Development of a SNP marker set for selection against left-sided displacement of the abomasum in German Holstein cattle

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Introduction

Left-sided displacement of the abomasum (LDA) is a common dairy cattle disease with a prevalence of about 2% in the German Holstein population (Hamann, H., Wolf, V., Scholz, H. *et al.* 2004). In the course of LDA the abomasum starts bloating and displaces from the abdominal floor to the abdominal wall. LDA has to be medically treated and often surgery is necessary to fix the abomasum in its correct position to the abdominal wall. However, even if the treatment succeeded without any incidents, a significantly reduced milk performance as well as conception problems increase the culling risk for cows (Hamann, H., Wolf, V., Scholz, H. *et al.* 2004; Geishauser, T., Reiche, D., Schemann, M. 1998). On the average, about one half of all cows affected by LDA were culled within the first year after surgery (Wolf, V., Hamann, H., Scholz, H. *et al.* 2001). LDA is a multifactorial disease, in which environmental effects play a role. However, the heritability of LDA reported in various studies ranged between 20% and 50% (Hamann, H., Wolf, V., Scholz, H. *et al.* 2004; Wolf, V., Hamann, H., Scholz, H. *et al.* 2001) and thus is much higher than in many other cattle diseases. Several QTL for LDA have already been identified in German Holstein cows (Mömke, S., Scholz, H., Doll, K. *et al.* 2008), among them a QTL proximal on bovine chromosome (BTA) 23. Since LDA is usually preceded by a decreased motility of the abomasum, impaired abomasal emptying, and malfunctions at the level of the intrinsic nervous system combined with impaired cholinergic muscle responses (Geishauser, T., Reiche, D., Schemann, M. 1998), the motilin (*MLN*) gene located within this QTL is an appropriate candidate gene and SNPs within this gene were developed and investigated. Furthermore, an analysis using the Illumina Bovine SNP50 Chip was performed. The objective of this study was to identify genetic markers for LDA. We were able to detect a total of five genome-wide significantly LDA-associated single nucleotide polymorphisms (SNPs) on BTA 1, 3, 6, 8, and 23.

Material and methods

Genome-wide association study using the bovine 50K Illumina Beadchip. We collected blood samples from more than 5,000 German Holstein cows. All cows were purebred German Holsteins. Of the sampled cows, 590 animals were chosen. Of these, 100 were affected by LDA and 490 were non-affected control cows. The animals within cases and controls as well as between cases and controls descended from different sires and were not

closely related with each other. Genotyping was performed using the Illumina Bovine SNP50 Chip (Illumina, San Diego, CA, USA).

Analysis of associated SNP markers using an extended data set. After the SNP chip analysis, markers which showed genome-wide significant association with LDA were evaluated in a larger material. This material included a total of 1,000 German Holstein cows with 388 of them affected by LDA. Genotyping was performed using a 7300 Realtime PCR system (Custom TaqMan® SNP Genotyping Assays, Applied Biosystems, Darmstadt, Germany) or restriction endonucleases. For investigation of putative restriction fragment length polymorphisms (RFLPs) the NEBcutter V2.0 (http://tools.neb.com/NEBcutter2/index.php) was used.

Candidate gene analysis. We investigated *MLN* as the most promising candidate gene for LDA within the QTL of BTA23. We were able to detect a genome-wide significantly LDA-associated SNP within the MLN gene. This SNP was analysed on the same material of 1,000 cows as mentioned above. An expression analysis was performed on abomasal tissue of 32 cows showing each genotype of this SNP. The quantitative reverse transcriptase (qRT)-PCR was carried out using an ABI7300 sequence detection system (Applied Biosystems). The MLN transcript specific expression was normalised by the bovine RPL4 expression level (ΔCT), and the relative expression level was calculated by the ΔΔCT method using the average ΔCT of the homozygous wildtype samples as calibrator (Livak *et al*. 2001). All assays were performed in duplicates.

Statistical analysis. For genome-wide mapping we performed an association analysis using the adaptive permutation procedure of PLINK version 1.07 (http://pngu.mgh.harvard.edu/ purcell/ plink/, Purcell *et al.,* 2007) where only SNPs with a minor allele frequency (MAF) >5% and a call rate >90% were included. Genome-wide significance was ascertained through adaptive permutations using 10,000,000 permutations per SNP.

Results and discussion

The five SNPs Hapmap43396-BTA-89742 (LDA1) on BTA1 (45.41 Mb), Hapmap49033-BTA-115567 (LDA2) on BTA3 (69.92 Mb), Hapmap40002-BTA-45500 (LDA3) on BTA6 (96.98 Mb), BTB-01163826 (LDA4) on BTA8 (71.34 Mb) and FN298674:g.1891insG (LDA5) on BTA23 (8.25 Mb) showed genome-wide significant associations with LDA (Table 1). These results are partly in concordance with the results of the previously performed linkage analysis for LDA (Mömke, S., Scholz, H., Doll, K. *et al.* 2008). The associated regions on BTA1 and BTA23 are located within QTL for LDA on these chromosomes. Furthermore, the family-dependent QTL on BTA6 is in good agreement with the findings in this association study.

Table 1. Marker names, official nomenclature, position and genome-wide error probabilities for the five genome-widely associated SNPs tested in a total of 1,000 German Holstein cows.

Marker name	Official nomenclature	BTA	Position (Mb)	p-value ($-\log_{10}$)
LDA1	Hapmap43396-BTA-89742	1	45.41	< 4.0
LDA2	Hapmap49033-BTA-115567	3	69.92	< 4.0
LDA3	Hapmap40002-BTA-45500	6	96.98	< 4.0
LDA4	BTB-01163826	8	71.34	< 4.0
LDA5	FN298674:g.1891insG	23	8.25	2.9

The SNP on BTA23 is located within the LDA-candidate gene *motilin (MLN)*. This SNP not only shows genome-wide significant association with LDA, but was also tested in an expression analysis. In heterozygote individuals, the expression level of *MLN* is decreased by 90%, in homozygote mutated individuals it is decreased by 93%. Therefore, this or a closely neighbored polymorphism seems to have an influence on the transcription rate of the motilin gene. The polymorphisms within *MLN* explain 4.2% of the phenotypic variance for LDA. In a study using the house musk screw as animal model for motilin studies, the contractile responses induced by motilin were dose-dependent (Tsutsui, C., Kajihara, K., Yanaka, T. *et al.* 2009). This study shows the relevance of *MLN* for the development of bovine LDA, a disease known to be initiated by a reduced peristalsis of the gastrointestinal tract (Geishauser, T., Reiche, D., Schemann, M. 1998).

An analysis of variance for the SNPs LDA1-5 and the sire effect showed an explained variance of 32 %. The LDA SNPs explained 12.9 % of the total phenotypic variance in this model. Further meaningful SNPs in linkage disequilibrium with LDA were located on BTA5, 18, 20, and 27. These genome-wide associated regions will be screened for positional candidate genes and polymorphisms in close linkage disequilibrium with LDA.

Conclusion

In conclusion, we performed a genome wide association study with following evaluation of five genome-wide significantly LDA-associated SNPs. One of these SNPs was identified within the *MLN* gene and was confirmed by expression analysis. Therefore, *MLN* seems to be one of the causal genes for LDA. Using the described set of five SNP markers it is now possible to explain 12.9 % of the phenotypic variance for LDA. This study enables the accomplishment of a first genetic test to decrease the risk of LDA in the German Holstein cattle breed.

References

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