Accuracy of direct genomic values for functional traits in Brown Swiss cattle

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ABSTRACT

In this study, direct genomic values for the functional traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat in Brown Swiss dairy cattle were estimated based on ~777,000 (777K) single nucleotide polymorphism (SNP) information from 1,126 animals. Accuracy of direct genomic values was assessed by a 5-fold cross-validation with 10 replicates. Correlations between deregressed proofs and direct genomic values were 0.63 for general temperament, 0.73 for milking temperament, 0.69 for aggressiveness, 0.65 for rank order in herd, 0.69 for milking speed, 0.71 for udder depth, 0.66 for position of labia, and 0.74 for days to first heat. Using the information of ~54,000 (54K) SNP led to only marginal deviations in the observed accuracy. Trying to predict the 20% youngest bulls led to correlations of 0.55, 0.77, 0.73, 0.55, 0.64, 0.59, 0.67, and 0.77, respectively, for the traits listed above. Using a novel method to estimate the accuracy of a direct genomic value (defined as correlation between direct genomic value and true breeding value and accounting for the correlation between direct genomic values and conventional breeding values) revealed accuracies of 0.37, 0.20, 0.19, 0.27, 0.48, 0.45, 0.36, and 0.12, respectively, for the traits listed above. These values are much smaller but probably also more realistic than accuracies based on correlations, given the heritabilities and samples sizes in this study. Annotation of the largest estimated SNP effects revealed 2 candidate genes affecting the traits general temperament and days to first heat.

Key words: direct genomic value prediction, accuracy of direct genomic value, functional trait, gene annotation

INTRODUCTION

Genomic selection is well established in dairy cattle breeding, and accuracies of direct genomic values (DGV) for traditional production or conformation traits are high (e.g., Segelke et al., 2012). To maximize the benefit, genomic selection should also be used for scarcely recorded functional traits, which are becoming increasingly relevant in dairy cattle breeding. For this reason, one aim of our study was to apply the estimation of DGV to a set of functional traits. High-density chips with information of more than 777,000 (777K) SNP are increasingly available in cattle breeding. We thus compared results from an estimation of DGV with 777K data with the results of estimation of DGV with ~54,000 (54K) SNP data. To assess the accuracy of DGV, it is easy to calculate the correlation between DGV and conventional breeding value (EBV), but this correlation is different from the correlation between DGV and true breeding value (TBV), which is normally used in practical breeding to reflect the accuracy of a breeding value. To account for this, the correlation between DGV and EBV is often divided by the accuracy of the EBV. As stated by Amer and Banos (2010), this correction leads to an overestimation of accuracy; therefore, we applied a new method proposed by Wellmann et al. (2013) to estimate more realistic accuracies. To date, only few genome-wide association studies for functional traits have been conducted in Brown Swiss (BS) cattle (e.g., Guo et al., 2012). Another aim of our work was thus to screen the genome for SNP with large effects on the observed traits to find genes that are associated with these SNP and could influence the respective trait.

MATERIALS AND METHODS

Genotypic Data

The 777K genotypes used in this study were derived by imputation from a data set of 880 BS animals genotyped with the Illumina BovineHD chip (727 cows, 153 bulls) and from a data set of 6,016 animals genotyped with the Illumina Bovine SNP50 chip (548

Received May 23, 2013.

Accepted November 25, 2013.

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cows, 5,468 bulls; both chips from Illumina Inc., San Diego, CA). Before imputation, both data sets were checked for quality separately: SNP with minor allele frequency <0.5%, a call rate <90%, missing position, or position on the sex chromosomes were excluded. In addition, within the 777K data set, mitochondrial SNP and 1 SNP of each of 55 pairs of SNP with identical position but different denomination, respectively, were excluded. Animals with a call rate <90% were excluded from both data sets. After quality checks, imputation was done by using FImpute (Sargolzaei et al., 2011) from 39,004 to 627,306 SNP, using a combination of family and population imputation. This method led to a correlation of 0.99 between true genotype and imputed genotype if both parents were genotyped with the 777K chip and to a correlation of 0.97 between true genotype and imputed genotype if no close relatives were genotyped with the 777K chip (Gredler et al., 2013). We used a subset of 1,126 animals (930 cows from Switzerland with phenotype information for the traits observed and 196 bulls with performance records of at least one daughter) for estimation of DGV and SNP effects in our study.

Phenotypic Data

Deregressed proofs (**DRPF**) for the traits general temperament, milking temperament, aggressiveness, rank order in herd, milking speed, udder depth, position of labia, and days to first heat of the 1,126 BS animals were used as quasi phenotypes. For detailed information on the trait definitions, see Kramer et al. (2013). Although some of these traits are routinely evaluated and multiple-trait across-country evaluation (MACE) proofs would have been available, we decided

to use our own breeding values to evaluate the possibilities of genomic estimation based on genotypes and phenotypes of a mixed data set of cows and bulls. The DRPF for individual i within a given trait were derived as described in Garrick et al. (2009):

$$DRPF_i = \frac{EBV_i}{r_{EBV_i}^2},$$

where $DRPF_i$ is the DRPF of individual i , EBV_i is the EBV for individual i , and $r_{EBV_i}^2$ is the squared accuracy of the EBV_i for individual i . In our study, EBV of cows with several phenotypic measurements as well as EBV of bulls with a different number of daughters (mean: 3.22; range: 1–31) were used. Distribution of birth year of animals is shown in Figure 1. Ninety-five percent of bulls were born between the years 1988 and 2004, and 95% of the cows were born between the years 1998 and 2007. Accuracies of conventional breeding values (r_{EBV}) were in a wide range and, on average, higher in cows than in bulls for most traits (Table 1).

Model

The DGV were estimated using the model $\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{g} + \mathbf{e}$, where $\mathbf{y}$ is a vector of DRPF, $\mathbf{1}$ is a vector of 1s, μ is the overall mean, $\mathbf{g}$ is a vector of additive genetic effects, $\mathbf{e}$ is a vector of residual effects, and $\mathbf{Z}$ is a design matrix allocating genetic effects to the observations. For random elements, the distributions $\mathbf{e} \sim N(0, \mathbf{R}\sigma_e^2)$ and $\mathbf{g} \sim N(0, \mathbf{G}\sigma_g^2)$ were assumed, where $\mathbf{R}$ is a diagonal matrix with $1/w_i$ on the diagonal and w_i is a weighting factor for the residual variance, and $\mathbf{G}$ is a genomic relationship matrix. Additive genetic variance

Figure 1. Distribution of birth year for genotyped cows and bulls.

Table 1. Heritabilities (h^2) with standard error (SE), mean accuracies (r_{EBV}) with standard deviation (SD) of estimated or conventional breeding values (EBV) and number of records used for estimation of EBV (n EBV) for the different groups of animals for the traits observed

Trait	$h^2 \pm \text{SE}$	$r_{EBV} \pm \text{SD}$			n EBV
		All animals (n = 1,126)	Cows (n = 930)	Bulls (n = 196)	
General temperament	0.38 $\pm$ 0.07	0.66 $\pm$ 0.08	0.68 $\pm$ 0.05	0.59 $\pm$ 0.12	2,312
Milking temperament	0.04 $\pm$ 0.04	0.30 $\pm$ 0.06	0.30 $\pm$ 0.05	0.29 $\pm$ 0.09	2,259
Aggressiveness	0.12 $\pm$ 0.08	0.34 $\pm$ 0.08	0.34 $\pm$ 0.08	0.33 $\pm$ 0.10	2,309
Rank order in herd	0.16 $\pm$ 0.06	0.51 $\pm$ 0.07	0.51 $\pm$ 0.05	0.47 $\pm$ 0.12	2,304
Milking speed	0.42 $\pm$ 0.06	0.72 $\pm$ 0.08	0.74 $\pm$ 0.05	0.62 $\pm$ 0.12	4,540
Udder depth	0.42 $\pm$ 0.06	0.66 $\pm$ 0.08	0.67 $\pm$ 0.06	0.58 $\pm$ 0.12	2,195
Position of labia	0.28 $\pm$ 0.06	0.61 $\pm$ 0.08	0.62 $\pm$ 0.07	0.54 $\pm$ 0.12	2,232
Days to first heat	0.02 $\pm$ 0.04	0.18 $\pm$ 0.04	0.19 $\pm$ 0.04	0.18 $\pm$ 0.06	1,678

(σ_g^2) and residual variance (σ_e^2) were estimated from the complete data set using ASReml (Gilmour et al., 2009).

Because of the wide range of r_{EBV} , residual errors were also in a wide range and it was thus necessary to account for different residual variances. The weighting factor for the reciprocal of the residual error of individual i (w_i) was calculated as described in Garrick et al. (2009):

$$w_i = \frac{1 - h^2}{h^2 \left(c + \frac{1 - r_{EBV_i}^2}{r_{EBV_i}^2} \right)},$$

where h^2 is the heritability of the observed trait, c is the fraction of genetic variance that is not explained by markers, and $r_{EBV_i}^2$ is the squared accuracy of the EBV. We assumed that the complete genetic variance was explained by the 627k SNP markers and so c was set to 0. This assumption is also made in routine breeding value estimation for functional traits in Simmental cattle in Germany and Austria (Edel et al., 2011).

The genomic relationship matrix $\mathbf{G}$ was set up from the SNP data as described in VanRaden (2008):

$$\mathbf{G} = \frac{(\mathbf{M} - \mathbf{P})(\mathbf{M} - \mathbf{P})'}{2 \sum_{j=1}^m p_j (1 - p_j)},$$

where p_j is the frequency of the second allele at locus j ; m is the total number of SNP; $\mathbf{M}$ is a matrix with genotypes coded as 0, 1, and 2 in columns and animals in rows; and $\mathbf{P}$ is a matrix with all elements in column j being $2p_j$.

The correlation between DGV and DRPF ($r_{DGV,DRPF}$) was assessed by random 5-fold cross-validation with 10

replicates and was calculated as an average over all 50 replicates of predicted folds.

To study the influence of the age structure, we also performed one cross-validation run with 777K SNP information with animals sorted by age, where the DGV of the youngest 177 animals from birth year 2007–2008 (approximately one-fifth) were predicted with use of the older animals (birth years 1985–2006).

Random cross-validation was further carried out with the 54K SNP panel. For cross-validation with 54K information, the data set was restricted to the 39,004 SNP from the 54K SNP chip after filtering, and σ_g^2 and σ_e^2 were estimated again using a $\mathbf{G}$ matrix based on 54K information only.

The SNP effects were derived for the 777K SNP data as described in, for example, Ober et al. (2012):

$$\hat{\mathbf{s}} = \frac{\hat{\sigma}_g^2}{2 \sum_{j=1}^m p_j (1 - p_j)} (\mathbf{M} - \mathbf{P})' (\hat{\sigma}_g^2 \mathbf{G} + \hat{\sigma}_e^2 \mathbf{R})^{-1} (\mathbf{y} - \mathbf{1}\hat{\mu}),$$

where $\hat{\mathbf{s}}$ is a vector of SNP effects, and all other factors are as described above. The SNP effects were then standardized as follows:

$$\hat{\mathbf{s}}_{STD} = \frac{\hat{\mathbf{s}}}{\sqrt{\hat{\sigma}_{SNP}^2}},$$

with

$$\hat{\sigma}_{SNP}^2 = \frac{\hat{\sigma}_g^2}{2 \sum_{j=1}^m p_j (1 - p_j)},$$

where $\hat{\mathbf{s}}_{STD}$ is a vector of standardized SNP effects and all other factors are described above. The SNP with an

absolute standardized effect >4 SD were considered SNP with a high influence on the specific trait. In the Manhattan plots (not shown), some distinct peaks formed by the high-influence SNP were obvious. All genes up to 100 kb upstream and downstream of the SNP position with the most extreme value within these peaks of high influence SNP were annotated using the map viewer option of the bovine genome sequence assembly (*Bos taurus* 6.1; http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9913&build=6.1) to find genes that might causatively influence the respective trait.

The correlation $r_{DGV,DRPF}$ is different from the correlation between DGV and TBV, which is the accuracy of genomic breeding values (r_{DGV}). Note that the often reported reliability of genomic breeding values is the square of the accuracy (r_{DGV}^2) used in our study. Often, r_{DGV} is estimated as $r_{DGV,DRPF}/r_{EBV}$, where it is further assumed that $r_{DGV,DRPF} = r_{DGV,EBV}$. It was noted by Amer and Banos (2010) that with this approach an overlap of testing and training sets in cross-validation may lead to an overestimation of r_{DGV} . Wellmann et al. (2013) suggested correcting for this bias by fitting the model

$$\hat{r}_{DGV} = r_{DGV,DRPF} - \hat{a}_1(r_{EBV}^{VS} - 1), \quad [1]$$

where $\hat{r}_{DGV}$ is the estimated accuracy of DGV defined as correlation between DGV and TBV, $r_{DGV,DRPF}$ is the estimated correlation between DGV and DRPF in 1 fold of a cross-validation, r_{EBV}^{VS} is the mean accuracy of EBV in the validation set of this fold, and $\hat{a}_1$ is a regression coefficient estimated from the equation

$$r_{DGV,DRPF} = a_0 + a_1 r_{EBV}^{VS} + a_2 r_{EBV}^{TS} + e, \quad [2]$$

where a_0 , a_1 , and a_2 are the intercept and fixed regression coefficients, e is the residual error, and r_{EBV}^{TS} is the mean accuracy of EBV in the training set of the specific fold. For further details, see Wellmann et al. (2013).

For this approach, data sets with different accuracies of conventional breeding values (r_{EBV}) are required to fit the regression model. As described in Wellmann et al. (2013), we performed this by fitting Eq. [2] for all traits simultaneously.

If the accuracy of DGV is compared with the accuracy of EBV, EBV should be based only on pedigree information because this is also available in an early stage of an animal's life. We thus approximated accuracies of EBV based on pedigree information from the

average accuracies of EBV of bulls and cows (Table 1) by using the formula

$$r = \sqrt{0.25(r_s^2 + r_d^2)},$$

where r_s^2 is the reliability of the sire's EBV and r_d^2 is the reliability of the dam's EBV. This formula is also used by, for example, Edel et al. (2010).

RESULTS AND DISCUSSION

Correlation Between DGV and DRPF

The values for $r_{DGV,DRPF}$ from 5-fold random cross-validation with 10 replicates with 777K information are shown in Figure 2. With random cross-validation, we obtained $r_{DGV,DRPF}$ ($\pm$ SD) of 0.63 ± 0.05 for general temperament, 0.73 ± 0.04 for milking temperament, 0.69 ± 0.06 for aggressiveness, 0.65 ± 0.04 for rank order in herd, 0.69 ± 0.03 for milking speed, 0.71 ± 0.03 for udder depth, 0.66 ± 0.03 for position of labia, and 0.74 ± 0.02 for days to first heat between DGV and DRPF. The correlations between DGV and DRPF after stratification by age were in a range of 0.55 and 0.77 and thus were slightly lower, on average, than correlations from random cross-validation (Figure 2). The $r_{DGV,DRPF}$ values for the youngest animals deviated from the average values from random cross-validation between $+0.04$ for milking temperament and aggressiveness and -0.12 for udder depth, but were mostly in the range of values obtained by random cross-validation. These results are in line with Saatchi et al. (2011), who compared random cross-validation and cross-validation by age with 16 traits of American Angus beef cattle and also found higher accuracy for most traits with random cross-validation compared with stratification by age. If dairy cattle breeding values are estimated in practice, information from older animals (EBV of bulls with a certain number of daughters in milk or phenotypic measurements of lactating cows) is used to predict DGV for young animals; for example, bull calves for purchase by AI organizations. Thus, the age-stratified approach reflects the practical relevant case. However, due to the lack of resampling, the point estimate obtained for $r_{DGV,DRPF}$ in the age-stratified scenario may have a large stochastic error and should be interpreted with caution. Erbe et al. (2011) have suggested an age-stratification with resampling, which provides a distribution of estimates for the accuracy; however, this approach could not be implemented here due to the limited sample size.

Figure 2. Correlations between direct genomic values (DGV) and deregressed proofs (DRPF) from cross-validation for general temperament (GT), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), milking speed (MS), udder depth (UD), position of labia (PL), and days to first heat (DH), based on 777K (~777,000) SNP information. Results from 5-fold random cross-validation with 10 replicates are indicated by boxes; results from stratification by age are indicated by ×.

In general, $r_{DGV,DRPF}$ values in our study were high, especially if the small number of animals in the data set ($n = 1,126$) and the low r_{EBV} (0.18–0.72) are taken into account. Erbe et al. (2012) found correlations between genomic breeding values and daughter trait deviations of 0.58, 0.58, and 0.56 for the traits milk yield, fat yield, and protein yield, respectively, in a data set of 2,257 Australian Holstein bulls. Thus, we reached apparently higher correlations between DGV and DRPF although our data set was smaller and r_{EBV} was lower. This might be due to overestimation of accuracy of DGV, which will be discussed later.

Using 54K genotype information (Figure 3), $r_{DGV,DRPF}$ were in a range between 0.74 ± 0.02 (days to first heat) and 0.63 ± 0.05 (general temperament) and thus show a difference from results from cross-validation with 777K genotype information of <0.01 for all traits. This finding is in line with results from Su et al. (2012) with Nordic Holstein and Red Dairy cattle. The authors found only slight differences in reliability for DGV between 54K genotype data and 777K genotype data of up to 0.03 for the traits protein, fertility, and udder health. Our results were also in line with results from Erbe et al. (2012), who found correlations between genomic breeding value and daughter trait deviation from 54K data being 0.01 to 0.03 less than those obtained with 777K data in Australian Holstein cattle. This indicates that marker density of the 54K SNP chip appears sufficient and using the more expensive 777K SNP chip provides little improvement in predictive ability. This finding is further supported by, for example, VanRaden et al. (2013), who found that the high-density SNP

chip provides only slightly better results. Those authors estimated a reliability of 61.1% when using the high-density SNP chip, and a reliability of 60.7% when using a 54K SNP chip, both as an average of 28 different traits.

Accuracy of DGV

The frequently used approach for calculating the accuracy of DGV as $r_{DGV,DRPF}/r_{EBV}$ would have resulted in accuracies >1.0 for most of the traits in our data set, which was characterized by high values of $r_{DGV,DRPF}$ and low but highly variable values for r_{EBV} (Table 2). This is a clear case of overestimation of accuracy, as described by Amer and Banos (2010). We thus applied the approach of Wellmann et al. (2013) for estimation of r_{DGV} . Values of r_{DGV} for the traits observed are shown in Table 2. The results indicate that it is generally possible to implement a system of genomic breeding value estimation for functional traits based on genotypes of phenotyped cows and sires with phenotyped daughters. Unfortunately, the limited number of individuals in our study was insufficient to achieve a large advantage of genomic prediction over parent average (r_{PA}). Further work is needed to evaluate how many individuals have to be genotyped and phenotyped to achieve higher accuracy of GBV.

Our results indicate that r_{DGV} and r_{PA} are functions of heritability and size of the training set for the respective trait. Milking speed had the highest heritability and the largest training set; the highest r_{DGV} and r_{PA} were obtained for this trait (Table 2). In contrast, days

Table 2. Correlation between direct genomic value and deregressed proof ($r_{DGV,DRPF}$), heritability (h^2), size of the training set (n EBV), accuracy of conventional breeding values from parent average (r_{PA}), accuracy of genomic breeding values following the common approach ($r_{DGV,DRPF}/r_{EBV}$), and accuracy of direct genomic values (r_{DGV}) from application of the formula by Wellmann et al. (2013) for the traits observed

Trait	$r_{DGV,DRPF}$	h^2	n EBV	r_{PA}	$r_{DGV,DRPF}/r_{EBV}$	r_{DGV}
General temperament	0.63	0.38	2,312	0.45	0.95	0.37
Milking temperament	0.73	0.04	2,259	0.21	>1	0.20
Aggressiveness	0.69	0.12	2,309	0.24	>1	0.19
Rank order in herd	0.65	0.16	2,304	0.35	>1	0.27
Milking speed	0.69	0.42	4,540	0.48	0.96	0.48
Udder depth	0.71	0.42	2,195	0.44	>1	0.45
Position of labia	0.66	0.28	2,232	0.41	>1	0.36
Days to first heat	0.74	0.02	1,678	0.13	>1	0.12

to first heat had the smallest r_{DGV} , the smallest r_{PA} , the smallest training set, and also the smallest heritability (Table 2). In general, computing the observed accuracy of PA can be very helpful; however, most cross-validation studies do not test the parent average predictions directly, whereas validations using time truncation do.

As can be seen in Figure 4, $r_{DGV,DRPF}$ varied strongly within trait, whereas mean r_{EBV} in the validation sets was trait-specifically quite consistent (from $r_{EBV} \approx 0.20$ for days to first heat to $r_{EBV} \approx 0.70$ for milking speed) across the samples. Fitting Eq. [2] to these data resulted in $a_0 = 0.75 \pm 0.01$, $a_1 = -0.76 \pm 0.46$, and $a_2 = 0.64 \pm 0.46$. Despite the large standard errors of the regression coefficients, our results were in line with the general findings of Wellmann et al. (2013) in another species (pigs); namely, that a_2 is positive and a_1 is negative. This latter result indicates that the accuracy of genomic breeding values, estimated as $r_{DGV,DRPF}/r_{EBV}$, is more biased upward, the smaller the accuracy of conventional breeding values r_{EBV} is for a given trait.

By shifting the estimates of accuracy from >0.9 to 0.12 (for days to first heat) to 0.48 (for milking speed), the applied correction provides more realistic estimates of the realized accuracy (Table 2).

As mentioned above, standard errors of the estimates for a_0 , a_1 , and a_2 were high. One way to prevent these high standard errors might be to split the data set in a nonrandom way into training and validation sets; for example, cross validation by age. This might be an option for larger data sets (e.g., Erbe et al., 2011) but in our limited data set, only one replicate would be possible. Estimates from a regression based on these values will also have large standard errors. This strategy of splitting the data thus has no advantages in our data. We further tried to reduce the standard error by including random effects for trait and cross validation fold in Eq. [2] to estimate a_0 , a_1 , and a_2 . This led to the same values for a_0 , a_1 , and a_2 and additionally to a slight increase of the standard error of a_0 as well as a slight decreases of the standard errors for a_1 and a_2 .

Figure 3. Correlations between direct genomic values (DGV) and deregressed proofs (DRPF) from cross-validation for general temperament (GT), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), milking speed (MS), udder depth (UD), position of labia (PL), and days to first heat (DH), based on 54K (~54,000) SNP information.

Figure 4. Linear regression of the correlation between direct genomic value and deregressed proofs ($r_{DGV,DRPF}$) on the accuracy of EBV in the validation set (r_{EBV}), simultaneously for all 8 traits: days to first heat (DH), milking temperament (MT), aggressiveness (AG), rank order in herd (RO), position of labia (PL), udder depth (UD), general temperament (GT), and milking speed (MS).

($a_0 = 0.75 \pm 0.03$; $a_1 = -0.76 \pm 0.38$, and $a_2 = 0.64 \pm 0.38$). Thus, the advantage of this second option seems to be limited.

Results for SNP Effects

Genomic regions with clusters of SNP with large effects (>4 SD above the mean) on the traits observed are listed in Table 3. When annotating these regions, we found 2 candidate genes that might influence the

functional traits observed. The *TAC1* (tachykinin, precursor 1) gene is located within a peak on chromosome 4 for general temperament. This gene encodes a member of the tachykinin peptide hormone family, which play a role in behavior response (Chiwakata et al., 1991) and might thus also affect the general temperament of an animal. For the trait days to first heat, we found a peak in the center of chromosome 21. Four genes are located here, one of which is *CYP11A1*, which encodes the cholesterol side-chain cleavage enzyme.

Table 3. Localization of regions with possible high influence on the observed traits, denomination of SNP with the greatest effect within that region (SNP ID), and genes up to 100 kb upstream and downstream of the SNP with the greatest effect within that region

Trait	SNP ID	BTA	Position (bp)	Gene 100 kb upstream or downstream ¹
General temperament	BovineHD0400004398	4	14,887,518	TAC1
	BovineHD0800030109	8	101,460,591	AKAP2, TXN, TXNDC8
Milking temperament	BovineHD1400015525	14	55,580,399	
	BovineHD0800017506	8	58,503,427	
Aggressiveness	BovineHD2000018921	20	65,702,103	ADCY2
	BovineHD0100035735	1	126,516,898	SLC9A9
Rank order in herd	BovineHD0600001952	6	8,448,579	
	BovineHD1800009080	18	29,383,349	
Milking speed	BovineHD1900003727	19	14,062,783	ACACA, TADA2A, DUSP14, SYNRG
	BovineHD0500031516	5	109,364,642	CACNA1C, IL17RA, CECR5, CECR1
Udder depth	BovineHD0900004541	9	16,815,501	
	BovineHD1100024011	11	83,456,178	
Position of labia	BovineHD1200003011	12	10,668,524	OLFM4
	BovineHD1400018332	14	65,573,091	YWHAZ
Days to first heat	BovineHD0300020780	3	70,574,840	TNNI3K
	ARSBFGLNGS13749	6	67,877,316	ATP10D, CORIN
Position of labia	BovineHD0800030481	8	102,549,325	ZNF483
	BovineHD2500002490	25	9,358,007	ATF7IP2, EMP2, TEK5
Days to first heat	BovineHD0400024614	4	88,917,242	WASL, SPAM1
	BovineHD1500021240	15	73,554,550	
	BovineHD4100015166	21	34,675,608	UBL7, SEMA7A, CYP11A1 , CCDC33

¹Genes in bold face could affect the observed trait.

This enzyme catalyzes the composition of pregnenolone from cholesterol, a steroid hormone involved in fertility (Heo et al., 2012). The *CYP11A1* gene might influence the level of estrogen and thus the time span between parturition and occurrence of first heat.

CONCLUSIONS

As stated above, limited work has evaluated genome-wide association of functional traits in Brown Swiss, especially for behavior traits or the new conformation trait position of labia. We found no biologically plausible candidate gene that might influence milking temperament, although we observed a distinct peak on chromosome 14. Larger studies and more collaboration between geneticists and molecular biologists are necessary to identify genes affecting the functional traits studied here.

ACKNOWLEDGMENTS

The authors gratefully acknowledge co-funding from the European Commission, under the Seventh Framework Program for Research and Technological Development, for the Collaborative Project LowInputBreeds (Grant agreement No 222623). However, the views expressed by the authors do not necessarily reflect the views of the European Commission, nor do they in any way anticipate the Commission's future policy in this area. Parts of this research were also funded by the German Federal Ministry of Education and Research (BMBF) within the AgroClustEr "Synbreed – Synergistic plant and animal breeding" (FKZ 0315528C). The authors thank Braunvieh Schweiz (Zug, Switzerland), the genotype pool Germany-Austria, Associazione Nazionale Allevatori Bovini della Razza Bruna (Bus-solengo, Italy), and Beltsville Agricultural Research Center (Beltsville, MD) for provision of genotypes.

REFERENCES

- Amer, P. R., and G. Banos. 2010. Implications of avoiding overlap between training and testing data sets when evaluating genomic predictions of genetic merit. *J. Dairy Sci.* 93:3320–3330.
- Chiwakata, C., B. Brackmann, N. Hunt, M. Davidoff, W. Schulze, and R. Ivell. 1991. Tachykinin (substance-P) gene expression in Leydig cells of the human and mouse testis. *Endocrinology* 128:2441–2448.
- Edel, C., R. Emmerling, and K.-U. Götz. 2010. A modification of VanRaden's index for the blending of genomic breeding values. P0324 in Proc. 9th World Congr. Genet. Appl. Livest. Prod., Leipzig, Germany. German Society for Animal Science, Neustadt, Germany.
- Edel, C., R. Emmerling, S. Neuner, and K. U. Götz. 2011. Genomische Selektion: Stand der Implementierung beim Fleckvieh. *Zuchtungs-kunde* 83:257–265.
- Erbe, M., B. J. Hayes, L. K. Matukumalli, S. Goswami, P. J. Bowman, C. M. Reich, B. A. Mason, and M. E. Goddard. 2012. Improving accuracy of genomic predictions within and between dairy cattle breeds with imputed high-density single nucleotide polymorphism panels. *J. Dairy Sci.* 95:4114–4129.
- Erbe, M., F. Seefried, and H. Simianer. 2011. Effect of relationship and age structure between training and validation set on the accuracy of genomic breeding value prediction using genomic BLUP. *Adv. Anim. Breed. Genet.* 19:287–290.
- Garrick, D. J., J. F. Taylor, and R. L. Fernando. 2009. Deregressing estimated breeding values and weighting information for genomic regression analyses. *Genet. Sel. Evol.* 41:55.
- Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml User Guide. Release 3.0. VSN International Ltd., Hemel Hempstead, UK.
- Gredler, B., M. Sargolzaei, B. Bapst, A. Bieber, H. Simianer, and F. R. Seefried. 2013. Accuracy of 54K to HD genotype imputation in Brown Swiss cattle. P0534 in Proc. Plant and Animal Genome XXI, San Diego, CA. Scherago International, Jersey City, NJ.
- Guo, J., H. Jorjani, and Ö. Carlborg. 2012. A genome-wide association study using international breeding-evaluation data identifies major loci affecting production traits and stature in the Brown Swiss cattle breed. *BMC Genet.* 13:82. <http://dx.doi.org/10.1186/1471-2156-13-82>.
- Heo, G. Y., W. L. Liao, I. V. Turko, and I. A. Pikuleva. 2012. Features of the retinal environment which affect the activities and product profile of cholesterol-metabolizing cytochromes P450 CYP27A1 and CYP11A1. *Arch. Biochem. Biophys.* 518:119–126.
- Kramer, M., M. Erbe, B. Bapst, A. Bieber, and H. Simianer. 2013. Estimation of genetic parameters for novel functional traits in Brown Swiss cattle. *J. Dairy Sci.* 96:5954–5964.
- Ober, U., F. Ayroles, E. A. Stone, S. Richards, D. Zhu, R. A. Gibbs, C. Stricker, D. Gianola, M. Schlather, T. F. C. Mackey, and H. Simianer. 2012. Using whole-genome sequence data to predict quantitative trait phenotypes in *Drosophila melanogaster*. *PLoS Genet.* 8:e1002685. <http://dx.doi.org/10.1371/journal.pgen.1002685>.
- Saatchi, M., M. C. McClure, S. D. McKay, M. M. Rolf, J. W. Kim, J. E. Decker, T. M. Taxis, R. H. Chapple, H. R. Ramey, S. L. Northcutt, S. Bauck, B. Woodward, J. C. M. Dekkers, R. L. Fernando, R. D. Schnabel, D. J. Garrick, and J. F. Taylor. 2011. Accuracies of genomic breeding values in American Angus beef cattle using K-means clustering for cross-validation. *Genet. Sel. Evol.* 43:40.
- Sargolzaei, M., J. P. Chesnais, and F. S. Schenkel. 2011. FImpute—An efficient imputation algorithm for dairy cattle populations. *J. Dairy Sci.* 94(E-Suppl. 1):421. (Abstr.)
- Segelke, D., J. Chen, Z. Liu, F. Reinhardt, G. Thaller, and R. Reents. 2012. Reliability of genomic prediction for German Holsteins using imputed genotypes from low-density chips. *J. Dairy Sci.* 95:5403–5411.
- Su, G., R. F. Brøndum, P. Ma, B. Guldbrandtsen, G. P. Aamand, and M. S. Lund. 2012. Comparison of genomic predictions using medium-density (~54,000) and high-density (~777,000) single nucleotide polymorphism marker panels in Nordic Holstein and Red Dairy cattle populations. *J. Dairy Sci.* 95:4657–4665.
- VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. *J. Dairy Sci.* 91:4414–4423.
- VanRaden, P. M., D. J. Null, M. Sargolzaei, G. R. Wiggans, M. E. Tooker, J. B. Cole, T. S. Sonstegard, E. E. Connor, M. Winters, J. B. C. H. M. van Kaam, A. Valentini, B. J. Doormaal, M. A. Faust, and G. A. Doak. 2013. Genomic imputation and evaluation using high-density Holstein genotypes. *J. Dairy Sci.* 96:668–678.
- Wellmann, R., S. Preuß, E. Tholen, J. Heinkel, K. Wimmers, and J. Bennewitz. 2013. Genomic selection using low density marker panels with application to sire line pig breeding. *Genet. Sel. Evol.* 45:28.