A New Holstein Haplotype Affecting Calf Survival

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Abstract

In recent years, the availability of phenotypic records and genomic data for cattle and the application of genomic tools revealed haplotypes affecting fertility and prenatal death. This study reports the identification of a new haplotype associated with calf survival in the Holstein population. Several calves from specific mating initially showed unspecific symptoms like chronic diarrhea and insufficient development. Affected animals died within the first months of life despite of symptomatic treatment. A genome-wide case-control-study based on 54K SNP Chip genotypes determined a causal region at BTA 11. Subsequent homozygosity mapping identified a haplotype affecting calf mortality in the homozygous state. Blood chemical analysis of affected calves revealed pronounced hypocholesterolemia indicating a disorder of the fat metabolism. Heterozygous animals without clinical manifestations show decreased levels of blood cholesterol suggesting a codominant inheritance for this genetic defect. Pedigree analyses revealed a prominent Canadian Holstein bull; MAUGHLIN STORM, as a carrier for this disorder. The widespread use of this bull and of its sons in the breeding program lead to a strong increase of the haplotype frequency in the German Holstein population within the last years. The occurrence of an identical healthy haplotype and the presence of several gaps within the bovine genome complicate the identification of a concordant variant.

Key words: calf survival, haplotype, cholesterol

1. Introduction

For cattle farmers, there is a strong demand in raising healthy calves as a basis for a persistent and highly productive cow. A diseased calf and its potential loss is associated with high costs for the farmer, including the value of this animal, the costs for medical treatment and the raising costs. Moreover calf mortality is an important animal welfare issue. Calf survival is mainly influenced by management factors. But there is also a genetic background for the mortality of young cattle implying its inclusion in future breeding strategies (Olesen *et al*., 2000).

In recent years, a number of recessive haplotypes and a few causative mutations have been discovered in cattle (VanRaden *et al*., 2011; Adams *et al*., 2012; McClure *et al*., 2014), most of them affecting fertility or increased embryonic mortality. Causative mutations for calf diseases and for the postnatal mortality of calves are less reported (e.g. Jung *et al*., 2014; Pausch *et al*., 2015).
Breeding organizations in Germany reported about Holstein calves who suffer from chronic diarrhea. The affected young animals were underdeveloped, especially in weight, and showed secondary diseases like pneumonia and edemas. Affected calves were unresponsive to medical treatment and died at the age from 3 weeks to 6 months. Diagnostic tests for common infectious viral, bacterial or parasitic pathogens yielded negative results. Two calves originating from a split embryo, but born and raised in two different herds revealed the same phenotype.

2. Material & Methods

Three calves were examined clinically at the Clinic for Cattle in Hanover and extensive blood biochemical analysis was performed. Euthanized animals were submitted to a diagnostic laboratory for post mortem examination.

For genomic analyses, the DNA of affected calves was genotyped. Genomic data of unaffected, control animals were used from routine genetic evaluation of the German Holstein population. After imputation and quality control, genotypes of 45,163 SNPs of 23 case and 11,177 control animals, who survived the first year of life, were available for genome-wide association study (GWAS). GWAS was performed by using GCTA (Yang *et al*., 2011). Haplotypes of 166,300 animals were inferred using default parameters of Beagle (Browning & Browning 2009).

The analysis of calf survival is possible because of the reporting system in Germany, including birth and death of every calf. The survival rate of descendants of risk-matings compared to those of non-risk-matings is based on data of female calves only, including in 6.9 million entries.

3. Results

Phenotype description

Three calves between the age of one and five months with severe emaciation and chronic recurrent diarrhea were admitted to the Clinic for Cattle in Hanover. All animals were underdeveloped in weight. At the time of death these calves weighted between 36.5 and 59 kg. The blood biochemical analysis of affected calves revealed markedly decreased plasma cholesterol concentrations. All affected animals had cholesterol concentrations below 0.5 mmol/L whereas non-affected control animals had values above 2 mmol/L, except of one animal showing intermediate values between affected and non-affected animals. The most remarkable finding of the post mortem examination was a severe emaciation with nearly complete absence of fat reserves, even in bone marrow and spinal canal.

Disease-associated region

Based on genotypes of 45,613 SNPs used for routine genomic evaluation in German Holstein population (vit, 2015), a GWAS was performed. The study delivered a strong significant association on BTA 11 (Fig.1).

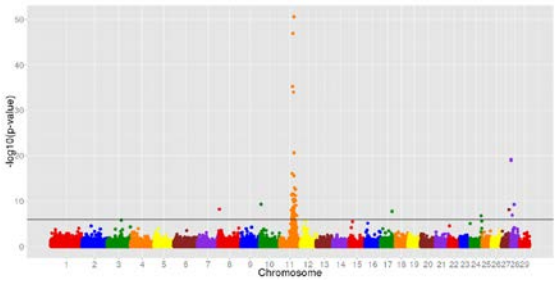

Figure 1 Association mapping of 45,613 SNPs of 23 affected and 11,177 non-affected animals.

Based on autozygosity mapping, we could identify a common homozygous segment in the 23 affected animals (74.5 – 77 Mb) within the associated region of BTA11 (Fig. 2).

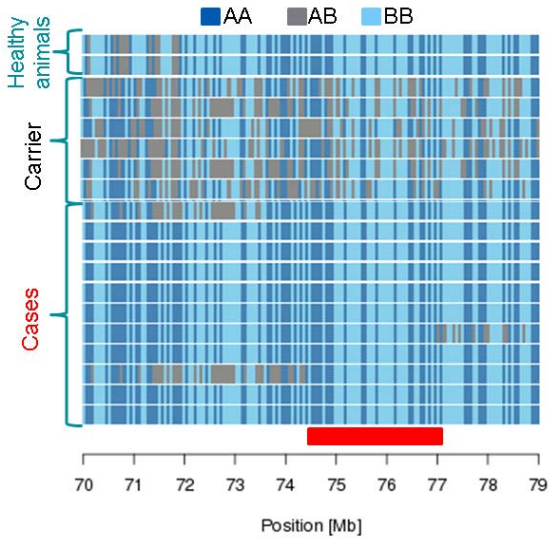

Figure 2 Autozygosity mapping of affected and unaffected animals.

However, we observed healthy animals out of the control group which have the identical homozygous haplotype like the affected animals.

Haplotype analysis

Haplotype analysis resulted in 234 animals which were homozygous for the haplotype indicating that the animals do not die immediately after birth. 80% of these homozygous animals died within the first year of life. 14.093 animals were heterozygous for the disease associated region. The frequency of the associated haplotype in the genotyped German Holstein population, mainly consisting of candidate bulls, daughter proven bulls and selected bulls for the reference population, was 8.7% (Fig. 3).

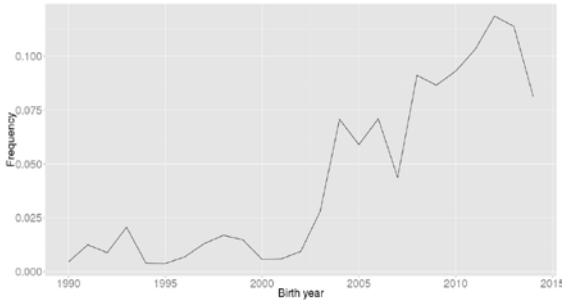

Figure 3 Haplotype frequency in the German Holstein population.

The availability of routinely analyzed blood values facilitated the comparison of cholesterol levels of animals carrying the haplotype to non-carrier. On average, the cholesterol levels of heterozygous carrier are clearly lower (1.65 mmol/L) compared to non-carrier animals (2.30 mmol/L).

Survival rate of the calves resulting from risk-matings, where the sire and maternal grandsire are carrier of the haplotype, compared to the survival rate of descendants of non-risk matings significantly decreases as from day 30 after birth (Fig. 4). At day 300, the mortality of calves of risk-matings is 16 % compared to 9 % of progenies of non-risk matings.

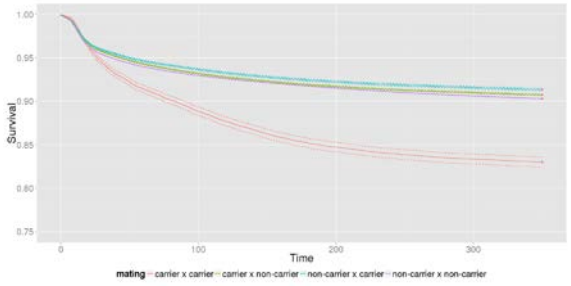

Figure 4 Survival analyses of calves from different matings.

Economic importance

In Germany, 3,400 homozygous animals are estimated to be born per year, assuming random mating of all bulls, a calculated carrier frequency of 8.7% and 1.8 Mio. Holstein calves born per year. The resulting economic loss mounts up to 1.3 Mio. € per year (value of each calf: 400€ including average lifetime of 85 days, raising costs and medical treatment).

Sequence-based analysis

In order to identify the causal mutation, we have embarked on a sequencing project that includes one affected animal which was homozygous for the haplotype. The results were compared to whole genome sequence data of 38 Holstein and five Red Dairy cattle which do not carry the haplotype. The resulting variable positions were compared to the 1000 bull genomes project data (Daetwyler *et al*., 2014). None of the detected variants could be postulated as being causal yet. The bovine reference genome within the disease-associated region includes many gaps (~ 6%). We are currently working on completing the genomic sequence within the associated region in order to detect the causal variant. The identification of the causal mutation is expected soon.

4. Discussion

Haplotype analysis revealed animals which were homozygous for the 54K-based

haplotype, but were unaffected. However, affected homozygous animals revealed the prominent Holstein bull MAUGHLIN STORM on maternal and paternal site of the pedigree. Homozygous animals showing no signs of illness had STORM not more than once in their pedigree. Healthy and affected homozygous animals, both, trace back to a common ancestor who himself is the maternal great grandsire of STORM most probably inheriting the identical haplotype. This phenomenon is suggestive for an existing causal mutation which probably firstly occurred within STORM and is not included in the 54K SNP Chip data. Based on array-derived data, one cannot distinguish between affected and unaffected haplotypes, similar to the arachnomelia syndrome in Brown Suisse (Drögemüller *et al.*, 2010) and Zinc deficiency-like syndrome in Fleckvieh (Jung *et al*., 2014). Most probably, the identical haplotypes differ for the causal mutation only.
Due to the fact that the 54K-based haplotype does not cover the causal mutation, the haplotype frequency does not reflect the frequency of the causal mutation and is therefore overestimated. The strong increase of the haplotype frequency since the 2000s is a result of the intense and widespread use of the prominent Holstein bull MAUGHLIN STORM carrying the haplotype in the heterozygous state. This bull produced many sons and grandsons becoming AI bulls and therefore inheriting the defective haplotype in following years. Despite the high haplotype frequency, the genetic defect remained undetected within the last years most possibly due to the unspecific phenotype characterized by diarrhea. Most of the cases have probably not been reported. This disorder requires careful examination which is not routinely carried out by the farmer or veterinarian.

The identified haplotype appears to be associated with cholesterol metabolism. One calf which did not suffer from chronic diarrhea showed intermediate cholesterol values between affected and non-affected animals. This animal carries the phenotype underlying haplotype in the heterozygous state indicating a codominant inheritance of this defect. This effect could be validated by comparing the blood cholesterol levels of animals carrying the haplotype to non-carrier whose blood were routinely analyzed. Actually, it is not clear, if heterozygous animals revealing reduced cholesterol levels, but do survive, could also have any disadvantages in any other traits which will be investigated in subsequent studies.

5. Conclusion

This study identified a genetic disposition for calf survival. The identified haplotype is clearly associated with calf mortality having a high impact on the worldwide Holstein population. Unfortunately, the accuracy of the haplotype test for the prediction of the carrier status is not 100% due to the existence of two identical haplotypes, a healthy and affected haplotype. Further investigation is needed to improve the haplotype test. However, the information about the haplotype status including the availability of pedigree data allows for the prevention of future risk-matings. Identifying carriers for this genetic disorder and considering this information in breeding programs can prevent calf mortality and improve animal welfare and health.

6. Acknowledgements

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