Molecular Genetic Dissection And Phenotypic Characterization Of Major Loci Affecting Pre- And Postnatal Growth In Steers

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Introduction

Pre- and postnatal growth is a major factor affecting cattle production. This is obvious for beef production, but also for dairy cattle populations, the efficiency and regulation of resource allocation is of substantial interest. Recently, a major locus affecting prenatal growth has been identified in the bovine *non-SMC condensin I complex, subunit G* (*NCAPG*) gene (Eberlein *et al*., 2009) on bovine chromosome 6 (BTA6). The potential causal role of the non-synonymous *I442M* mutation in exon 9 of the gene was corroborated by several lines of evidence including linkage and association studies and gene expression analyses in fetal placenta. The mutation explained 16% of the variance in an experimental F_2-population established from Charolais and German Holstein by consistent application of embryo transfer. The conserved QTL – *NCAPG I442M* haplotype had also been confirmed for a purebred German Holstein sire segregating for the respective QTL. A large whole genome association study in a beef cattle crossbred population representing several major beef cattle breeds and kept under substantially divergent conditions, compared to our resource population, provided compelling confirmation for a pivotal role of variation in the *NCAPG* gene being a causal modulator of fetal growth (Snelling *et al*., 2010). The authors found a five SNP-haplotype with highly significant effect on birth weight comprising the *NCAPG* gene as the only gene in the respective genomic interval.

Another major locus affecting resource allocation in cattle is the *growth differentiation factor 8 (GDF8)* gene, for which numerous mutations have been described (Grobet *et al*., 1998, Dunner *et al*., 2003), some of which have been associated with major differences in carcass traits (Grobet *et al*., 1997, Morris *et al*., 2009, Allais *et al*., 2010). However, there are some controversial studies, whether and to what extent the *GDF8* mutations also affect body weight gain during development (Casas *et al*., 2004, Allais *et al*., 2010).

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In our resource population, the *NCAPG I442M* locus and also the disrupting mutation *Q204X* of the *GDF8* gene segregated, which enabled evaluating the effects of both loci on postnatal growth on an identical genetic background.

Recent progress in targeted metabolomic analyses facilitates parallel screening of several hundreds of metabolites to establish specific metabolic phenotypes, which offer new insights into the genetic determination of complex traits, as recently successfully demonstrated in human and mouse (Altmaier *et al*., 2008, Gieger *et al*., 2009, Illig *et al*., 2010). Consequently, for the first time in livestock, this new powerful technology was applied to investigate the physiological pathways affected by the *NCAPG I442M* and *GDF8 Q204X* mutation. The results were expected to provide novel insights especially into the NCAPG function in mammals, for which no experimental data exist up to now, although our studies and studies in human indicate its prominent role in modulation of pre- and/or postnatal growth (Weedon *et al*., 2008, Gudbjartsson *et al*., 2008).

Material and methods

Animals. For our studies, we investigated 155 male F_2 individuals from a Charolais x German Holstein resource population. All individuals had been generated by means of embryo transfer to virgin German Holstein heifers. After birth, calves were fed a milk replacer/hay/concentrate diet until day 121 followed by a semi *ad libitum* feed ration of concentrates and chaffed hay until slaughter at 18 months of age.

Genotypes. All P_0, F_1 and F_2 individuals of the resource population were genotyped for the *NCAPG I442M* and the *GDF8 Q204X* locus.

Phenotypes. Body weight was recorded monthly until 18 months of age. At slaughter, a comprehensive, detailed dissection of carcass traits was obtained as described by Pfuhl *et al.* (2007). Targeted metabolic profiling on plasma samples was performed essentially as described by Gieger *et al.* (2008).

Statistical analyses. The target mutations *NCAPG I442M* and *GDF8 Q204X* were tested for association with growth, lipid deposition and metabolic profile by association linkage disequilibrium (LD) studies, essentially as described by Eberlein *et al*. (2009) fitting a fixed effect of slaughter year, an additive effect of the SNP tested and a infinitesimal genetic animal effect in a one or two locus model.

Results and discussion

The *NCAPG 442M* allele that has been associated with increased birth weight, also showed a significant effect increasing total body weight at all intervals of age (Table 1). The most prominent effects, however, were obtained during puberty in the interval 182 to 365 days of age, whereas differences in body weight were substantially less pronounced at the early or late periods of life. The locus explained 3.9 – 15.8% of the variance for postnatal body weight in our resource population. The effects correspond to results (Setoguchi *et al*., 2009) obtained in Japanese Black and Japanese Brown cattle, two populations of substantially different origin compared to European cattle breeds (McKay *et al*., 2008). For the *GDF8*

Q204X locus, we obtained only a trend of effects on birth weight, which is in line with recent results in literature for mutations at the bovine *GDF8* locus (Allais *et al.*, 2010). It has to be considered that these results were obtained for calves all generated by embryo transfer to virgin German Holstein heifers. Thus, any prenatal maternal effect related to differences between beef or dairy breeds could be excluded. In contrast to birth weight, the effect of the loss-of-function allele *GDF8 204X* on body weight was significant at all later time points. The *GDF8 Q204X* locus explained 4.3 – 7.7 % of the variance for postnatal body weight in the model. When analysed jointly in a two-locus model, essentially identical effects were obtained for the individual loci explaining 21.4 % of the variance at day 365. These results indicate that both loci act independently and primarily modulate postnatal growth in a key interval comprising the onset of puberty. However, in contrast to *NCAPG I442M*, the *GDF8 Q204X* mutation does not seem to exert major effects on prenatal body weight.

Table 1: Estimates of additive genetic effects (kg) of the *NCAPG I442M* and the *GDF8 Q204X* mutation on body weight at different postnatal time points

Trait	*NCAPG 442M*		*GDF8 204X*		Joint analysis % variance explained[b]
	a[a]	p-value	a[a]	p-value	
Birth weight	2.88 ±0.64	1.46×10^{-5}	2.41 ±1.29	0.06	14.6
BW 121 days	4.60 ±1.89	0.016	8.73 ±3.63	0.018	7.7
BW 182 days	7.97 ±2.43	1.41×10^{-3}	13.77 ±4.66	4.02×10^{-3}	12.7
BW 273 days	17.00 ±3.44	2.72×10^{-6}	23.03 ±6.85	1.11×10^{-3}	20.5
BW 365 days	23.56 ±4.39	3.23×10^{-7}	28.34 ±8.83	1.79×10^{-3}	21.4
BW 456 days	26.31 ±5.88	1.51×10^{-5}	33.77 ±11.50	4.26×10^{-3}	16.1
BW 547 days	25.42 ±6.37	1.04×10^{-4}	29.95 ±12.48	0.019	12.3

[α]additive genetic effect of the respective alleles (±s.e.) from a single locus analysis, [b] results from the joint two-locus analysis; BW: body weight

In the key interval of divergent growth at 240 days of age, both loci were associated with distinct, clearly divergent metabolic pattern with no overlap in significantly divergent metabolites between *NCAPG I442M* and *GDF8 Q204X*. These results indicate that indeed these metabolic patterns associated with *NCAPG I442M* and *GDF8 Q204X* are not simply effects of overall growth modulation, but represent specific metabotypes associated with the specific distinct action of the different mutations.

Conclusion

The *NCAPG I442M* and the *GDF8 Q204X* locus both affected postnatal growth substantially, however, contributed differently to genetic modulation of prenatal growth. Specific metabolic patterns obtained for each respective locus by targeted metabolomic analyses can serve as biomarkers of definite differential metabolic pathways addressed by the mutations in *NCAPG* and *GDF8*, respectively.

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