A CORTICOTROPHIN-RELEASING HORMONE POLYMORPHISM ASSOCIATED WITH POST-NATAL GROWTH IN BEEF CATTLE

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INTRODUCTION

There is interest to increase growth rates in beef cattle through various means such as hormone implants (Bagley *et al*., 1989) or by selective breeding based on EPDs (Kress *et al.,* 1977). Improved growth rates are typically associated with higher economic returns to beef producers. High marker density genomic maps have greatly facilitated the detection of quantitative trait loci (QTLs). Chromosomal regions identified as harbouring QTLs can be screened for candidate genes with physiological roles influencing the trait with the goal of isolating the actual QTL for the trait under investigation.

Corticotrophin-releasing hormone (*CRH)* is an obvious physiological candidate gene for growth as it influences the release of glucocorticoids (Dunn and Berridge, 1990) which are considered to be growth inhibitors (Sharpe *et al*., 1986). *CRH* from the hypothalamus stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) from the pituitary gland ; ACTH subsequently stimulates the synthesis and release of glucocorticoids from the adrenal cortex (Roche *et al*., 1988). *CRH* has been linkage mapped to cattle chromosome 14 (Barendse *et al*., 1997) and maps in the vicinity of a QTL affecting post-natal growth (Buchanan *et al*., 2000). The *CRH* gene is comprised of two exons, however only exon 2 is translated and codes for the prepro-*CRH* in sheep and humans (Roche *et al*., 1988 ; Shibahara *et al.,* 1983). Using a positional and physiological approach, *CRH* was then selected as a candidate for screening to uncover genetic variation that might affect growth.

MATERIALS AND METHODS

Cattle. The Canadian beef reference herd (Schmutz et al., 2001) was developed for gene mapping. Individual animals were selected from this herd to screen for genetic variation. A group of 429 unrelated bulls were selected randomly from the 2001 Canadian sire summaries (n = 100 Angus, n = 98 Hereford, n = 68 Charolais, n = 86 Limousin, and n = 77 Simmental). DNA was extracted with standard protocols.

SNP identification. Primers were designed to amplify exon 2 of CRH by aligning ovine (Roche et al., 1988 ; Genbank accession number M22853) to human (Genbank accession number V00571) sequence where exon boundaries were delineated (CRH forward = atg cga ctg ccg ctg ctc g and CRH reverse = aga gag ggg agc agc ccg). A 254 bp fragment was amplified by PCR using a GeneE Thermocycler (Techne Co.). A total volume of 20 µl was amplified and contained : 100 ng of DNA, 10x PCR buffer, 5x Q-solution (Qiagen), 0.2 mM dNTPs, 0.5 units Taq polymerase, 4 pmol forward primer, and 4 pmol reverse primer. Conditions for amplification were as follows : 1 cycle of 95^{o}C for 2 minutes, followed by 35

cycles of denaturation at 95°C for 45s, annealing at 55°C for 30s and extension at 72°C for 45s, followed by a final cycle of extension at 72°C for 3 minutes.
Sequencing of nine PCR products (representing 3 light and 6 heavy weaning weight animals) was performed on an ABI373 sequencer (Applied Biosystems) at the Canadian National Research Council Plant Biotechnology Institute, Saskatoon, SK. Sequence was aligned and compared for variation using Sequencher™ 3.1.1 (Gene Codes Corporation).

PCR-RFLP analysis. CRH forward and reverse primers were used to amplify the final product for RFLP analysis. Fifteen microliters of the PCR product were digested for 2 hours at 65°C with 10 U Taq I (MBI Fermentas) in a total reaction volume of 20 µl following the manufacturer's recommendations. The fragments were separated on a 4% agarose gel by electrophoresis.

Regression analysis. A regression analysis was carried out to determine if the dosage of the "C" allele of the CRH gene had an effect on weaning weight and yearling weight of the bulls examined in this study as well as the EPDs for weaning and yearling weight of these bulls. The analysis was carried out by breed, with a fixed breed effect included in the analytical model.

RESULTS

We selected animals from the Canadian beef reference herd based on adjusted weaning weight to screen for any polymorphism in *CRH* that could explain the variation in phenotypes. A SNP was identified at 231 bp (amino acid 77) into exon 2 of *CRH* where a cytosine was substituted with a guanine. This caused a non-conservative mutation where histidine is substituted with aspartic acid. The G allele is cleaved by *Taq* I into two fragments of 226 and 28 bp (not visible on gel) while the C allele is not cleaved. This SNP is inherited in a Mendelian fashion (data not shown).
A group of 429 unrelated beef bulls were subsequently genotyped using the PCR-RFLP. Allele frequencies in the five beef breeds are reported (Table 1). The results of our analysis indicated that "C" allele dosage was significant ($P<0.05$) for both weaning weight and yearling weight EPDs of the bulls studied. This effect was not observed for individual weaning and yearling weights of the bulls, however, only a limited number of the bulls in this study had weaning and yearling weight records available.

Table 1. Allele frequencies of the *CRH* SNP for the five beef breeds

Breed	n	G	C
Angus	100	0.60	0.40
Hereford	98	0.77	0.23
Charolais	68	0.70	0.30
Limousin	86	0.63	0.37
Simmental	77	0.47	0.53

DISCUSSION

A SNP was identified in the propeptide region of *CRH* that could affect the bioactivity of the mature peptide as this amino acid substitution (His to Asp) is likely to change the folding of the

prepropeptide. The altered folding could affect secretion, preclude proprotein convertases (PC1 and PC2) from cleaving the mature protein from the inactive proprotein (Benjannet *et al*., 1991) or it could affect further processing of the protein hormone. Mutations in the proprotein region of several genes have been cited as causative. Szabo *et al.* (1998) found a 12 bp deletion in the proprotein region (preceding the proteolytic processing site) of mouse myostatin that is associated with the "compact" mouse phenotype. Grobet *et al.* (1998) found seven mutations in the bovine myostatin gene, five of which were in the proprotein region. Three of the five mutations were disrupting mutations and were found in Charolais, Limousin and Maine Anjou breeds.

Upon testing for an association between this missense mutation in *CRH* and post-natal growth, significance was found. Since both weaning weight and yearling weight were associated with *CRH* genotypes, both the cow-calf producer and the feedlot owner could benefit from selection based on this SNP.

Corticotrophin-releasing hormone is a neural peptide with numerous roles. Most frequently *CRH* is cited as being associated with stress and appetite behaviours (Dunn and Berridge, 1990; Glowa *et al*., 1992). *CRH* appears to play a role in post-natal growth which is intricately linked to the above mentioned appetite and stress associations. A causative mutation altering the bioactivity and/or level of functioning of *CRH* could prove useful as a selection tool for increasing post-natal growth traits.

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