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Genome-Wide Association Study Identifies Two Major Loci Affecting Calving Ease and Growth-Related Traits in Cattle

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ABSTRACT

Identifying quantitative trait loci (QTL) underlying complex, low-heritability traits is notoriously difficult. Prototypical for such traits, calving ease is an important breeding objective of cattle (*Bos taurus*)-improving programs. To identify QTL underlying calving ease, we performed a genome-wide association study using estimated breeding values (EBVs) as highly heritable phenotypes for paternal calving ease (pCE) and related traits. The massively structured study population consisted of 1800 bulls of the German Fleckvieh (FV) breed. Two pCE-associated regions on bovine chromosomes (BTA) 14 and 21 ($P = 5.72 \times 10^{-15}$ and $P = 2.27 \times 10^{-8}$, respectively) were identified using principal components analysis to correct for population stratification. The two most significantly associated SNPs explain 10% of the EBV variation. Since marker alleles with negative effect on pCE have positive effects on growth-related traits, the QTL may exert their effects on the birthing process through fetal growth traits. The QTL region on BTA14 corresponds to a human chromosome (HSA) region that is associated with growth characteristics. The HSA region corresponding to the BTA21 pCE QTL is maternally imprinted and involved in the Prader–Willi and Angelman syndromes. Resequencing of positional candidate genes on BTA14 revealed a highly significantly ($P = 1.96 \times 10^{-14}$) associated polymorphism ablating a polyadenylation signal of the gene encoding ribosomal protein S20 (*RPS20*). Our study demonstrates the leverage potential of EBVs in unraveling the genetic architecture of lowly heritable traits.

THE recent availability of genome-wide SNP panels in cattle and other livestock species enables the mapping of quantitative trait loci (QTL) as well as the prediction of an animal's genetic merit without relying on phenotypic information (Goddard and Hayes 2009). However, the complex genetic architecture of agriculturally important traits renders the systematic identification and characterization of individual QTL a difficult task. The proportion of trait variance explained by an average QTL is very small. First mapping results in cattle seem to validate the classical quantitative genetic model of a large number of loci of small additive effects (Barendse *et al.* 2007, Daetwyler *et al.* 2008, Cole *et al.* 2009) and agree with findings from mapping QTL in the human genome (Manolio *et al.* 2009). In addition to the relative contribution of a QTL to the trait variation, the heritability of the trait is a major determinant of the mapping power (Goddard and Hayes 2009).

The heritability of calving traits, *i. e.* traits that describe the birthing process (dystocia in the case of difficulties) and the perinatal viability (stillbirth) of the calf as affected by the birthing process, are low, ranging from 0.04 to 0.15 (Lin *et al.* 1989, Steinbock *et al.* 2003, Seidenspinner *et al.* 2009). Calving traits are of considerable economic importance due to veterinary treatment costs, calf loss and lower production of cows affected by dystocia. Estimated breeding values (EBVs) for calving traits are used as selection criteria in attempts to reduce calving problems both in dairy and beef breeds (*e.g.,* Van Tassell *et al.* 2003, Freer 2008)). Calving traits are complex since they are influenced by a sire-effect through the size of the calf as well as dam effects consisting mainly of the pelvic dimensions. Routine progeny testing results in highly reliable EBVs for calving traits and thereby boosts the heritability to levels that make them amenable to QTL mapping even with medium-sized samples.

An important prerequisite for unbiased QTL mapping based on linkage disequilibrium (LD) is homogeneity of the mapping population (Devlin and Roeder 1999). The heavy use of genetically superior bulls, facilitated by artificial insemination, and introgression lead to massively stratified populations. We attempted to correct for population stratification by principal components analysis (PCA)-based approaches that have been successful in human genome-wide QTL mapping (Price *et al.* 2006).

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Here we report the mapping of two loci affecting very low heritability calving traits in a heavily structured dual purpose (dairy, beef) cattle population. The mapping approach was facilitated by the use of EBVs and consequent correction of population stratification.

MATERIAL AND METHODS

Animals and phenotypes: Bulls of the dual purpose breed Fleckvieh (FV, $n = 1829$) were genotyped using the Illumina BovineSNP 50K Bead chip composed of 54,001 single nucleotide polymorphisms (SNPs). Phenotypes in the form of EBVs for beef (daily gain, DG) and conformation traits (body size, BS) as well as functionality traits such as paternal calving ease (pCE) and paternal stillbirth incidence (pSB) were obtained from the Bavarian State Research Center for Agriculture (http://www.lfl.bayern.de/bazi-rind) (November 2009 version, supporting information, Table S1). Breeding value estimation was based on best linear unbiased prediction (BLUP) animal model. The calving process is described by a score ranging from 1 (unassisted delivery) to 4 (surgical delivery, fetotomy). Stillbirth is recorded as categorical trait (alive or not 48 hr postpartum). Paternal and maternal effects on calving ease and stillbirth incidence are estimated multivariately for the first *vs.* later parities. Parity-specific EBVs are combined to produce paternal and maternal EBVs, respectively.

Genotypes and quality control: Of 1829 genotyped FV animals, 6 were excluded from further analyses due to genotype call rates below 90%. The remaining samples exhibited an average genotyping rate of 99.14%. A total of 549 SNPs were omitted because their chromosome position was not known. A total of 728 SNPs were discarded because genotyping failed in more than 10% of animals, 8480 SNPs were excluded due to a minor allele frequency smaller than 1%, and 810 SNPs showed a significant ($P < 1 \times 10^{-3}$) deviation from the Hardy–Weinberg equilibrium, indicating genotyping errors, and were thus not considered for further analyses. Pairwise identity-by-descent (IBD) was calculated on the basis of identity-by-state (IBS) information derived from the remaining 43,863 SNPs using the method-of-moments approach implemented in *PLINK* (Purcell *et al.* 2007). The IBD relationship of each pair of animals was compared with the corresponding pedigree relationship calculated using the *PyPedal* package (Cole 2007). Comparison of the marker with the pedigree relationship revealed several inconsistencies, most likely resulting from mislabeling of DNA samples and false relationships. Unresolved inconsistencies led to the exclusion of 23 animals (Figure S1). The final set consisted of 1800 animals. The phenotype and genotype data are available from the authors upon request.

Single-marker analysis: Single-marker analysis was first carried out without considering population stratification. The EBVs were regressed on the number of copies of one of the alleles as implemented by the *PLINK–assoc* option. Quantile–quantile plots of the expected *vs.* the observed *P*-values were inspected for an inflation of small *P*-values indicating false positive association signals due to a structured population. The genome-wide inflation factor was computed according to Devlin and Roeder (1999).

We next applied a PCA-based approach, implemented in the EIGENSOFT 3.0 package (Price *et al.* 2006), for eliminating false positive association signals due to ancestry differences and resulting population stratification. One SNP of a pair in LD with $r^2 > 0.25$ was excluded using the *PLINK–indep-pairwise* option (500 SNP window, shifted at 50 SNP intervals). A *smartpca* version of the EIGENSOFT 3.0 package (compiled from source code with modifications for the bovine chromosome complement) was run on the pruned data set consisting of 20,000 autosomal SNPs with the following option: the value of each marker is replaced with the residuals from a multivariate regression without intercept on the five preceding markers to further reduce redundancies due to LD. Eigenvalues (λ) and eigenvectors were calculated for all axes of variation. Correlation of ancestry adjusted EBVs and genotypes was calculated using the previously obtained eigenvectors with a *smarteigenstrat* version of EIGENSOFT 3.0 compiled for the bovine chromosome complement. The resulting test statistic is equal to $(N - K - 1)$ times the squared correlation and χ^2 distributed, where N is the number of samples and K the number of axes with an eigenvalue that amounts to at least 70% of the mean eigenvalue (Jolliffe's criterion, Jolliffe 2002) used to adjust for ancestry (Price *et al.* 2006). Quantile–quantile plots were inspected and the genomic inflation factors were calculated (see above) to judge the extent of false positive signals. SNPs were considered as significantly associated for *P*-values below the 5% Bonferroni-corrected type I error threshold for 43,863 independent tests. Allele substitution effects were estimated for each significant marker in a linear regression model implemented in *R* (http://www.r-project.org) with axes of variation with $\lambda \geq 0.7$ as covariables.

Haplotype analysis: Haplotypes for each chromosome region with significant association signals were reconstructed using default parameters in *fastPHASE* (Scheet and Stephens 2006) and inspected by means of bifurcation plots obtained with *sweep* (Sabeti *et al.* 2002) to visualize recombination events and to define the length of haplotypes. The resulting haplotypes were analyzed for association in a multilinear regression model implemented in *R* (see above).

Estimating the power of the genome-wide association study: According to Goddard and Hayes (2009) the correlation (r) between marker and trait, $r_{t \cdot m}$, is equal to $r_{m \cdot q} \cdot r_{q \cdot g} \cdot r_{g \cdot t}$, with m representing the marker genotype, q the QTL, g the genotypic value, and t the phenotypic value (EBV) of an animal. $r^2_{m \cdot q}$ measures the LD between marker and trait, $r^2_{q \cdot g}$ the variance explained by the QTL, and $r^2_{g \cdot t}$ the reliability of the EBV. Using this equation and the formula for the standard error of the correlation coefficient, the number of animals (N) required for identifying a QTL can be calculated as

$$N = \left(\frac{1 - r^2_{t \cdot m}}{r_{t \cdot m} \left(1 / z_{(1-\alpha)} \right)} \right)^2,$$

where z is the normal score and α the Bonferroni-corrected type I error rate for 43,863 independent tests. Assuming a reliability of the EBV of 0.9, a LD between marker and QTL of $r^2 = 0.35$, and the QTL to explain 4% of the genetic variance, the required number of animals amounts to about 1700. Thus the power of our study with $N = 1800$ should allow identification of QTL, explaining at least 4% of the genetic variance using EBVs of high reliability.

Annotation and polymorphism analysis of candidate genes: The *GENOMETHREADER* software tool (Gremme *et al.* 2005) was used to predict the genomic structure and localization of the candidate genes based on the University of Maryland *UMD3.1* assembly of the bovine genome sequence (Zimin *et al.* 2009) and the Dana–Farber Cancer Institute bovine gene index release 12.0 (Quackenbush *et al.* 2001) together with the annotated RNA sequences of the *UMD3.1* assembly (Zimin *et al.* 2009). The *GENOMETHREADER* output was viewed and edited using the *Apollo* sequence annotation editor (Lewis *et al.* 2002). The exons and the promoter regions of the candidate genes were PCR amplified (the primers are listed in Table S2) and resequenced in 12 FV bulls with specific genotypes for the

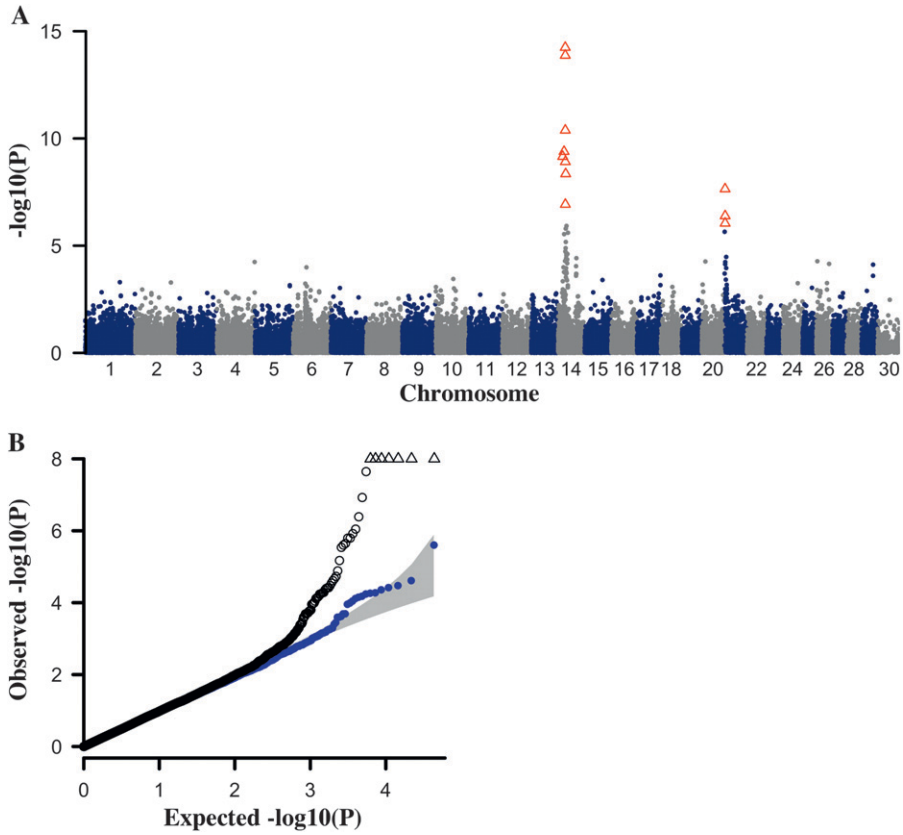

FIGURE 1.—Association of 43,863 SNPs with the estimated breeding value (EBV) for paternal calving ease (pCE) in the Fleckvieh breed. (A) Manhattan plot. Red triangles represent SNPs with $P < 1.14 \times 10^{-6}$ (Bonferroni corrected significance level). (B) Quantile–quantile plot. The shaded area represents the 95% concentration band under the null hypothesis of no association. The open black dots represent the *P*-values of the entire study, open triangles represent SNPs with $P < 1 \times 10^{-8}$, and the solid blue dots indicate the *P*-values excluding those from the associated regions on chromosomes 14 and 21.

SNP with the most significant signal for the pCE EBV (*BTA14–ARS–BFGL–NGS-104268*), *i.e.*, in 1 bull with GG and in 11 bulls with AG genotypes.

Genotyping of candidate gene polymorphisms: Genotypes of selected SNPs were determined by TaqMan genotyping assays (Applied Biosystems Applera, Darmstadt, Germany). DNA samples were available for 810 FV animals only. Candidate gene polymorphisms were genotyped in these animals, and the genotypes of the remaining 990 animals of the study population were inferred using the EM algorithm implemented in *fastPHASE*.

RESULTS

Single-marker analysis: In a first attempt to identify QTL for pCE, we applied a linear regression model that did not account for the covariance of related animals. This model yielded 1146 autosomal SNPs exceeding the significance threshold and a genome-wide inflation factor of 4.75. However, an apparent association signal was observed on chromosome 14 ($P = 1.64 \times 10^{-55}$; Figure S2). The inflation of significant association signals most likely results from relatedness of animals leading to a massively structured population. The 1800 bulls within our study descend from 234 sires and 328 maternal grand sires. The paternal half sib families and the maternal grand sire families encompass up to 81 and 137 members, respectively. This is manifested by an average coefficient of relationship of 0.047 and distinct clusters of related animals (Figure S3A and Figure S3B). Recent introgression of Holstein-Friesian (HF) into FV can be uncovered by PCA. A 50% HF sire was broadly used within the FV population in the early 1980s to improve milk performance and udder quality of cows. Of 1800 FV bulls within the study population, 1050 exhibit HF ancestry via two of his sons (both 25% HF), as can be visualized by contrasting the top two axes of variation of the PCA (Figure S3C). Thus, HF admixture and the paternal and maternal sire families lead to a massively structured study population and concomitant inflation of significant association signals.

Therefore, the association study was repeated, now correcting for population stratification using a PCA-based approach implemented in the EIGENSOFT 3.0 package. The correction was based on 773 axes of variation that met the Jolliffe's criterion. In addition to the highly significant association with the pCE EBV on chromosome 14 that was already observed in the analysis without correction for stratification, the PCA-based analysis now also revealed significant association on chromosome 21 (Figure 1A). The Q–Q plot (Figure 1B) and an inflation factor of 0.97 document that the PCA-based analysis successfully eliminated association artifacts resulting from population stratification.

Eight SNPs on chromosome 14 and three SNPs on chromosome 21 meet the Bonferroni-corrected significance threshold (Table 1). Of the eight significant SNPs on chromosome 14, six lie within a 1.4-Mb interval (from 24.06 to 25.4 Mb). Two significant SNPs outside this interval are in LD ($r^2 = 0.48$ and 0.68) with the most

TABLE 1

SNPs showing significant association with pCE, pSB, DG, and BS EBVs in 1800 Fleckvieh animals

SNP	Chromosome	Minor allele (minor allele frequency)	Physical position (bp)	EBV	Eigenstrat statistic	*P*-value	α
ARS-BFGL-NGS-104268	14	A (0.12)	24,057,354	pCE	60.99	5.72×10^{-15}	−0.58
				pSB	47.12	6.69×10^{-12}	−0.54
BTA-91250-no-rs	14	A (0.10)	24,145,838	pCE	59.32	1.34×10^{-14}	−0.62
				pSB	47.51	5.47×10^{-12}	0.58
				BS	26.89	2.15×10^{-7}	0.45
BTB-01417924	14	G (0.13)	24,182,406	pCE	43.54	4.15×10^{-11}	−0.46
				pSB	33.79	6.13×10^{-9}	−0.42
Hapmap59686-rs29020689	14	A (0.14)	24,365,162	pCE	39.07	4.08×10^{-10}	−0.40
				pSB	38.27	6.18×10^{-10}	−0.42
ARS-BFGL-NGS-28867	14	G (0.10)	20,323,857	pCE	38.03	6.96×10^{-10}	−0.50
				pSB	33.78	6.17×10^{-9}	−0.49
				DG	25.50	4.42×10^{-7}	0.41
UA-IFASA-7112	14	G (0.09)	16,109,986	pCE	36.94	1.22×10^{-9}	−0.51
				pSB	32.09	1.47×10^{-8}	−0.49
Hapmap46735-BTA-86653	14	G (0.20)	25,401,722	pCE	34.40	4.48×10^{-9}	−0.36
				pSB	28.89	7.65×10^{-8}	−0.34
ARS-BFGL-NGS-53975	21	G (0.27)	2,151,256	pCE	31.25	2.27×10^{-8}	0.24
BTB-01532239	14	A (0.28)	24,437,778	pCE	28.05	1.19×10^{-7}	−0.26
				pSB	26.94	2.10×10^{-7}	−0.27
ARS-BFGL-NGS-114372	21	C (0.22)	2,381,941	pCE	25.65	4.09×10^{-7}	0.24
Hapmap52072-rs29018920	21	A (0.22)	2,333,804	pCE	24.12	9.01×10^{-7}	0.23

Eleven SNPs meet the genome-wide significance level of $P < 1.14 \times 10^{-6}$. SNPs are arranged in the order of increasing *P*-values for the association with the paternal calving ease EBV. The *P*-value for each trait x genotype combination is obtained by a principal components analysis - based approach to account for population stratification. The allelic substitution effect (α) is given for the minor allele in additive genetic standard deviations of the EBV. Physical positions are based on the UMD3.1 assembly of the bovine genome sequence.

significantly associated SNP on chromosome 14. Three significantly associated SNPs in high LD define a second pCE QTL region on chromosome 21 (2.15–2.39 Mb). While the minor allele of the significant SNPs on chromosome 14 has a negative effect on the pCE EBV, it is the major allele of the significant SNPs on chromosome 21 that lowers the pCE EBV. The most significant SNP on chromosome 14 exhibits an allele substitution effect of −7.01, corresponding to 58% of the standard deviation of the EBV. The substitution effect of the major allele of the most significant marker on chromosome 21 is −2.93, *i.e.,* 24% of the standard deviation of the EBV (Figure 2A).

pCE is highly correlated with the paternal stillbirth incidence (pSB) as well as with growth-related EBVs such as for DG and BS (Table S3). Consequently, association signals can also be observed for these EBVs, particularly on chromosome 14 (Table 1 and Figure S4). The QTL alleles that lower the pCE and pSB EBVs have a positive effect on the growth-related EBVs.

Several chromosome regions show suggestive association ($P < 1 \times 10^{-3}$, Table S4), most prominently a second region on chromosome 14 with 5 SNPs located between 58.3 and 59.3 Mb.

Haplotype analysis: Haplotype analysis was carried out for the associated regions on chromosomes 14 and 21 in an attempt to delineate the chromosomal segment carrying the pCE QTL. On chromosome 14, the allele that lowers the pCE EBV could be pinpointed to a specific haplotype that spans 1.58 Mb (starting at 23.82 Mb) and encompasses 23 SNPs (Table 2). This haplotype version occurs in a frequency of 10% in the study population. Its negative effect on the pCE EBV ($P = 1.56 \times 10^{-16}$) is more prominent than any of the associated SNPs ($-0.66\sigma_A$ *vs.* $-0.62\sigma_A$, Figure 2B). This is a strong indication for the causal variant lowering the pCE EBV to exclusively reside on this haplotype version.

On chromosome 21, the associated SNPs are contained within a haplotype spanning 0.6 Mb (starting at 1.78 Mb). The most frequent haplotype version occurs in a frequency of 66% and has a negative effect on the pCE EBV ($P = 3.15 \times 10^{-7}$). However, it explains less of the genetic variance than the most significant SNP does ($-0.18\sigma_A$ *vs.* $-0.24\sigma_A$).

Identification and analysis of candidate genes: The assessment of the transcriptional content of the pCE EBV-associated regions was based on the *UMD3.1* assembly and annotation of the bovine genome (Zimin *et al.* 2009). The 23.82–25.40 Mb interval on chromosome 14 encompasses 13 transcripts/genes (Figure 3A). The associated region on bovine chromosome 14 is conserved in human chromosome 8q21, which has been shown to be associated with adult height (Gudbjartsson *et al.* 2008). Since adult stature is positively correlated with fetal size and

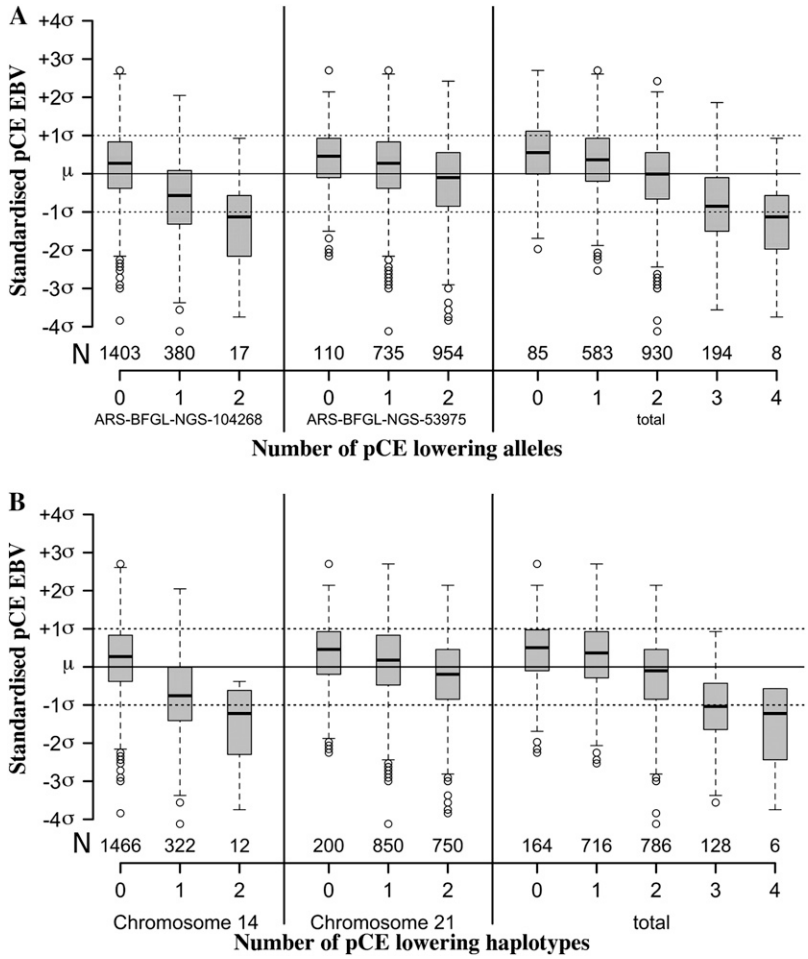

FIGURE 2.—Effect of the most significantly associated markers on the EBV for pCE in the Fleckvieh breed. The boxplots show the effects of the most significantly associated SNPs (A) and haplotypes (B) on chromosomes 14 and 21 separately and combined. The solid line represents the population mean, and the dotted lines indicate one standard deviation of the EBV.

fetal size is an important determinant of the birthing process, we considered *PLAG1, MOS, CHCHD7, RDHE2* (alias *SDR16C5*), *RPS20, LYN, TGS1, PENK,* as proposed by GUDBJARTSSON *et al.* (2008) as positional and functional candidate genes for the pCE QTL in cattle. Of this list, *PLAG1*, *TGS1, RPS20,* and *LYN* together with *SOX17,* another gene in the critical region that we considered a functional candidate, were resequenced in a panel of 12 animals of our study population. In total, we screened 30.3 kb resulting in the detection of 48 polymorphisms (Table S5). We decided to genotype four putatively functional SNPs, located in *SOX17* (*ss250608762*), *RPS20* (*ss250608720*, *ss250608721*), and *TGS1* (*ss250608741*), in 810 animals and analyzed the association with the pCE EBV in the complete study population using genotype imputation (Figure 3, B and C). Only *ss250608721* produced a highly significant signal ($P = 1.96 \times 10^{-14}$). The polymorphism affects a polyadenylation signal of a cistron encompassing the genes for the ribosomal protein S20 (*RPS20*, a ribosomal component) and the small nucleolar RNA U54 (*SNORD54*, a ribosomal RNA modifying RNA) (Figure 4).

The association signals on chromosome 21 result from the most proximal region on the chromosome (Figure S5). The region contains, among other transcripts, those encoding *SNURF–SNRPN* and *UBE3A*. These two transcripts are encoded in the human chromosome interval 15q11–15q13 that is subject to imprinting. The lack of a functional paternal copy of 15q11–15q13 causes the Prader–Willi syndrome, while the lack of a functional maternal copy of *UBE3A* is implicated in the Angelman syndrome (HORSTHEMKE and WAGSTAFF 2008). The *SNURF–SNRPN* mRNA is derived from a single large transcriptional unit of which more than 70 snoRNAs of the C/D box type are processed (BACHELLERIE *et al.* 2002). Preliminary BLAST analyses indicate the presence of a snoRNA cluster in the proximal region of bovine chromosome 21. However, a systematic annotation has not been attempted. The lack of detailed knowledge of the genomic organization, the imprinting status and transcriptional content of the associated region on chromosome 21 precluded the analysis of candidate genes, although a functional implication of the region in fetal growth and thus pCE seems obvious when considering that fetal growth retardation is symptomatic for the Prader–Willi syndrome.

DISCUSSION

Our genome-wide association study based on a dense SNP marker map provides strong evidence for two QTL

TABLE 2

SNPs within the haplotype associated with the estimated breeding value (EBV) for paternal calving ease (pCE) on bovine chromosome 14

SNP	Physical position (bp)	Haplotype allele	Minor allele (allele frequency)	Eigenstrat statistic	*P* value	α
BTB-01953819	23,817,572	A	G (0.26)	0.37	0.54	0.03
Hapmap45796-BTA-25271	23,853,811	T	A (0.07)	5.39	0.02	−0.18
ss250608741*	23,884,989	G	A (0.09)	1.06	0.3	0.06
ARS-BFGL-BAC-8052	23,893,220	G	A (0.01)	6.72	9.55×10^{-3}	−0.45
ARS-BFGL-NGS-97821	23,946,436	G	A (0.1)	0.98	0.32	0.07
ARS-BFGL-NGS-104268	24,057,354	A	A (0.12)	61.00	5.71×10^{-15}	−0.58
BTA-91250-no-rs	24,145,838	A	A (0.1)	59.32	1.34×10^{-14}	−0.62
BTB-01417924	24,182,406	G	G (0.13)	43.54	4.15×10^{-11}	−0.46
ARS-BFGL-NGS-110427	24,326,513	A	G (0.11)	0.02	0.89	−0.01
Hapmap59686-rs29020689	24,365,162	A	A (0.14)	36.94	1.22×10^{-9}	−0.40
ARS-BFGL-NGS-102351	24,407,125	G	G (0.25)	18.34	1.85×10^{-5}	−0.21
BTB-01532239	24,437,778	A	A (0.28)	28.04	1.19×10^{-7}	−0.26
BTB-01530788	24,524,205	A	G (0.34)	8.65	3.27×10^{-3}	0.12
BTB-01530836	24,573,257	G	A (0.35)	4.30	0.04	0.07
BTB-00557585	24,607,527	A	G (0.35)	4.75	0.04	0.08
BTB-00557532	24,643,266	A	G (0.35)	4.53	0.03	0.07
ss250608762*	24,759,177	G	T (0.01)	1.00	0.32	−0.14
Hapmap40120-BTA-34288	24,787,245	C	A (0.09)	0.28	0.6	−0.05
ss250608721*	24,954,981	A	A (0.16)	58.57	1.96×10^{-14}	−0.47
ss250608720*	24,955,318	T	C (0.32)	3.56	0.06	0.06
Hapmap41234-BTA-34285	25,107,556	G	A (0.04)	13.89	1.94×10^{-4}	−0.42
BTB-02056709	25,175,950	A	G (0.18)	2.55	0.11	−0.08
BTB-00559128	25,215,027	A	G (0.21)	0.01	0.92	0.00
BTB-00557354	25,254,540	G	A (0.12)	1.63	0.2	0.09
Hapmap46986-BTA-34282	25,307,116	A	G (0.46)	9.62	1.93×10^{-3}	0.13
BTB-01779799	25,351,733	G	A (0.44)	19.00	1.30×10^{-5}	0.19
Hapmap46735-BTA-86653	25,401,722	G	G (0.2)	34.40	4.48×10^{-9}	−0.36

Twenty-three SNPs belong to the BovineSNP50 Bead chip collection and four additional SNPs designated by * result from resequencing. The *P*-values were obtained by using a principal components analysis-based approach to account for population stratification. Genotypes for SNPs resulting from resequencing were determined in 810 animals and imputed for the remaining 990 animals of the study population. The allelic substitution effect (α) is given for the minor allele in additive genetic standard deviations of the pCE EBV. SNPs are arranged according to their physical position, on the basis of UMD3.1 assembly of the bovine genome sequence.

on chromosomes 14 and 21, respectively, that together explain at least 10% of the variation of the pCE EBV in the German FV breed. The two QTL also explain a substantial fraction of the pSB EBV as well as of EBVs of postnatal growth such as DG and BS. Stillbirth can be considered as the dichotomic manifestation of the calving-ease score, as dystocia is a major cause of perinatal mortality. The correlation of pCE with growth-related traits and the coincident QTL point to fetal growth and the resulting birth weight as major determinant for the ease of delivery (Meijering 1984, Johanson and Berger 2003). Thus, the two QTL might primarily affect fetal growth. One could expect that they would explain a larger fraction of the genetic variation of birth weight, a trait that is not routinely measured in dairy cattle. Improving postnatal growth along with lactation traits is a major breeding objective of the FV breed. This dual purpose selection is likely to act on the two QTL identified in our study. Animals known to carry favorable alleles for the chromosome 14 and 21 QTL could now be more stringently selected with regard to beef traits. However, the identification of QTL that either affect prenatal or postnatal growth but not both would facilitate the efficient improvement of postnatal beef performance without antagonistically compromising calving ease. In any case, conventional selection schemes seem to allow favorable selection responses for calving ease and postnatal growth despite the genetic antagonism (MacNeil 2003, Bennett 2008, Bennett *et al.* 2008).

A key factor for successfully mapping a QTL for a complex trait with very low heritability such as pCE was the use of reliably estimated breeding values for calving traits. If one assumes a heritability of 0.08, a LD between marker SNPs and QTL of $r^2 = 0.35$ and 4% of the genetic variation explained by the QTL, one would require approximately 20,000 individuals for the successful identification of a QTL (see MATERIAL AND METHODS). Using EBVs with a reliability of 90%, *i.e.,* a *quasi*-heritability of 0.9, requires less than 1800 animals to detect association. Breeding values are routinely estimated for many traits

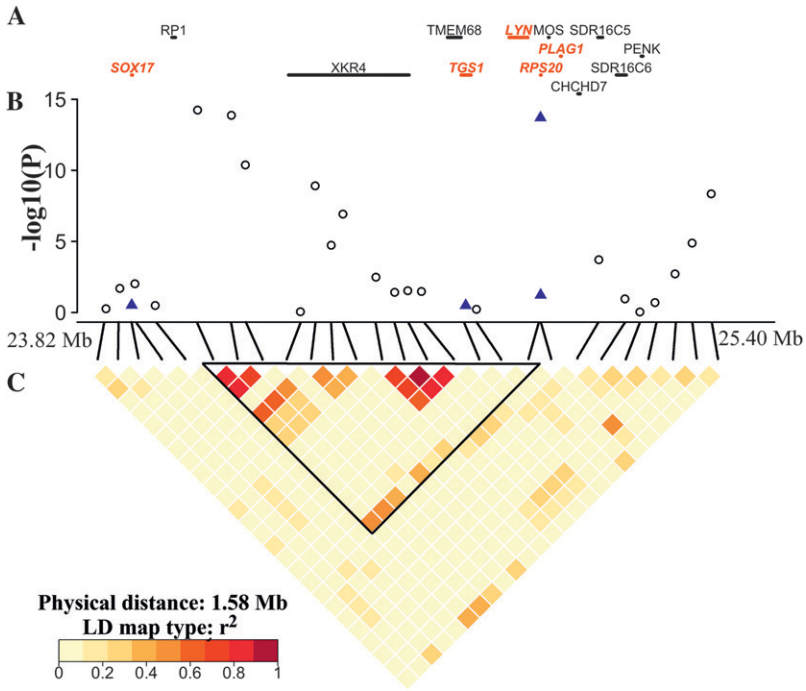

Figure 3.—Detailed view of the region on chromosome 14 delineated by the haplotype associated with the EBV for pCE. (A) Map of genes contained in this region. Red symbols indicate genes resequenced in this study. (B) *P*-values of 27 SNPs from analysis of association with the pCE EBV. The open black dots indicate results from genotyping of the entire study population, and the blue triangles represent *P*-values resulting from imputation based on 810 genotyped animals. (C) Heatmap of the pairwise linkage disequilibrium (r^2). The triangle delineates a linkage disequilibrium block containing the most significantly associated SNPs, including the potentially functional *ss250608721* variant in *RPS20*.

and are thus indispensable for dissecting complex trait variation in livestock species.

Another key factor for successfully mapping the two QTL was careful correction for extensive relationship among the study animals. The adjustment along 773 axes of variation allowed us to account for major as well as for more subtle relationships that can possibly not be revealed by pedigree analyses. The association signal on chromosome 21 became apparent only when population structure was corrected for. Thus, PCA-based elimination of false positive association signals might enable the detection of QTL with a smaller impact on the trait variation that would otherwise be "buried" in the false positive signals. Suggestive signals ($P < 1 \times 10^{-3}$, Table S4) are thus more likely to represent real QTL.

Our findings about two highly significant QTL for pCE as well as about additional suggestive QTL are supported by several previous studies on calving ease and growth trait QTL, based on microsatellite marker analyses. Kneeland *et al.* (2004) identified three regions on chromosome 14 to affect birth weight in a composite breed. The proximal region from 26.0 to 26.7 cM most likely corresponds to the highly significant QTL region identified in our study, the more distal region between 36.2 and 46.2 cM may corroborate a suggestive QTL region resulting from our study. Davis *et al.* (1998) also identified a QTL affecting birth weight at 42 cM. Koshkoih *et al.* (2006) provide additional evidence for two birth weight QTL on chromosome 14 at 26 and 50 cM, respectively, in a cross of Limousin and Jersey animals. Maltecca *et al.* (2009) recently identified a birth weight QTL at 19 cM on chromosome 14 in a Jersey–Holstein cross. There are also reports on QTL for postnatally measured growth traits in Wagyu (Mizoshita *et al.* 2004, Takasuga *et al.* 2007) and a Jersey–Limousin cross (Morris *et al.* 2002), indirectly supporting our suggestive evidence for a secondary pCE QTL on chromosome 14. Casas *et al.* (2003) and Davis *et al.* (1998) identified a QTL for birth weight in the very proximal region of chromosome 21 in crosses of Brahman with Hereford and Charolais, respectively, providing supportive evidence for the pCE QTL identified in this study.

There is also support in the literature for suggestive QTL on other chromosomes: Olsen *et al.* (2009) and Holmberg and Andersson-Eklund (2006) identified in a Swedish and Norwegian dairy cattle population, respectively, a dystocia/stillbirth QTL at 36–37 cM on chromosome 6. We observe a suggestive pCE QTL at

```
   ACTCTGAG AATAACTACG AGGAAAACTC CTTGTGGTGA AGGTTCTAAG
ACTTGGGATC GATTCCAAAT GAGGATCCAC AAGCGACTCA TTGACCTGCA
CAGTCCTTCT GAAATTGTCA AGCAGATCAC TTCCATCAGT ATTGAGCCGG
GAGTCGAGGT GGAAGTCACC ATTGCTGATG CCTAAATCAA CCTTTTTAAT
AAATCGATAA TCAGTTGTTA AATTTTGTTG ACTTTTATTT ATAATACTGA
CAAGTCTTAG GAATAGAGTT GTGTTATGGA ATTGATTCCA CCTTTTAAGT
                  ★ ss250608721 [A/G]
AGAATAATCA GAATTAAATG GATAGCCTGT GGTGGTTGAC CCTGTTTCCA
TTGTAAGCTA GTTTACACGT GACAGCATTT GAAAATGGAA TTGTGTTAAC
TGCCACATGA GATCTTGCTA TTGATGAATA TTGGGGTTTT TGAACATGTG
TGTGGGTTTA ACTTTGGAAC TGTCAGCAGT TTCTGGGATG ATTCTAATGT
TACCAGCTGT GGTTAGAAAC CTAGTGTGGT AAAAGGCAAG GAACTCAAAA
TCTAGGTCTT AATATCTGAC CCTGCCATTT GTCATAACTC CTTTTAAACT
TGGCAAATTT CAGTAAGTTG TGGAAACTTT TATAGATACT GGTGCCTTAA
TTTTAGATAA GAAAATACAA TAAAATATAA AACAATCCTA GTACTTAGGT
TATATCTATA GAATGTGTTT TATATATACT CCTTGGAATC ACTTTTCACT
TTATGTATAT AAGTCATTCC GTACGAAGCT AAGTTAAGCA TTTATATGTT
```

Figure 4.—Predicted 3′-UTR of cattle *RPS20*. The gray-shaded sequence designates the predicted exon 4, while the predicted polyadenylation [poly(A)] sites are denoted by underscoring. The star locates the candidate quantitative trait nucleotide position, ablating a poly(A) site.

about 40 Mb on chromosome 6. Gutierrez-Gil *et al.* (2009) identified a fetal growth/birth weight QTL in the same region on the basis of a Charolais–Holstein cross. Eberlein *et al.* (2009) provide evidence for the gene (*NCAPG*) encoding the Non-SMC Condensin I Complex, Subunit G, to encompass this QTL, also based on a Charolais–Holstein cross. However, a prominent calving-ease QTL in the Holstein breed on chromosome 18 (Cole *et al.* 2009) could not be detected in this study or is not segregating in the Fleckvieh breed.

A preliminary candidate gene analysis identified a highly significantly pCE-associated SNP in a cistron encoding a ribosomal protein (*RPS20*) and an internally nested small nucleolar RNA (*SNORD54*). The SNP affects a polyadenylation site. Alternative polyadenylation at tandem poly(A) sites yields transcripts with different 3′-UTR sequences providing the potential of differential regulation of mRNA expression by RNA binding proteins and/or miRNAs (Sandberg *et al.* 2008, Licatalosi and Darnell 2010). The marker allele causing the gain of an upstream polyadenylation signal is associated with a lower pCE EBV, *i.e.,* a higher incidence of calving difficulties. This is hypothetically compatible with a shorter and more highly expressed mRNA encoding ribosomal components, leading to a higher ribosome assembly rate and concomitantly stronger fetal growth. Thus we consider the polymorphism as a candidate quantitative trait nucleotide position. Interestingly, the pCE QTL on BTA21 is also in a chromosome region encoding factors involved in ribosomal assembly, specifically small nucleolar RNAs. It is therefore possible that both QTL affect ribosomal biogenesis. Mutations disturbing the ribosome assembly are often associated with abnormal fetal growth (Lempiäinen and Shore 2009, Freed *et al.* 2010).

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GENETICS

Supporting Information
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Genome-Wide Association Study Identifies Two Major Loci Affecting Calving Ease and Growth-Related Traits in Cattle

Hubert Pausch, Krzysztof Flisikowski, Simone Jung, Reiner Emmerling, Christian Edel, Kay-Uwe Götz and Ruedi Fries

DOI: 10.1534/genetics.110.124057

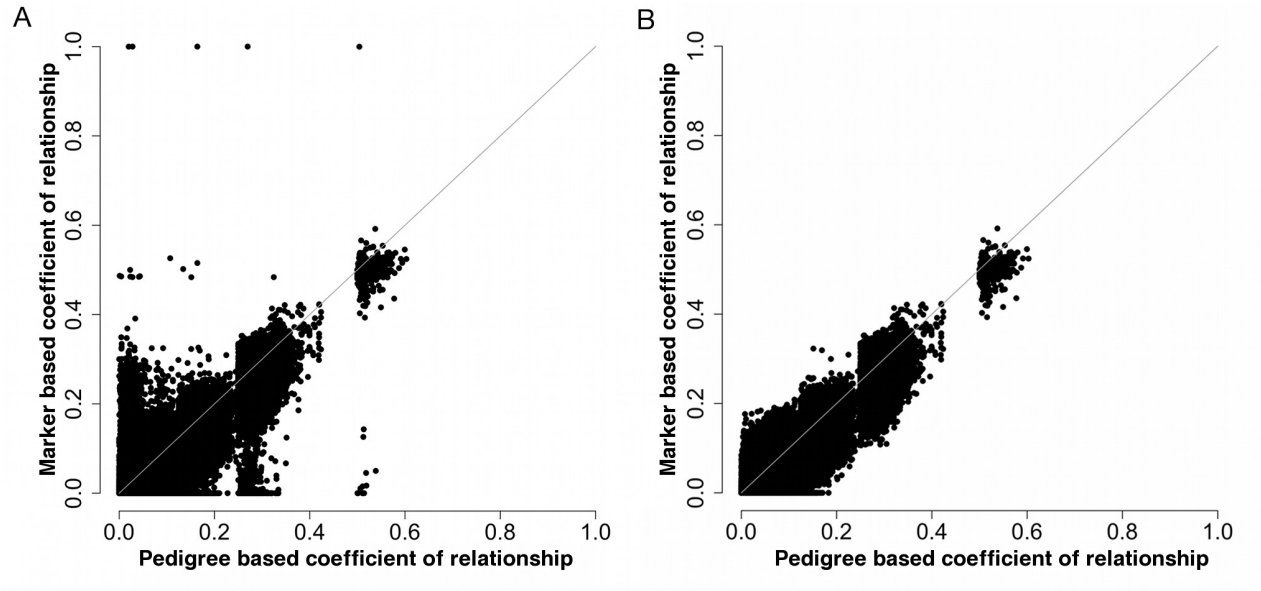

FIGURE S1.—Pairwise pedigree *vs.* IBD relationship for 1823 Fleckvieh bulls before (A) and after (B) the exclusion of 23 animals with inconsistencies.

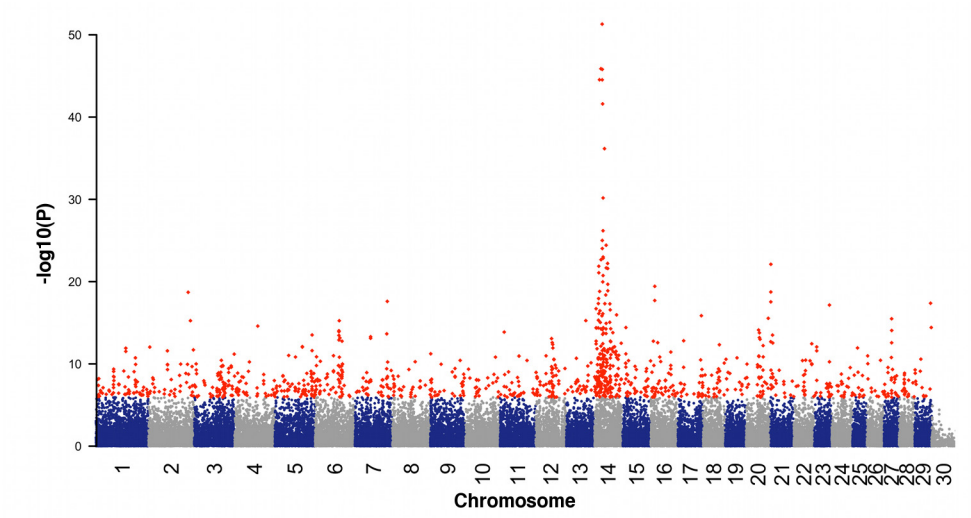

FIGURE S2.—Manhattan plot for association of 43,863 SNPs with the estimated breeding value (EBV) for paternal calving ease (pCE) without considering population stratification. The red dots represent SNPs with $P < 1.14 \times 10^{-6}$ (Bonferroni corrected significance level).

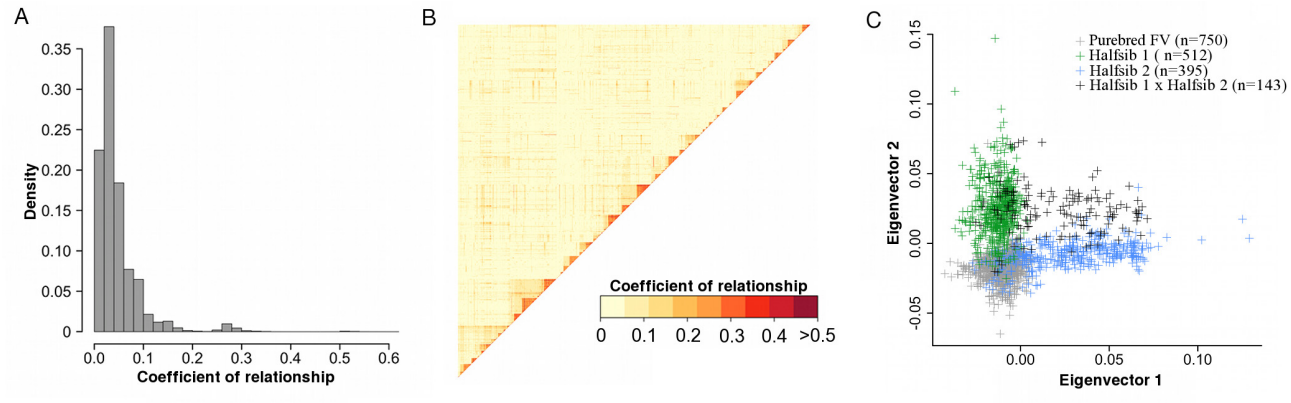

FIGURE S3.—Population stratification within the study population. (A) Distribution of the coefficients of relationship of 1800 Fleckvieh animals. (B) Heatmap of the coefficients of relationship presents cluster of related individuals. (C) Plot of the first two eigenvectors visualizing recent introgression of Holstein-Friesian (HF) into the Fleckvieh breed (through two 25% HF halfsibs) and resulting stratification.

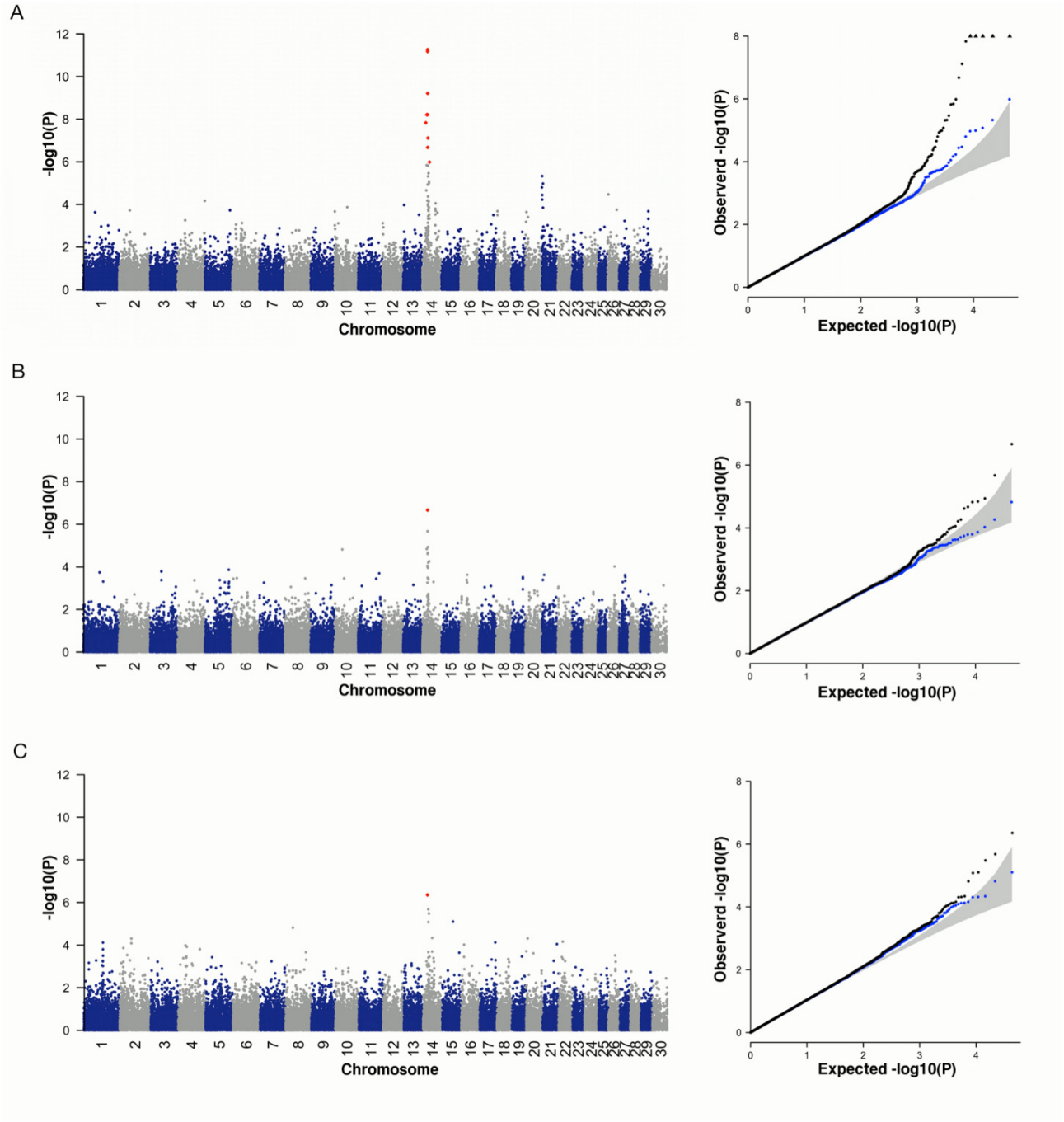

FIGURE S4.—Manhattan plots and corresponding Quantile-quantile plots for association of 43,863 SNPs with the expected breeding value for paternal stillbirth incidence (A), body size (B) and daily gain (C) after correction for population stratification. The red dots represent SNPs with $P < 1.14 \times 10^{-6}$ (Bonferroni corrected significance level). The 95% concentration band under the null hypothesis of no association is indicated by the shaded area in the QQ plots. The black symbols represent the P values of the entire study (triangles represent SNPs with $P < 1 \times 10^{-8}$). The blue dots indicate the P values excluding those from the associated regions on chromosome 14.

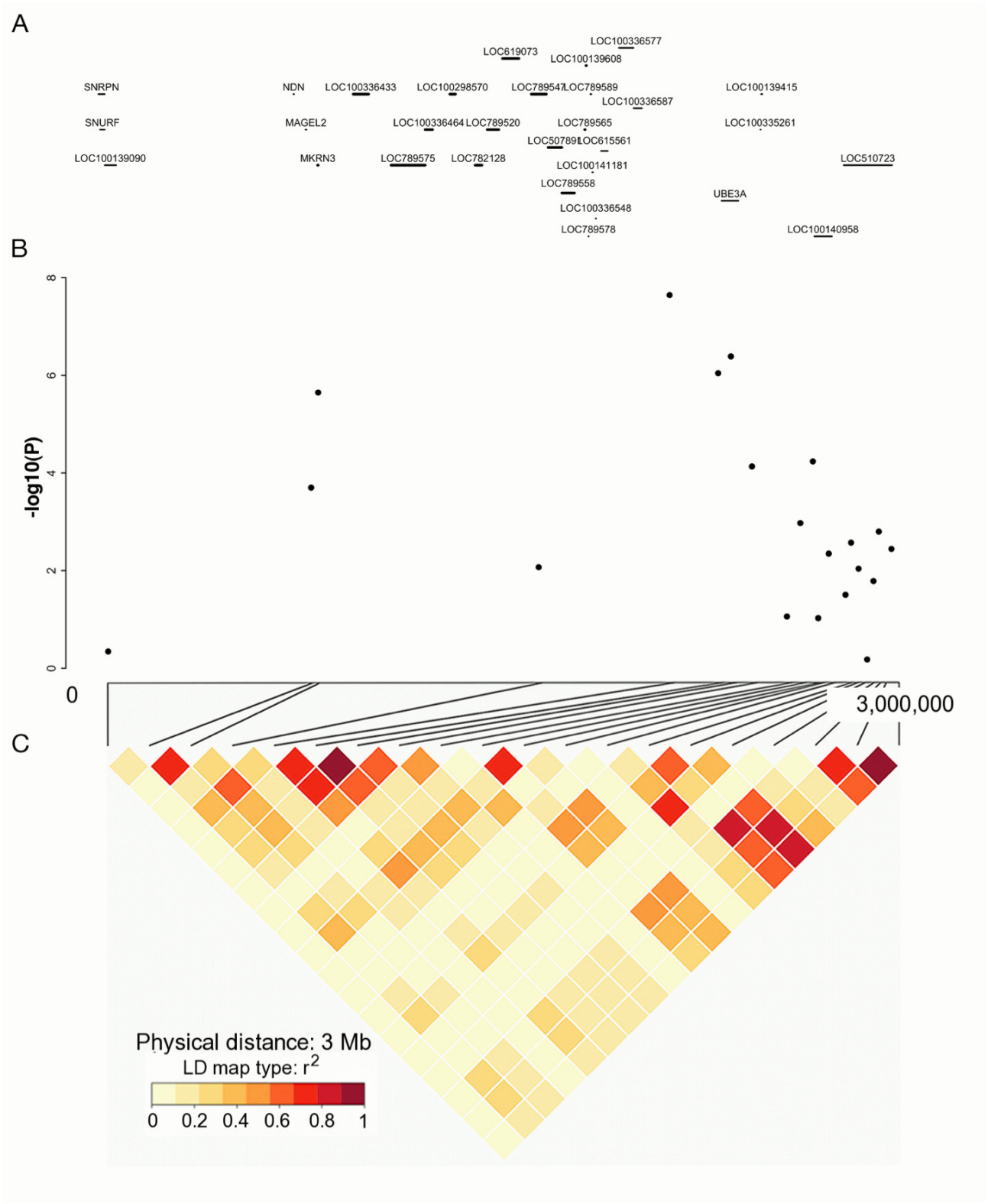

FIGURE S5.—Detailed view of the region on chromosome 21 delineated by the haplotype associated with the estimated breeding value (EBV) for paternal calving ease (pCE). (A) Map of genes contained in this region. (B) P values of 20 SNPs from analysis of association with the pCE – EBV in 1800 Fleckvieh animals. (C) Heatmap of the pairwise linkage disequilibrium (r^2).

TABLE S1

Characteristics of the considered estimated breeding values (EBVs) of 1800 Fleckvieh bulls

EBV	Standard deviation (σ)	Mean reliability (r^2)
Daily gain (**DG**)	11.68	0.92
Paternal calving ease (**pCE**)	10.07	0.92
Paternal stillbirth incidence (**pSB**)	9.39	0.83
Body size (**BS**)	9.97	0.90

TABLE S2

Primers used for the re-sequencing of bovine *LYN*, *PLAG1*, *RPS20*, *SOX17* and *TGS1* genes

Gene	Primer_id	Region	Direction	Sequence
LYN	6964	PROM	forward	GGCCAGTACTTTGCATGTGA
	6965	EX1	reverse	ATTACGCAGCCATGTTTTGA
	6966	EX2	forward	GCTCTGCAGGACTGTTCCTC
	6967	EX2	reverse	GATGGAGAGATGGACGGATG
	6968	EX3	forward	GAACAGGGAAGGTGAACGAA
	6969	EX3	reverse	GGCAGCACAGATGGATAAGG
	6970	EX4	forward	CCCATGGTATGCAGGATCTTA
	6971	EX5	reverse	TCACTTGGCTGTAAAGCTGAAA
	6972	EX6	forward	AGGGCCATGTTGTTTATCCA
	6973	EX6	reverse	ATGGACTGTAGCCCACCAAG
	7010	EX6	forward	CCCCATAATGCCAATCTTGT
	7011	EX6	reverse	TGATCCTGCAACTTTATCCAAA
	6974	EX7	forward	CTTGGCGAGTTGGAAATGAT
	6975	EX7	reverse	CTGGAAGAGGGCATGACAAC
	6976	EX8	forward	CCAGGGAAGTCCCTAAAGGT
	6977	EX8	reverse	TTCTCCAGGCAAGAATACCG
	7006	EX8	forward	TCCCTTCTTTTCCCTCCCTA
	7007	EX8	reverse	CGAGCCTGCTGTTGATAGTCT
	6978	EX9	forward	GTCAAAGGGGACAGGTCAGA
	6979	EX9	reverse	GGGGTAGACAGGGAAGGAAA
	6980	EX10	forward	GAAAAGCTGGGACAATGACG
	6981	EX10	reverse	TGCCTGTTGTAATCGCTTTG
	6982	EX11	forward	TCCTTCTCCAGGGGATCTTT
	6983	EX11	reverse	GAGGAGCCCTGTGTCTTGTC
	7012	EX11	forward	CCGCAAAGAAGGAAAGTGTG
	7013	EX11	reverse	GACAAGAAGGCGGAGAAGTG
	6984	EX12	forward	CTTGGGGCTAGGTCTTCAGT
	6985	3'UTR	reverse	TCTGCGACTCACTGAAATGG
PLAG1	6986	PROM	forward	TTCTCTGGGCCTCTCACTTT
	6987	EX1	reverse	AGCTTCTCCGATGACAGGTT
	6988	EX2	forward	GGATCTCAGGGGATCTGTGA

	6989	EX2	reverse	GCGGAAAGAGGTGGATACAA
	6990	EX3	forward	GGTCTGCGGTGTTTAGGTGT
	6991	EX3	reverse	GGAGGAGTTCGTCCTTGATG
	6992	EX3	forward	GCACATGAAGAAGAGCCACA
	6993	EX3	reverse	CCGTGGGACTCTACTGGAAA
	6994	EX3	forward	AGGAGGAGGCACACTCTTCA
	6995	3'UTR	reverse	CAGCAAACATTTGAGCCAGA
RPS20	6996	PROM	forward	TGCAGATGACACCACCCTTA
	6997	PROM	reverse	CGGAGTTCACCCAAACTCAT
	7016	PROM	forward	AGATGGGCATACCAGACCAC
	7017	PROM	reverse	GGCCAAGTAATGTCTCTGCTTT
	6998	EX1	forward	ACCTCATGCGAAGAGCTGAC
	7014	EX1	reverse	CCTTACGCCTTCCTCTTTGA
	6999	EX2	forward	CCTGGAGGCATCTCATAAGC
	7015	EX2	reverse	AACACGGCACACACCAAGT
	7000	EX3	forward	CAGGGAATGGGCTTATGAGA
	7001	EX3	reverse	GCCAAAGCTCCAGATGTTTC
	7002	EX4	forward	CCGGTTGCTTTTAAACATGG
	7003	3'UTR	reverse	TGAGTTCCTTGCCTTTTACCA
SOX17	7138	PROM	forward	GTTGGCTGATGTTTGGTGTG
	7139	PROM	reverse	CAGGTCCCAAGTTTCAGCTC
	7413	PROM	forward	CCAAGCATCGAAACACAAAA
	7414	PROM	reverse	GGTGTCTCTCCACCCCCTAC
	7415	PROM	forward	TCCATCCTATGCATCCTGTG
	7416	PROM	reverse	TGGCCAAAAAGTGGTTGTAG
	7417	PROM	forward	TGAATCTCAGAGACCCAGGAA
	7418	PROM	reverse	TTCGAGAGGCCTTCTTTGTG
	7419	PROM	forward	GGGCAAGGTCCTTAACGTCT
	7420	PROM	reverse	ACTCAACCTGGAGCTGAGGA
	7140	EX1	forward	TTTTCTTAGGGGCAGGTGTC
	7141	EX1	reverse	ACTCACCCAGCATCTTGCTC
	7532	EX1	forward	TGAGCTGAAACTTGGGACCT
	7533	EX1	reverse	CTCGCCCTTCATCTTCATGT
	7534	EX1	forward	GTACGCCAGTGACGAGCAGA

	7535	EX1	reverse	GCCGCTTGGAGAGTAGGAGA
	7142	EX2	forward	CCCCAGCCTTCAACCTTT
	7143	EX2	reverse	CGGGGCGTAGCTGTAAGG
	7144	EX2	forward	CCCTGGGCCTTACAGCTAC
	7145	EX2	reverse	TCCTTGGGGAGGTGTGTAAC
	7146	3'UTR	forward	AACTATCCCGACGTGTGAGC
	7147	3'UTR	reverse	GGGTCACCTGAAATGCATAAG
	7421	3'UTR	forward	AGGGGAAGCCCTCAAATAAA
	7422	3'UTR	reverse	TGCCCATTGTAAATCACCTG
	7423	3'UTR	forward	ATCACTGTCCTGCCCTGTCA
	7424	3'UTR	reverse	CCATTGCCTTCTCCGATAGT
	7425	3'UTR	forward	CATTTGATGTGCAAACCTTCA
	7426	3'UTR	reverse	TATGGCAACAGCATGCAGAA
	7427	3'UTR	forward	TCTCTGGTGGTCCAGTGGTT
	7428	3'UTR	reverse	TATGCTTCCCAACGAACCTT
TGS1	6884	PROM	forward	CCGTAAGACCAGACGCACAG
	6885	EX1	reverse	CCCCTTTTTCGTAAGCATCA
	6886	EX2	forward	TCAATCCTTGTTAGAACCCTGT
	6887	EX2	reverse	AGGCCAGACTGTGGATGTTC
	6888	EX3	forward	TGCACACCTTTACTTTGAGCA
	6889	EX3	reverse	AATCCTCACGCACGAGACAT
	6890	EX4	forward	AGTCCATACGGTCGCAGAGT
	6891	EX4	reverse	TGTGAGGCATCAAAAGTCCA
	6892	EX4	forward	CATGCAGATCAGACCCTGTG
	6893	EX4	reverse	TGTATCCGACTCCTAGCAACC
	6894	EX5	forward	GGTCTGCCATGCAGTTCTTT
	6895	EX5	reverse	CTTCTTGACCCAGGAATGGA
	6896	EX6	forward	TCCCAAACACTGCTAGGTAAT
	6897	EX6	reverse	CAATGAAATTACATGTGGCTAGA
	6898	EX7	forward	TGCAGTCCTCTGCATGTTTA
	6899	EX7	reverse	GGCCTCCAGGATGGTACTTA
	6900	EX8	forward	GCAGCTTGTCAGGTCAAAAA
	6901	EX8	reverse	CAGAACACGCAGCCTACAGA
	6902	EX9	forward	TCTGTAGGCTGCGTGTTCTG

6903	EX9	reverse	AAATGCTGCAAAGGACATGA
6904	EX10	forward	GAAAATTGGGACTGGGGATA
6905	EX10	reverse	AAACACAACAGTACCCAAAGTG
6906	EX11	forward	CTGCTCAGAAGATGCAGTCG
6907	EX11	reverse	CCAGGAACAGGTTCTGAGGA
6908	EX12	forward	AGGAACCTGGAGGGCTAGAG
6909	EX12	reverse	GCTATGTCAGGTGTGCAGGA
6910	EX13	forward	TGAACATTTGAGATGCCTCATT
6911	3'UTR	reverse	GCCAAAGCCATGTTTTGTTT

TABLE S3

Correlation between the estimated breeding values EBVs for daily gain (DG), paternal calving ease (pCE), paternal stillbirth incidence (pSB) and body size (BS) of 1800 animals

	pCE	pSB	BS
DG	-0.21	-0.18	0.39
pCE		0.86	-0.36
pSB			-0.23

TABLE S4

SNPs showing suggestive associations ($1.14 \times 10^{-6} < P < 1 \times 10^{-3}$) with the estimated breeding value (EBV) for paternal calving ease (pCE)

SNP	Chromosome	Minor allele and MAF	Physical position (BP)	Trait	Eigenstrat statistic	P value	α
ARS-BFGL-NGS-93455	1	A (0.24)	109,649,036	pCE	12.10	5.10×10^{-4}	0.17
BTA-49059-no-rs	2	A (0.02)	112,990,834	pCE	12.04	5.22×10^{-4}	0.47
ARS-BFGL-NGS-19373	4	G (0.4)	119,924,805	pCE	16.18	5.75×10^{-5}	0.18
ARS-BFGL-NGS-32612	5	A (0.42)	110,671,789	pCE	11.49	7.00×10^{-4}	0.15
ARS-BFGL-NGS-13748	5	A (0.42)	110,704,158	pCE	11.32	7.66×10^{-4}	0.15
Hapmap26308-BTC-057761	6	G (0.22)	38,576,012	pCE	11.81	5.91×10^{-4}	0.18
BTB-00251059	6	G (0.06)	42,190,501	pCE	15.09	1.02×10^{-4}	0.35
Hapmap47224-BTA-24614	6	G (0.44)	43,303,952	pCE	11.29	7.81×10^{-4}	-0.15
Hapmap23217-BTA-152007	7	A (0.42)	28,940,286	pCE	10.94	9.40×10^{-4}	-0.17
ARS-BFGL-NGS-104767	10	A (0.17)	1,361,856	pCE	11.14	8.45×10^{-4}	-0.21
BTA-70225-no-rs	10	G (0.39)	56,285,758	pCE	12.75	3.57×10^{-4}	-0.16
ARS-BFGL-NGS-55539	10	A (0.31)	58,488,593	pCE	10.86	9.82×10^{-4}	0.18
BTB-01518485	14	G (0.14)	58,203,661	pCE	13.40	2.52×10^{-4}	0.26
BTB-01518486	14	A (0.1)	58,262,807	pCE	15.33	9.03×10^{-5}	0.28
BTB-01289984	14	G (0.2)	58,491,253	pCE	13.79	2.04×10^{-4}	0.18
BTB-00574555	14	A (0.1)	59,225,067	pCE	13.78	2.05×10^{-4}	0.28
UA-IFASA-7897	14	A (0.12)	59,280,392	pCE	16.96	3.81×10^{-5}	0.29

ARS-BFGL-NGS-27017	15	A (0.18)	57,333,896	pCE	12.54	3.97×10^{-4}	0.20
ARS-BFGL-NGS-94657	17	G (0.47)	74,234,279	pCE	13.48	2.42×10^{-4}	-0.13
UA-IFASA-6850	17	G (0.34)	74,256,192	pCE	11.51	6.91×10^{-4}	-0.13
Hapmap51998-BTA-43053	18	G (0.27)	36,985,552	pCE	11.15	8.39×10^{-4}	-0.17
BTB-01393816	20	A (0.21)	2,754,521	pCE	11.62	6.52×10^{-4}	-0.19
Hapmap40409-BTA-26097	20	G (0.27)	11,576,011	pCE	16.28	5.47×10^{-5}	0.21
ARS-BFGL-NGS-42400	24	A (0.47)	47,413,118	pCE	11.90	5.61×10^{-4}	0.15
BTB-01710538	25	G (0.1)	29,635,262	pCE	10.98	9.23×10^{-4}	0.23
BTB-00920322	26	C (0.47)	3,930,593	pCE	16.33	5.33×10^{-5}	-0.14
ARS-BFGL-NGS-16336	26	A (0.35)	34,398,368	pCE	11.92	5.54×10^{-4}	-0.59
Hapmap42269-BTA-61597	26	A (0.29)	41,041,883	pCE	15.80	7.06×10^{-5}	-0.42
UA-IFASA-6120	29	G (0.27)	37,014,709	pCE	13.38	2.54 x 10-4	-0.19
ARS-BFGL-NGS-104213	29	G (0.02)	37,152,168	pCE	15.64	7.68 x 10-5	-0.59

TABLE S5

Characterization of the *PLAG1*, *RPS20*, *LYN*, *SOX17* and *TGS1* polymorphisms

Gene	SNP_ID	Localization	SNP	AminoAcid
PLAG1	ss250608717	INT2	AT	
	ss250608718	PROM	CT	
RPS20	ss250608719	EX3	CT	G42
	ss250608720*	INT3	CT	
	ss250608721*	3'UTR	AG	
	ss250608722	3'UTR	AG	
	ss250608723	PROM	AC	
	ss250608724	PROM	AG	
	ss250608725	PROM	AG	
	ss250608726	PROM	CG	
LYN	ss250608727	INT11	CG	
	ss250608728	INT11	CT	
	ss250608729	EX12	CT	T454
	ss250608730	EX12	AG	T489
	ss250608731	3'UTR	CT	
	ss250608732	INT10	CT	
	ss250608733	INT4	CT	
	ss250608734	INT5	AG	
	ss250608735	INT1	AG	
	ss250608736	INT2	GT	
	ss250608737	INT11	AG	
	ss250608738	EX6	CT	G177
	ss250608739	EX6	CT	S205
SOX17	ss250608740	3'END	AG	
	ss250608741*	EX1	AG	A50T
TGS1	ss250608742	EX3	CT	S90
	ss250608743	INT3	CT	
	ss250608744	INT3	AT	
	ss250608745	EX4	CT	S297T
	ss250608746	INT4	CT	
	ss250608747	INT2	AG	

ss250608748	INT5	CT	
ss250608749	INT6	AG	
ss250608750	INT6	AG	
ss250608751	INT6	CT	
ss250608752	3'UTR	CG	
ss250608753	INT11	GT	
ss250608754	INT12	INS T	
ss250608755	INT10	CG	
ss250608756	EX9	DEL GAA	K626-
ss250608757	INT10	GT	
ss250608758	INT7	CT	
ss250608759	EX11	AT	I733
ss250608760	EX11	CT	V758
ss250608761	EX8	AG	P561
ss250608762*	EX8	GT	P594S
ss250608763	INT8	CT	
ss250608764	PROM	CG	

The * indicates SNPs that were genotyped in 810 animals of the study population.