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Genetic Aspects of Lactation

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Summary

Genetic analysis of milk production on the animal level is a complex issue, which may be elucidated on the basis of a few aspects. Numerous genes are involved in lactation; their gene variants were however rarely identified to be responsible for the variation of trait values between animals. Associations between carriers of genotypes or alleles and trait values in populations were extensively studied and merely seem to indicate variable effects of linked loci. QTL analyses regarding lactation traits mapped numerous effects in more or less large chromosome intervals. A number of polymorphic promoter variants were identified and some of them were associated with the quantity of allele specific mRNA in mammary gland or allele specific proteins in milk. However, even if the results look very promising, no discrimination between the analysed polymorphic sites and the context of the respective gene will be possible from studies of associations with the target traits in different individuals. Functional sites of milk protein coding genes may be separately analysed in transgenic animals or cells, but up to now very few comparisons of allelic regulatory sites were reported. New methods available from the field of biotechnology provide enough power and flexibility which is necessary to improve the causal analysis of function of numerous genes as well as of specific intragenic sites. Consequently more and more knowledge accumulates for lactation genetics which can be exploited for economic profit. However, also the concerns are being discussed, e. g. for genetically modified milk. Concerns need also to be considered regarding the application of markers or causative alleles in selection of animals (e. g. diminishing of genetic diversity).

Introduction

As reflected in many reports, increases in milk production per cow during the last 50 years have been dramatic. Some of the improvements in lactation can probably be explained by changes in the structure and function of genes which act on the mammary gland. In principle, complex traits like lactation are under homeostatic control, where alterations in the expression of one gene are counterbalanced by the endogenous expression of other genes. This means that identifying single causative genes for lactation traits is difficult, bearing the danger of misinterpretation as milk production on the animal level depends on more than only the function of the mammary gland.

Some of the genetic aspects of lactation in dairy cattle however can be focused on the involved genes, studies of association between gene variants and trait values, QTL mapping regarding lactation traits, effects of promoter variants, and analyses in transgenic cells or animals.

1 Genes involved in lactation and "milk traits"

Table 1 lists some of the major genes which are involved in lactation.

Milk proteins are the main source of nutrition for the neonate mammal. All genes which code for secreted proteins are expressed in epithelial tissues like mammary and salivary glands and have functions in host defence, nutrition and immunomodulation. In placental mammals caseins are the major milk proteins. Depending on the species, three to four evolutionary related genes, coding for the so-called "Calcium-sensitive" caseins, and a functionally related κ-casein coding gene are located in a casein gene cluster region of between 250 and 350 kb. Comparative analysis of this cluster shows the unusual high divergence of the coding regions of the casein coding genes within species and even within breeds. This variation contrasts with the conservation observed regarding the structure of the entire casein gene cluster and for a number of non-coding regions between species. A more actual topic of research is orientated to the analysis of regulation of milk protein gene expression. The genes influence the intracellular transport, accumulation as well as secretion of their products. The tissue specific expression is caused by gene promoter sequences containing binding sites (response elements) for ubiquitous as well as mammary gland specific transcription factors.

Lactose is synthesized in the golgi apparatus of the mammary secretory cells by the lactose synthetase complex. This complex is composed of the α-lactalbumin and the enzyme α-1,4-galactosyltransferase. The production of lactose is critical in the control of milk secretion and consequently for milk volume. Of all the bovine milk protein genes, the expression of *LALBA* is the most lactation-specific and strictly controlled.

Equivalent knowledge as for the milk protein coding genes exists for further classes of lactation connected genes. Table 1 lists some genes which are involved in milk fat synthesis and secretion. At least two enzymes catalyze the reaction in which triacylglycerol is covalently joined to long-chain fatty acyl-CoA in order to form triglycerides as major constituents of fat. *DGAT1* (diacyl-glycerol O-acyltransferase homolog 1) encodes one of these enzymes. Knockout mice that lack both allelic copies of *DGAT1* showed deficient lactation. In cattle the locus was mapped in the region of a major milk fat content QTL (Grisat et al. 2002, Winter et al. 2002). Triglycerides in the VLDL (very low density lipoprotein) are hydrolyzed in the mammary capillaries by an enzyme called lipoprotein lipase (LPL). The resulting products are taken up by the mammary epithelial cells and used for the triglyceride synthesis. Acetyl-CoA carboxylase (ACACA) is the key enzyme for the fatty acid synthesis pathway and milk fat synthesis during lactogenesis. The gene for the Fatty Acid Synthetase (FASN) codes for an enzyme responsible for the chain elongation of the fatty acid chains.

Among the many genes which influence mammary gland development, growth and apoptosis probably those coding for hormones, receptors or DNA binding proteins belong to the most important. Members of the GH (GH1, GHRH) and IGF family (IGF-1, IGF-II, Insulin) together with their respective receptors (e. g. GHR) were shown to act as central regulators of energy metabolism, mammalian growth and development. Six different IFG binding proteins (IGFBP) and five different classes of membrane receptors may interact with ligands in the IGF family. STAT5A and STAT5B convert extracellular signals into gene transcription that determines the functions and phenotypes of mammary gland cells (Groner and Hennighausen 2000). *STAT*

factors comprise a family of seven genes encoding proteins of similar domain structure and modes of activation.

Thus, some knowledge is available about the structure and function of genes and how they control metabolic pathways. Many genes have significant functions for lactation traits; however information about the structure and function of genes and how they control metabolic pathways does not necessarily indicate the importance of gene variants for the variation of trait values between individuals. There are only few DNA sequence data on genomic level available for cattle. We have to hope for a sequencing of the total bovine genome and for more data regarding DNA variability of individual gene regions, i. e. about the conserved and less conserved genomic DNA regions in the species cattle.

2 Association between carriers of gene variants (or markers) and lactation traits

Identified alleles at single loci can be used to mark animals which carry distinct allelic variants and to analyse their association with trait values, either through a direct test for the desired variant or by tests for linked markers. Over 30 years studies focused on relationships between carriers of genotypes of very different loci and traits of lactation. As example **Table 2** summarizes the results on associations between carriers of different milk protein coding alleles and traits of milk composition, yield and processing. However, such associations pertain not to an effect of a single gene, but to that of a chromosome interval (cluster of genes, haplotype). Therefore the results were not consistent across studies. Several reasons may be responsible for the conflicting data, e. g. breeds, design of experimental studies, origins of individuals, statistical methods and variable haplotypes. However, new methods can be used for much improved analyses of associations, i. e. the regarding of families, phylogeny, typing of several linked genes or quantification of specific gene products (allele specific mRNAs or proteins).

3 QTL mapping regarding lactation traits

More advanced studies were performed within families, include several linked loci and so allow a mapping of QTL (quantitative trait loci) which affect the trait values of lactation. Much of the QTL analysis in dairy cattle was performed in order to map loci associated with milk production within breeds (mainly Holstein), rather than generating crosses between populations in order to take advantage of differences between populations, as it is performed in pig QTL mapping. Usually a large number of polymorphic loci were typed for alleles; they covered the entire genome, a chromosome or a section within a chromosome. Almost all QTL studies in cattle use the half-sib structure available in the industry and include the offspring from a number of sires. Genes that are heterozygous in individual sires will segregate in the offspring and allow a mapping of gene effects. If trait values of the daughter performances depend on the marked chromosome sections which they inherited from the sire, they will be associated to the marker intervals (“daughter design”). The “granddaughter design” uses sons instead of daughters. The sons can be evaluated on the basis of records from large numbers of their daughters, and breeding values are associated with the marker loci. Confidence intervals of QTLs can be narrowed with the help of linkage disequilibrium mapping in populations.

In between more than 20 experimental studies were reported, however not all with independent material. **Fig. 1** assembles the results from studies for bovine chromosomes which harboured the most frequently described QTLs. A QTL identified in such a scan has a confidence

interval of several cM (mostly >> 10 cM), and the causative genes are expected to be located within the marker interval, but are not directly known. Almost none of the major candidate genes (compare **Table 1** with the Type 1 genes in **Fig. 1**) were detected within QTL intervals.

QTL measure net-effects between haplotypes of a chromosome section which may be different between individuals (sires) included. QTL analyses are affected by epistasis, stratification, assortative mating, effects of age, heterogenous environment and other influences which therefore led to different results between studies. QTL mapping results from population (linkage disequilibrium mapping) possibly bear misleading effects when complex quantitative traits are used for which several causative sites of different gene loci but also within a distinct locus may have influence on the trait values. Therefore parameters for a population need high efforts, should carefully be interpreted and might have less relevance for application. Consequently QTL mapping have to be supported by further approaches.

4 Effects of promoter variants on protein binding and gene expression using population data

Moving from a situation in which the interval of a QTL allows the identification of the underlying gene or gene cluster, we may be interested in analysing the causative nucleotide variants. However, eukaryotic genes can be rather complex and usually include numerous polymorphic nucleotides. About 1 % of the nucleotide positions of the bovine milk protein coding genes in cattle were observed to be variable in 13 animals from different breeds (Geldermann et al. 1996), resulting in an expectation of roughly 100 polymorphic sites within a gene of 10 kb, and the question is which of the several allelic positions alters the function of the regarded gene most.

A first approach was the analysis of associations between promoter variants and protein binding as well as gene expression based on samples from populations. Polymorphisms within the protein binding sites (response elements) of milk protein coding genes were studied in different breeds. A number of polymorphic sites were identified and some of them were associated with the quantity of allele specific proteins in the milk (**Table 3**). Mobility shift assay and DNaseI footprinting confirmed differential binding affinity due to an allele specific mutation. Only few association studies, mostly covering a small number of animals, are available concerning allele specific mRNAs, and the in vivo relevance of allele specific protein binding is not proven. However, even if the results look very promising, no discrimination between the analysed polymorphic sites and the context of the respective gene will be possible in regard to the association with target trait values in different individuals since together with a single polymorphic nucleotide (SNP) in a breed and even in a species we always have to expect linked SNPs in disequilibrium.

5 Analysis of functional sites within genes in transgenic cells or animals

The transgene technology allows an isolated analysis of functional sites, their reaction with ligands, and a comparison between allelic variants of a gene. The required pre-condition however is that the causative SNP is known or at least strongly assumed. In principle the experimental approach includes a generation of animals or cell lines which carry a transgen containing a regulatory sequence in ligation with a reporter gene, the quantification of reporter gene expression, and the analysis of influences on gene expression, e. g. hormones.

Expression studies in transgenic mammary gland cells or animals were performed for several of the milk protein coding genes and indicated hormone induced activation of gene promoters.

RNA extracted from somatic cells in milk has been shown to be representative of gene expression in the mammary gland and thus provides a source for gene expression studies (Boutinaud und Jammes 2002). In vitro expression systems allow the analysis of regulatory mechanisms and the study of the lactoprotein gene expression under simplified and standardized conditions. However, the study of complex spatial and temporal expression patterns of genes requires the application of experimental animals. As shown in **Table 4**, different promoter sequences for lactoprotein coding genes were tested. As in many fields of research, transgenic mice or murine cell lines are used as test systems for investigations of regulatory DNA sites involved in lactoprotein expression. The results obtained show that function of evolutionary conserved regulatory sites involved in bovine milk protein expression can be investigated and allowed a mapping of functional sites (cis-acting regulatory elements) within genes that are relevant for lactation. However, results from very few comparisons of allelic regulatory sites are reported so far. Moreover, actually the test systems are time consuming and insufficiently sensitive. There is a demand for development of homologous cell systems, their use under defined conditions and the quantification of gene expression with help of multiplex methods like DNA- and protein-arrays.

6 Aspects for application and further research

Only some chromosome regions and genes were analysed for their effects on lactation (Debeljak et al. 2000). However, high milk performance is more than simply the result of the mammary gland capacity and regulation. Features of e. g. body size, metabolism, udder composition as well as adaptation are involved. This means that the identification of single-gene approaches in order to enhance production characteristics is a difficult challenge and needs to regard the complex biological control of the entire organism. However, there are new methods available from the field of biotechnology seem to have the necessary power and can be adjusted for a causal analysis for function of numerous genes as well as of the specific sites within the genes. Those methods of structural and functional genomics, like the microarrays, are well described in literature.

Consequently more and more knowledge in lactation genetics can be applied for economic profit. On one hand, direct and accurate DNA tests identify the presence of distinct alleles and can be performed using very small samples that can be collected at any time. So e. g. an animal can be tested and selected for breeding early in life, before information of milk performance is available. On the other hand, impact of application rises from the generation of transgenic cells and animals. In view of application many examples are discussed in literature. They may be divided into the following strategies:

- Improvement of yield and composition of milk components with the target to maximize milk performance, technological quality of milk, and / or offspring growth.
- Use of animals as a model for human mammary diseases and oncogenesis.
- Development of promoter sequences for production of recombinant proteins in mammary gland cells or in transgenic animals.
- Analyses of allelic variants for selection assisted by markers or causal gene variants.
- Introgression and composing of chromosome sections marked by allelic variants from donor populations in a recipient population.

As in other fields of research, there are concerns to be considered. They are being well discussed for genetically modified milk (public acceptance, animal welfare, safety of the product, profitability). However, further concerns for using markers or causative alleles in selection of animals may rise from insufficient knowledge of allelic compositions that are responsible for the

variance of a performance trait in the regarded population. For example, a strong selection on a distinct allele will lead to a relatively large homozygous chromosome interval in the population. Several closely linked loci will be affected as well and the genetic diversity will be diminished without knowing the importance of the there located DNA variants for the trait values. For chromosome intervals with fixed alleles further selection will produce no response and only very rarely mutations will generate new variants. Therefore the genotype assisted selection can cause irreversible disadvantage and should not be used for breeding before the causal DNA variants within the influenced chromosome section are known. Moreover, simultaneous selection may be essential in order to keep the flanking gene regions variable during selection on a distinct allele; however that would require additional knowledge and efforts. Thus more basic research is required, but also advanced regulations for practical breeding.

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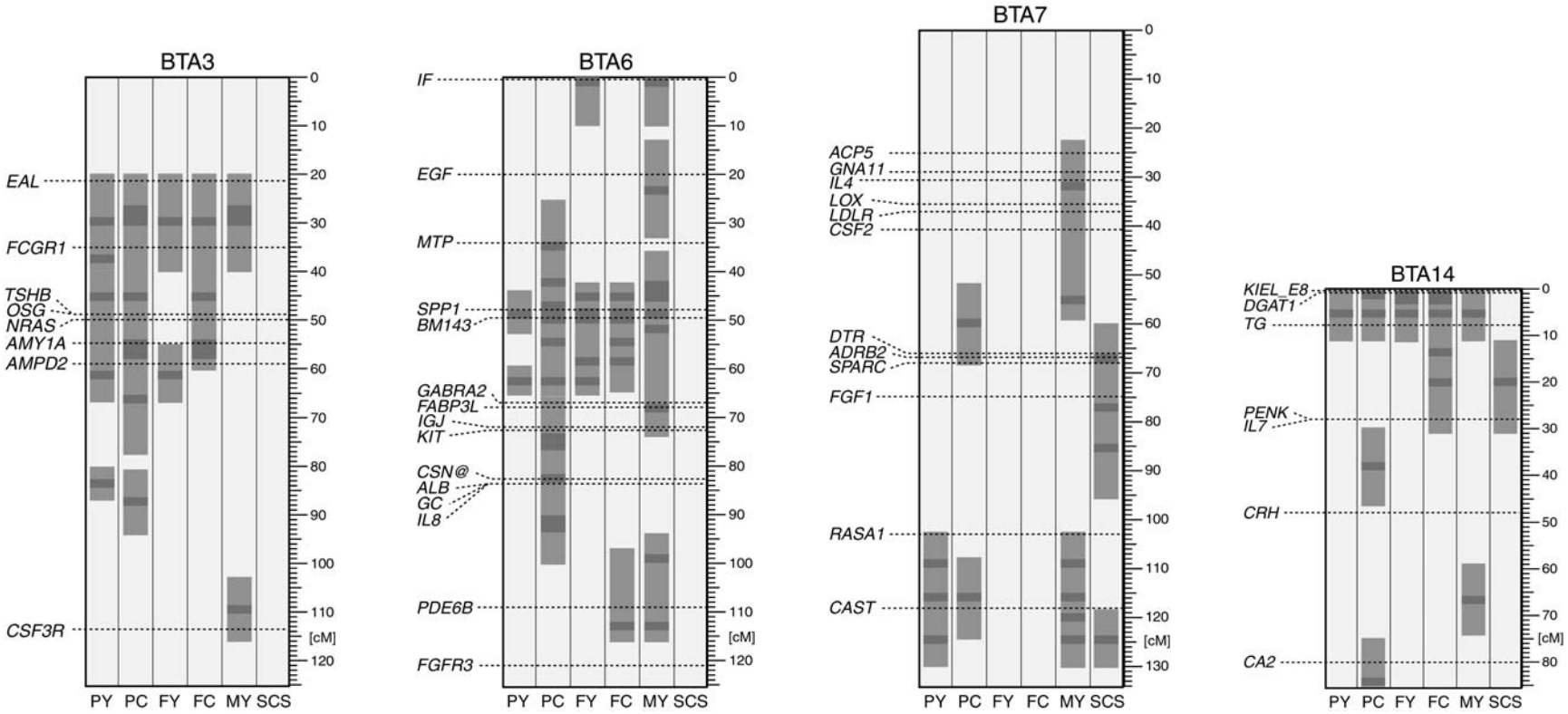
Fig. 1: Intervals of Quantitative Trait Loci (QTL) for lactation traits on bovine Chromosomes which were most frequently described
PY, protein yield; PC, protein content ; FY, fat yield; FC; fat content; MY, Milk yield; SCS, somatic cell score.

References: Ashwell et al. 2004, Ashwell and Tassell 1999, Bennewitz et al. 2004, Farnir et al. 2002, Freyer et al. 2003, Geldermann et al. 1985, Grisart et al. 2002, Heyen et al. 1999, Kühn et al. 2003, Mosig et al. 2001, Olsen et al. 2002, Plante et al. 2001, Riquet et al. 1999, Rodriguez-Zas et al. 2002b, 2002a, Ron et al. 2001, Velmala et al. 1999, Viitala et al. 2003, Zhang et al. 1998.



cM, MARC97 map units; Type I marker loci mapped in cattle are indicated.
BTA3: EAL, erythrocyte antigen L. FCGR1, Fc fragment of IgG, receptor for CD64. TSHB, thyroid stimulating hormone, beta polypeptide. OSG, oviduct specific glycoprotein. NRAS, neuroblastoma RAS oncogene homolog. AMY1A, amylase 1, alpha. AMPD2, adenosine monophosphate deaminase 2. CSF3R, colony stimulating factor 3, receptor. **BTA6:** IF, complement component 1. EGF, epidermal growth factor. MTP, microsomal triglyceride transfer protein. SPP1, secreted phosphoprotein 1 (osteopontin). BM143, microsatellite locus. GABRA2, gamma-amino butyric acid A receptor alpha 2. FABP3L, fatty acid binding protein (heart) like. IGJ, immunglobulin J polypeptide. KIT, Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog. CSN@, casein gene cluster. ALB, albumin. GC, group-specific component (vitamin D binding protein). IL8, interleukin 8. PDE6B, phosphodiesterase, cyclic GMP. FGFR3, fibroblast growth factor receptor 3. **BTA7:** ACP5, tartrate-resistant acid phosphatase type 5 precursor. GNA11, guanine nucleotide binding protein, alpha 11. IL4, interleukin 4. LOX, lysyl oxidase. LDLR, low density lipoprotein receptor. CSF2,colony stimulating factor 2. DTR, diphtheria toxin receptor. ADRB2, adrenergic, beta-2, receptor. SPARC, secreted protein, acidic, cysteine-rich. FGF1, fibroblast growth factor 1, acidic. RASA1, RAS p21 protein activator (GTPase activating protein). CAST, calpastatin. **BTA14:** KIEL_E8, cysteine and histidine-rich protein. DGAT1,diacylglycerol O-acyltransferase 1. TG, thyroglobulin. PENK, pro-enkephalin. IL7, interleukin 7. CRH, corticotropin releasing hormone. CA2, carbonic anhydrase II.

Table 1: Examples of candidate genes for lactation

Gene	Gene product	Map position Cytogenetic	Map position MARC 97[cM]	Major expression[1)]	Function	Exemplary references for mapping information
Milk protein coding						
CSN1S1	α_{s1}-Casein	6q31	82.6	MG	Nutrient, host defense, immunomodulation	Threadgill and Womack 1990
CSN1S2	α_{s2}-Casein			MG		Gallagher et al. 1994
CSN2	β-Casein			MG		Threadgill and Womack 1990
CSN3	κ-Casein			MG	Stabilisation of micelles	"
LALBA	α-Lactalbumin	5q21	44.5-50.5	MG	Regulation of lactose synthesis and therefore milk volume	„
LGB	β-Lactoglobulin	11q28	108.7	MG	Nutrient	Hayes and Petit 1993
Fatty acid synthesis and secretion						
DGAT1	Diacylglycerol O-acyltransferase	14q11	0.3	VT	Triglyceride synthesis	Winter et al. 2002, Grisart et al. 2002, Bennewitz et al. 2004
BTN	Butyrophilin	23q21-q23	n.m.	MG	Fat secretion	Brunner et al. 1996
LEP (OB, OBS)	Leptin (obesity)	4q32	82.8	AT	Feeding behaviour and energy metabolism	Pfister-Genskow et al. 1996
LEPR	Leptin receptor	3q33	n.m.	VT		Pfister-Genskow et al. 1997
FASN	Fatty acid synthetase	19q22	n.m.	VT	Fatty acid chain elongation	Roy et al. 2001
ACACA	Acetyl-CoA-carboxylase α	19q13-q14	n.m.	VT	Fatty acid synthesis (rate limiting)	Mao et al. 2001
LPL	Lipoprotein lipase	8	63.0	VT	Triglyceride hydrolisation	Threadgill and Womack 1991
Mammary gland development, growth and apoptosis						
GH1 (GH)	Growth hormone	19q22prox.	65.7	VT	Regulation of energy metabolism, growth and development	Hediger et al. 1990
GHR	Growth hormone receptor	20q17	50.0	VT		Moody et al. 1995
GHRH	Growth hormone releasing hormone	13q22-q23	71.0	VT		Barendse et al. 1997
IGF1	Insulin-like growth factor 1	5	73.0	VT		Bishop et al. 1991
IGF1R	Insulin-like growth factor 1 receptor	21	13.0	VT		Moody et al. 1996
IGFBP3	Insulin-like growth factor binding protein 3	4	68.0	VT		Maciulla et al. 1997
IGFBP4	Insulin-like growth factor binding protein 4	19	60.0	VT		Moore et al. 2003
PRL	Prolactin	23q23med.	43.2	VT	Lacto- and lipogenesis	Hallerman et al. 1988
PRLR	Prolactin receptor	20q17	52.5	VT		Hayes et al. 1996
STAT5A	Signal transducer and activator of transcription 5A	19q17	n.m.	VT	Transcription factor	Seyfert et al. 2000
CSNK2A2	Casein kinase 2 alpha	18	40.0	VT	Protein secretion	Aasland et al. 2000, Lasa-Benito et al. 1996, Meggio et al. 1988
Others						
FMO3	Flavin-containing mono-oxygenase-3	n.m.	n.m.	VT	Nitrogen-oxide-forming activity, fishy flavour of milk	Lunden et al. 2002
BOLA	MHC class molecules	23	35.4	APC	Antigen presentation	Andersson and Rask 1988

[1)] MG: mammary gland; AT: adipose tissue; VT: various tissues; APC: antigen presenting cells.

Table 2: Summary from literature reports on associations between carriers of different milk protein genotypes and traits of milk production for the breed Holstein

References e. g. Bobe et al. 1999, FitzGerald et al. 1999, Ng-Kwai-Hang et al. 1990, Ojala et al. 1997.

a Milk processing traits

Trait	Gene locus	Superior allele or genotype
Size of micelles	CSN3	*BB, BC*
Curd coagulation time	CSN1S1	*CC*
	CSN2	*BB*
	CSN3	*BB, BC*
Curd firmness	CSN2	*BB*
	CSN3	*BB, BC*
	LGB	*BB*
Cheese yield	CSN1S1	*BB*
	CSN3	*BB*
	LGB	*BB*

b Composition and yield of milk protein

Trait	Gene locus	Superior allele or genotype
Milk protein content	CSN1S1	*BC, CC*
	CSN2	A_2A_3
	CSN3	*BB*
	LGB	*AA, AB*
Milk protein yield	CSN1S1	*BC*
	CSN2	A_3B
	CSN3	*BB*
	LGB	*AA*
Casein content	CSN1S1	*BB, BC*
	CSN3	*BB*
	LGB	*BB*
Whey protein content (~ β-lactoglobulin content)	CSN1S1	*AB*
	CSN2	A_1A_3
	CSN3	*AA*
	LGB	*AA*

Table 3: Effects of promoter variants in milk protein coding genes on protein binding and association with gene expression

Analysed position / Response element	Experimental approach			Reference
	DNA tests	Protein binding	Association with / Number of animals[1)]	
CSN1S1				
-175 (a/g) / AP-1	RFLP	-	Total protein / 135	Szymanowska et al. 2003
"	RFLP	EMSA	Single proteins / 142	Kuss et al. 2004
-728 (t/-)	?	-	mRNA / 3	Szymanowska et al. 2004
"	RFLP	-	Total protein / 135	Szymanowska et al. 2003
"	RFLP	-	Single proteins / 3	Martin et al. 2002
-733 (t/c)	-	EMSA	-	Martin et al. 2002 ; Szymanowska et al. 2004
655 bp (5')	SSCP	-	Total protein / 678	Prinzenberg et al. 2003
CSN1S2				
+7 (a/c) and -7 (c/t)	-	EMSA	-	Szymanowska et al. 2004
-186 (c/t)	-	EMSA	-	"
"	RFLP	EMSA	-	Martin et al. 2002
-1084 (c/t)	RFLP	-	Total protein / 135	Szymanowska et al. 2003
"	RFLP	-	Single proteins + mRNA / 3	Martin et al. 2002
-1100 (ac/ct)	?	EMSA	mRNA / 3	Szymanowska et al. 2004
"	-	EMSA	-	Martin et al. 2002
CSN2				
-109 (g/c)	-	EMSA	-	Szymanowska et al. 2004
"	RFLP	EMSA	-	Martin et al. 2002
CSN3				
5' region	SSCP	-	Total protein	Kaminski 2000
LGB				
-22 (g/a); -209 (g/c) / STAT5; -662 (g/c) / STAT5	RFLP	-	Single proteins / 71	Ehrmann et al. 1997
-435 (g/c) / AP-2	RFLP	Footprint / EMSA	Single proteins / ?	Lum et al. 1997
"	RFLP	-	Single proteins / 142	Kuss et al. 2003
208 bp (5')	SSCP	-	Total protein / 104	Kaminski and Zabolewicz 2000

[1)] Single proteins: allele or locus specific proteins quantified; total protein: milk protein content and yield from records of industry.
-: Not investigated.
?: No information.

Table 4: Analysis of bovine regulatory DNA sequences from milk protein coding genes

a. Examples of studies in transgenic cells in vitro

Gene	Analysed region	Mammary epithelial cell line (species)	Major results	Reference
CSN2	-5.3/+1.6 kb	Primary cells (mouse)	Hormone and matrix dependent expression, localization in regulatory promoter elements.	Yoshimura und Oka 1990
CSN2	-3815/+42 bp	CID9 (mouse)	Multiple regulatory promoter elements between -2605 and +42 bp.	Schmidhauser et al. 1990
CSN2	-1790/+42 bp	CID9	Localization of an enhancer element (BCE1) at ca. -1.5 kb.	Schmidhauser et al. 1992
CSN2	-930/+20 bp	Bovine	No expression.	Ahn et al. 1995
CSN1S1	-681/+18 bp		Induction not hormone-dependent.	"
CSN2	-16 kb/+8 kb	HC11 (mouse)	Tissue-specific and developmentally regulated expression, integration-site-dependent.	Rijnkels et al. 1995
CSN1S2	-8/+1.5 kb	HC11	No hormone dependent expression.	"
CSN3	-5/+19 kb	HC11	"	"
CSN3	-552/+18 bp	HC11	Localization of regulatory elements between -439 and -125 bp.	Adachi et al. 1996
LGB	-759/+59 bp	HC11	Higher reporter gene expression of promoter haplotype A compared to B.	Geldermann et al. 1996
CSN2	5´ (1.8 kb) + intron 1 (2 kb)	HC11	Prolactin-inducable enhancer activity of several elements in intron 1.	Kang et al. 1998
LGB	5' (-753 bp) + exon 1	HC11	Different transcriptional activity of two promoter variants.	Folch et al. 1999

b. Examples of studies in transgenic animals

Gene	Analysed region	Species of transgenic recipient	Major results	Reference
LALBA	-477/-220 bp	Mouse	Location of important cis-acting elements	Soulier et al. 1992
CSN2	-16 kb/+8 kb	Mouse	Expression tissue-specific and developmentally regulated	Rijnkels et al. 1995
CSN1S2	-8/+1.5 kb	Mouse	No proper expression.	"
CSN3	-5 to + 19 kb	Mouse	"	"
CSN1S1	5´ (14.2 kb) + coding DNA	Mouse	Tissue-specific and developmental regulated expression.	Rijnkels et al. 1998
LALBA	5´ (2.0 kb) + exons (2.0 kb) + 3´ (329 bp)	Pig	Secretion of the translated protein into milk.	Bleck et al. 1998
CSN2	5' (3.8 kb)	Mouse	Accurate spatial and temporal expression in mammary gland.	Cerdan et al. 1998
LGB	5´ (1.2 kb) + coding DNA (1 kb)	Mouse	Position dependent, copy-number-related expression.	Gutierrez-Adan et al. 1999
CSN2	5' (1.8 kb)	Mouse	Proper regulation in mammary gland; constitutive and sex independent expression in lung; no expression in other tissues.	Oh et al. 1999
CSN2	5'(15 kb)	Mouse	Constitutive and sex independent expression in mammary gland and lung; no expression in other tissues.	"
CSN2	5´ (6.6 kb) + transcribed DNA + 3´ (2.6 kb)	Mouse	Secretion of the translated protein into milk.	Brophy et al. 2003
CSN2	5´ (6.6 kb) + transcribed DNA 3´ (2.6 kb)	Mouse	Secretion of the translated hybrid protein into milk.	"