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Identification of genetic variants in differentially expressed sequences in cattle of different metabolic type – potential genetic markers of nutrient utilization

Dedicated to Prof. Dr. Dr. h.c. mult. Ernst Kalm on the occasion of his 65th birthday

Abstract

Two cattle breeds serve as a model to identify genes and genetic variants, respectively, that are potentially associated with nutrient transformation: Holsteins bred for high milk production mainly, and Charolais bred for high body weight with outstanding muscular growth. The major differences between Charolais and Holstein regarding many general body parameters originate from differences in pathways and deposition of nutrients. In an initial experiment, expressed sequence tags (ESTs) differentially displayed between both cattle breeds were isolated by mRNA differential display in liver and intestine. Of the in total identified 277 ESTs, 79 showing the most prominent differences, were screened for single nucleotide polymorphisms (SNPs). Thirty four SNPs were detected in 30 ESTs In a direct sequencing approach based on the comparative sequencing of the corresponding amplicons generated by PCR from genomic DNA pools of 20 animals each of both cattle breeds,. Eighteen of these SNPs showed breed specific distribution of allelic variants. Occurrence of ESTs with a breed specific SNP distribution and localisation of the respective ESTs to chromosome regions known to be affecting carcass and growth traits in cattle suggest a trait association of the respective SNPs. The polymorphic nature of the SNP markers suggests that they will be useful for evaluating whether variation in these genomic regions influences nutrient pathways in cattle.

Key Words: single nucleotide polymorphism, differentially expressed sequences, metabolic type, cattle

Zusammenfassung

Titel der Arbeit: **Identifizierung von genetischen Varianten in merkmalsassoziiert exprimierten Sequenzen bei Rindern unterschiedlichen Stoffwechseltyps – Potentielle genetische Marker für die Nährstoffverwertung**

Tiere zweier Rinderrassen dienten als Modell, um Gene bzw. Genvarianten zu identifizieren, die potentiell mit der Nährstofftransformation assoziiert sind: Holsteins, die hauptsächlich auf hohe Milchleistung, und Charolais, die auf großes Körpergewicht mit herausragendem Muskelwachstum gezüchtet wurden. Die Hauptunterschiede in den wesentlichen Körpermerkmalen beider Rassen basieren auf der unterschiedlichen Transformation und Speicherung der Nährstoffe. In einem früheren Experiment wurden different zwischen beiden Rinderrassen exprimierte 'expressed sequence tags' (ESTs) in Leber bzw. Dünndarmepithel mittels ‚mRNA differential display' isoliert. Von insgesamt 277 identifizierten ESTs wurden 79 mit den ausgeprägtesten Unterschieden auf Einzelbasenpaarpolymorphismen (SNPs) untersucht. In einem Direktsequenzierungsansatz basierend auf der vergleichenden Sequenzierung der entsprechenden Amplicons, die mittels PCR genomischer DNA-Pools von jeweils 20 Tieren beider Rinderrassen generiert wurden, konnten in 30 ESTs insgesamt 34 SNPs nachgewiesen werden. Von diesen SNPs zeigten 18 eine rassenspezifische Verteilung der Allelvarianten. Das Auftreten von ESTs mit einer rassenspezifischen SNP-Verteilung und die Lokalisation der entsprechenden ESTs in Chromosomenregionen, von denen bekannt ist, dass sie Schlachtkörper- und Wachstumsmerkmale beim Rind beeinflussen, weist auf eine Merkmalsassoziation der entsprechenden SNPs hin. Aufgrund ihrer polymorphen Natur scheinen diese SNP-Marker geeignet zu überprüfen, ob Variation in diesen Chromosomenregionen den Nährstoffumsatz beim Rind beeinflusst.

Schlüsselwörter: Einzelbasenpaarpolymorphismen, different exprimierte Sequenzen, Stoffwechseltypen, Rind

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Introduction

Identifying the genetic and physiological background of variation in nutrient turnover of organisms is a well recognised prerequisite for optimised nutrition in relation to health and performance and also for efficient breeding strategies in livestock (HESKETH et al., 1998). Cattle breeds being primarily selected for either milk or meat production and strikingly differing in their respective phenotype represent a suitable model for study the genetic and physiological reasons of variances in nutritional turnover (KUEHN et al., 2002). The causative reasons why ruminants transform feed components preferentially in body tissues (accretion type) or milk (secretion type) are to a great extent unknown. So far systematic characterisation of metabolic types was limited to a descriptive level only.

During the past 5 years many experiments have identified a high number of different quantitative trait loci (QTL) regions in cattle affecting milk performance, growth and meat quality (e. g., STONE et al., 1999; CASAS et., 2001, 2003, 2004; KIM et al., 2003; KHATKAR et al., 2004). QTL positions and highly significant QTL effects repeatedly confirmed in independent studies emphasize the potential value of mapped QTL in selection for growth and milk performance using marker-assisted selection programs. However, efficient utilisation of mapped QTL in breeding programs requires a higher mapping resolution or the ultimate positional cloning of the corresponding genes or genetic variants, but also a better understanding of the complex physiological process at the cellular level.

To obtain a better understanding of the underlying causative interactions a suitable animal model for the genetic and physiological investigations intended, an F_2-resource family has been designed by crossing Charolais bulls and German Holstein cows. These cattle breeds representing the accretion and the secretion metabolic type, respectively, are the background for a maximum phenotypic variation introduced into a large number of traits including lactation, growth, carcass composition, and meat quality, as well as physiological characteristics (KUEHN et al., 2002). By the application of QTL mapping within this F_2-design genomic localisation and characterisation of genetic variation of complex traits like nutrient transformation for accretion and secretion will be studied. One of the major efforts to identify the causal gene or gene variant itself is the identification of coding sequences or transcript units (COLLINS, 1995), especially those that are localized in the QTL region of interest and that show trait-associated expression. We hypothesize that allelic variation in ESTs differentially expressed between the two metabolic types may be associated with variation in nutrient turnover in cattle. Testing this hypothesis requires genetic markers for identifying allelic variants at each gene locus.

In initial experiments, expressed sequence tags (ESTs) differentially displayed between Charolais and Holstein were identified, isolated by mRNA differential display (DDRT-PCR) and studied for their trait-associated expression by real-time RT-PCR (SCHWERIN et al., 1999; DORROCH et al., 2001). This paper describes the identification of 34 single nucleotide polymorphism (SNP) markers of a set of 30 of differentially expressed ESTs and characterisation of their distribution among each 20 cows of the Charolais and German Holstein breed. According to their localisation in QTL regions based on established integrated marker/gene-maps and their trait-associated expression, candidate genes were suggested.

Materials and Methods

SNP screening

Amplicons were generated using genomic DNA pools of each 20 bulls of both cattle breeds and amplified by PCR using EST-specific primers. A combined Charolais and Holstein DNA pool was established and used as an intermediate control. Amplicons were comparatively sequenced by Taq cycle sequencing with a model 310C sequencer (Perkin Elmer/Applied Biosystems, Weiterstadt, Germany). Potential SNPs identified according to the occurrence of two nucleotide peaks at the same position in the sequence profile were confirmed by sequencing of DNA samples of individual animals. Figure 1 demonstrates exemplarily the sequencing approach used. Primer sequences, PCR conditions, accession number of polymorphic sequences, length of overlapping fragments, sequence similarity percentage, potential homologous gene, and chromosome assignements are reported within Tables 1 and 2.

Statistical analysis

For all analyses the SAS/STAT package (SAS, 1999) was used. Distribution of allele frequencies between breeds and co-incidence of breed specific gene expression and allelic distribution were compared by χ^2 test using 2x2 contingence table.

Results

Screening for single nucleotide polymorphisms in differently expressed sequences

In a previous study, ESTs differentially expressed between both cattle breeds were identified in liver and intestine by DDRT-PCR (DORROCH et al., 2001). Of the in total identified 277 ESTs 79 showing the most prominent differences, were screened for SNP using a direct sequencing approach based on comparative sequencing of PCR generated DNA fragments. Amplicons were generated using genomic DNA pools of each 20 unrelated bulls of both cattle breeds. A combined Charolais and Holstein DNA pool was established and used as an intermediate control. Potential SNPs identified according to the occurrence of two nucleotide peaks at the same position in the sequence profile were proved by sequencing of DNA samples of individual animals. Figure 1 demonstrates exemplarily the sequencing approach used.

Table 1

Bovine intestinal (fbn-i...) and hepatic (fbn-l...) expressed sequence tags: Homologies, PCR conditions (Bovine exprimierte Sequenzen des Dünndarms (fbn-i...) und der Leber (fbn-l...): Homologien und PCR-Bedingungen)

Name	**GenBank**	**Sequences showing similarities with the bovine EST**			**Similarity**		**PCR-Amplification** (40 cycles)			
of EST	**Acc.-No.**				overlap		Primer sequences		Anneal T [°C]	Length [bp]
		Name of sequence	Species	Acc.- No.	[%]	bp	Sense (5'-3')	Antisense (5'-3')		
fbn-i112	BE217404	BAC clone RP42-354B6	*B. taurus*	AC091660	93.0	64	GAATAATGGGAATAGGAGAA	TGAGAATGGCTGCTTGTTTT	50°	275
fbn-i107	BE217407	no homology					CTGTGTGGCTAACCTGTGAC	CGTCCTCTCCTGGGCAAACA	60°	100
fbn-i102	BE217410	Werner syndrome (WRN) gene, cDNA	*H. sapiens*	AY442327	75.0	220	AACGCCATATATAGCAAGAC	GATCCAAAACAAAGCAAGAA	55°	269
fbn-i046	BE217425	BAC clone RP11-695P1	*H. sapiens*	AC025105	86.0	208	ATTCAGGGCAATTGTCAGTC	AAGAGCATTTGGCCTTTTAC	55°	226
fbn-i039	BE217430	Apolipoprotein AI and CIII mRNA	*H. sapiens*	NM_000040	83.0	165	GGCTGCCCCCAAAGGTCACT	TATTGGAAGGCCAGCACATT	60°	95
fbn-i033	BE217434	BAC clone R-102C24	*H. sapiens*	AL137818	85.0	389	CAGCAAAAACGAGTTAGATA	AGTTGTTTACATTTTTCAGA	55°	280
fbn-i080	BE217438	BAC clone RP11-77P3	*H. sapiens*	AL139803	87.0	56	TCGCAGCCTAGAGGGAGAAG	TTTGTCCTAGAATGCGTTAC	60°	228
fbn-i062	BE217449	FUS/TLS protein gene	*H. sapiens*	AF071213	89.4	292	GATCAAAACCAGCTCAGTAG	AGCAGTTGCCAACCCAGTAG	55°	234
fbn-i011	BE217464	no homology					TTAAGACTCTGCACTTTCAC	GCAGAGCAATTTCTGTAGGA	55°	400
fbn-i010	BE217465	BAC clone RP42-158g13	*B. taurus*	AC105306	88.0	626	GGGCACCATGATGATACTTC	TCTTGTTGGGGCCCTGATAA	55°	262
fbn-i009	BE217466	BAC clone RP11-128L5	*H. sapiens*	AC100860	84.0	59	CAGTGGAGTGCTGTGTAAAT	GCAGAAGCCACCAGGAGAGG	55°	264
fbn-i003	BE217472	BAC clone RP11-134L4	*H. sapiens*	AL591419	86.0	58	CTGAAAACCACCAGCAAAAA	CCATGCTCTTCTGGCTTACA	60°	127
fbn-l173	BE217476	SA gene product, mRNA	*H. sapiens*	NM_005622	88.0	180	GAATGGATAACAAAACACTT	CCTAAAATTAATTCACACTT	55°	196
fbn-l170	BE217478	BAC clone RP11-71A24	*H. sapiens*	AL359997	79.0	88	TTCCAGTTCAGAAGAAAATG	TTCAACCCCAAAGTTTATTC	55°	239
fbn-l165	BE217481	NADH dehydrogenase (ubiquinone)	*B. taurus*	X63209	98.1	160	GGCGATCCCAACAAAGAACC	TTTCTAGGATTGAAGGAGTC	55°	99
fbn-l150	BE217491	BAC clone RP11-394I13	*H. sapiens*	AC016737	88.0	147	ATCTGAGCAGCAGATTTAGC	TTCCCACCATGAAGAATAGA	50°	154
fbn-l146	BE217493	BAC clone RP11-542O24	*H. sapiens*	AC009241	100.0	26	TAAATACCCTAATGACACTG	TTTAAAATGAAAATCCACTA	50°	161
fbn-l125	BE217503	UDP-N-acetylglucosaminepyrophosphorylase 1	*H. sapiens*	NM_003115	86.0	240	CCTGAGACAAGAAGAAAAGT	TCCAAGCTAAGGAAAACATT	55°	243
fbn-l107	BE217514	Apolipoprotein CIII mRNA	*H. sapiens*	NM-000040	81.0	178	CAGCCAGAGACTGGATGACT	TGGGGAGGGCACTGAGAATG	60°	242
fbn-l102	BE217518	Alpha NAC mRNA	*H. sapiens*	NM_005594	95.0	521	GCTGCAGTTACTCTTTTGAA	AGATACCTACATTGTTTTTG	45°	349
fbn-l099	BE217521	SCC6 EST	*S. scrofa*	U89057	81.5	108	TCAATGAGCTTTAGTTTTTA	CTCTTTTGTGATAGCTGGTA	50°	313
fbn-l091	BE217525	no homology					ACACATGGAAGGTGGATTGA	AGAGCTCCATAGAGCACCTG	55°	296
fbn-l079	BE217533	no homology					CGCCCTTCTTTCTCTCACAG	TGGAAAACACAGACCCTATT	50°	265
fbn-l044	BE217549	no homology					ATTTTTCTAAACACCACAGG	AAGGGGATTTGAATGCTCTC	55°	198
fbn-l039	BE217553	no homology					TCTGAATCTTAACCAAAAAT	CAAAGCTGAGACCCCTACAT	50°	189
fbn-l036	BE217556	Tyrosin3-monooxygenase/tryptophan (YWHAB)	*H. sapiens*	NM_003404	92.0	179	GCAGCTGCTTAGGGACAATC	TCACAAAGCACGGTAGACAT	60°	400
fbn-l065	BE217570	BAC clone RP11-321A17	*H. sapiens*	AC024619	89.0	164	GCTGGCCTCCTTGCACTAAT	TCCTGCTTGGGTTTCATCTG	55°	130
fbn-l057	BE217577	BAC clone RP11-72B4	*H. sapiens*	AL161454	89.0	56	AGTTAAAGGCCTCAACAATA	ACAGTTAAGAGGATACAATG	55°	263
fbn-l008	BE217590	BAC clone RP11-425A23	*H. sapiens*	AC093835	90.0	43	TTTGAAGTCCCTTGGTGATA	TTTCGCAACTAAACAACAGC	55°	157
fbn-l063	BF146288	BAC clone RP11-746H2	*H. sapiens*	AL109953	90.0	42	ACTAAAGGGCACCATCTGTT	TGGTCAACGATGTGATAAAC	60°	369

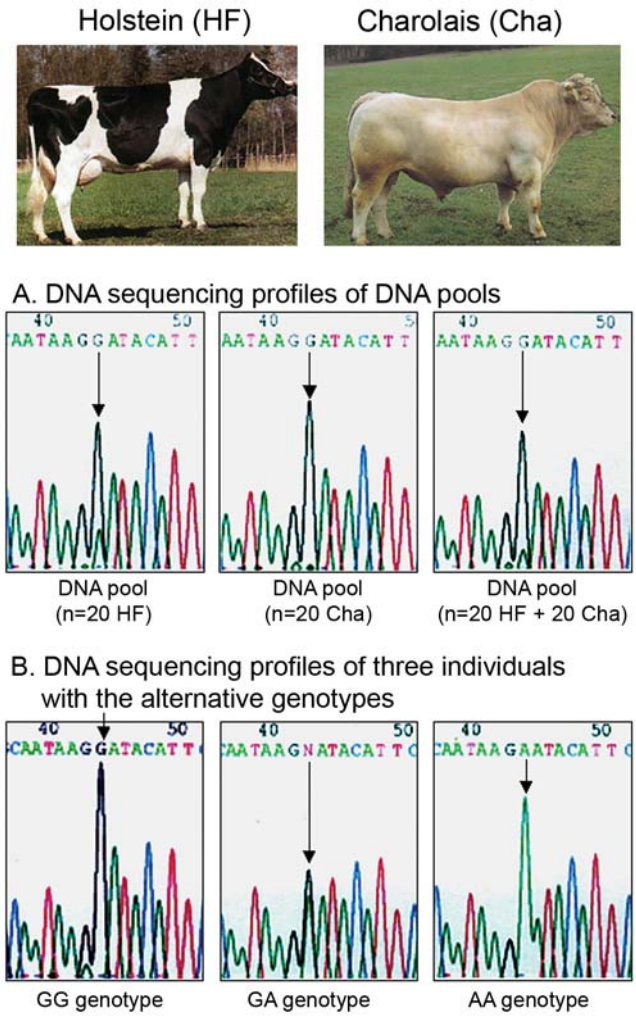

Fig. 1: DNA sequencing profiles of the expressed sequence tag fbn-l057 harbouring a G/A transition generated by Taq Cycle sequencing of A) DNA pools involving 20 unrelated Holstein and Charolais cattle and B) individuals with the alternative genotypes. Arrows indicate the variant nucleotide position. In the sequence profile, the green "A" peak under the black "G" peak in the Holstein pool (Figure 1A) represents a allelic frequency of 22 per cent proved by sequencing of the individual animals. Figure 1B shows the sequence profiles of the variant EST region of individuals of the two alternative homozygous (GG, AA) and the heterozygous genotypes (GA) (DNA-Sequenzierprofile der exprimierten Sequenz fbn-l057, die eine GA-Transition aufweist, nach Taq-Cycle-Sequenzierung von A) DNA-Pools aus 20 unverwandten Holstein- und Charolais-Rindern und B) Individuen mit den alternativen Genotypen. Pfeile markieren die variable Nukleotidposition. In den Sequenzprofilen repräsentiert der grüne „A"-Peak unter dem schwarzen „G"-Peak im Holstein-Pool (Abb. 1A), verifiziert durch Sequenzierung der Einzeltiere, eine Allelfrequenz von 22 Prozent. Abb. 1B zeigt die Sequenzprofile der variablen EST-Region von Individuen mit den alternativen homozygoten (GG, AA) und dem heterozygoten Genotyp (GA))

The use of this sequencing approach resulted in the identification of one or more SNPs in 30 ESTs of the 79 ESTs screened (Table 2). Of the identified 34 SNPs, allele distribution of 33 SNPs were comparatively analysed in each 20 cows of the Holstein and the Charolais breed, respectively. Nineteen SNPs showed significantly different distribution between both breeds. The assumed mutant variants (allele with overall lower frequency) were more frequent in the Charolais breed in 12 SNPs and in the Holstein breed in 7 SNPs, respectively.

Table 2
Definition, physical localization and genotype frequency of single nucleotide polymorphisms (SNPs) identified in bovine expressed sequence tags (ESTs) in German Holstein (HF) and Charolais (Cha) cattle breeds (Definition, physikalische Lokalisation und Genotypenfrequenz von Einzelbasenpaarpolymorhismen (SNPs), die bovinen exprimierten Sequenzen (ESTs) in den Deutsche Holstein (HF) und Charolais Rinderrassen)

Name of EST	Marker	Definition of SNPs			Allele frequency [p]				SHC mapping[c]
		Position (bp)	Allele A	Allele B	HF n = 20		Cha n = 20		
					A	B	A	B	BTA
fbn-l008	SNP-l008	85-86	CA	- -	0.80	0.20	0.42	0.58 [a]	17
fbn-l036	SNP-l036	72-98	+	-	1.00	0.00	0.90	0.10	13
fbn-l039	SNP-l039	157	T	C	n. a.[b]	n. a.[b]	n. a.[b]	n. a.[b]	3
fbn-l044	SNP-l044	68-70	CAA	TAC	0.70	0.30	0.82	0.18	5
fbn-l057	SNP-l057	65	G	A	0.78	0.22	0.94	0.06 [a]	8
fbn-l063	SNP-l063	160	$(T)_{10}$	$(T)_{11}$	0.58	0.42	0.70	0.30 [a]	13
fbn-l065	SNP-l065	62	C	T	0.42	0.58	0.70	0.30 [a]	19
fbn-l079	SNP-l079a	55	C	T	0.68	0.32	0.48	0.52 [a]	18
fbn-l079	SNP-l079b	234	G	-	0.4	0.6	0.68	0.32 [a]	18
fbn-l091	SNP-l091	82	T	C	0.88	0.12	0.52	0.48 [a]	1
fbn-l099	SNP-l099	212	G	A	0.74	0.26	0.80	0.20	3
fbn-l102	SNP-l102	147	G	A	0.98	0.02	0.88	0.12 [a]	8
fbn-l107	SNP-l107	71	-	G	0.98	0.02	1.00	0.00	15
fbn-l125	SNP-l125a	125	A	G	0.95	0.05	0.70	0.30 [a]	3
fbn-l125	SNP-l125b	178	T	C	0.90	0.10	0.82	0.18	3
fbn-l146	SNP-l146	119	C	T	1.00	0.00	0.69	0.31 [a]	2
fbn-l150	SNP-l150	76	T	C	0.67	0.33	0.92	0.08 [a]	2
fbn-l165	SNP-l165	63	C	T	1.00	0.00	0.75	0.25 [a]	26
fbn-l170	SNP-l170	199	T	C	0.76	0.24	0.95	0.05 [a]	8
fbn-l173	SNP-l173	167	C	T	0.82	0.18	0.82	0.18	23
fbn-i003	SNP-i003	104	G	A	0.89	0.11	0.88	0.12	23
fbn-i009	SNP-i009	250	C	T	0.78	0.22	0.85	0.15	14
fbn-i010	SNP-i010	203	C	G	0.80	0.20	0.88	0.12	29
fbn-i011	SNP-i011	96	$(A)_{10}$	$(A)_{11}$	1.00	0.00	0.82	0.18 [a]	6
fbn-i033	SNP-i033a	127	T	C	0.61	0.39	0.72	0.28	21
fbn-i033	SNP-i033b	130	C	A	0.86	0.14	1.00	0.00 [a]	21
fbn-i039	SNP-i039	75	C	T	0.72	0.28	0.83	0.17	15
fbn-i046	SNP-i046	192	A	G	0.78	0.22	0.50	0.50 [a]	24
fbn-i062	SNP-i062	124	$(A)_{14}$	$(A)_{15}$	0.78	0.22	0.50	0.50 [a]	25
fbn-i080	SNP-i080	200	A	C	0.50	0.50	0.60	0.40	12
fbn-i102	SNP-i102	62	C	T	0.95	0.05	0.42	0.58 [a]	27
fbn-i107	SNP-i107a	132	T	C	0.80	0.20	0.85	0.15	3
fbn-i107	SNP-i107b	200	A	C	1.00	0.00	0.92	0.08	3
fbn-i112	SNP-i112	165	T	C	0.90	0.10	0.60	0.40 [a]	8

[a] significantly different allele distribution compared to German Holstein ($P < 0.05$)
[b] not analyzed
[c] according to DORROCH et al. (2001) and GOLDAMMER et al. (2002)

ESTs with breed specific SNP distribution map within the vicinity of quantitative trait affecting growth and beef quality traits in cattle

Comparative analysis of chromosomal position of the 18 ESTs with SNPs showing breed-specific distribution (DORROCH et al., 2001; GOLDAMMER et al., 2002), and chromosomal localisation of quantitative trait loci affecting carcass and growth traits in cattle (STONE et al., 1999; CASAS et., 2001, 2003, 2004; KIM et al., 2003;) indicated that about 80 % of the polymorphic ESTs identified are located in chromosome regions known to be affecting carcass and growth traits in cattle, supporting the putative candidate gene character of the ESTs identified.

FASTA search of GenBank/EMBL database revealed that 10 ESTs showed similarity with BAC sequences, 3 ESTs did not show any similarity with database entries, whereas 4 of the polymorphic ESTs were similar to the previously described genes *NACA* (nascent-polypeptide-associated complex alpha polypeptide; fbn-l102), *NDUFB8* (NADH-ubiquinone oxidoreductase ASHI subunit; fbn-l165), *FUS/TLS* (FUS/TLS gene product; fbn-i062), and *UAP1* (UDP-N-acetylglucosamine pyrophosphorylase 1; fbn-l125).

Table 3
Results from regional assignment of loci metabolic type-associated expressed by somatic cell genetics using somatic cell panel (SCP) and whole-genome radiation hybrid panel (WGRH) and from studies on detection of quantitative trait loci (QTL) for growth and beef quality traits in cattle (Ergebnisse der chromosomalen Zuordnung von Genorten, die Stoffwechseltyp-spezifisch exprimiert sind, mittels Somatischer Zellgenetik unter Verwendung des somatischen Zellpanels (SCP) und des Gesamtgenom-Strahlungs-Hybridpanels, und von Untersuchungen des Nachweises von merkmalsbeeinflussenden Genorten (QTL) für Wachstum und Fleischqualität beim Rind)

Locus name	Regional assignment			Position of neighbouring micro-satellite marker within the genetic map (IHARA et al., 2005) [cM]	QTL positions[1]	
	Chromo-some BTA	$WGRH_{5000}$-Mapping Neighbouring micro-satellite marker	Reference		[cM]	Reference
UAP1	3	INRA006	GOLDAMMER et al., 2002	17.5	28-70	STONE et al., 1999; CASAS et al., 2001, 2004
NACA	8	IDVGA11	GOLDAMMER et al., 2002	11.0	15	CASAS et al., 2001
FUS/TLS	25	ILSTS046	DORROCH et al., 2001	33.3	44	KIM et al., 2003
NDUFB8	26	INRA81	GOLDAMMER et al., 2002	29.6	26	CASAS et al., 2004

[1]Position of the maximum of the test statistic along the chromosome

In Table 3 chromosomal localisation of the loci *NACA, NDUFB8, FUS/TLS, and UAP1* (DORROCH et al., 2001; GOLDAMMER et al., 2002) is accompanied with results from studies on detection of QTLs for carcass and growth traits in cattle (STONE et al., 1999; CASA et al., 2003, 2004). The somatic cell panel mapping data represent the ESTs assignment to a chromosome and the radiation hybrid mapping data the loci of ESTs characterised by the neighbouring microsatellite markers in the RH_{5000} second-generation cattle map (EVERTS VAN DER WIND et al., 2004). In addition, the position of the corresponding microsatellite markers in the bovine linkage map (IHARA et al., 2005) is given.

Discussion

This paper presents data on identification and distribution of genetic variants of DNA sequences potentially associated with energy transformation in cattle, because of their trait-associated expression. Based on trait-associated mRNA pattern identified by

DDRT-PCR (SCHWERIN et al., 1999; DORROCH et al., 2001) of 79 ESTs screened for potential trait-associated SNPs 30 ESTs were identified exhibiting one or more SNPs, of which 19 SNPs (18 ESTs) showed significantly different distribution between cows of Holstein and Charolais breed, respectively. Localisation of 15 of the 18 ESTs with SNPs showing breed-specific distribution (DORROCH et al., 2001; GOLDAMMER et al., 2002) in chromosomal regions known to harbour quantitative trait loci affecting carcass and growth traits in cattle (TAYLOR et al., 1998; STONE et al., 1999; REXROAD et al., 2001; CASAS et., 2003, 2004; KIM et al., 2003; LI et al., 2004) support the putative candidate gene character of these ESTs. The identified SNPs represent potential informative genetic markers to test our hypothesis that allelic variation in ESTs differentially expressed between the two distinct metabolic cattle types may be associated with variation in nutrient turnover. The SNPs identified in this study provide a pool of genetic polymorphisms, which can be exploited in detailed investigations of a F_2 resource population between Charolais and Holstein (KUEHN et al., 2002) as well as in further commercial populations for their potential use as direct genetic markers.

Holstein and Charolais cattle differ in process of growth and nutrition accretion. In comparison to Charolais the maximum of daily gain is earlier in Holstein cattle and the daily body gain drops stronger after reached maximum. From energetic feed evaluation systems (INRA, 1988; AfB, 1995), it can be concluded that the energetic requirements for the same body weight gain is significantly higher in German Holstein in comparison with Charolais. The major differences between Charolais and German Holstein regarding many general production parameters originate from differences in nutrient pathways and storage. Genetic differences in partitioning and utilisation of nutrients and nutrition-gene interactions seem to be main factors for differences in metabolic type of ruminants (BAUMAN and CURRIE, 1980; CRONJÉ, 2000). Growth, lactation, and metabolism are controlled by multiple hormones and factors acting in an endocrine and an autocrine manner (CANT et al., 1999; BREIER, et al., 2000). Both, endocrine and autocrine mechanisms, control the partitioning of absorbed nutrients between various body tissues and organs.

According to sequence identity with known genes physiological function of four ESTs showing breed-specific distribution of alleles can be allocated: *NACA*, *NDUFB8*, *FUS/TLS*, and *UAP1*. *NACA* is acting as a transcriptional coactivator complexing with newly synthesized polypeptide chains and showing chaperone effects (WIEDMANN et al., 1994; HAMMERLE et al., 2003). *NDUFB8* belongs to a family of eukaryotic NADH-ubiquinone oxidoreductase *ASHI* subunits proteins. Its main function is the transport of electrons from NADH to ubiquinone, which is accompanied by translocation of proteins from mitochondrial matrix to the intermembrane space (LOEFFEN et al., 1998). *UAP1* catalyses the synthesis of N-acyl-alpha-D-glucosamine 1-phosphate into diphosphate UDP-N-acetyl-D-glucosamine in the presence of UTP (MIO et al., 1998). The *FUS/TLS* gene product is a member of the serine-arginine (SR) family of proteins, which is involved in constitutive and regulated RNA splicing. It interacts with the oncoprotein TLS and abrogates the influence of TLS on E1A pre-mRNA splicing (MEISSNER et al., 2003).

Genetic variations in genes involved in energy metabolism are assumed considering the presumable physiological function of the ESTs exhibiting single nucleotide polymorphisms and their breed-specific distribution.. In a further study based on the

polymorphic nature of SNP markers, it will be proved whether variation in the genomic regions influences nutrient turn over in cattle.

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