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Analysis of association of two SNP in cholecystokinin B receptor gene with behaviour scores in German Angus and German Simmental cattle (Brief Report)

Assoziationsanalyse zweier SNP im Cholecystokininrezeptor B Gen mit Verhaltensmerkmalen bei Deutsche Angus und Deutsches Fleckvieh (Brief Report)

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Background

Behaviour including temperament of cattle especially suckler cows is important for the safety of stockmen and animals. The temperament of cattle can be defined as the answer to human handling situations (BURROW 1997) and can be measured for calves in different tests like tethering test, weighing test or separation- and restraint test (TULLOH 1961, BOISSY and BOUISSOU 1988, LE NEINDRE *et al.* 1995). The heritabilities for behaviour-traits ranged from 0.02 to 0.33 (GAULY *et al.* 2002). In this study the cholecystokinin B receptor gene is analysed. This candidate gene is important in the regulation of anxiety in rats (WANG *et al.* 2005) and in humans (HÖSING *et al.* 2004). In different tests with CCKBR-knockout mice they show more exploratory activity than the wild type mice (RAUD *et al.* 2003) and less anxious behaviour (HORINOUCHI *et al.* 2004).

Procedures

Phenotypes

German Angus (GA) and German Simmental (GS) calves were evaluated in three behaviour tests as mentioned within background. In total 962 calves of six GA-sires (545 calves) and eight GS-sires (417 calves) born in four consecutive years were included. Four scores were distributed in the three behaviour tests: one for tethering test (TT, three weeks of age), one for separation- and restraint test (ST, seven to eight month of age) and two for the weighing test at three month of age. The first score for weighing test was given while entering the scale (WT1) and the second during weighing process (WT2). All scores ranged from 1=docile, calm to 5=very excited and were nearly normally distributed with a left tendency.

Detection of single nucleotide polymorphisms

Two PCR-fragments of bovine *CCKBR* (GenBank NW_001493314) were sequenced; one fragment including the region from exon III to exon IV (688 bp, primer f1: 5'-GGG TGT CTG TGA GCG TGT C-3', r1: 5'-GCA GCC CTC CCT GAC TTC-3') and the other one covered the 3'UTR (790 bp; primer f2: 5'-TCA CTG TCC AGG CTG AGC TA-3', r2: 5'-GCG TAT TCC ACC CCT AAC CT-3').

In both fragments synonymous *C/T*-SNPs were identified and used to establish different methods for genotyping. The SNP in intron 3 was genotyped by PCR-RFLP-analysis with *Bse*NI (primer in3f: 5'-ACC CAG AAT CTT GCT CCA AC-3', in3r: 5'-AGT CAG TGC GGA TCC CTG T-3') and the one in 3'UTR with tetra-primer ARMS-PCR-analysis (primer outF: 5'-GTA TAG CAG GGG CAT TGA ATC TTT CAG G-3', outR: 5'-AAA AAG GAA ATT GAG GGG GAA ACC AAG T-3', inF: 5'-CAC CAA CCT GCC TAA TCT CAC ACT CAC T-3', inR: 5'-TCT TAG TGC AAA ACA GCT CGT TGG TAC G-3').

Statistical analyses

Association analyses between genotypes at the SNPs and behaviour scores were done within breeds using a variance analysis including sex and genotype as fixed effects, age at test as covariate and sire and residual as random effects. Furthermore the allele frequencies for the SNPs between animals with extreme scores (1 versus 4+5) were compared using the chi^2-test procedures.

Results and discussion

Compared to GenBank NW_001493314 the SNP in intron 3 is at position 311 275 and the other one in the 3'UTR at position 309 831. In the case of allele C in intron 3 the binding site for transcription factor MZF1 (myeloid zinc finger 1) is completed. The genotype- and allele-frequencies for these SNP in the calves are shown in table 1. Noticeable are the missing of genotype *TT* in intron 3 and 3'UTR in GS and GA, respectively. The missing *TT* genotype in GS for intron 3 is expected because all GS sires are homozygous *CC*. The missing TT genotype in GA for 3'UTR can be explained by the low frequency of the T-allele in GA. Only one of the six GA-sires is heterozygous, while the others are homozygous *CC*. Only the SNP in intron 3 in GA is not in Hardy-Weinberg-equilibrium.

Table 1
Genotype- and allele-frequencies of both SNPs in *CCKBR* separated by German Angus (GA) and German Simmental (GS)
Genotyp- und Allelfrequenzen der beiden SNPs im CCKBR getrennt nach den untersuchten Rassen Deutsche Angus (GA) und Deutsches Fleckvieh (GS)

SNP positions in NW_001493314	Breed	N of tested animals	Genotype-frequency			Allele-frequency	
			CC	*CT*	*TT*	*C*	*T*
pos. 311 275 (intron 3)	GA	542	0.55	0.35	0.10	0.73	0.27
	GS	412	0.95	0.05	0.00	0.97	0.03
pos. 309 831 (3'UTR)	GA	543	0.88	0.12	0.00	0.93	0.07
	GS	417	0.60	0.35	0.05	0.78	0.22

With the chi^2-test procedure only an influence by the intron 3 SNP on score WT2 in GA could be detected with a *P*-value of 0.0431. In addition a higher frequency of allele C in score 5 than in score 1 was seen. This result could not be verified with the variance analysis and also no association was detected for the 3'UTR SNP.

In humans there are several studies about different SNPs and their influence on behaviour (TACHIKAWA *et al.* 1999, HATTORI *et al.* 2001, HÖSING *et al.* 2004). So in cattle the sequencing

of the whole gene should follow giving the chance to detect more mutations with possible influence on behaviour.

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