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On the genomic regions associated with milk lactose in Fleckvieh cattle

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ABSTRACT

Lactose is a sugar uniquely found in mammals' milk and it is the major milk solid in bovines. Lactose yield (LY, kg/d) is responsible for milk volume, whereas lactose percentage (LP) is thought to be more related to epithelial integrity and thus to udder health. There is a paucity of studies that have investigated lactose at the genomic level in dairy cows. This paper aimed to improve our knowledge on LP and LY, providing new insights into the significant genomic regions affecting these traits. A genome-wide association study for LP and LY was carried out in Fleckvieh cattle by using bulls' deregressed estimated breeding values of first lactation as pseudo-phenotypes. Heritabilities of first-lactation test-day LP and LY estimated using linear animal models were 0.38 and 0.25, respectively. A total of 2,854 bulls genotyped with a 54K SNP chip were available for the genome-wide association study; a linear mixed model approach was adopted for the analysis. The significant SNP of LP were scattered across the whole genome, with signals on chromosomes 1, 2, 3, 7, 12, 16, 18, 19, 20, 28, and 29; the top 4 significant SNP explained 4.90% of the LP genetic variance. The signals were mostly in regions or genes with involvement in molecular intra- or extracellular transport; for example, *CDH5*, *RASGEF1C*, *ABCA6*, and *SLC35F3*. A significant region within chromosome 20 was previously shown to affect mastitis or somatic cell score in cattle. As regards LY, the significant SNP were concentrated in fewer regions (chromosomes 6 and 14), related to mastitis/somatic cell score, immune response, and transport mechanisms. The 5 most significant SNP for LY explained 8.45% of genetic variance and more than one-quarter of this value has to be attributed to the variant within *ADGRB1*. Significant peaks in target regions remained even after adjustment for the 2 most significant variants previously detected on BTA6 and BTA14. The present study is a prelude for deeper investigations into the biological role of lactose for milk secretion and volume determination, stressing the connection with genes regulating intra- or extracellular trafficking and immune and inflammatory responses in dairy cows. Also, these results improve the knowledge on the relationship between lactose and udder health; they support the idea that LP and its derived traits are potential candidates as indicators of udder health in breeding programs aimed to enhance cows' resistance to mastitis.

Key words: lactose, genome-wide association study (GWAS), inflammatory response, molecular transport, bovine milk

INTRODUCTION

Lactose is the natural sugar present in milk of mammals and its concentration is predicted using mid-infrared spectroscopy in individual and bulk milk samples during routine recording schemes. Lactose percentage (**LP**) is associated with udder health in cattle (Ebrahimie et al., 2018; Costa et al., 2019a); in fact, as soon as IMI and inflammation occur, milk LP decreases. Overall, moderate correlations between LP and SCS, the most adopted indicator of IMI worldwide, have been reported in the literature, with peaks of −0.44 (Stoop et al., 2007) and −0.66 (Vilas Boas et al., 2017) for genetic and phenotypic correlations, respectively. Moreover, Costa et al. (2019a) estimated a negative genetic correlation (−0.18) between LP and mastitis in Fleckvieh (**FV**) cows in early to mid lactation. In the presence of IMI, the permeability of epithelial cells changes and lactose is partly lost in the bloodstream (Bansal et al., 2005); this explains why plasma LP is an indicator of epithelial integrity in cattle (Herve et al., 2019). As regards lactose yield (**LY**), this trait is closely related to milk volume, because it is the major milk osmole; in

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fact, the amount of synthesized lactose directly determines water uptake in the alveoli (Fox et al., 2015). This means that the higher the LY upstream, the higher the milk yield. The ability to synthesize a high amount of lactose might be related to greater expression of genes encoding glucose transporters, which are responsible for glucose uptake from blood (Zhao, 2014), and also to greater blood sugar availability. The mammary gland is the first target tissue for the delivery of substrates as glucose; this supports the fact that the metabolic priority of dairy cows is milk synthesis and that the udder is subjected to homeorhesis (Bauman and Currie, 1980). Moreover, the Golgi is the cellular environment specialized for lactose synthesis, and greater genetic expression of its enzymes in mammary tissue could further affect LP, LY, or both. Despite this, little is known about the physiological and biological paths behind conversion of glucose to lactose in the mammary gland and the association of LP with mastitis or IMI. In fact, interest in this milk component has only recently increased in the scientific community (Sneddon et al., 2015, 2016; Haile-Mariam and Pryce, 2017; Costa et al., 2018, 2019b). Milk yield, and fat and protein content and yield are usually included in the most common routine genetic or genomic evaluation schemes in Europe and are thus the target phenotypes of genome-wide association studies (**GWAS**) in dairy cattle (Wang and Bovenhuis, 2018). Conversely, only a few GWAS for LP and LY have been performed in cattle (Lopdell et al., 2017; Wang and Bovenhuis, 2018). Thus, an opportunity exists to investigate genomic regions that affect LP and LY to better understand which genes influence these traits.

The FV breed has a share of about 75% of Austrian controlled dairy cows (ZAR, 2018), and a joint genetic evaluation is carried out for FV by Austria, Germany, and the Czech Republic. This cooperative system allows generation of both big data and accurate genomic EBV, whose routine estimation was recognized by the International Committee for Animal Recording in 2011. Although LP and LY have recently been reported to be genetically associated with mastitis and ketosis in FV (Costa et al., 2019a), information on genomic regions affecting LP and LY is lacking for this breed. Therefore, in this study, we aimed to perform a GWAS to better understand the genetic background of LP and LY, and to evaluate accordance with genomic regions affecting udder health traits, such as mastitis, IMI, and SCS.

MATERIALS AND METHODS

Genotypes

Genomic data of 7,003 purebred FV bulls were jointly provided by Austria, Germany, and the Czech Republic. All individuals were genotyped with the 54K Illumina BovineSNP50 BeadChip (Illumina Inc., San Diego, CA).

Pseudo-Phenotypes

The bulls' deregressed EBV of first lactation were used as pseudo-phenotypes in the GWAS. First, variance components and heritability of LP and LY (Table 1) were estimated using the VCE6 software, version 6.0 (Neumaier and Groeneveld, 1998). For this purpose, 198,038 test-day milk yield and infrared-predicted LP records of 54,878 cows representing a subset of the entire data (>16 million milk test-day records and >1 million cows) were used; LY was calculated as (LP/100 × milk yield). The single-trait test-day repeatability animal model included the fixed effects of region-year-month of sampling, herd-year-season of sampling, age at calving, and DIM (linear and quadratic covariate), and the random effects of permanent environment, additive genetic animal, and residual. Bulls' EBV for first lactation were then estimated using the MiX99 software (Lidauer et al., 2015) by applying the above-mentioned model on all test-day records with reliable information on LP and LY (n = 7,065,937; Table 1). The pedigree included 1,824,262 animals; that is, cows with phenotypic observations and all available generations of ancestors. A deregression was carried out based on the approach proposed by Jairath et al. (1998) and Schaeffer (2001), implemented in the software MiX99 (Lidauer et al., 2015). Finally, only bulls with an estimated daughter contribution ≥10 were considered for further investigation, which led to 3,566 and 3,558 bulls for LP and LY, respectively.

Table 1. Descriptive statistics (7,065,937 test-day records) and heritability[1] of lactose percentage and lactose yield in first-parity cows

Trait	Mean	CV (%)	Minimum	Maximum	Heritability (SE)
Lactose percentage (%)	4.86	3.29	4.00	5.49	0.38 (0.01)
Lactose yield (kg/d)	0.98	28.57	0.12	3.74	0.25 (0.01)

[1]Estimated using a subset of 198,038 test-day records.

Quality Control

The quality control was performed with the software PLINK 1.9 (Purcell et al., 2007; Chang et al., 2015). The quality of SNP was ensured by removing variants with call rate <0.90, minor allele frequency <1%, and deviating significantly (P-value <1.00E-6) from Hardy-Weinberg equilibrium. A call rate ≥0.90 was required for bulls to be included. After editing, 40,486 SNP and 2,854 bulls for LP, and 40,094 SNP and 2,853 bulls for LY were available for the GWAS.

Association Study

The linear mixed model approach implemented in GEMMA software (Zhou and Stephens, 2012) for the GWAS was

$$\mathbf{y} = \mu + \mathbf{X}\beta + \mathbf{u} + \boldsymbol{\varepsilon},$$

where $\mathbf{y}$ is the vector of phenotypes (deregressed EBV of LP or LY); μ is the intercept; $\mathbf{X}$ is a vector of marker genotypes; β is the effect size of the markers; $\mathbf{u}$ is a vector of random individual effects; and $\boldsymbol{\varepsilon}$ is a vector of errors. Log-likelihood ratio statistics were adopted to test the null hypothesis that a polymorphism did not affect the phenotype; that is, H_0: $\beta = 0$ was tested against H_1: $\beta \neq 0$ for each SNP. In addition, the genomic relationship matrix was included in the analysis to account for population structure and avoid the presence of systematic bias. Because the Bonferroni correction is usually too restrictive and does not account for the fact that several SNP may trace the same QTL because of linkage disequilibrium (Goddard and Hayes, 2012), the false discovery rate with a cut-off at 0.20 (P-value <0.00013) was used as criterion to fulfill the P-value thresholds for significance discrimination (Efron, 2007). Graphical representations of Manhattan and quantile-quantile plots were obtained in the R software using the "CMplot" (Yin, 2016) and "qqman" packages (Turner, 2018), respectively. The proportion of genetic variance explained by a target SNP (**SNP-t**) and its variants in linkage disequilibrium was derived in the GCTA software (Yang et al., 2011). In particular, genetic variance was estimated using 2 different genomic relationship matrices: one for the SNP-t (with variants in linkage disequilibrium) and one for the remaining SNP. Then, the proportion of variance explained by the SNP-t was derived as the ratio between the 2 variances. Finally, significant genes were identified on the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/; NCBI, 2018) by mapping polymorphisms referring to the genome assembly ARS-UCD 1.2. "Nearby" genes were defined to be at most ±0.1 Mb distant from the significant SNP.

RESULTS AND DISCUSSION

Lactose Percentage

The significant variants detected in the GWAS for LP are listed in Table 2 and the quantile-quantile plot is shown in Figure 1. Within the genome, signals of LP were spread across several chromosomes (Figure 2), which confirms that LP is a polygenic trait controlled by multiple regions with cumulative effects (Goddard et al., 2016). The amount of genetic variance explained by the 4 most significant SNP was 4.90%, of which one-third was attributed to ARS-BFGL-NGS-39978 (P = 4.132E-08) that is close to (≤0.1 Mb) *NEMP2*, *NAB1*, *MFSD6*, and *LOC104971101* (BTA2). Table 3 provides an overview of genes detected and Table 4 summarizes their known major functions. Several polymorphisms were in mastitis and inflammation response-related regions; in particular, a variant (154.087 Mb, BTA1) was detected within the gene *PLCL2*, which includes leukocyte B proliferation and immune response regulation among its functions. Additionally, a significant SNP (15.526 Mb, BTA3) was detected near *EFNA1*, which encodes ephrin, a protein related to the inflammatory response in mammary cells and the integrity of mammary epithelial cells (Kang et al., 2018). The gene *ANKH* in BTA20 was close to 2 significant variants previously detected by Tiezzi et al. (2015) in a GWAS for mastitis in first-parity US Holsteins. Similarly, Meredith et al. (2013) found variants within the window 57.65 to 57.75 Mb of BTA20 that affected SCS in Holstein cows in Ireland. This was confirmed by Lopdell et al. (2017), who reported a significant signal for LP at 58.45 Mb (BTA20). A significant polymorphism in BTA1 was found within *PAK2*, regulator of signal transduction, and near *NRROS*, involved in immune response and oxidative activity. Significant signals, including that with the lowest P-value, were in the region from 5.75 to 6.70 Mb of BTA2, known to be related to transmembrane transport activity and including *MFSD6*, *NEMP2*, and *SLC40A1*. In particular, among the functions of *MFSD6*, it is worth mentioning the roles in reception of macrophages and facilitation of intra- or extracellular transport (NCBI, 2018). A SNP on BTA7 was within *RASGEF1C*, regulator of membrane-associated activity and vesicle trafficking. The *CDH5* gene, regulator of cell polarity, was detected on BTA18, whereas the membrane-associated protein *ABCA6* and the junction protein *GJC1* were within BTA19. Among all functions of the *ABCA* gene family, it is worth mentioning the

molecular transport within and among cells, as for *RASGEF1C* (BTA7) and *SLC35F3* (BTA28); moreover, *ABCA6* is related to leukocytes and antimicrobial activity (Wathes et al., 2019) and it has been found significant for mastitis in Holstein cows, together with its neighbors (*ABCA5*, *ABCA9*, and *ABCA10*; Tiezzi et al., 2015). The *KCNK1* gene was close to 2 signals on BTA28; it codes for proteins involved in potassium and sodium channel activities and for stabilization of membrane potential in epithelial cells. In addition, *KCNK1* is well expressed in mammary tissue and is related to mastitis in mice (Ogorevc et al., 2009). A couple of significant SNP on BTA20 were near *ANKH*, a mastitis-related gene in Holsteins (Tiezzi et al., 2015). Finally, a few variants were within genes without functions related to either IMI or transport mechanisms: *LOC112449084* and *LOC784305* (BTA12), *LOC101904639* (BTA16), and *LOC112442737* (BTA19). These signals were also detected for LP by Lopdell et al. (2017) in Holstein and Jersey cows in New Zealand. The region within BTA19 (33.51 to 61.13 Mb), known to affect LP (Lopdell et al., 2017; Wang and Bovenhuis, 2018), was here picked up by 3 significant SNP, 2 of them located at 61.017 and 61.441 Mb, which were also neighboring the SNP (58.45 Mb) detected by Lopdell et al. (2017). The effect of BTA28 region (at around 6.56 Mb) was confirmed in the present study by the presence of 3 significant variants (6.55 to 6.89 Mb). The SNP on BTA29 (9.32 Mb) was not far from the one (9.61 Mb) detected by Lopdell et al. (2017), and Wang and Bovenhuis (2018). Finally, no signals were detected at 8.70 Mb of BTA28 or 37.1 Mb of BTA6, both thought to significantly affect LP (Lopdell et al., 2017; Wang and Bovenhuis, 2018).

These findings make LP an interesting trait for genetic purposes in cattle and may be used to better understand causality between IMI and this milk component. In fact, the present GWAS helped increase the knowledge of the genetic architecture of such a complex trait, which relies on several factors such as blood glucose availability and uptake, blood–milk barrier (i.e., osmotic equilibrium), and the epithelial integrity of alveoli (i.e., udder health).

Lactose Yield

Findings related to GWAS for LY are shown in Table 5 and the quantile-quantile plot is reported in Figure 1. Visually, 2 major significant peaks were detected for LY (Figure 3); indeed, around 74% of significant variants were within BTA6 and BTA14, and the amount of genetic variance explained by the top 5 significant SNP was 8.45%, with the variant within *ADGRB1* accounting for 2.44% (Figure 4). The latter is a regulator of transmembrane signaling receptor activity, promotor of microbicidal activity of macrophages and associated with udder morphology in cows (Marete et al., 2018), stressing the strong biological dependence of LY on udder health and immune response. The same variant was the most significant in a GWAS for mastitis (Wang et al., 2015) and for milk yield and fat and protein percentage in Chinese Holsteins (Wang et al., 2019). Sahana et al. (2013) found the above-mentioned SNP

Table 2. Significant SNP for deregressed EBV of lactose percentage, their position on *Bos taurus* autosomes, and *P*-values

BTA	SNP	Position (Mb)	*P*-value
1	ARS-BFGL-NGS-5124	71.263427	9.293E-05
	Hapmap42521-BTA-35582	72.736590	4.123E-06
	ARS-BFGL-NGS-95240	154.087389	3.260E-05
2	ARS-BFGL-NGS-39978	5.757355	4.132E-08
	Hapmap49624-BTA-47893	6.700805	4.093E-05
3	ARS-BFGL-NGS-64215	15.525599	4.367E-05
7	ARS-BFGL-NGS-110962	1.009369	5.051E-07
12	BTA-123122-no-rs	69.319934	1.920E-07
	Hapmap50646-BTA-29027	70.140996	2.090E-05
	ARS-BFGL-NGS-57541	77.315938	9.680E-05
16	ARS-BFGL-NGS-74373	51.811400	9.342E-05
	BTA-26576-no-rs	67.703949	2.502E-05
18	ARS-BFGL-NGS-119782	34.126956	2.138E-06
19	ARS-BFGL-NGS-19774	44.547216	5.576E-05
	Hapmap25852-BTA-148919	61.016756	1.027E-05
	ARS-BFGL-NGS-55564	61.441042	1.371E-05
20	BTB-01648514	58.240835	2.366E-06
	BTB-01648552	58.264762	9.257E-05
28	BTB-00874839	6.547497	6.825E-05
	BTB-00874898	6.575192	4.084E-07
	ARS-BFGL-NGS-40170	6.888276	1.618E-05
29	Hapmap32898-BTA-66437	9.319793	4.730E-06

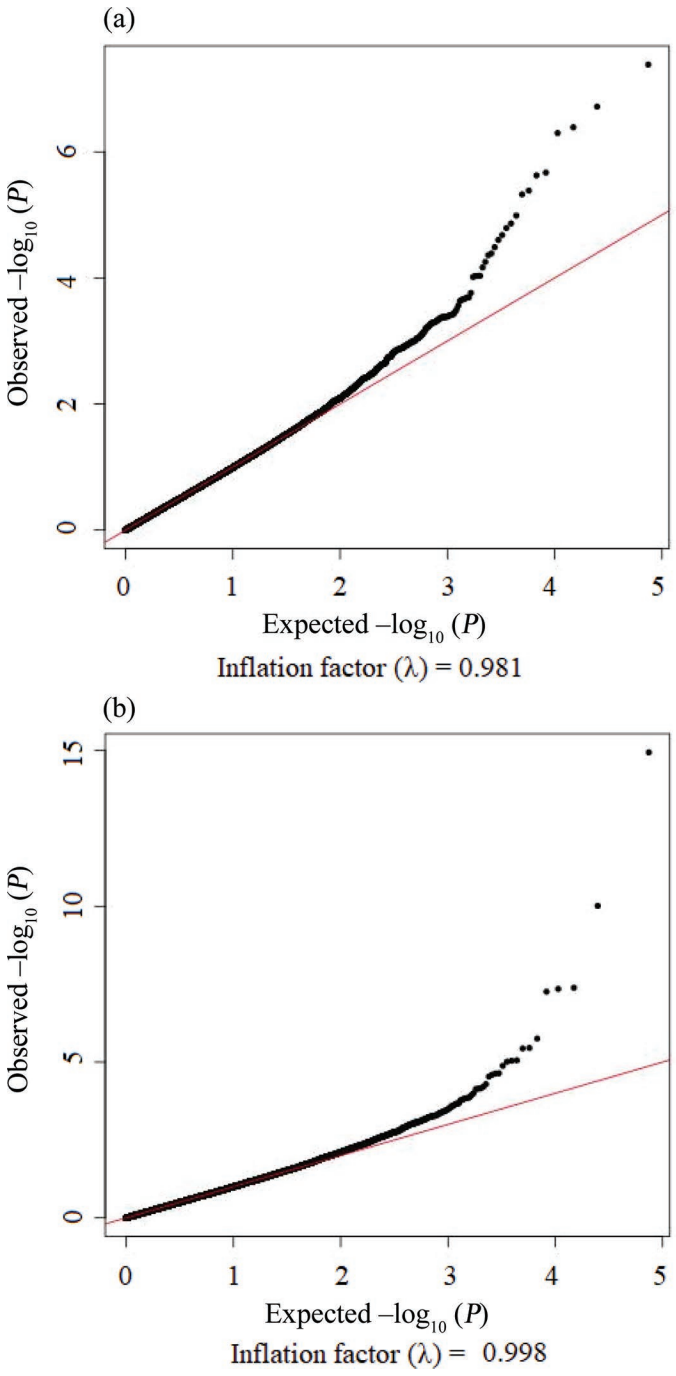

Figure 1. Quantile–quantile plot of the expected *P*-values under null hypothesis versus the observed *P*-values distribution for (a) lactose percentage, and (b) lactose yield.

to be significant for mastitis in second-parity cows. Interestingly, the *DGAT1* gene was found to influence LY in the GWAS of Lopdell et al. (2017), based on the UMD3.1.1 assembly. Because the current GWAS was based on the ARS-UCD1.2 genome assembly, *DGAT1* was not identified in this study; however, the presence of such a significant signal confirmed previous findings on the presence of significant QTL for LY around 1.77 Mb of BTA14 (tag variant rs134364612; Lopdell et al., 2017).

A list of all detected genes is provided in Table 3, with their main functions listed in Table 6. Few regions on BTA6 and BTA14 are known to affect SCS and mastitis (Ogorevc et al., 2009; Wang et al., 2015). Within BTA6, several signals covered a region from 84.57 to 88.82 Mb, coincident to the window already reported to affect mastitis or SCS in US Holsteins (Cole et al., 2011), Nordic Holsteins (Sahana et al., 2013), Danish Holsteins (Wu et al., 2015), and German Holsteins (Abdel-Shafy et al., 2018). Furthermore, a variant in position 88.82 Mb of BTA6 was very close to *CXCL8* (88.81 Mb), a gene related to mastitis, IMI, immune and inflammatory responses, and neutrophil activation (Youngerman et al., 2004; NCBI, 2018). Moreover, this significant region (87.11 to 88.82 Mb, BTA6) included *NPFFR2* (87.25 to 87.33 Mb), which is involved in macrophage activity stimulation, and it is related to udder health. In fact, several authors (Sahana et al., 2013; Wu et al., 2015; Cai et al., 2018) reported a relationship between mastitis and *NPFFR2* in bovines. Pausch et al. (2016) found this gene to affect both the morphology of mammary gland and mastitis resistance in FV cows. Within the above-mentioned window of BTA6, 3 other important genes were detected: *MGC152010*, associated with blood NEFA and metabolic status of cow (Ha et al., 2015), and *ANKRD17* and *LOC781441*, which are related to neutrophil count and activity and to glucuronosyltransferase activity, respectively. The gene *ANKRD17* was less than 0.1 Mb from *COX18*, located in an area significant for SCS in dairy cows (Chen et al., 2015). The significant gene *SLC4A4* (also known as *NBCe1*) is a sodium-cotransporter solute carrier, patented as a genetic marker for mastitis resistance (Yamaguchi and Ishikawa, 2008; Fang et al., 2017, 2018) and was flanking the region on BTA6 detected in this GWAS. Our results are supported by those of Fang et al. (2017), who reported a significant region for mastitis resistance at 88.84 and 88.72 Mb (BTA6) for Holstein and Nordic Red cows, respectively. The variant Hapmap25708-BTC-043671 (87.11 Mb) was less than 1 Mb from *DCK*, which is related to udder health (Wu et al., 2015; Cai et al., 2018), milk protein (Strucken et al., 2012), and milk casein (Dadousis et al., 2017).

On BTA14, a significant SNP (4.34 Mb) was close to *FAM135B*, involved in cellular lipid metabolic processes, and *COL22A1*, a gene that was significant for milk and protein yield and fat percentage in the study of Jiang et al. (2010) in Chinese Holstein cows. Furthermore, on the same chromosome, the sugar transport regulating gene *SLC45A4* and a regulator of ketone body metabo-

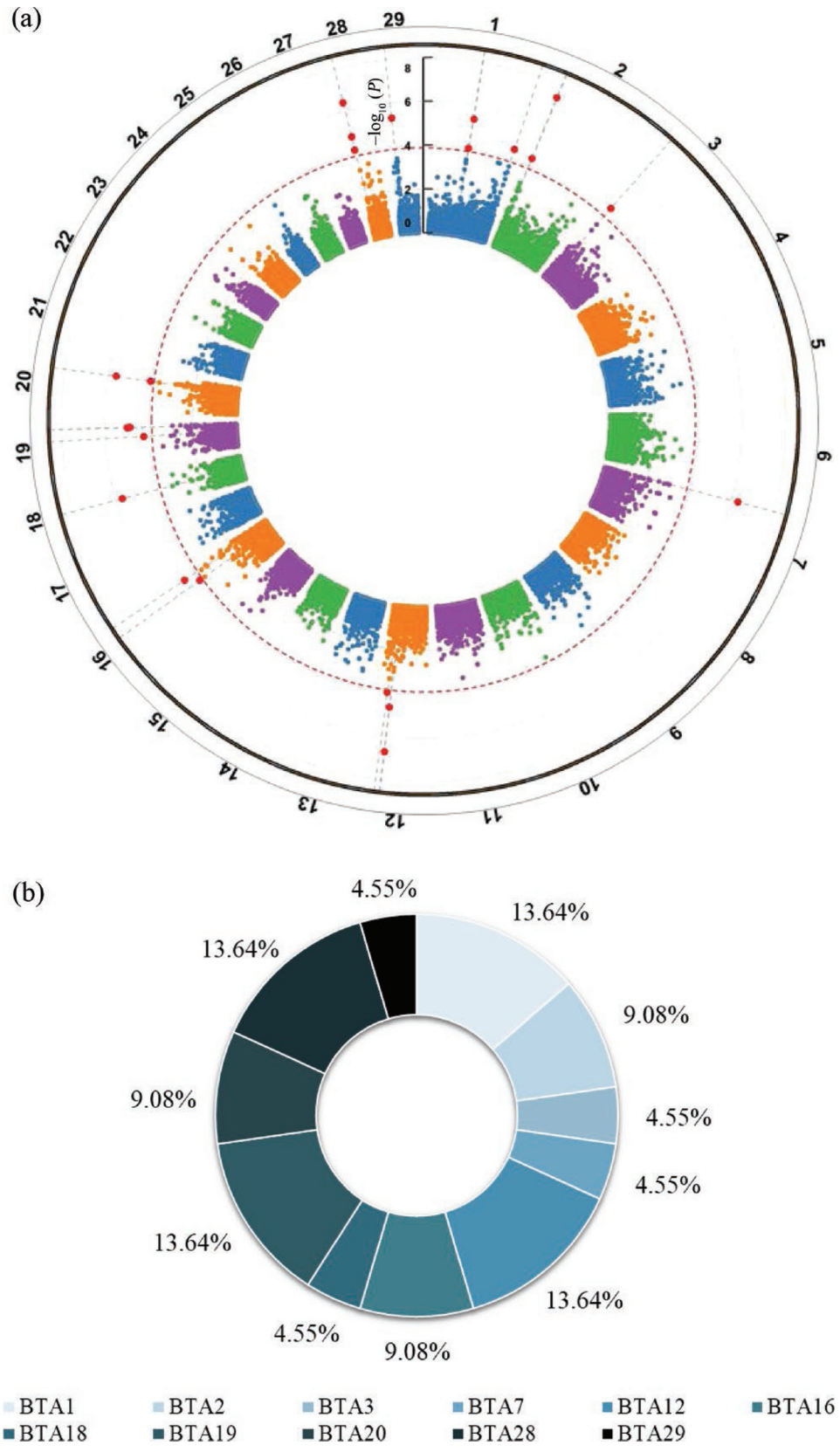

Figure 2. (a) Manhattan plot for lactose percentage, and (b) distribution of significant variants across BTA. The gray solid and red dashed lines in the Manhattan plots indicate BTA and false discovery rate threshold ($P < 0.00013$), respectively.

Table 3. Gene harboring the SNP ($Gene_H$) and nearby genes (left and right) for each significant SNP for lactose percentage (LP) and lactose yield (LY)

Trait	BTA	SNP	$Gene_H$	Nearby genes (±0.1 Mb from SNP)
LP	1	ARS-BFGL-NGS-5124	*PAK2*	*NRROS*, *CEP19*, *LOC112447342*, *PIGX*, *SENP5*, *LOC112447342*, *LOC104970886*
LP	1	ARS-BFGL-NGS-95240	*PLCL2*	*TBC1D5*
LP	1	Hapmap42521-BTA-35582	—	*LOC104970891*
LP	2	ARS-BFGL-NGS-39978	—	*NEMP2*, *NAB1*, *MFSD6*, *LOC104971101*
LP	2	Hapmap49624-BTA-47893	—	*ANKAR*, *OSGELP1*, *ASNSD1*, *SLC40A1*
LP	3	ARS-BFGL-NGS-64215	—	*EFNA1*, *EFNA3*, *EFNA4*, *ADAM15*, *DCST1*, *DCST2*, *ZBTB7B*, *LENEP*, *FLAD1*, *CKS1B*, *SHC1*, *LOC107132270*, *MIR92B*, *MUC1*, *TRIM46*, *KRTCAP2*, *DPM3*, *SLC50A1*
LY	6	ARS-BFGL-NGS-17376	—	*LOC112447100*, *LOC112447101*, *LOC107132573*, *CXCL8*, *LOC534181*, *LOC112447102*, *CXCL5*, *PPBP*
LY	6	ARS-BFGL-NGS-27958	*MGC152010*	*LOC781441*, *LOC540544*, *LOC781478*, *LOC530553*, *LOC530356*
LY	6	BTA-111700-no-rs	*LOC781441*	*LOC104968908*, *LOC100296421*, *LOC781649*, *LOC540544*, *LOC781478*, *MGC152010*
LY	6	BTA-77173-no-rs	*ANKRD17*	*COX18*
LY	6	Hapmap25708-BTC-043671	—	—
LY	6	Hapmap42671-BTA-77163	—	*LOC112447164*, *LOC112447182*
LY	6	Hapmap54336-rs29010419	—	*COX18*, *LOC112447164*, *ANKRD17*
LY	6	Hapmap57014-rs29019575	—	*ADAMTS3*, *TRNAC-GCA*
LP	7	ARS-BFGL-NGS-110962	*RASGEF1C*	*MAPK9*, *GFPT2*, *LOC107132588*, *LOC100848388*
LP	12	ARS-BFGL-NGS-57541	*LOC112449084*	*TMTC4*, *GGACT*, *MIR2892*
LP	12	BTA-123122-no-rs	—	—
LP	12	Hapmap50646-BTA-29027	*LOC784305*	—
LY	14	ARS-BFGL-NGS-112858	—	*KHDRBS3*
LY	14	ARS-BFGL-NGS-3122	—	*SLC45A4*, *DENND3*, *LOC112449594*, *LOC112449595*
LY	14	ARS-BFGL-NGS-34135	*LYNX1*	*LOC112441461*, *LOC112449566*, *LOC112449590*, *LOC100848939*, *LOC104973965*, *LY6D*, *LOC112449567*, *LYPD2*, *SLURP1*, *THEM6*, *PSCA*, *JRK*, *ARC*, *LOC101905222*
LY	14	ARS-BFGL-NGS-4939	*ADGRB1*	*PSCA*, *JRK*, *ARC*, *LOC101905222*
LY	14	ARS-BFGL-NGS-56327	—	*COL22A1*, *FAM135B*, *LOC112449648*
LY	14	BTA-35941-no-rs	—	*LOC101905853*, *LOC101901918*, *TRNAC-GCA*
LY	14	Hapmap23302-BTC-052123	—	—
LY	14	Hapmap23454-BTC-046932	—	—
LY	14	Hapmap26283-BTC-048098	—	—
LY	16	ARS-BFGL-NGS-15423	—	*LOC112441794*
LY	16	ARS-BFGL-NGS-2382	—	*DSTYK*, *CNTN2*, *TMEM81*, *RBBP5*, *TMCC2*, *LOC112441810*
LP	16	ARS-BFGL-NGS-74373	*LOC101904639*	*SPEN*, *LOC507787*, *LOC789035*, *FBLIM1*, *TMEM82*, *SLC25A34*, *LOC112441770*, *PLEKHM2*, *LOC515551*, *LOC112441934*
LP	16	BTA-26576-no-rs	—	*PTGS2*, *LOC107133257*, *LOC112441859*
LY	17	ARS-BFGL-NGS-14166	*ARHGAP10*	—
LY	17	BTB-00689316	—	*RSPH14*, *GNAZ*, *LOC531152*, *VPREB1*, *TOP3B*, *PPM1F*, *MAPK1*, *LOC112442124*
LY	18	ARS-BFGL-NGS-114779	*ZNF423*	*TRNAG-CCC*, *LOC112442280*
LP	18	ARS-BFGL-NGS-119782	*CDH5*	*BEAN1*
LP	19	ARS-BFGL-NGS-19774	*GJC1*	*DBF4B*, *HIGD1B*, *EFTUD2*, *MIR2343*, *CCDC103*, *FAM187A*, *GFAP*, *KIF18B*, *LOC104975097*
LP	19	ARS-BFGL-NGS-55564	*ABCA6*	*ABCA5*, *ABCA9*, *ABCA10*
LP	19	Hapmap25852-BTA-148919	*LOC112442737*	—
LP	20	BTB-01648514	—	*ANKH*, *LOC107131578*, *LOC112443072*
LP	20	BTB-01648552	—	*ANKH*, *LOC107131578*, *LOC112443072*
LY	21	Hapmap58004-rs29023371	—	*LOC112443345*
LP	28	ARS-BFGL-NGS-40170	*SLC35F3*	—
LP	28	BTB-00874839	—	*KCNK1*, *TRNAC-ACA*
LP	28	BTB-00874898	—	*KCNK1*, *TRNAC-ACA*
LP	29	Hapmap32898-BTA-66437	—	*ACTN2*, *HEATR1*, *LOC112444786*, *LOC101908221*

lism, *DENND3*, were both close to the variant ARS-BFGL-NGS-3122 (Sigdel et al., 2017). According to Ogorevc et al. (2009), several mastitis and SCS-related regions are spread across the bovine genome and many are concentrated on BTA14; in fact, *DENND3* was also significant in a GWAS of SCS in dairy cows (Chen et al., 2015). One significant variant was within *LYNX1* and near *LY6D*, *THEM6*, *PSCA*, *SLURP*, and *LYPD2*;

Table 4. Location and main functions of detected genes in genome-wide association study of lactose percentage (sources: Gene Cards, 2018, https://www.genecards.org/; NCBI, 2018)

BTA	Gene	Window (Mb)	Function
1	*PAK2*	71.257814–71.350400	Signal transduction; ATP binding
1	*PLCL2*	153.882096–154.095595	Leukocyte B proliferation; intracellular signal transduction; immune response regulation
7	*RASGEF1C*	1.004381–1.109795	Regulation of membrane-associated molecular activity; intracellular signaling pathways; cell differentiation and proliferation; cytoskeletal organization; vesicle trafficking; nuclear transport
12	*LOC112449084*	77.289796–77.346416	—
12	*LOC784305*	69.969856–70.238238	Multidrug resistance-associated protein
16	*LOC101904639*	51.805664–51.815138	—
18	*CDH5*	34.122721–34.161590	Calcium-dependent cell adhesion protein; regulation of cell polarity
19	*ABCA6*	61.413594–61.483297	Membrane-associated protein; regulation of extra-/intracellular transport of various molecules; macrophage lipid homeostasis; antimicrobial activity
19	*GJC1*	44.522640–44.555661	Junction protein; cell communication
19	*LOC112442737*	60.983169–61.075519	—
28	*SLC35F3*	6.720734–7.155385	Solute carrier; thiamine transport

all of these genes overlapped with those reported by Tiezzi et al. (2015) for clinical mastitis in dairy cows and are involved in regulation of neutrophil activity. These genes are known as globally as the lymphocyte-antigen-6 complex. This finding supported the idea that LY is strongly dependent on mammary epithelial cell number and functionality. It is worth noting that LY and mastitis are positively associated in FV cows (genetic correlation of 0.52; Costa et al., 2019a), suggesting that high-producing cows are more susceptible to IMI and udder health problems than low-producing cows. One variant was found within *ARHGAP10*, a gene related to mastitis resistance in cows (Kurz et al., 2019), whereas the transcription factor *ZNF423* was detected in BTA18. No coding genes were present in other significant regions of BTA16 and BTA17.

To adjust for the effects of the 2 highest significant signals for LY (*P*-value <1.00E-10); that is, ARS-BFGL-NGS-4939 (BTA14) and Hapmap25708-BTC-043671 (BTA6), and to check for the presence of effective causal variants, an additional GWAS was performed by fixing the 2 variants in the original model using GEMMA software (Zhou and Stephens, 2012). The significance of variants was checked following the

Table 5. Significant SNP[1] for deregressed EBV of lactose yield, their position on *Bos taurus* autosomes, and *P*-value

BTA	SNP	Position (Mb)	*P*-value
6	BTA-111700-no-rs	84.575241	1.763E-06
	ARS-BFGL-NGS-27958	84.689991	3.652E-06
	Hapmap25708-BTC-043671	87.113639	9.683E-11
	Hapmap57014-rs29019575	87.801255	2.923E-05
	Hapmap42671-BTA-77163	88.006286	5.119E-05
	Hapmap54336-rs29010419	88.132026	9.712E-06
	BTA-77173-no-rs	88.242415	1.319E-05
	ARS-BFGL-NGS-17376	88.822266	9.083E-06
14	ARS-BFGL-NGS-34135	1.675278	4.123E-08
	ARS-BFGL-NGS-4939	1.801116	1.152E-15
	BTA-35941-no-rs	2.276443	4.492E-08
	ARS-BFGL-NGS-3122	2.721633	6.219E-05
	ARS-BFGL-NGS-56327	4.336714	3.533E-06
	Hapmap23302-BTC-052123	4.848750	5.512E-08
	Hapmap23454-BTC-046932	5.831267	8.790E-06
	ARS-BFGL-NGS-112858	6.589274	2.578E-05
	Hapmap26283-BTC-048098	7.371252	7.145E-05
16	*ARS-BFGL-NGS-2382*	2.933483	6.967E-05
	ARS-BFGL-NGS-15423	74.158269	2.290E-05
17	ARS-BFGL-NGS-14166	10.283664	2.350E-05
	BTB-00689316	71.925055	7.369E-05
18	ARS-BFGL-NGS-114779	18.192027	9.987E-05
21	*Hapmap58004-rs29023371*	62.069305	1.146E-04

[1]SNP in italics were significant in the additional genome-wide association study adjusted for the 2 most significant (*P*-value <1.00E-10) variants: Hapmap25708-BTC-043671 and ARS-BFGL-NGS-4939.

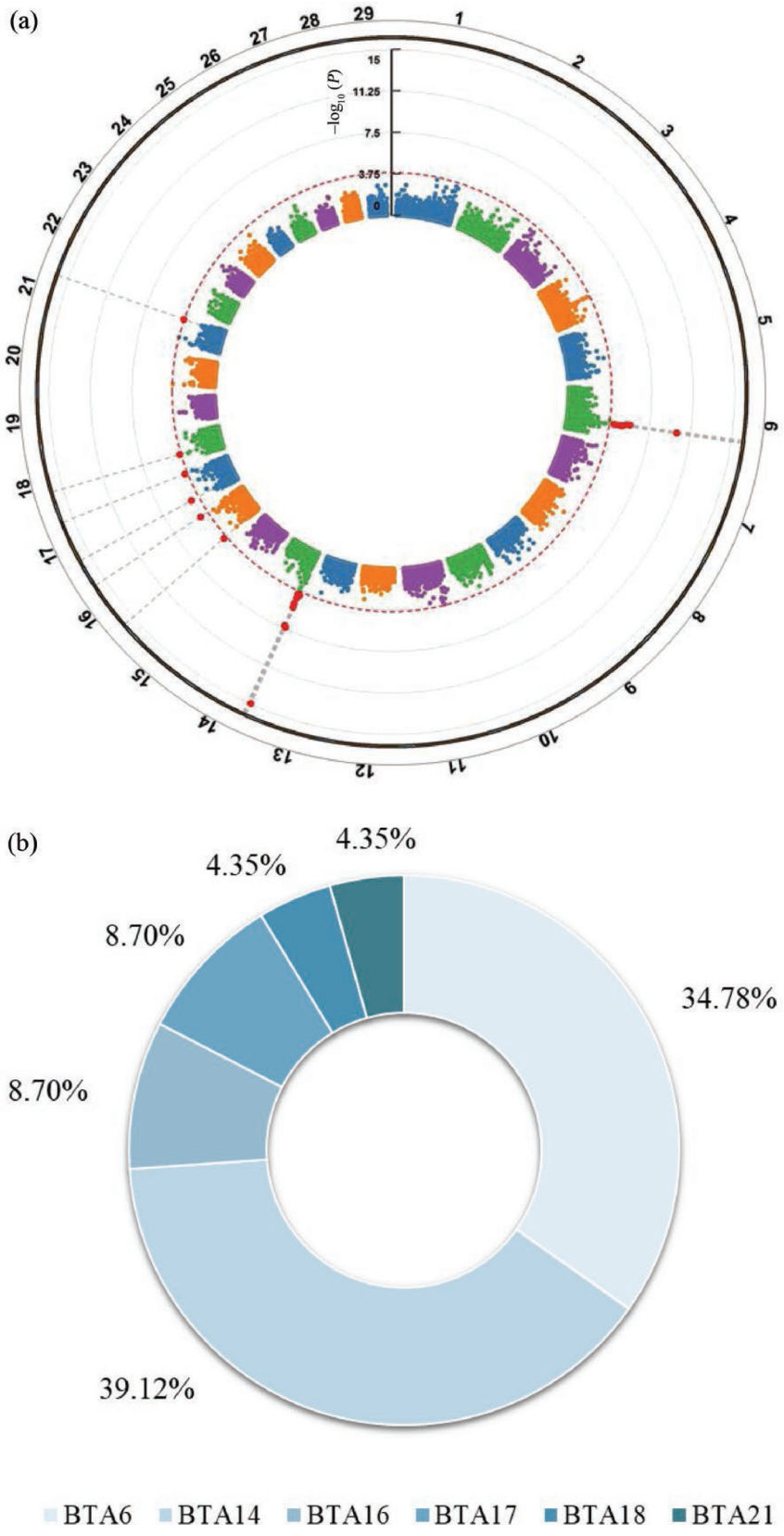

Figure 3. (a) Manhattan plot for lactose yield, and (b) distribution of significant variants across BTA. The gray solid and red dashed lines in the Manhattan plots indicate BTA and false discovery rate threshold ($P < 0.00013$), respectively.

Table 6. Location and main functions of detected genes in genome-wide association study of lactose yield (sources: Gene Cards, 2018, https://www.genecards.org/; NCBI, 2018)

BTA	Gene	Window (Mb)	Functions
6	*LOC781441*	84.563427–84.592360	Glucuronosyltransferase activity
6	*MGC152010*	84.674163–84.712898	Glucuronosyltransferase activity
6	*ANKRD17*	88.187894–88.355145	Ankyrin repeat-containing proteins; related to neutrophils count and activity
14	*LYNX1*	1.669740–1.675364	Acetylcholine receptor binding and regulation; ion channel inhibitor activity
14	*ADGRB1*	1.797345–1.874927	Adhesion G protein-coupled receptor B1, transmembrane signaling receptor activity
17	*ARHGAP10*	10.019895–10.404767	Activation of GTPases RhoA and Cdc42; PTKB2 (Rho GTPase activating protein 10) regulation of cytoskeletal organization via Rho family GTPases
18	*ZNF423*	17.963192–18.309039	Transcription factor, differentiation of white and brown adipocytes; regulation of transforming growth factor (TGF)-β receptor signaling

previously described criteria. Although the number of significant SNP decreased (Table 5), the significant regions detected mirrored those of previous GWAS, with the only exception of a new variant in a noncoding region of BTA20 (4.40 Mb), Hapmap54098-rs29010434. The smaller number of significant polymorphisms was expected, because the 2 strongest signals are thought to be in linkage disequilibrium with other neutral SNP and are also thought to affect the expression of other genes. However, these findings indicated the presence of multiple causal variants segregating in both BTA6 and BTA14 for LY.

CONCLUSIONS

In the present study, we report significant regions of the bovine genome affecting milk lactose in Fleckvieh cattle, the major dairy breed in Austria. The ultimate goal of this study was to detect causal variants and overlapping regions with mastitis and SCS, because lactose percentage and its derived traits have the potential to be used as indicator traits in breeding programs to improve cow udder health. Signals of lactose percentage are scattered across several BTA and connected to some previously identified regions related to intra- or extracellular transport mechanisms, mastitis, and immune response. Conversely, the significant SNP for lactose yield are mainly concentrated on BTA6 and BTA14, always within or close to genes with functions related to transport mechanisms, mastitis, IMI, and inflammatory response. These findings highlight that lactose percentage is affected by several regions of the genome, whereas lactose yield is influenced by fewer regions with larger effects. Lactose yield and percentage do not share common regions, likely because of the different nature of these traits; in fact, the amount of lactose produced is only moderately genetically correlated with the final percentage in milk. Even though lactose percentage and yield are influenced by different regions of the genome, both traits have strong connections with intra- or extracellular transport mechanisms and immune response of dairy cows. Finally, some regions with unknown functions and not previously detected by other GWAS show high significance in the present study.

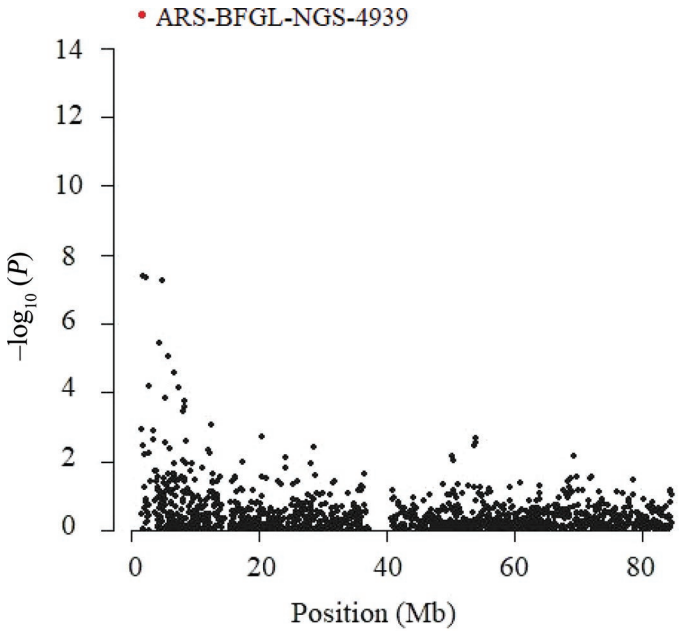

Figure 4. Manhattan plot focused on BTA14 of lactose yield with identification of the SNP (red dot) within *ADGRB1*.

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