Comparison of genomic architecture for gestation length, stillbirth and calving ease in Fleckvieh cattle

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Summary

Breeding values from 7416 Austrian and German Fleckvieh bulls were used to determine similarities and differences in the genomic architecture of stillbirth (SB), calving ease (CE) and gestation length (GL). The comparison was done using genome wide associations based on 42,041 SNPs after quality control and the full data set.
The genomic region on BTA21 (2.4Mb) appeared to be significant for all three traits, containing the UBE3A gene connected to known syndromes impairing reproductive functions. The region on BTA14 (24Mb) was harbouring PLAG1, TGS1, RPS20, LYN and SOX17 connected to growth and body size was also significant for SB and CE, but was far from significance for GL. An additional peak was located on BTA6 (38Mb) significant for CE and approaching significance for SB. The genomic region contained the LCORL gene associated with both intrauterine growth and adult height. Based on the apparent significance of growth and body size related genomic regions for SB and CE, and the lack thereof for GL we concluded that the size of the calf does not affect gestation length.
The genomic regions influencing only GL, but not SB or CE were located on BTA4 (94-95Mb) and BTA7 (53Mb). Both genomic regions were rich in genes, but without any strong candidates based on the described gene functions. Genes of similar functions appeared in both however, which might provide some lead for further investigations. These included genes connected to immune response, particularly viral resistance, early embryogenesis, myogenesis and prenatal development.

Keywords: cattle, GWAS, gestation length, stillbirth, calving ease, genetic architecture

Introduction

Calvings are crucial events in cattle breeding with complications leading to potential loss or impaired performance of the cow and calf and compromised animal welfare (Eaglen et al., 2013). The frequently studied calving traits are calving ease (CE) and stillbirth (SB), recently amended by gestation length (GL). The importance of the aforementioned traits is considerable in both dairy and beef cattle, with direct and indirect economic outcomes via cow and calf survival, decreased milk production, reduced fertility and overall health problems (Heins et al., 2006; Eaglen et al., 2011; Barrier et al., 2012).

The heritability for direct SB was estimated to $h^2 = 0.03$ (Cole et al., 2016), for direct CE $h^2 = 0.10$, while the heritability of GL was in the range of $h^2 = 0.49$ (Eaglen et al. 2013). The clear differences between heritabilities are also implying the GL to be more responsive to selection. The correlations between GL and the other two traits are also variable, with $r = 0.19$ (SB – GL) and $r = 0.45$ (CE - GL) (Hansen et al., 2004). The correlations between CE and SB were found to be negative, in the range of $r = -0.59$ (Luo et al. 1999). Indirect

causative effects of GL to CE via the increased birth weight of the calf were shown by Inoue et al. (2017).

From the perspective of trait architecture the genomic regions on BTA14 and BTA21 were shown to influence SB and CE in Fleckvieh cattle (Pausch et al. 2011). Several candidate genes were identified on BTA6 in Norwegian Red cattle with a potential effect on bone formation, thus influencing SB (Olsen et al. 2010). Genomic regions affecting SB and CE were identified on BTA7 and BTA28 (Maltecca et al. 2011) and BTA18 in Holstein cattle (Cole et al. 2009). Additional regions affecting GL were found on BTA18 (Maltecca et al. 2011) based on SNP and on BTA6, 7, 14 and 21 based on microsatellite markers (Maltecca et al. 2009).

The aim of the study was to further investigate the genomic regions influencing GL, SB and CE, including any potentially influential genes, and to compare them between traits to derive connections between the underlying genome architecture.

Material and methods

Single nucleotide polymorphism (SNP) data of the German-Austrian genotype pool of Fleckvieh (dual purpose Simmental) bulls was analysed, merged from Illumina BovineSNP50v1, BovineSNP50v2 and BovineHD BeadChips. The data was subjected to standard quality control, allowing no more than 10% missing per animal and per SNP, no less than 1% minor allele frequency and a Hardy-Weinberg equilibrium threshold of 10^{-9}. After quality control 7,416 animals and 42,041 SNPs remained for the analysis.

Breeding values for GL, SB and CE (Fuerst et al, 2017) provided by ZuchtData GmbH were used as phenotypes. The animals with reliabilities of breeding values below 0.3 were excluded from the analyses.

The genome wide association study (GWAS) was done using the GEMMA software (Zhou & Stephens, 2012) with univariate linear mixed model. The relatedness matrix was used to correct for the population structure. The GWAS was performed for the whole data set as well its subsets. Two subsets were created based on low (1296 animals with less than 88, i.e. mean minus genetic one sd) and high (733 animals with more than 112, i.e. mean plus one genetic sd) breeding values for the gestation length.

The genomic regions around the most significant hits were searched 0.5 Mb up and downwards (1 Mb in total) to identify any potentially influential genes using the National Center for Biotechnology Information (NCBI) database.

Results and Discussion

The correlation coefficients between the direct breeding values were r = 0.55 between SB and CE; r = 0.19 between SB and GL and r = 0.45 between CE and GL, well corresponding with findings of Hansen et al. (2004) and Luo et al. (1999).

The summary of the genome wide association results using all available animals is shown in Figure 1. For ease of comparison the –log(p) values were limited to 20 in Figure 1, with the real values over 40 in case of peaks on BTA14 for SB and CE; BTA21 for CE; and over 60 for the peak at BTA21 for GL.

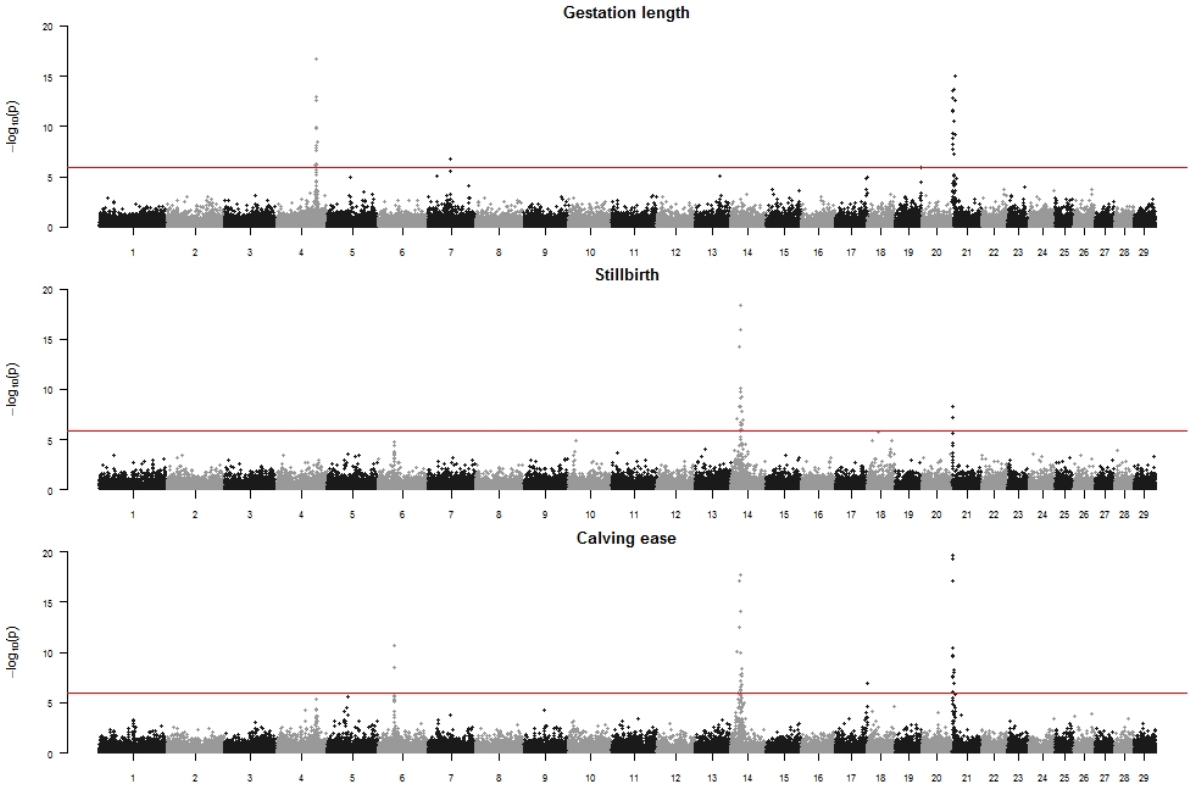

Figure 1: Genome wide associations for gestation length, stillbirth and calving ease. The horizontal line denotes the Bonferroni threshold with a significance level p = 0.05.

The most prominent peak was detected at around 2.4 Mb on BTA21 common for all three traits. The region corresponds to the finding of Pausch et al. (2011) identifying the same region for SB and CE. We amend the earlier findings with stating the major influence of the 2.4 Mb region of BTA21 also in case of GL. The most prominent SNP was directly on top of the UBE3A gene connected to Prader-Willi and Angelman syndromes, impairing reproductive functions (Bischof et al., 2007). We note however the number of unidentified pseudogenes in the peak's immediate vicinity. Although the pseudogenes do not influence any trait directly, they might be essential in regulation of other genes (Tutar, 2012), thus potentially providing other explanations.

The second most significant region for both SB and CE was the region around 24 Mb on BTA14. The region contains several growth and body size related genes such as PLAG1, TGS1, RPS20, LYN and SOX17 (Pausch et al., 2011; Utsunomiya et al., 2013). While the enlarged body size has a significant effect on the calving process, it does not affect the whole gestation period, as shown by the lack of the signal for the GL trait.

The same applies to the 38.5 Mb region of BTA6 containing the gene LCORL associated with both intrauterine growth and adult height (Horikoshi et al., 2013). The same region was found by Olsen et al. (2010) pointing to genes SPP1, IBSP and MEPE connected to bone and cartilage formation, thus potentially affecting SB. Also in this case the genomic region is significant for SB and CE, but not for GL. Based on these findings we hypothesize that while body size related traits have a major influence on the outcome of calving, but they do not affect the length of gestation as such. This interpretation does not both ways however. Short gestations tend to result into lighter calves, as shown for the Angus cattle by Reynolds et al. (1980). Similarly, an increased GL was shown increase calf weight, with a follow up effect on CE (Inoue et al., 2017).

The most significant region for GL that does not appear for SB or CE was a relatively wide peak, located at 94-95Mb on BTA4. The region is rich in genes, without any obvious

connection to GL. The lack of our ability to identify any such connection might be due to our imperfect understanding of the physiology as well as the likely complex nature of gestation. Several genes on the region were connected to immune and viral resistance traits, early embryogenesis and myogenesis, which might be used as starting points for further investigations.

A smaller signal from GL was detected in the 53 Mb region of BTA7. Again the region appears to be gene rich. From the genes present in the immediate vicinity the NRG2 was connected to implantation and prenatal development, CYSTM1 and IGIP to immunity response, particularly against viruses. Another interesting gene in the region is HB-EGF, which together with prostaglandins and interferon-τ regulate uterine function for pregnancy establishment in ruminants (Takatsu and Acosta, 2015).

With sub-setting the data based on low and high breeding values for gestation length (Figure 2 and 3) the peak on BTA6 and BTA21 has vanished in all but one case. The loss of signals point towards uniformity of the animals within these genomic regions when the GL based variability decreased. The signal on BTA14 however remains strong in all cases, confirming its importance for SB and CE.

Conclusions

Overall the genetic architecture was more similar between SB and CE, with three of the largest peaks corresponding between the two traits. The significance of genomic regions influencing body height and weight was notably missing for GL, suggesting a major influence for SB and CE, but not for GL. With this we conclude that the size of the calf does not affect the length of gestation. Genomic regions significant only for GL appear to be gene rich, containing genes related to immune response, viral resistance, embryogenesis and prenatal development.

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Appendix 1

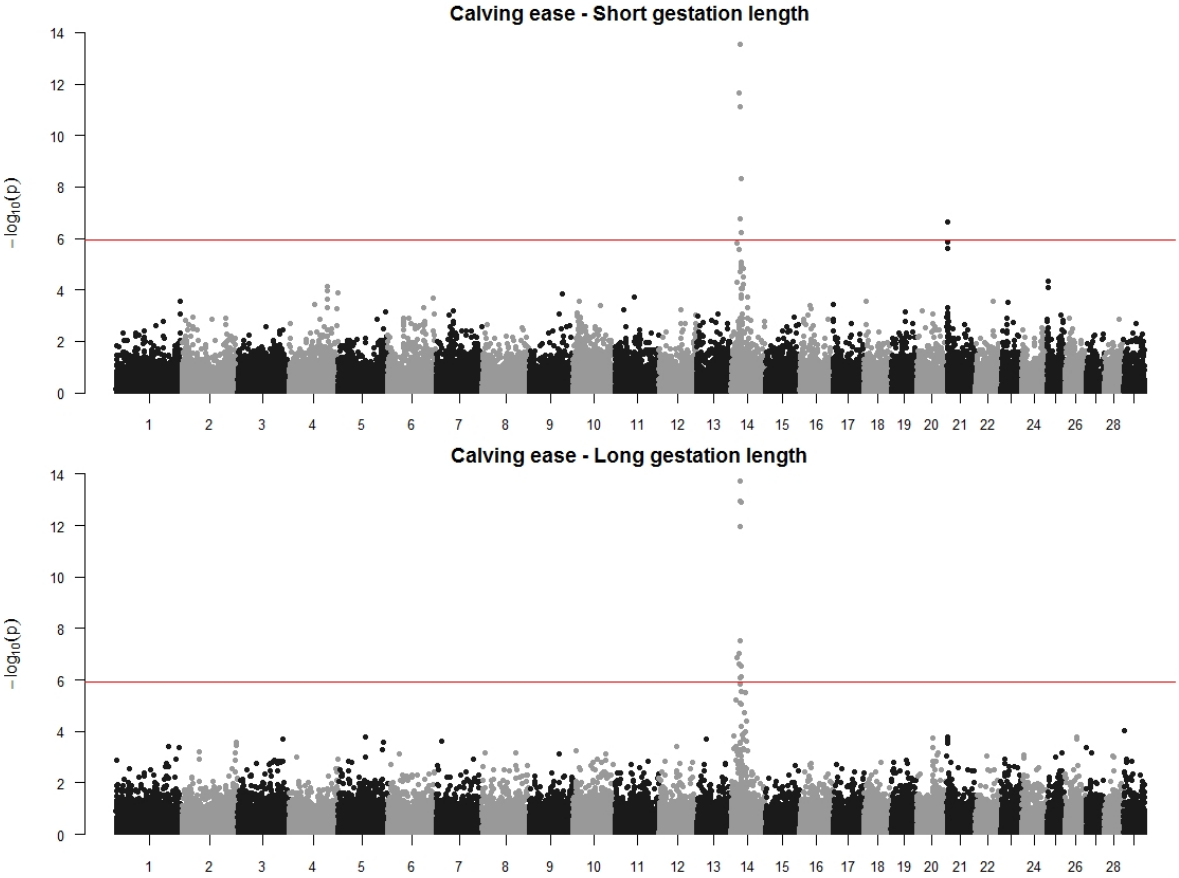

Figure 2: Genome wide associations for calving ease for subsets of animals the low (less than mean – 1 standard deviation) and high (more than mean + 1 standard deviation) breeding values for gestation length. The horizontal line denotes the Bonferroni threshold with a significance level p = 0.05.

Appendix 2

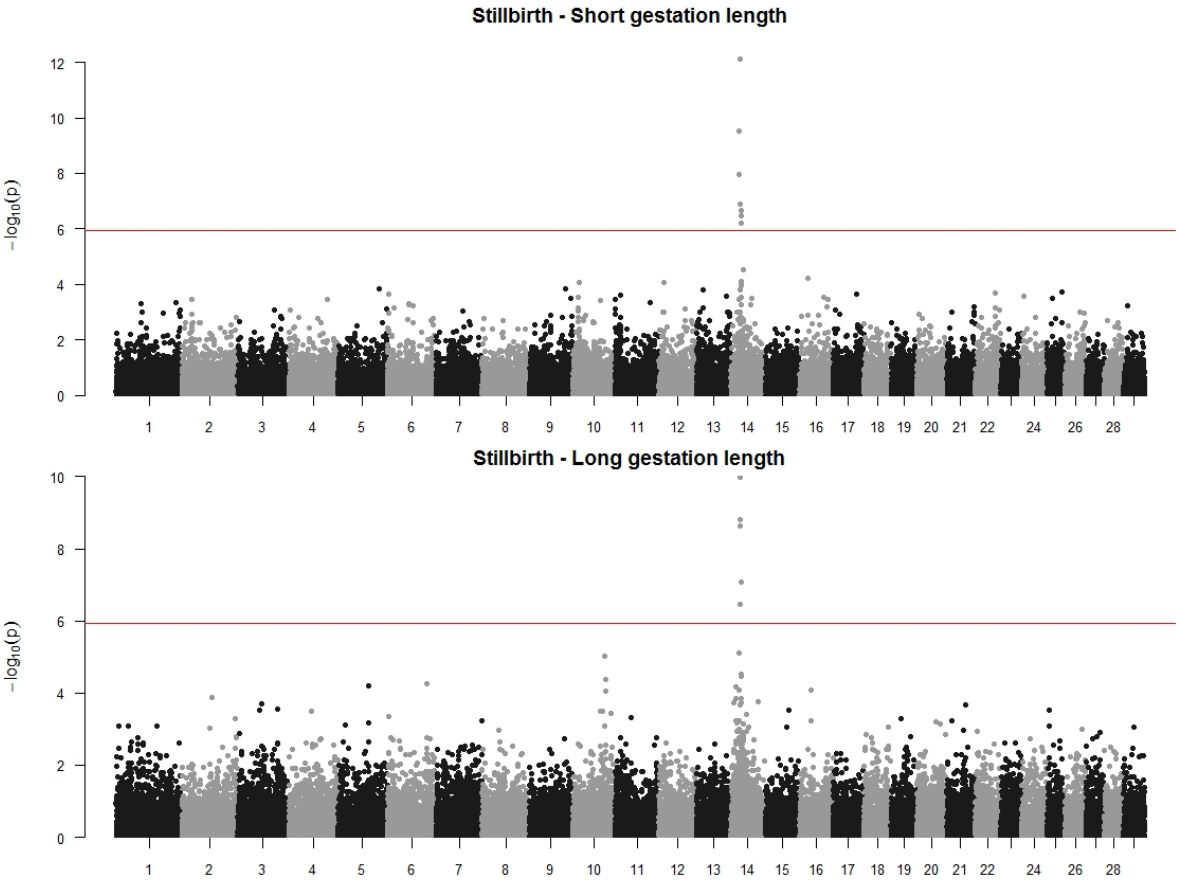

Figure 3: Genome wide associations for stillbirth for subsets of animals the low (less than mean – 1 standard deviation) and high (more than mean + 1 standard deviation) breeding values for gestation length. The horizontal line denotes the Bonferroni threshold with a significance level p = 0.05.