

Dissection of Genetic Factors Modulating Fetal Growth in Cattle Indicates a Substantial Role of the Non-SMC Condensin I Complex, Subunit G (*NCAPG*) Gene

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Manuscript received June 20, 2009
Accepted for publication August 21, 2009

ABSTRACT

The increasing evidence of fetal developmental effects on postnatal life, the still unknown fetal growth mechanisms impairing offspring generated by somatic nuclear transfer techniques, and the impact on stillbirth and dystocia in conventional reproduction have generated increasing attention toward mammalian fetal growth. We identified a highly significant quantitative trait locus (QTL) affecting fetal growth on bovine chromosome 6 in a specific resource population, which was set up by consistent use of embryo transfer and foster mothers and, thus, enabled dissection of fetal-specific genetic components of fetal growth. Merging our data with results from other cattle populations differing in historical and geographical origin and with comparative data from human whole-genome association mapping suggests that a nonsynonymous polymorphism in the non-SMC condensin I complex, subunit G (*NCAPG*) gene, *NCAPG* c.1326T>G, is the potential cause of the identified QTL resulting in divergent bovine fetal growth. *NCAPG* gene expression data in fetal placentomes with different *NCAPG* c.1326T>G genotypes, which are in line with recent results about differential *NCAPG* expression in placentomes from studies on assisted reproduction techniques, indicate that the *NCAPG* locus may give valuable information on the specific mechanisms regulating fetal growth in mammals.

FARM animal species are receiving a growing interest as animal models, because increasingly whole-genome sequences have become available (*e.g.*, for cattle, horses, or chicken) as well as specific resource populations managed under standardized conditions (ANDERSSON and GEORGES 2004). Fetal development plays a major role in postnatal physiology in mammals as demonstrated for humans, laboratory animals, and livestock (PARK *et al.* 2008; CORSON *et al.* 2009; FREATHY *et al.* 2009). Additionally, in cattle, fetal growth has raised attention due to the still unknown fetal growth mechanisms impairing calves generated by somatic nuclear transfer techniques and because of its impact on stillbirth and dystocia in conventional reproduction. Calves generated by somatic cloning techniques are frequently affected by the large offspring syndrome, which is characterized by abnormally large fetal growth (HILL *et al.* 1999). The underlying mechanisms are still

poorly understood. Besides artificially induced variation in fetal development, there is also substantial conventional genetic variation in fetal growth with a medium heritability in cattle ($h^2 = 0.2\text{--}0.6$) (HANSEN *et al.* 2004; PHOCAS and LALOE 2004). In conventional cattle production, fetal growth has an important impact, because it is highly correlated to incidence of dystocia and stillbirth (HANSEN *et al.* 2004; PHOCAS and LALOE 2004), raising animal welfare issues. Thus, the identification of the factors modulating fetal growth would assist artificial reproduction and conventional animal breeding and also would contribute to an increased knowledge of pathways regulating fetal development. As exemplified by CANDILLE *et al.* (2007), the detection of the background of naturally occurring genetic variation can elucidate previously unknown physiological pathways.

In the literature, several studies in different dairy and beef cattle populations concurrently reported that bovine chromosome 6 (BTA6) harbors quantitative trait loci (QTL) affecting fetal growth (DAVIS *et al.* 1998; CASAS *et al.* 2000; KNEELAND *et al.* 2004; GUTIERREZ-GIL *et al.* 2009; MALTECCA *et al.* 2009). These results were in line with other studies providing evidence for QTL

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.109.106476/DC1>.

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affecting stillbirth, dystocia, or calving ease on the same chromosome (SCHROOTEN *et al.* 2000; KÜHN *et al.* 2003; HOLMBERG and ANDERSSON-EKLUND 2006; KOLBEHDARI *et al.* 2008; OLSEN *et al.* 2008; SCHULMAN *et al.* 2008). This initiated our attempt to scrutinize BTA6 for the genetic background of variation in the fetal component regulating fetal growth. Fetal growth is affected by maternal factors providing resources for the fetus, by fetal factors determining the development of the fetus itself, and by feto–maternal interaction (MURPHY *et al.* 2006). For dissecting these complex developmental processes, specific animal models are required, and use of foster mothers enables the specific investigation of the fetal component influencing fetal growth. Therefore, we took specific advantage of a unique cattle resource population (KÜHN *et al.* 2002) generated from Charolais and German Holstein, representatives of beef and dairy cattle, respectively, which are known to differ substantially regarding fetal growth (MARLOWE *et al.* 1984; NOAKES 2001). This resource population was created by consistent application of embryo transfer to foster mothers, which separates genetically determined exclusively fetal effects from genetically determined maternal or embryo–maternal factors. Using this animal model, we identified a genetic locus affecting the fetal component of fetal growth on BTA6 by linkage and association analysis. Furthermore, merging our data with respective intraspecies and across-species information highlighted the non-SMC condensin I complex, subunit G (*NCAPG*) gene as strongly associated with fetal growth.

MATERIALS AND METHODS

Animals: Our study included 733 individuals from a resource population (KÜHN *et al.* 2002) generated from the founder breeds Charolais and German Holstein: 5 Charolais males, 94 German Holstein females, 168 F₁, and 466 F₂. All F₁ and F₂ animals were generated by multiple ovulation and embryo transfer, which created full- and half-sib F₁ and F₂ families. Embryos were transferred randomly to virgin heifers kept in the experimental herd of the FBN Dummerstorf according to a standard protocol. Foster mothers were taken randomly from the German Holstein production population with no further specific genetic relationship between them. Female F₂'s were mated back to a German Holstein sire, which created a backcross (BC) population comprising 139 offspring available for genotyping.

During the postnatal period, the calves were housed in a loose stall barn and were fed a milk replacer diet according to requirements, which was gradually replaced by *ad libitum* access to hay and concentrates. For all calves, birth weight and subsequently weight at 121 days of age were recorded (Table 1). Effects that affected fetal and postnatal growth within the F₂ generation or the BC generation were calculated by applying a linear model

$$\text{trait}_{ij} = \text{sex}_i + \text{birth year}_j + e_{ij},$$

where traits investigated were birth weight, weight at 121 days of age, and average daily gain in the time period birth to day 121; sex_{*i*} is the fixed effect of sex_{*i*}; and birth year_{*j*} is the fixed

effect of the *j*th year of birth. The resulting residuals *e_{ij}* for the F₂ individuals were obtained and employed in all further QTL and association analyses. For the analysis of the BC individuals, the parity of dam (first or second parity) was added to the model as a fixed effect and the respective residuals were used.

Markers, genotypes, and haplotypes: Initially, the individuals from the P₀, F₁, and F₂ generation were genotyped for 16 microsatellite markers (see supporting information, Table S1) evenly distributed across the chromosome according to standard protocols (KÜHN and WEIKARD 2007). In the initial QTL confidence interval for fetal growth determined by bootstrapping (see below), 23 candidate SNPs were added along with 13 intragenic SNPs far outside of the targeted QTL region serving as negative controls (see Table S1). Genotyping of SNPs was performed on an Illumina Bead station (OLIPHANT *et al.* 2002) (<http://www.illumina.com>) as part of a targeted 384-SNP GoldenGate assay. The *NCAPG* c.1326T>G SNP was genotyped by PCR–RFLP, using restriction enzyme *TasI* (Fermentas) (amplification primers: forward 5'-ATTTAGGAAACGACTACTGG-3' and reverse 5'-ATTTGTA TTCTCTTATTATCATC-3'). First, all microsatellite markers and then additionally, all SNP markers were included to calculate a genetic map using CRIMAP Version 2.50 options [GREEN *et al.* 1990, incorporating modifications by Ian Evans and Jill Maddox (University of Melbourne)]. Markers unseparated by crossovers were ordered according to WEIKARD *et al.* (2006) and according to the bovine BTA6 assembly Build 4.0 (NCBI accession no. NC_007304). For those marker pairs sharing an identical position on our genetic map, a distance of 0.01 cM was assumed to provide a minimal distance required for QTL linkage mapping. Minor allele frequencies and tests for deviation from Hardy–Weinberg equilibrium for the SNPs were calculated in founder breeds (see Table S1). The most likely haplotypes of the individuals comprising microsatellites and SNPs were determined by a Markov chain Monte Carlo (MCMC) algorithm implemented in Qxpak (PEREZ-ENCISO and MISZTAL 2004). Pairwise linkage disequilibrium between the 23 SNPs distributed across the initial QTL confidence interval was determined by calculating pairwise *r*² values, using the Haploview program (BARRETT *et al.* 2005).

QTL analyses: As a robust starting point for QTL analysis, we applied a QTL regression analysis [QTL express (SEATON *et al.* 2002)] in the line cross model assuming alternatively fixed QTL alleles in the founder breeds. The significance thresholds were determined by 10,000 permutations and 95% confidence intervals by 10,000 bootstraps. Significantly segregating F₁ individuals were determined by performing within-F₁ parental half-sib analyses for all F₁ sires and dams exceeding 20 offspring in a regression QTL model (QTL express) and permutation tests. All families exceeding a 5% chromosome-wide threshold were considered as segregating at the QTL.

In addition to the QTL regression analysis, a variance component QTL model as implemented in Qxpak (PEREZ-ENCISO and MISZTAL 2004) including an infinitesimal animal effect was calculated,

$$y = Zu + Qg + e,$$

where *y* is a vector of phenotypes, *u* represents a vector of individual infinitesimal polygenic effects, *Z* and *Q* represent the incidence matrices for the polygenic and the QTL effect, respectively, *g* is a vector of QTL effects (either random or fixed effects within lines depending on the model applied), and *e* is the vector of random residuals. Identity-by-descent probabilities were calculated on the basis of a MCMC algorithm implemented in Qxpak (PEREZ-ENCISO *et al.* 2000). A likelihood-ratio test (LRT) was calculated to test for

TABLE 1

Birth weight, weight at 121 days of age, and average daily gain in the time period birth to day 121 in the P₀, F₁, F₂, and backcross generation of a Charolais × German Holstein resource population

Generation	<i>n</i>	Birth weight			Weight at day 121			Average daily gain, birth to day 121	
		Mean (SD) [kg]	Min [kg]	Max [kg]	Mean (SD) [kg]	Min [kg]	Max [kg]	Min [kg]	Max [kg]
GH	70	43.31 (4.75)	33.0	56.0					
Charolais	43	50.79 (8.08)	29.0	66.0					
F ₁	168	46.95 (6.09)	30.0	69.0					
F ₂	489	45.15 (6.64)	25.0	71.0	144.6 (16.04)	94.1	189.0	0.441	1.124
BC	238 ^a	40.85 (5.27)	25.0	54.0					

GH, German Holstein; BC, backcross generation of individuals born to F₂ dams from a German Holstein sire.

^aA total of 139 individuals were sampled for genotyping.

statistical significance. Significance thresholds for the LRT were determined according to NEZER *et al.* (2002), considering one chromosome with a length of 1.2 M and an average marker density for the variance component analyses of 0.02927 M. LRT significance thresholds for $\alpha = 0.05$ and $\alpha = 0.0001$ were 10.5 and 24.

The variance component approach used to model a random QTL effect (PEREZ-ENCISO and MISZTAL 2004) further complemented the initial QTL analysis with the more likely relaxed hypothesis regarding QTL allele distribution in the founder breeds. For the variance component QTL model, only markers with >200 informative meioses in the F₂ design were included, because it was indicated that the random QTL model may be unstable, when information gaps occur in the genotype file. The confidence interval for the QTL investigated by the variance component model was determined by the LOD drop-off method as suggested by LANDER and BOTSTEIN (1989), with one LOD interval corresponding to a 96% confidence interval (MANGIN *et al.* 1994).

For the two-QTL analyses we used a variance component method, because this enabled modeling of the QTL effects as random or fixed. For the two-QTL analyses, we assumed a fixed QTL effect at a specific position at the maximum in the one-QTL test statistic and tested in 1-cM intervals across the chromosome, whether there was a second QTL position for which the resulting two-QTL model had a statistically significant increased likelihood compared to the one-QTL model with the fixed QTL effect. The second QTL was modeled with a fixed or a random QTL effect, respectively. LRT tests were performed to assess the statistical significance of the two-QTL hypothesis (LRT model with two QTL *vs.* model with one QTL).

Association studies: Association linkage disequilibrium (LD) studies were performed to fine map the QTL in the initial QTL confidence interval 44–56 cM, applying the following model as implemented in the LD association analysis in Qxpak,

$$y_i = \sum_k \sum_h \lambda_{ikh} g_k + u_i + e_{ikh},$$

where y_i is the record of individual i , λ_{ikh} is an indicator variable, which is 1 when the allele at the h th haplotype (1 or 2) of the i th individual is k and otherwise 0, g_k is the allelic effect of allele k , u_i is the infinitesimal genetic effect of individual i , and e_{ikh} is the residual. Multiple testing of a total of 52 markers had to be accounted for to avoid false positive associations. To avoid false positive associations due to multiple testing of 52 markers, a Bonferroni correction was calculated, a most

stringent procedure given the fact that there was linkage and also linkage disequilibrium between markers. Those SNPs with the most significant effect were tested in an extended two-marker model, whether they still showed a significant association after accounting for the effects of one of the other significantly associated SNPs,

$$y_i = \sum_k \sum_h \lambda_{ikh} g_k + \sum_m \sum_n \lambda_{imn} g_m + u_i + e_{ihkmn},$$

where y_i is the record of individual i , λ_{ikh} is an indicator variable for locus 1, which is 1 when the allele at the h th haplotype (1 or 2) of the i th individual is k and otherwise 0, λ_{imn} is a respective indicator variable for locus 2, and g_k and g_m are the respective allelic effects for locus 1 and locus 2. A likelihood-ratio test (likelihood of model with locus 1 and locus 2 *vs.* likelihood of model with locus 1) was calculated to test for statistical significance.

Furthermore, SNPs that were significant in the association analyses were tested, if they had an effect on the QTL test statistic, when included in the fixed QTL model as implemented in Qxpak,

$$y_i = \sum_k \sum_h \lambda_{ikh} g_k + \beta_i a + u_i + e_{ikh},$$

where y_i is the record of individual i , λ_{ikh} is an indicator variable, which is 1 when the allele at the h th haplotype (1 or 2) of the i th individual is k and otherwise 0, g_k is the allelic effect of allele k , β_i is the additive QTL coefficient, and a is the additive QTL effect.

Haplotype analyses: The most likely haplotypes of the Qq segregating F₁ individuals were taken from the MCMC algorithm (see above) and assigned to the respective QTL alleles Q and q according to the effect estimation from the half-sib regression model. For this purpose, we selected offspring with extremely different transmission probabilities at position 50 cM for the parental target chromosome as calculated in the QTL Express analysis. Then, we determined from the flanking parental marker alleles, which of the two parental haplotypes the respective offspring inherited at the respective position. The haplotype, for which a positive effect on fetal growth was calculated in the regression analysis, was denoted “ Q ” and the haplotype, for which a negative effect was calculated, was denoted “ q .” All Q -carrying and all q -carrying haplotypes, respectively, were aligned and tested for concordance between QTL status and the candidate polymorphisms. The allele frequencies for the NCAPG *c.1326T>G* locus were assumed

according to the respective allele count for the alleles in the founder populations (*NCAPG c.1326G*, 0.9 and *NCAPG c.1326T*, 0.1 for Charolais and *NCAPG c.1326G*, 0.2 and *NCAPG c.1326T*, 0.8 for German Holsteins; expected allele frequency in the F_1 population, 0.55 for allele *NCAPG c.1326G* and 0.45 for allele *NCAPG c.1326T*). Following SEROUSSI (2009), the likelihood of the observed concordance would be $p_G^{N_Q} \times p_T^{N_q}$, where p_G is the allele frequency of the allele *NCAPG c.1326G* in the F_1 population, p_T is the allele frequency of the allele *NCAPG c.1326T* in the F_1 population, and N_Q and N_q are the number of concordant chromosomes carrying the Q or the q QTL allele, respectively. Furthermore, the concordance analysis in the significantly segregating F_1 parents was complemented by analysis of individuals very likely homozygous QQ or qq , respectively, at the QTL for fetal growth. For this purpose, the random QTL effect at position 55 cM as well as the fixed QTL effect at position 50 cM for each individual was taken from the two-QTL model. The 10 individuals with the highest and lowest QTL effects were assumed QQ or qq at the respective QTL positions. Binomial tests were performed to test whether there was a significant concordance of divergent marker alleles in the two groups,

$$P[(X_1 = a) \cup (X_2 = b)] \\ = \binom{n_Q}{a} p_1^a (1 - p_1)^{n_Q - a} \binom{n_q}{b} p_2^b (1 - p_2)^{n_q - b},$$

where $P[(X_1 = a) \cup (X_2 = b)]$ is the probability that allele 1 is observed at least a times in the sample of n Q -carrying chromosomes and allele 2 is observed at least b times in the n q -carrying chromosomes. Due to 10 individuals tested in each group, n_Q and $n_q = 20$; allele 1 is the marker allele concordant with the Q allele in the significantly segregating F_1 parents, allele 2 is the marker allele concordant with the q allele in the significantly segregating F_1 parents, a is the number of chromosomes carrying marker allele 1, and b is the number of chromosomes carrying the marker allele 2. The allele frequencies of the two different marker alleles are denoted p_1 and p_2 for alleles 1 and 2, respectively.

Resequencing and refined haplotypes: To obtain additional information on the haplotype structure and linkage disequilibrium in the *NCAPG* region we resequenced 4916 bp comprising the region proximal to the *NCAPG* gene and several regions from the *NCAPG* and the *LCORL* gene. Both genes are located in the confidence interval of this study and are also highlighted in a QTL analysis on calving difficulty in another cattle breed and in whole-genome association studies addressing human height (OLSEN *et al.* 2008; WEEDON *et al.* 2008). Information on PCR primers is indicated in Table S2. Genomic PCR products were generated and subsequently sequenced with the DYEnamic ET Terminator Cycle Sequencing reaction and the MegaBACE 1000 DNA Analysis System (GE Healthcare). For sequencing, PCR primers were used. Targets for resequencing were the four F_1 individuals, which showed statistically significant segregation of the QTL (probably Qq at the QTL), and the 20 individuals with the highest and lowest estimated QTL effects (supposed to be QQ and qq , respectively) selected as described in the previous paragraph. To obtain further information on the *NCAPG c.1326T>G* mutation history, we additionally included 6 unrelated individuals from the *Bos indicus* subspecies for genotyping by resequencing in the vicinity of the *NCAPG c.1326T>G* locus.

Fetal *NCAPG* expression pattern: Fetal expression patterns of the *NCAPG* gene were analyzed by reverse transcription (RT)-PCR on a multitissue panel consisting of six fetal bovine tissues (skeletal muscle, kidney, liver, lung, brain, and placenta, all tissues from 123 days of pregnancy). Principally, total RNA was extracted using the Nucleospin II RNA kit (Macherey-

Nagel). For muscle tissue, an initial purification step using Trizol (Invitrogen) was added. Genomic DNA was carefully eliminated from RNA preparations by repeated on-column RNase-free DNaseI digestion. The cDNAs were synthesized using the Superscript III RNase H-reverse transcriptase (Invitrogen) with oligo(dT) primer according to the manufacturer's instructions. Expression of the *NCAPG* gene was analyzed using gene-specific primers (forward primer, 5'-TGGAGCAAGTAA AAGATAGAAC-3'; reverse primer, 5'-ACCACTTTTCTCCGTC CTCTG-3'), producing a DNA fragment of 244 bp.

Quantitative *NCAPG* expression analysis: Quantitative *NCAPG* gene expression was analyzed by quantitative real-time PCR (qRT-PCR), using the LightCycler 480 system (Roche Diagnostics), on total RNA from fetal placenta taken at days 120–128 of gestation of six purebred German Holstein fetuses with different genotypes at the *NCAPG c.1326T>G* polymorphism (three with genotype *NCAPG c.1326T/NCAPG c.1326T*, two with genotype *NCAPG c.1326T/NCAPG c.1326G*, and one with the rare homozygous *NCAPG c.1326G/NCAPG c.1326G* genotype). The fetuses were all paternal half-sibs generated by ordinary artificial insemination to lactating first parity cows. The RNA samples (1 μ g) were reverse transcribed to cDNAs, using Superscript III RNase H-reverse transcriptase (Invitrogen) and the gene-specific primer 5'-CTCCATCATTTCTTCTCTCTAA C-3'. The cDNA samples equivalent to 25 ng total RNA in the reverse transcription assay were subjected to qRT-PCR in duplicate, using the LightCycler 480 SYBR Green I Master kit (Roche Diagnostics) and gene-specific exon-spanning primers (forward primer, 5'-CTGGAGCAAGTAAAGATAGAAC-3'; reverse primer, 5'-ACCACTTTTCTCC GTCCTCTG-3') generating an amplicon of 245 bp. To generate a standard calibration curve a standard transcript was prepared from the 245-bp PCR amplicon and purified with the Nucleospin Extract kit II (Macherey-Nagel). The quantity of the *NCAPG* mRNA in the analyzed cDNA samples of fetal placenta was calculated from the standard calibration curve on the basis of a serial dilution of the standard amplicon covering four orders of magnitude (10^3 – 10^6 copies), which ran simultaneously with the samples.

Statistical analysis of mRNA abundance was performed by regressing the amount of transcript against the number of copies of the *NCAPG c.1326T* allele,

$$y_i = b \times NCAPG_i + e_i,$$

where y_i is the number of *NCAPG* transcripts in the equivalent of 25 ng fetal placental RNA of fetus i , b is the regression coefficient of the *NCAPG c.1326T* allele, $NCAPG_i$ is the number of copies of the *NCAPG c.1326T* allele of individual i , and e_i is the residual effect.

RESULTS

Detection of a QTL affecting fetal growth: Initially, a line cross QTL regression model including 16 micro-satellite markers revealed a highly significant QTL (F value 90.16, $P < 0.0001$) affecting fetal growth in a 12-cM 95% confidence interval (44–56 cM) on BTA6 (Figure 1). The QTL accounted for 16.3% of the variance in the model as calculated as the relative reduction of the residual variance due to including the QTL in the model (KNOTT *et al.* 1996). The QTL seems to act in a strictly additive mode, because we did not find any evidence for dominance or imprinting effects. The QTL allele assumed to be originating from the

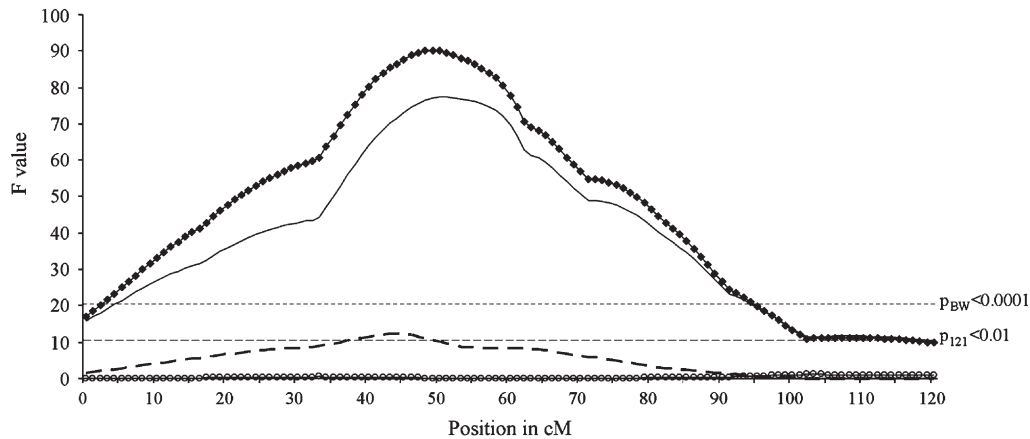


FIGURE 1.—QTL test statistic for fetal growth. A line cross model with an additive QTL effect was assumed for regression QTL mapping including information from 16 microsatellite markers (for information on marker position see Table S1). ♦, birth weight, all F_2 individuals, $n = 465$; —, birth weight, F_2 individuals with data for day 121, $n = 352$; ---, live weight at day 121, F_2 individuals with data for day 121, $n = 352$; ○, average daily gain in the interval birth to day 121, F_2

individuals with data for day 121, $n = 352$. Significance thresholds for birth weight (p_{BW} , $n = 465$ individuals) and live weight at day 121 (p_{121} , $n = 352$ individuals) are indicated.

Charolais breed was estimated to increase birth weight by 3.78 kg (SE 0.40). Whereas the QTL region exhibited a substantial effect on prenatal growth, the test statistic dropped substantially for the trait “weight at 121 days of age” and did not reveal any significant QTL for “average daily gain in the time period birth to day 121.” In consecutive half-sib regression analyses, four of eight F_1 parents tested (three sires, one dam) proved to be significantly heterozygous at the QTL affecting fetal birth with the peaks of the test statistics between 48 and 52 cM. The QTL test statistic for the other four F_1 half-sib families with >20 offspring did not exceed the statistical significance threshold. However, it has to be considered that those F_1 families all had a lower number of offspring than those four F_1 families with significant segregation of the QTL.

Association study of targeted SNPs with fetal and early postnatal growth: Targeted candidate SNPs and control SNPs were used to refine the QTL for fetal growth in association analyses. In addition to the initially genotyped 16 microsatellites, we tested 23 SNPs (see Table S1) in and adjacent to the initial QTL confidence interval for their LD association with fetal growth. Whereas 16 loci were nominally significant, adjustment for multiple testing maintained 7 SNPs and 1 microsatellite showing a 5% experimentwise significance (Figure 2). The SNP showing the strongest association was *NCAPG c.1326T>G* with a nominal P -value $< 10^{-16}$. Because *SPP1 c.621+338A>G* is located adjacent to *NCAPG c.1326T>G* and displays LD to *NCAPG c.1326T>G*, the observed trait association ($P < 10^{-10}$) of *SPP1 c.621+338A>G* was to be expected. Interestingly, there is a cluster of markers (*rs29025709–DIK82*) located 8 cM telomeric of *NCAPG c.1326T>G* comprising four markers that all also show a significant association with fetal growth, although three of them, e.g., *DIK82*, are not contained in the initial 95% confidence interval 44–56 cM. To test if the marker associations in the cluster *SEPSECS–DIK82* were due

to linkage disequilibrium generated by the pedigree relationships in our data set, five markers (the four markers with the highest significance from the one-marker association and also *PPARGC1A c.1892+19T>C*) were separately tested in a two-marker model for an association additional to the effect of one of the four other markers. We found that associations for *SPP1 c.621+338A>G* and *STIM2 (rs29014935)*, but not for *DIK82*, vanished, when tested jointly with *NCAPG c.1326T>G* (Figure 2). Taking *SPP1 c.621+338A>G* into the two-marker model still yielded significant effects for *NCAPG c.1326T>G*, *STIM2 (rs29014935)*, and *DIK82*. Joint analysis with *DIK82* in a two-marker model showed no statistically significant effect for *SPP1 c.621+338A>G*, but *STIM2 (rs29014935)* and *NCAPG c.1326T>G* were still significantly associated. These results indicated that there might be more than one locus affecting fetal growth in the targeted chromosomal region of BTA6.

Combined linkage and association study: In a second QTL mapping step, we included the additional SNPs into the QTL analysis to increase the mapping resolution and obtained a QTL with fixed effect at position 50 cM (LRT = 92.7) (Figure 3). Its 1-LOD drop-off interval spanned from 47 to 51 cM. The extension of the QTL model including one of the four markers with the most significant association to fetal growth tested whether one of those markers accounts for the QTL with fixed effect in the region 47–51 cM. The drop in the QTL test statistic was most prominent for *NCAPG c.1326T>G* and shifted the peak of the test statistic telomeric toward 58 cM (Figure 3). Marker *DIK82* also resulted in a marked decrease of the test statistic when included in the model and, in combination with *NCAPG c.1326T>G*, resulted in an almost flat test statistic with a maximum of 1.74 in the region 40–65 cM. Thus, the combined LD information from *NCAPG c.1326T>G* and *DIK82* completely erases the QTL with fixed effect in the region 44–56 cM. In contrast, *SPP1 c.621+338A>G*

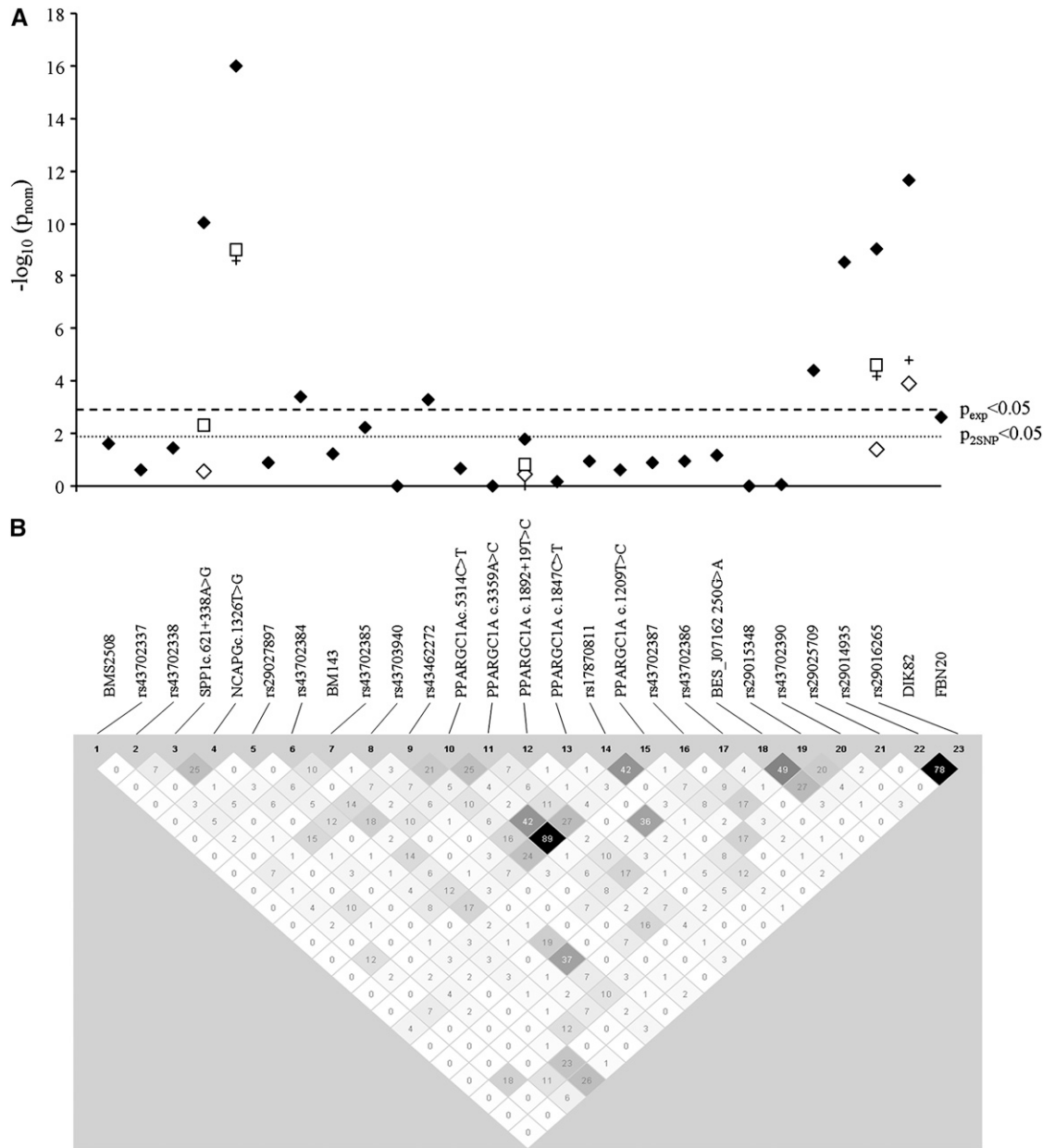


FIGURE 2.—(A) Association study between markers in the interval *BMS2508–FBN20* and fetal growth. On the y-axis the $-\log_{10}$ of the nominal P -value of the test is indicated. Experimentwise thresholds for one-marker (p_{exp}) and two-marker-association studies ($p_{2\text{SNP}}$) are indicated. ♦, one-marker association analysis; +, association analysis testing individually the two-marker model with the *SPP1* c.621+338A>G SNP against a one-marker model without the *SPP1* c.621+338A>G SNP. ◇, association analysis testing individually the two-marker model with the *NCAPG* c.1326T>G SNP against a one marker-model without the *NCAPG* c.1326T>G SNP; □, association analysis testing individually the two-marker model with marker *DIK82* against a one-marker model without marker *DIK82*. (B) Pairwise linkage disequilibrium calculated as r^2 between all SNPs in the BTA6 target interval 37.4–47.8 Mb.

did not further decrease the test statistic when jointly included with *NCAPG* c.1326T>G in the QTL model (data not shown). *STIM2* (rs29016265), like *SPP1* c.621+338A>G, resulted in a substantial decrease in the test statistic, although the maximum of the test statistic still showed a statistically significant QTL (data not shown).

Two-QTL analyses: Initial data from association mapping suggested that two QTL affecting fetal growth could be segregating in the middle part of BTA6. This

was supported by results from a QTL analysis assuming a QTL with random effect, which indicated a highly significant QTL (Figure 4) with a confidence interval spanning the region 55–57 cM. Thus, the confidence intervals obtained from the fixed effect and the random QTL effect analyses did not overlap. Although this incongruence obtained by the LOD drop-off methods should not be overinterpreted, it justified the consecutive two-QTL analyses. When the second QTL was modeled with a line-specific fixed QTL effect in addition

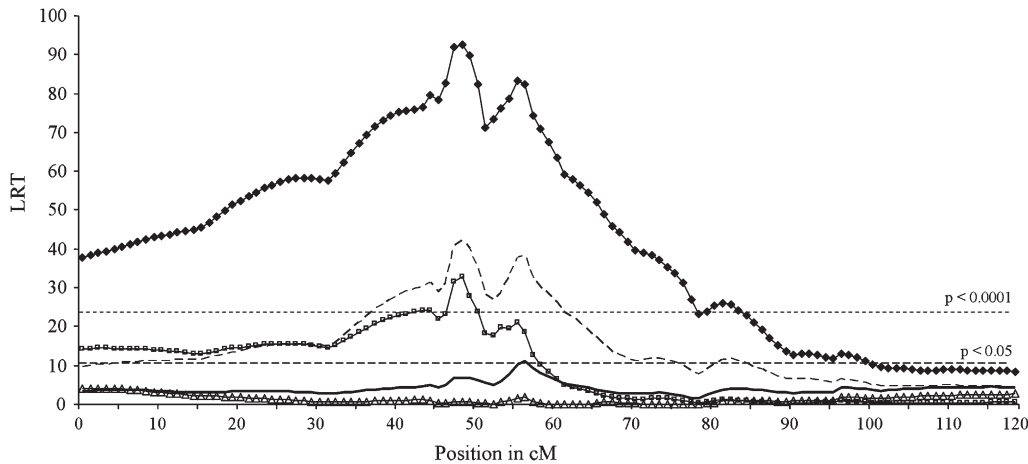


FIGURE 3.—QTL test statistic for variance component QTL mapping/association analysis of fetal growth. For information on markers included see Table S1. ♦, fixed-effect QTL model. ---, fixed-effect QTL model accounting for the effect of the *SPP1* c.621+338A>G SNP; □, fixed-effect QTL model accounting for the effect of the marker *DIK82*; —, fixed-effect QTL model accounting for the effect of the *NCAPG* c.1326T>G SNP; ▲, fixed-effect QTL model accounting for the

joint effects of the *NCAPG* c.1326T>G SNP and marker *DIK82*. Thresholds for the significance of the model including marker and QTL *vs.* the model with marker only are indicated.

to a first QTL with a line-specific fixed effect at 50 cM, no significant second QTL was observed (Figure 4). However, when the second QTL was modeled as random in addition to a first QTL with a line-specific fixed effect at 50 cM, the test statistic showed a highly significant QTL (LRT two QTL *vs.* one QTL = 70.4) in the interval 54–56 cM and further peaks at positions 58, 63, and 72 cM. The confidence interval for the second QTL with presumably random QTL effects could be narrowed down to 2 cM. Further extending the model to include three different QTL did not provide any significant proof that a third QTL is segregating in the interval 40–65 cM.

Haplotype analyses: To obtain further information on the position and the potential causal background of the QTL detected, we performed concordance analyses of the parental haplotypes from those F_1 parents that were significantly segregating *Qq* for the fetal growth QTL. All four parental F_1 chromosomes carrying the *Q* allele shared the allele *NCAPG* c.1326G, whereas all F_1

chromosomes carrying the *q* allele shared the allele *NCAPG* c.1326T (Figure 5). The likelihood of this concordance being observed by chance is 0.36×10^{-3} . It has to be highlighted that all four segregating F_1 parents are unrelated. The other four F_1 parents with >20 offspring that did not reach the chromosomewise statistical significance threshold in the QTL regression analysis all shared a *NCAPG* c.1326G allele at the parental haplotype associated with increased fetal growth. Additionally, they also shared a *NCAPG* c.1326T allele at the parental haplotype associated with decreased fetal growth. Thus, the *NCAPG* c.1326T>G-QTL haplotypes in the not significantly segregating F_1 parents showed the same concordance as the respective haplotypes in those F_1 parents, for which we had obtained statistically significant segregation. Looking at all P_0 individuals, we found that no *NCAPG* c.1326T>G allele is fixed either in Charolais or in German Holsteins, although the allele frequencies in Charolais and German Holstein for

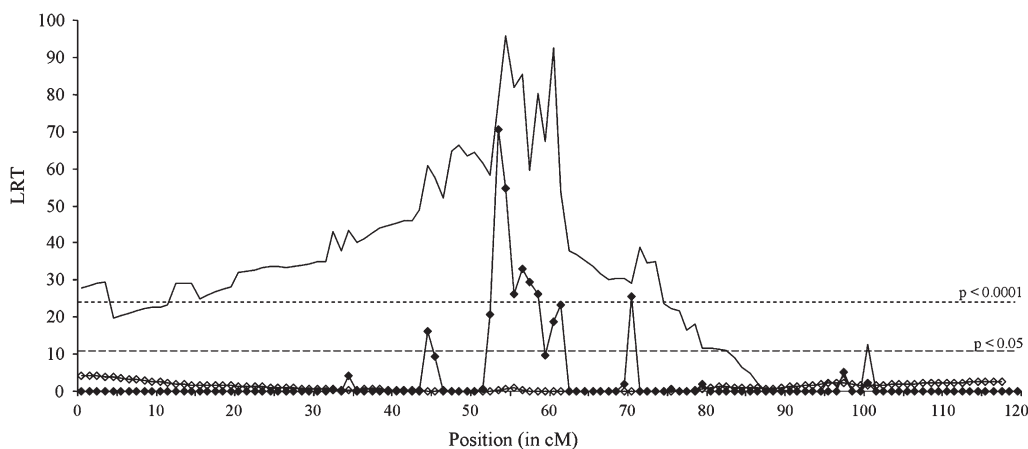


FIGURE 4.—QTL test statistic for variance component QTL mapping of fetal growth. For information on markers included see Table S1. —, one-QTL model assuming a random QTL effect. ♦, two-QTL model assuming a fixed-effect QTL at position 50 cM and scanning the chromosome for a second QTL with random effect (LRT: two-QTL model *vs.* one-QTL model). □, two-QTL model assuming a fixed-effect QTL at position 50 cM and scanning the

chromosome for a second QTL with fixed effect (LRT: two-QTL model *vs.* one-QTL model). Significance thresholds for the LRT two-QTL model *vs.* one-QTL model are indicated.

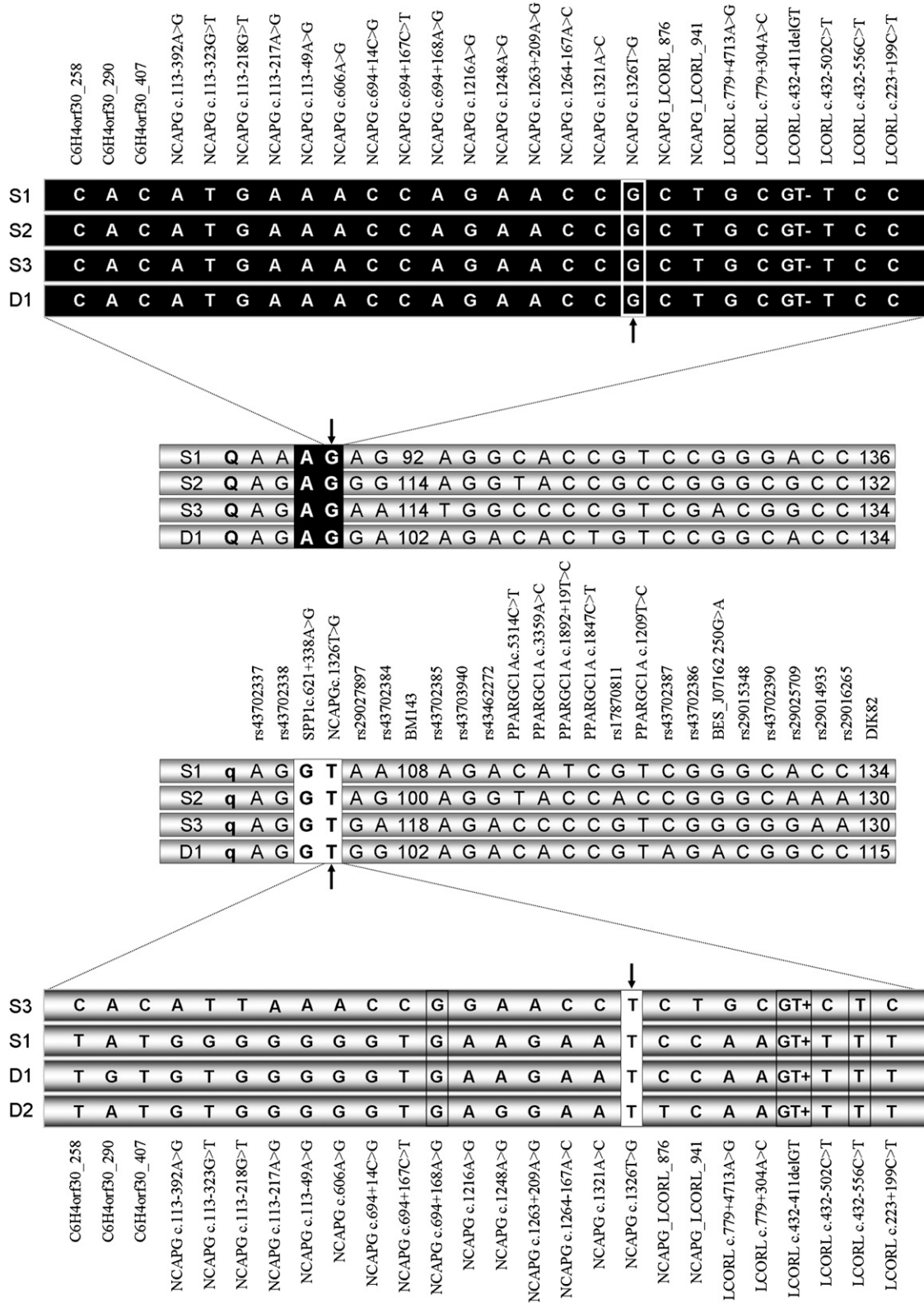


FIGURE 5.—Haplotypes of F₁ individuals (sires S1–S3, dam D1) with confirmed statistically significant segregation at the BTA6 locus affecting fetal growth in the region 37.4–47.9 Mb and the refined segment 38.13–38.33 Mb, respectively. *Q* (increased fetal growth) and *q* (decreased fetal growth) denote the QTL alleles assigned to the respective haplotypes as determined by within-family regression QTL analysis. The *NCAPG* c.1326T>G SNP is indicated by an arrow. For information on markers and marker positions see Table S1 and Table S3.

NCAPG *c.1326T>G* differed substantially. This is in contrast to *SPP1* *c.621+338A>G*, which also displayed a discordant distribution of alleles on chromosomes carrying alternative QTL alleles. As outlined in the previous sections, however, QTL and association studies showed that *NCAPG* *c.1326T>G* erased the effects of *SPP1* *c.621+338A>G* when included into the respective analyses, while this was not observed vice versa.

To further extend the concordance analyses, we obtained the putative QTL effects for the fixed effect and the random effect QTL at 50 and 55 cM, respectively, from the two-QTL analyses. From the 10 F_2 individuals with the lowest fixed QTL effects (presumably *qq*) at position 50 cM, 9 had a homozygous *NCAPG* *c.1326T/T* and 1 had a heterozygous *NCAPG* *c.1326T/G* genotype. Eight of the 10 F_2 individuals with the highest fixed QTL effects (presumably *QQ*) at position 50 cM had a homozygous *NCAPG* *c.1326G/G* and 2 had a heterozygous *NCAPG* *c.1326T/G* genotype. No other locus including *SPP1* *c.621+338A>G* showed a similarly biased distribution of genotypes. Statistical analysis revealed that the likelihood to obtain the *NCAPG* *c.1326T>G* distribution of alleles by chance is 2.31×10^{-9} . For the QTL with a random effect at position 55 cM, no analogously discordant allele distribution was observed for any marker.

Finally, backcross calves generated from mating a German Holstein sire to all female F_2 's for their first and second parity were recruited for an additional paternal half-sib regression QTL analysis. It showed a statistically significant QTL for fetal growth ($F = 10.43$, $P < 0.05$) within the family, indicating that the German Holstein sire should be *Qq* at a QTL affecting fetal growth. Further genotyping revealed that the sire was heterozygous *NCAPG* *c.1326T/G*, with the allele *NCAPG* *c.1326G* on the paternal haplotype increasing birth weight.

Resequencing and refined haplotypes: In the chromosomal segments that were included in resequencing, we found 26 polymorphisms in the F_1 and F_2 individuals (see Table S3). Alignment of haplotypes for the *Qq* individuals and the supposedly *QQ* and *qq* individuals, respectively, demonstrated that there is a large common haplotype block for the *Q*-carrying chromosomes encompassing the *NCAPG* *c.1326G* allele. This haplotype block spans at least 193 kb. This block is shared by all *Q*-carrying F_1 chromosomes (see Figure 5) and by 18 of 20 supposedly *Q*-carrying F_2 chromosomes. In contrast, no continuous shared identity-by-state haplotype block could be identified across all the *q*-carrying F_1 chromosomes. We observed different haplotypes for all four *q*-carrying F_1 chromosomes and 10 different haplotypes in the supposedly *q*-carrying F_2 chromosomes. None of the polymorphisms, except *NCAPG* *c.1326T>G*, which were heterozygous in all segregating F_1 parents and that showed a concordance with the QTL alleles, affected the amino acid sequence of the encoded protein or are located in a prominent regulatory region of the target

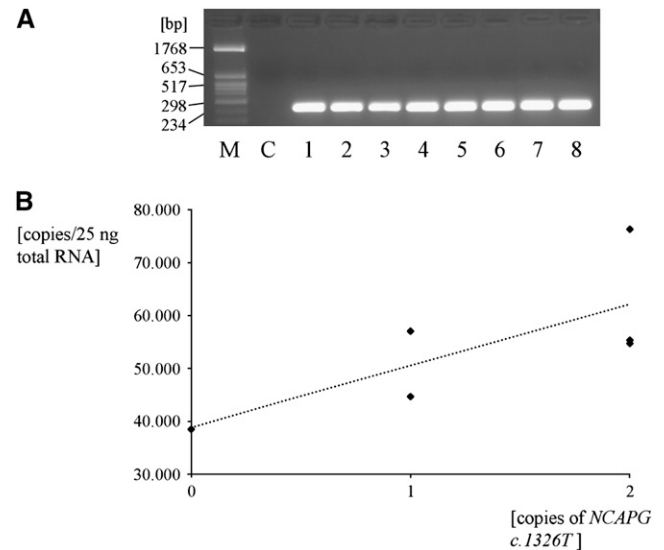


FIGURE 6.—(A) *NCAPG* gene mRNA expression in bovine fetal tissues. M, marker; C, nontemplate control; 1, skeletal muscle; 2, kidney; 3, liver; 4, lung; 5, brain; 6, bone; 7, cotyledon (fetal placenta); 8, caruncle (maternal placenta). (B) Quantitative *NCAPG* expression in the fetal placenta at days 120–128 from fetuses with differential numbers of *NCAPG* *c.1326T* alleles.

gene. Resequencing in six unrelated *B. indicus* individuals showed that all were homozygous for the *NCAPG* *c.1326T* allele (see Table S3).

Expression analysis: Expression analysis in a multi-tissue panel demonstrated *NCAPG* mRNA expression in all fetal tissues investigated including skeletal muscle, kidney, liver, lung, and brain and also in the fetal and maternal part of the placentomes (Figure 6A). Quantitative real-time analysis in the fetal part of placentomes originating from paternal half-sib fetuses with different *NCAPG* *c.1326T>G* genotypes suggested a differential *NCAPG* gene expression (Figure 6B). Regression of the number of *NCAPG* transcripts on the number of *NCAPG* *c.1326T* alleles showed an allele substitution effect of 11,675 copies ($P = 0.09$), suggesting that the *NCAPG* *c.1326T* allele is associated with increased *NCAPG* expression level.

DISCUSSION

In our study, we provide evidence for a QTL with highly significant effect on fetal growth in a unique cattle resource population. Systematic use of foster mothers and embryo transfer clearly breaks down any genetically determined correlation between biological mother and fetus and enables a stringent dissection of genetic factors modulating exclusively the fetal component of fetal growth.

Confirmation of a locus affecting fetal growth on BTA6: The QTL position affecting fetal growth on BTA6 determined in our study matches the suggested position of former studies on fetal growth and correlated traits

very well (CASAS *et al.* 2000; KÜHN *et al.* 2003; KNEELAND *et al.* 2004; HOLMBERG and ANDERSSON-EKLUND 2006). Recently, GUTIERREZ-GIL *et al.* (2009) mapped a highly significant, additively acting QTL for birth weight and body length at birth on BTA6 in a cross from Charolais and Holsteins explaining 27% of the variance with the Charolais originating QTL allele associated with increased birth weight. The authors reported a confidence interval from position 27 to 65 cM, which includes the *NCAPG* gene and is very similar to the confidence interval from another recent QTL analysis on bovine fetal growth in an experimental dairy cattle population (MALTECCA *et al.* 2009).

Collecting evidence for a causal role of the *NCAPG* locus on fetal growth: The final aim of studies dissecting the genetic background of complex traits is the detection of genetic variants that might be of causal effect. Whereas causality in livestock species is difficult to obtain, several lines of evidence can be collected to support any assumption on causal variants (GLAZIER *et al.* 2002; REBBECK *et al.* 2004). The results described in this study merged with several other reports from the literature provide substantial arguments in favor of a functional role of the *NCAPG* locus on fetal growth:

1. *Coincidence of QTL position and position of trait-associated *NCAPG* c.1326T>G SNP:* Within the confidence interval of the highly significant QTL affecting fetal growth, we identified the *NCAPG* c.1326T>G locus displaying a highly significant association with the phenotype.
2. *Concordance between QTL and *NCAPG* c.1326T>G alleles:* There is a significant concordance of QTL alleles and *NCAPG* c.1326T>G alleles in segregating F₁ individuals and in the extreme F₂ individuals with presumably *QQ* and *qq* QTL genotypes. As outlined by SEROUSSI (2009), full haplotype concordance of a representative sample of chromosomes is likely to point to the causative gene. The concordance between QTL alleles and *NCAPG* c.1326T>G alleles is further confirmed by an analogous concordance also in those F₁ parents that did not reach the formal statistical significance threshold in the within-family QTL analyses. Lack of full concordance between the assumed QTL genotype and the *NCAPG* c.1326T>G genotype in the F₂ individuals might be due to the effects of the second QTL affecting fetal growth in close proximity to the *NCAPG* gene.
3. **NCAPG* c.1326T>G explained a major proportion of the QTL:* The QTL model including *NCAPG* c.1326T>G decreased the QTL test statistic at the position 50 cM below the significance threshold. In combination with locus *DIK82*, *NCAPG* c.1326T>G completely erases the QTL effect in the fixed QTL model.
4. *Concordant segregating QTL–*NCAPG* c.1326T>G haplotypes within breed:* Within the German Holstein population, we observed a purebred sire significantly segregating for a QTL affecting fetal growth. This sire also showed a concordance between the QTL allele and the *NCAPG* c.1326T>G allele identical to that of the F₁ parents of the resource population.
5. **NCAPG* c.1326T>G–QTL linkage disequilibrium conserved across distantly related breeds:* Setoguchi and colleagues (SETOGUCHI *et al.* 2009) provided strong evidence for a highly significant association of the *NCAPG* c.1326T>G locus with growth in two unrelated Japanese cattle breeds. These populations have entirely different historical and geographical origin compared to each other (MINEZAWA 2003) and also compared to the European Charolais and German Holstein breeds used in our study (MCKAY *et al.* 2008). Conservation of linkage disequilibrium between alleles of the *NCAPG* c.1326T>G locus and QTL alleles affecting bovine growth across such divergent populations provides further support for a functional role of the *NCAPG* locus.
6. *The *NCAPG* c.1326T allele associated with low fetal growth is presumably the ancient allele:* The large haplotype block shared by the *Q*-carrying haplotypes compared to the lack of continuous haplotype block in the *q*-carrying haplotypes demonstrates that the *q* allele should be the ancient variant. This fits into the results from resequencing *B. indicus* individuals, where only the *NCAPG* c.1326T allele was found. Thus, during evolution presumably the *NCAPG* c.1326T allele had already been present, when the *B. taurus* and *B. indicus* subspecies separated. *NCAPG* c.1326G has been demonstrated to be associated with increased fetal growth in our population and should be associated with increased calving problems. Thus, in natural populations the mutation would be particularly disadvantageous due to lack of assistance during delivery of the calf.
7. *Fine mapping in other breeds confirmed positional candidacy of *NCAPG*:* Further positional indication advocating *NCAPG* as a background for divergent fetal growth comes from a recent fine-mapping study in yet another cattle breed. OLSEN *et al.* (2008) identified a 150-kb candidate QTL interval for calving difficulty, which is highly correlated to fetal growth, 20 kb adjacent to the *NCAPG* gene in Norwegian Red dairy cattle.
8. *Across-species support for positional candidacy of *NCAPG*:* Comparative data provide further support for the hypothesis that the *NCAPG* locus will affect mammalian growth. Concurrently, the *NCAPG/LCORN* region was one of only seven loci, which showed a highly significant effect on human height in two powerful major genomewide association metaanalyses in humans (GUDBJARTSSON *et al.* 2008; WEEDON *et al.* 2008).

		HEAT	HEAT
B. taurus 442I	423	RLGLILQEILTLTP-TTPISLSFLVERLLHIIID---DNKRIQIVTEIISE	
B. taurus 422M	423	RLGLILQEILTLTP-TTPISLSFLVERLLHIIID---DNKRIQIVTEIISE	
H. sapiens	422	KLLAVLQEILTLTP-TIPISLSFLVERLLHIIID---DNKRTQIVTEIISE	
P. troglodytes	422	KLLAVLQEILTLTP-TIPISLSFLVERLLHIIID---DNKRTQIVTEIISE	
E. caballus	422	RLAILQEILTLTP-TTPISLSFLVERLLHIIID---DNKRTEIVTEIISE	
C. lupus	424	RLAILQEILTLTP-TTPISLSFLVERLLCIIIVD---DNKRIQIVTEIISE	
M. musculus	422	RLAILQETLTLTP-TTPISLSFLVERLLHIIID---DNERIQIVTEIISE	
R. norvegicus	422	RLAILQETLTLTP-TTPISLSFLVERLLHIVTD---DSKRIQIVTEIISE	
G. gallus	424	HLVLLKKILTLTP-ATSTALVSLAERLLRIIKD---DARRIQTIAETVSE	
D. rerio	426	RMVAVLQEILVMP-NTPVSLIGLVEKLVNLRD---DAQIQMVAEIIISD	
D. melanogaster	454	NMRLLITNLLKDC-LLDHKIVRLVRCMEQLITD---TNDRIQYFIEIIE	
A. gambiae	406	SLKQVLSHMLCQE-TLFEMVRLIMETFEKIIITD---VESRLTFFVELVSS	
S. pombe	383	KLLKSLTNSLSTM-ALPDSLISLHIELLRKLCSS---ENDFCSLLVEIITE	
S. cerevisiae	442	SMLTVVRNMLALT-TLSEPLIKIGIRVMKLSLSIN---EKDFVTMAEIIIND	
K. lactis	507	DMLNIVRNFIGAVMLNDDLIRISLKVLIKLSIN---ERDFVTMTVEIIND	
A. gossypii	440	DMLNIVRNLLFTN-RPTEALINIGMLVKLVLSIN---EKDFITMSVEIITD	
M. grisea	521	KMFALLRQTLSTIP-ELPDEVTKLTVQVLRDLCPDAAAGEREFCSVVLEAVAD	
N. crassa	503	KMFALLRQTLSTIP-ELPDEVTKLTVQVLRDLCPDAAAGEKEFCSIVLEAVAD	

FIGURE 7.—Alignment of amino acid 423–469 of the bovine NCAPG protein to the respective region in other eukaryotes. The *B. taurus* NCAPG variant I442M due to the *NCAPG c.1326T>G* mutation is indicated. Regions corresponding to HEAT repeats in the bovine protein sequence are indicated.

9. *Indication of a functional role of NCAPG in fetal development:* The lines of support for an effect of the *NCAPG* locus on (fetal) growth accumulating from genetic studies are finally complemented by physiological data. NCAPG is a non-structural maintenance of chromosomes (SMC) subunit, which has a catalytic function in the mammalian condensin I complex and is important during mitotic cell division (DEJ *et al.* 2004). The *NCAPG c.1326T>G* SNP is a nonsynonymous mutation in exon 9 of the *NCAPG* gene generating the replacement of isoleucine by methionine at amino acid position 442 of the NCAPG protein. Comparison to other species supports the hypothesis that *NCAPG c.1326T* indeed is the ancient variant (Figure 7). The respective part of the NCAPG protein is highly conserved in mammals compared to nonmammalian species and is part of a HEAT repeat (Protsweep, <http://genius.embnnet.dk/fz-heidberg.de/menu/biounit/open-husar>) (DEL VAL *et al.* 2007), which is suggested to play important roles in chromosome dynamics (NEUWALD and HIRANO 2000). Furthermore, NCAPG interacts with DNA methyltransferase DNMT3B and thus links regulation of DNA methylation and mitotic chromosome condensation in mammalian cells (GEIMAN *et al.* 2004). Our own results demonstrated that *NCAPG* is indeed expressed in a variety of fetal tissues including the cotyledons of the placenta, which is a prerequisite for *NCAPG* modulating the fetal component of fetal growth.
10. *Suggestive differential NCAPG expression in fetuses with different NCAPG c.1326T>G genotype:* Our results, although not statistically significant, suggest a decreased *NCAPG* expression level associated with the *NCAPG c.1326G* allele, which increases fetal growth. A reduced *NCAPG* mRNA expression associated with the *NCAPG c.1326G* is in good agreement with transcriptome analyses in placentomes from cloned fetuses: *NCAPG* is one of the genes most differentially expressed in the placentomes of embryos

originating from somatic nuclear cloning *vs.* placentomes originating from embryos generated by conventional artificial insemination (EVERTS *et al.* 2008). Somatic cloning of bovine embryos is known to frequently result in extremely large offspring and significant abnormalities of the placenta (HILL *et al.* 1999; CONSTANT *et al.* 2006). Thus, our expression data add a first functional indication that the *NCAPG* gene may indeed represent a causal gene affecting fetal growth.

Exclusion of other functional and positional candidate SNPs and genes: Two prominent mutations (*ABCG2c.1743A>G* and *OPN3907*), in genes [ATP-binding cassette, sub-family G (WHITE), member 2 (*ABCG2*), and secreted phosphoprotein 1 (*SPP1*)] that closely neighbor *NCAPG* and are located within or near the QTL confidence interval ranging from 47 to 51 cM are controversially discussed as a causal background for genetic variation in milk composition (COHEN-ZINDER *et al.* 2005; SCHNABEL *et al.* 2005; DE KONING 2006). Furthermore, ALLAN *et al.* (2007) described a significant association of the *OPN3907* polymorphism with birth weight in an experimental cattle population. However, genotypes of the four F₁ parents significantly segregating for the QTL affecting fetal growth in our study revealed that all individuals are homozygous for both polymorphisms. Thus, both polymorphisms can be excluded as a cause for the QTL investigated in this study.

GUTIERREZ-GIL *et al.* (2009) pointed out that the QTL affecting fetal growth they described in the interval 27–65 cM on BTA6 seems to primarily affect bone growth. This would support homology of the bovine QTL on BTA6 to the *NCAPG/LCORL* locus on human chromosome 4 (HSA4) affecting height (GUDBJARTSSON *et al.* 2008; WEEDON *et al.* 2008), which is also determined by bone growth. Interestingly, there is an evolutionary conserved chromosomal breakpoint between BTA6 genes leucine aminopeptidase 3 (*LAP3*) and integrin-binding sialoprotein (*IBSP*) (WEIKARD *et al.* 2006). While on BTA6 (NCBI Build 4.0) both loci are located

immediately next to each other in close vicinity to *NCAPG*, *LAP3* was mapped near *NCAPG* on HSA4, but *IBSP* is located very distantly from *LAP3* at the telomeric end of the chromosome (NCBI build 36.3). Thus, if indeed there is a species-spanning homologous locus affecting mammalian bone growth, *IBSP* as well as secreted phosphoprotein 1 (*SPPI*, also known as osteopontin) and matrix extracellular phosphoglycoprotein (*MEPE*), all well described for their effects on bone metabolism (BECK *et al.* 2000; NAMPEI *et al.* 2004; KARADAG and FISHER 2006), but located centromeric of *IBSP* on BTA6, should be excluded as candidate genes for this locus. Our results demonstrate that comparative QTL information originating from different species can help to refine the genetic background of a complex trait in the target species as suggested by BURGESS-HERBERT *et al.* (2008).

Resequencing in the vicinity of the *NCAPG* *c.1326T>G* revealed a large number of variants within the *NCAPG* and the neighboring *LCORL* gene. However, none showed both concordance with the QTL alleles and a potential functional relevance. It has to be granted that in spite of many positional and functional indicators in favor of *NCAPG* *c.1326T>G*, it still cannot formally be excluded that variants other than *NCAPG* *c.1326T>G* might represent the causal background of the QTL affecting fetal growth. Next-generation sequencing (SHENDURE and JI 2008) of the entire genomic region in a well-characterized data set from divergent bovine populations might add further evidence in this respect. This will have to be complemented by refined functional *in vivo* and *in vitro* analyses in specific cell models.

Indication of a second locus affecting fetal growth:

Association analyses combined with QTL/association analyses and also one-QTL mapping suggested a second QTL with effect on fetal growth distinct from the *NCAPG* locus. The variance component model provided statistically significant detection of a second QTL, but only if this QTL was modeled with random effects. In contrast, the two-QTL model was not statistically more likely than a one-QTL model, when a fixed effect was assumed for the second QTL. Interestingly, the test statistics provided by SETOGUCHI *et al.* (2009) also give an indication that a second QTL might be segregating in the Japanese Brown population at the position 60 cM. Whereas for the locus at 50 cM, there is a mounting indication that the causal mutation might be located within or near the *NCAPG* gene, the genetic background of the putative second QTL at position 55 cM remains open. Although peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A*) and stromal interaction molecule 2 (*STIM2*) are candidate genes for fetal growth due to their function in energy metabolism and embryonic development (PUIGSERVER and SPIEGELMAN 2003; EID *et al.* 2008), our data do not convincingly support a causal role of either gene regarding genetic modulation of fetal growth. Further

research is necessary to elucidate the background of the second QTL affecting fetal growth. However, our results together with a wealth of other data on lactation suggest that the middle part of BTA6 seems to represent a true hotspot of genetic variation affecting growth and lactation traits.

While the QTL at positions 50 and 55 cM obviously exert strong effects on fetal growth, we could not detect effects on growth in the consecutive life period until day 121. HADJIPAVLOU and BISHOP (2009) described that QTL for growth might act at distinct times of life and may have no effect at other times. Analogously, we found an indication of *NCAPG* effects only in later life in our resource population (data not shown). Thus, a comprehensive analysis of the *NCAPG* locus regarding effects on growth and associated metabolic traits will shed further light on *NCAPG* *c.1326T>G* effects in postnatal life. This knowledge will be a prerequisite for applying *NCAPG* *c.1326T>G* in selection programs. Our results demonstrate that in spite of probably large differences in allele frequencies, the causal QTL alleles affecting fetal growth are still segregating within the Charolais and German Holsteins representing beef and dairy cattle breeds, respectively. Thus, both breeds may benefit from a targeted selection on *NCAPG* *c.1326T>G*.

Our QTL and association studies showed that the causal mutation underlying the identified locus modulating fetal growth has a major effect. The substantial size of the effect will facilitate the further functional investigation of *NCAPG* for its role in affecting growth during normal and aberrant fetal development in conventional reproduction or in assisted reproduction techniques.

We thank Jill Maddox (University of Melbourne, Australia) for providing the modified CRIMAP Version 2.50. Also, we thank our colleagues at the FBN Dummerstorf involved in the generation and care of the SEGfam F₂ resource population for their continuous support of our work. We thank the German Federal Ministry of Education and Research for the financial support of this work within the scope of the FUGATO QUALIPID project (FKZ 0313391C).

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Communicating editor: T. R. MAGNUSON

GENETICS

Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.109.106476/DC1>

Dissection of Genetic Factors Modulating Fetal Growth in Cattle Indicates a Substantial Role of the Non-SMC Condensin I Complex, Subunit G (*NCAPG*) Gene

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DOI: 10.1534/genetics.109.106476

TABLE S1**Markers included in fine mapping of QTL for fetal growth on BTA6**

Table S1 is available for download as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.109.106476/DC1>.

TABLE S2**Description of primers for resequencing in the NCAPG/LCORL region on BTA6**

Primers	Forward	Reverse	size (bp)	fragment
BTA114677_F1/R1	TATTAGTAAGAGCCCCATGTCC	CAATGAAAGATGAAGACCAAAAC	389	intergenic, centromeric to <i>C6H4orf30</i>
NCAPG_In1_F1/R1	CATTCTTGCCCTGGTATTCTC	GACCACCATAGCATATTTAAGG	535	<i>NCAPG</i> intron 1
NCAPG_In3_F1/E5_R1	TATGGGCTATCTTATGACTATG	TCAGCTAAAACCTTTAAGAGAC	511	NCAPG intron 3 to exon 5
NCAPG_Ex_F2/NCAPG_RFLP_R1	AGTTATATTCAAAGCATTCCAG	ATTTGTATTCTCTTATTATCATC	829	<i>NCAPG</i> exon 8 to exon 9
NCAPG_F1/R1	GTTGATTTATGAAAGATTATTAGG	AGCCAAAAGCACTGAAAACAC	412	<i>NCAPG</i> intron 8 to exon 9
BTC041166_F1/R1	TTCTCAAATGTCTGCCTCTCC	CTCTTCTCCCTTTTATCCTC	511	intergenic, telomeric to NCAPG gene
BTC041134_F1/R1	AAGTAGAAAGCACATAAGGATTG	AATCAGGGATCATTTTGCTATTG	409	<i>LCORL</i> intron 6*
BTC041133_F1/R1	CACCACACCTAAAACACAATG	CTTTCATACCTTTTCATTTGTCC	312	<i>LCORL</i> intron 6
BTC041097_F1/R1	AACTAGACATAAAAGGAGACAGC	CATGTTTTGGGATCAAGGACTG	501	<i>LCORL</i> intron 4
BTC041022_F1/R1	AATTTTCTACCAGGATTTCTGC	GACCACGGCTACGAAGAGAC	507	<i>LCORL</i> intron 2 to exon 2
Total bp resequenced			4916	

LCORL intron 6*: comparative in-silico analysis indicates that the annotation in the current bovine assembly Build4.0 (fragment annotated as 3'UTR) is incorrect

TABLE S3**Characterization of polymorphisms identified within the NCAPG/LCORL region on BTA6**

Table S3 is available for download as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.109.106476/DC1>.