits. However, despite those dramatic improvements concerning some of the economically important traits in several species of domestic animals, several limitations of traditional breeding methods are becoming more and more apparent, particularly concerning traits that are difficult to measure, appear late during ontogenesis, are genetically negatively correlated with other economically important traits, or have a low heretability.

Molecular analysis of the genomes of farm animals may help to solve some of these problems (GEORGES, ANDERSSON 1996). The call for targeted phenotypic changes of farm animals by breeding and/or keeping strategies requires a detailed knowledge of the action and interaction of genetic, physiological and environmental factors, including regulatory processes at different levels of the formation of the phenotype. Although most of economically important traits in livestock are multi-factorial traits, there are a number of phenotypic traits that are affected significantly by only one or few genes (mono- or oligogenetic traits). Nucleotide polymorphisms within a gene sequence may directly affect the quality or quantity of the corresponding protein. Additionally, mutations resulting from fault replication, recombination of DNA or from mutagenic effects of noxae, may alter the expression and function of the corresponding protein.

Primary and secondary gene effects are modified by interacting endogenous and exogenous factors. However, whether a gene or a genetic variant is advantageous or detrimental for its carrier depends on the context of genes and environmental factors in which it is integrated. In this sense it is expected that the molecular genome analysis will significantly contribute to the identification of the genetic variations underlying a specific phenotypic variation (structural genome analysis) and to the definition of trait-associated and environment-affected gene expression (functional genome analysis) as an important prerequisite to understanding the formation of the phenotype.

Structural genomics

In animal breeding this molecular approach is directed towards the identification of genome variants that contribute to variation in performance traits and to their use as direct or indirect genetic markers in marker assisted selection programmes.

Gene variants significantly affect the phenotype

The past decade has witnessed an explosion of not only molecular but also computational technology, which has enabled the identification of genes responsible for a number of inherited disorders and for affecting single gene traits. The majority of these gene variants affect the coding sequences resulting in the formation of non-functional proteins or of proteins with altered biochemical features.

Table 1. Genes and gene variants significantly affecting the phenotype in cattle (selection)

Trait	Locus	Phenotype	Mutation	Reference
Genetic disorders	CD18 (β_2-integrin)	BLAD	A/G transition	KEHRLI et al. 1990
	ASS	citrullinaemia	C/T transition	DENNIS et al. 1989
	UMPS	DUMPS	C/T transition	SCHWENGER et al. 1993
	keto acid dehydrogenase	MSUD	T/C transition	ZHANG et al. 1990
	TG (thyroglobuline)	congenital hypothyroidism	C/T transition	RICKETTS et al. 1987
	CHS	Chediak-Higashi syndrome	point mutation	YAMAGUCHI et al. 2000
	MANA (α-mannosidase)	(α-mannosidase)	n.g.*	HEALY et al. 1996
	MANB (α-mannosidase)	(α-mannosidase)	n.g.	CHEN et al. 1995
	PYGM	McArdle disease	n.g.	TSUJINO et al. 1996
	EPB3	sperocytosis	n.g.	INABA et al. 1996
	PRG (proteoglycan)	dermatosparaxia	n.g.	TAJIMA et al. 1999
	pro-collagenase I proteinase	dermatosparaxia	n.g.	COLIGE et al. 1999
	STAT5A	roan	transition	SEITZ et al. 1999
Milk performance	CASK (κ-casein)	cheese-making properties	several point mutations	PRINZENBERG et al. 1999
	LGB (β-lactoglobulin)	allergenic potential	several point mutations	KLUNGLAND et al. 1995
	PRL (prolactin)	milk yield	n.g.	COWAN et al. 1990
Coat colour	MC1R	red and black coat colour		KLUNGLAND et al. 1995
Growth, carcass composition	MH (myostatin)	muscle hypertrophy	deletion, insertion	GROBET et al. 1997

* not given.

Tables 1 and 2 present basic information on genes that affect significantly the phenotype in cattle and pigs and which are currently used in diagnostic gene assays using direct and indirect genetic markers.

Table 2. Genes and gene variants significantly affecting the phenotype in pigs (selection)

Trait	Locus	Phenotype	Mutation	Reference
Disease	FUT1	oedema disease	point mutation	MEIJERINK et al. 2000
Coat colour	KIT	white coat colour	duplication	JOHANSSON MOLLER et al. 1996
	MC1R	black coat colour	point mutation	KIJAS et al. 1998
Stress susceptibility	RYR1	malignant hyperthermia syndrome	C/T transition	FUJI et al. 1991
Growth	MC4R	growth, fat content	point mutation	KIM et al. 2000
Carcass composition and meat quality	H-FABP	intra-muscle fat content	n.g.*	GERBENS et al. 1997
	PRKAG3	excessive glycogen content	point mutation	MILAN et al. 2000
	RYR1	decreased meat quality	C/T transition	FUJI et al. 1991
	AGRP	meat quality and growth	n.g.	KIM et al. 2000
	LEP	fat proportion, lean meat content	n.g.	HARDGE et al. 2000
	LEPR	fat proportion, lean meat content	n.g.	HARDGE et al. 2000
Fertility	RARG	litter size	point mutation	MESSER et al. 1996
	FSHB	litter size	point mutation	ZHAO et al. 1998; LI et al. 1998
	RBP4	litter size	point mutation	MESSER et al. 1996 ; ROTHSCHILD et al. 2000
	ESR	litter size	point mutation	ROTHSCHILD et al. 1996
	PRLR	litter size	point mutation	VINCENT et al. 1998

* not given.

This success has been restricted mainly to simple but rare Mendelian cases. Although important to carriers, they are of limited significance with regard to the population. As already mentioned, most economically important traits in livestock are multifactorial, influenced by environmental factors and by an undefined number of genes.

Genetic mapping of loci underlying complex production traits

Description of microsatellites as an abundant source of polymorphic and well-dispersed markers has encouraged the generation of primary maps in live-

stock species. Individual and internationally coordinated efforts have resulted in the generation of linkage maps for various species: cattle (e.g. BARENDSE et al. 1994, 1997, BISHOP et al. 1994, FERRETTI et al. 1997), pigs (e.g. ROHRER et al. 1994, ARCHIBALD et al. 1995), and sheep (e.g. CRAWFORD et al. 1995). These maps provide adequate genome coverage with an average marker interval of 2.5-5 cM. The ability to generate complete genetic maps includes the capacity to evaluate entire genomes in order to dissect complex genetic traits into their individual Medelian entities and to channel all this information into breeding programmes. The identification of the quantitative trait loci (QTL) underlying the genetic variations for such traits will contribute both to dissecting these traits into their Mendelian components and to understanding interactions between various loci (LANDER, KRUGLYAK 1995).

However, in dealing with complex traits one must be aware that it is time-consuming to improve breeding via a variant analysis of a small, limited subset of loci that are thought to contribute significantly to a given trait. Furthermore, this strategy bears the risk of choosing the wrong locus as a phenotypically relevant marker. Nevertheless, the use of knowledge of QTL will help to develop an improved understanding of the genetic regulation of complex traits. In the past few years there was a remarkable progress in detecting genomic regions containing QTL, especially those concerning milk performance, growth, fertility, and genetic diseases in cattle and pigs. In the last 5 years many experiments have identified a high number of different QTL regions in cattle and pigs, affecting milk performance, growth, meat quality, exterior, and fertility. In Table 3 bovine and porcine chromosomes are summarised, on which confirmed and putative QTL regions have been mapped. Many chromosomes harbour different QTL regions for the same trait or QTL regions for different traits.

These results demonstrate that loci underlying complex production traits can be genetically mapped. QTL positions, and highly significant QTL effects repeatedly confirmed in independent studies emphasize the potential value of mapped QTL for marker-assisted selection programmes (SPELMAN, BOVENHUIS 1998). The advantage of DNA-marker-assisted selection techniques is due to their unprecedented accuracy, the increased intensity of selection and the decreased generation interval. However, efficient utilisation of mapped QTL or ultimate positional cloning of the corresponding causal genes, require a higher mapping resolution.

Large fragment libraries (e.g. LIBERT et al. 1993, LEEB 1995) and 'radiation hybrid panels' (e.g. WOMACK et al. 1997) are now available for the isolation, identification, and physical fine mapping and ordering of these genes or markers. For example, advanced approaches, such as comparative mapping of evolutionary conserved gene sequences (e.g. O'BRIEN et al. 1993, BAND et al. 2000) and procedures for microdissecting chromosomal fragments (e.g. GOLDAMMER et al. 1996), have already been used successfully for targeted generation of chromosome region-specific microsatellite markers in cattle (WEIKARD et al. 1997) and goats (VAIMAN et al. 1999).

Table 3. Chromosomes on which QTL regions underlying milk performances, growth, exterior and functional traits have been mapped in cattle and pigs: an overview

Species	Trait	Chromosomes with mapped QTL region/s	References
Cattle	Milk performances	BTA1, BTA2, BTA3, BTA4, BTA5, BTA6, BTA7, BTA9, BTA10, BTA11, BTA13, BTA14, BTA16, BTA17, BTA18, BTA19, BTA20, BTA23	e.g. ARRANZ et al. 1998; ASHWELL et al. 1996; DAVIS et al. 1998; GEORGES et al. 1995; KÜHN et al. 1996, 1999; MÄKI-TANILA et al. 1998; REINSCH et al. 1998; RON et al. 1994, 1998; ZHANG et al. 1998
	Growth	BTA1, BTA2, BTA3, BTA5, BTA6, BTA7, BTA8, BTA9, BTA12, BTA14, BTA18, BTA19, BTA21, BTA22, BTA23	
	Exterior	BTA6, BTA27	
	Functional traits	BTA5, BTA7, BTA18, BTA19, BTA21, BTA23	
Pig	Meat quality	SSC1, SSC2, SSC3, SSC4, SSC5, SSC6, SSC7, SSC8, SSC9, SSC10, SSC11, SSC12, SSC13, SSC14, SSC15, SSC17, SSC18	e.g. ANDERSSON et al. 1994; GELDERMANN et al. 1999; MALEK et al. 2000; KERR et al. 2000; GENET et al. 2000
	Carcass composition	SSC1, SSC2, SSC3, SSC4, SSC6, SSC7, SSC8, SSC12, SSC13, SSC17, SSCX	
	Fattening traits	SSC1, SSC3, SSC4, SSC5, SSC6, SSC7, SSC8, SSC12, SSC13, SSC14, SSC18	

However, a higher resolution map only partly resolves the problem for efficient application of genetic markers in selection programmes. For polygenic traits, another complicating factor is the number of genes that may underlie a single QTL. If a QTL itself is genetically complex because several genes within the locus affect the trait, the effect of each gene on the phenotype becomes difficult to measure (LEGARE et al. 2000). Therefore, the ultimate target of QTL analysis beyond fine mapping is the identification of the gene itself. Candidate genes can be directly tested for their association with particular traits. However, with growing knowledge on sequences of entire genomes and on sets of already known genes, the usefulness of QTL fine mapping strategies is expected to increase even further by using a positional candidate gene approach. Identified causal mutations are superior for breeding purposes to secondary markers. The validity of identified QTL-marker haplotypes is restricted to the families or populations in which they were mapped because linkage phases can vary between families. In contrast,

information on gene variants can be used directly for selection without restriction to families, because gene variants directly affect performance-traits.

However, it will be difficult to map livestock QTL with the precision needed for positional cloning, except for QTL with very large effects. The main reasons are that the effects of a given QTL on the phenotype cannot be considered in isolation because of multiple interference with other QTL and environmental factors. In addition, the potential causal mutation usually does not inactivate the gene but only alters the coding or regulatory region (GEORGES, ANDERSSON 1996). In the past few years, two loci with major gene effects have been successfully cloned in livestock: a deletion in the bovine myostatin gene causing the double-muscled phenotype in cattle (GROBET et al. 1997) and a mutation in the porcine regulatory subunit of the adenosine monophosphate-activated protein kinase associated with excessive glycogen content of skeletal muscle in pigs (MILAN et al. 2000). Both experiments could successfully combine enhanced fine mapping with comparative mapping approaches under consideration of known physiological functions of homologous positional candidate genes. However, this success has been restricted largely to the relatively simple Mendelian inheritance of the double-muscled phenotype in cattle and of the excessive glycogen content of skeletal muscle in pigs. But, with increasing knowledge of entire genomes, the usefulness of QTL fine mapping strategies is expected to increase even further with application of a positional candidate gene approach.

The final target of marker-assisted selection consists in the expansion of animals carrying marker and/or gene variants associated with the phenotype of interest in the population. Structural genomics will improve the understanding of the genetic basis of expression and of variability of phenotypes and will significantly contribute to breeding progress in farm animals by marker-assisted selection programmes. However, structural genomics does not or only partly considers interaction of genes as well as effects of environmental conditions on the formation of the phenotype. But as shown in mice, interaction between loci plays an important role in phenotype formation.

Interaction of genetic loci significantly contributes to phenotype formation

The effect of interaction between loci was tested, for example, for body weight, abdominal fat weight, and serum concentrations of leptin, and IGF-I in an intercross between a high-body-weight selected and a commercial inbred mice line (BROCKMANN et al. 2000). The estimates provide evidence that direct QTL and interaction effects together account for about 60% of the phenotypic F2 variance of body weight, abdominal fat weight, and for about 40% of the phenotypic F2 variance of leptin and IGF-I serum concentration. The net effects of all interactions found at the 0.01 significance level contributed 33%, 36%, 30% and 33% of the phenotypic F2 variance of body weight, abdominal fat weight, leptin and IGF-I serum concentration, respectively. As shown in this study, interaction

between loci significantly affects formation of complex phenotypic traits but also of single gene products. Interestingly, the structural gene itself must not essentially contribute to the phenotypic variance of the gene product. Thus loci that significantly contribute directly or by interaction with other loci to the phenotypic variance of leptin serum concentration were mapped to mouse chromosomes 1, 2, 3, 4, 5, 11, 12, 13, 14, 15, 16, 17, 18, 19 , and X, but the Leptin gene is physically mapped to mouse chromosome 6.

The functional genomics, i.e. combined application of technologies for whole-genome gene expression studies, such as arrays of peptides, proteins, small molecules or mRNAs, along with sequence information, computational tools, integrated knowledge databases, and traditional approaches, will contribute to understanding the function and regulation of all genes and proteins, deciphering the underlying workings of the cells, and formation of the phenotype.

Functional genomics

Depending on the developmental stage of an organism under the influence of the corresponding genetic and environmental factors, a specific set of genes is expressed in each tissue. Identification of patterns of gene expression in corresponding organs associated with the phenotype of interest is an essential prerequisite to facilitating interpretation and analysis of QTL and to defining complex phenotypic traits by complex 'epigenetic' networks of interacting genes, proteins and environmental signals (ZWEIGER, SCOTT 1997, MARRA et al. 1998).

DNA-based as well as more recently invented protein-based techniques offer new ways to delineate in detail the function of previously insufficiently characterised genes, to identify new genes and to study regulation of trait-associated gene expression. Efficient methods to study gene expression at the RNA level have been developed recently. These tools include Northern blots, polymerase chain reaction after reverse transcription, subtractive hybridisation, DNA micro/macroarrays (e.g. FERGUSON et al. 1996, BOWTELL 1999), and differential display RT-PCR (LIANG, PARDEE 1992).

Expressed sequence tags (ESTs) represent a simple but enormously powerful new tool. The power of the EST approach is due to the fact that in a given cell only a subset of the genome is actively transcribed. By isolating mRNA and producing a cDNA library one has the tool to analyse the fraction of all active genes of this cell. The rapid access to many expressed genes, their utilisation in constructing physical maps and their importance for the analysis of genomic DNA sequence have firmly established the value of EST data sets generated on a large scale (e.g. KONONEN et al. 1998, DARVASI 1998, LOCKHART, WINZELER 2000, ALIZADEH et al. 2000). In perhaps their most fundamental application, EST databases provide a resource for rapid identification of novel gene homologues by the sequence

homology which extends over any significant portion of the gene, implies a common ancestor and to some extent functional relations (ZWEIGER, SCOTT 1997). Armed with such novel discoveries, researchers can more rapidly develop an understanding of gene families, and of molecular processes in which they are involved. Genes which are differentially expressed in tissues affected by a disease or not, are of interest, as they may provide insight into the process of disease development. With transcript profiles from a sufficiently large set of tissues, statistically significant correlations may emerge between tissue-source information (such as disease states, treatment outcomes, exposure to various environmental factors, or genotypes), and the expression levels of particular genes or groups of genes (BOGUSKI, SCHULER 1995, TOUCHMAN et al. 1997). One of the most important applications for arrays of ESTs so far is the monitoring of gene expression (mRNA abundance). The collection of genes that are expressed or transcribed from genomic DNA, also referred to as the expression profile or the 'transcriptome', is a major determinant of cellular phenotype and function. The transcription of genomic DNA to produce mRNA is the first step in the process of protein synthesis, and differences in gene expression are responsible for both morphological and phenotypic differences as well as indicative of cellular response to environmental stimuli and perturbations. Unlike the genome, the transcriptome is highly dynamic and changes rapidly and dramatically in response to perturbations or to cellular development. The knowledge of regulation and extent of expression of a gene is central to understanding the activity and biological role of its encoded protein. Additionally, changes in the multi-gene patterns of expression can provide clues about regulatory mechanisms and broader cellular functions and biochemical pathways. However, RNA-based measurements have to be applied in combination with a protein-based method. Protein-based methods are important as they describe the final product of expression, post-translational protein modifications and protein complexes. Because of their importance many methods have been developed for monitoring protein levels, including Western blots, two-dimensional gels, methods based on protein or peptide chromatographic separation and mass spectrometric detection (e. g. MANN 1999, ODA et al. 1999), methods that use specific protein-fusion reporter constructs and colorimetric readouts (e. g. ROSS-McDONALD et al. 1997), and methods based on characterisation of actively translated, polysomal mRNA (ZONG et al. 1999, DIEHN et al. 2000). Messenger RNA levels, however, are informative for describing cell state and activity of genes, and for most genes changes in mRNA abundance are related to changes in protein abundance (LOCKHART, WINZELER 2000). The combined use of all these methods of functional genome analysis, along with sequence information, computational tools, integrated databases, and the traditional biological approaches, will significantly contribute to a better understanding of the formation of the phenotype of farm animals in order to improve breeding strategies and keeping conditions, as well as the well-being of farm animals.

Conclusion

The integration of QTL mapping approaches and developing technologies (such as high-throughput gene expression analysis, novel molecular genetic tools for genome manipulation, comparative mapping and sophisticated bioinformatics applications) will facilitate efficient large-scale functional mapping of genes to complex traits, and to estimate the effects of environmental factors. Thereby, a better framework for connecting genotypes with phenotypes will be achieved.

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