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Original Paper

Epistatic interactions on chromosome 14 influencing stillbirth in Fleckvieh cattle

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Single nucleotide polymorphism (SNP) data of 7384 Fleckvieh bulls was analyzed to identify epistatic interactions influencing stillbirth. Deregressed breeding values were used as phenotypes. The epistatic effects were identified as significant interaction terms from pairwise linear regressions performed for each SNP after accounting for multiple testing. Majority of the detected epistatic effects were located in the 9–31 Mb region of chromosome 14, corresponding to the most significant region from the genome wide association. Additional epistatic SNPs at 50.5 Mb and 80.5 Mb at the same chromosome were detected. The region around 25 Mb contained genes connected to height and body size such as PLAG1, CHCHD7, LYN, RDHE2 (SDR16C5) and PENK. The other interesting region at 50.5Mb contained the TRPS1 gene influencing bone malformations. Both regions have been identified as candidates influencing stillbirth.

Keywords: cattle, Fleckvieh, SNP, stillbirth, epistasis

1 Introduction

Epistasis is commonly defined as interaction between loci, when the phenotypic effect of a locus is influenced by the genotype of the second locus. These interactions could be present between two or more loci (Carlborg and Haley 2004). Although these interactions are known to influence a plethora of quantitative traits, see Cordell (2002) and Mackay (2014) for review, the topic is not extensively studied in animal genetics. Thus, our paper is intended to extend the understanding of epistasis in animal breeding. Our particular aim was to analyze epistatic effects influencing stillbirth in cattle.

2 Material and methods

Single nucleotide polymorphism (SNP) genotypes from 7384 Fleckvieh bulls were considered in the study, initially from both versions of BovineSNP50 BeadChips and the Illumina HD chip. For the final analysis only autosomal SNPs present on all chips were used. The genotypes were subjected to quality control using PLINK1.9 (Chang et al. 2015), with maximal limits for missingness per animal 10%, missingness per SNP 10%, at least 0.01 minor allele frequency and Hardy-Weinberg equilibrium threshold of 1E-9. After all quality control steps the number of remaining SNPs was 41,884.

Deregressed breeding values for stillbirth rate with minimum reliability of 0.3 provided by ZuchtData EDV-Dienstleistungen GbmH were used as phenotypes. Stillbirth was defined as death occurring just before or immediately after the delivery.

Testing all SNP combinations would have excessive computational capacity and time requirements. In order to limit the number of tests, a preliminary genome wide association was performed and only SNPs with GWAS significance of at least 0.01 were kept, following Ali et al. (2015). Total of 492 SNPs from all chromosomes satisfied this condition, resulting in 120,786 possible interactions.

The epistasis command of PLINK1.9 was used to analyze interactions between SNPs. The program fits a linear regression model using the quantitative trait, as:

$$Y = \beta_0 + \beta_1 g_A + \beta_2 g_B + \beta_3 g_A g_B$$

where:
g_A and g_B – allele counts and the β_3 coefficients are tested for significance for each allele pair (*A* and *B*)

The Bonferroni-Holm procedure (Holm 1979) considering the 120,786 performed pairwise tests with a p value limit of 0.05 was used to account for multiple testing.

3 Results and discussion

From the large number of tests only 744 SNP pairs showed significant interactions at the $P < 0.05$ level, after

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correction for multiple testing. Already at this point the results were dominated by interactions within BTA14. This outcome could be expected based on the highly significant GWAS peak around 24–25 megabases (Mb) on BTA14.

Among the 744 pairs there were a number of cases of duplicated results. Since all pairwise combinations for all SNPs were analyzed, it was common that neighboring SNPs from a given genomic region were showing significant interaction to other neighboring, or even the same SNP in another region, most likely describing the same connection. For this reason another filtering step was implemented to remove such cases. The filtering procedure rounded SNP base pair locations to whole Mb and kept only one for each chromosome and Mb combination considering both SNPs in the pair.

Among the remaining 185 genome wide pairs the vast majority was still located on BTA14, confirming the importance of this chromosome for the stillbirth trait in cattle (Figure 1). Within the 24–25 Mb region involved in the most pleiotropic interactions there were several interesting genes connected to calving ease and stillbirth, such as PLAG1, SOX17, CHCHD7, MOS, RPS20, LYN, XKR4 and others. Several of these genes, such as PLAG1, CHCHD7, MOS, RPS20, LYN, RDHE2 (SDR16C5) and PENK have been known to influence human and adult height (Utsunomiya et al. 2013), thus a possible effect on calving traits. With increased body size there is a higher chance of difficult calving that might lead to increased stillbirth rate.

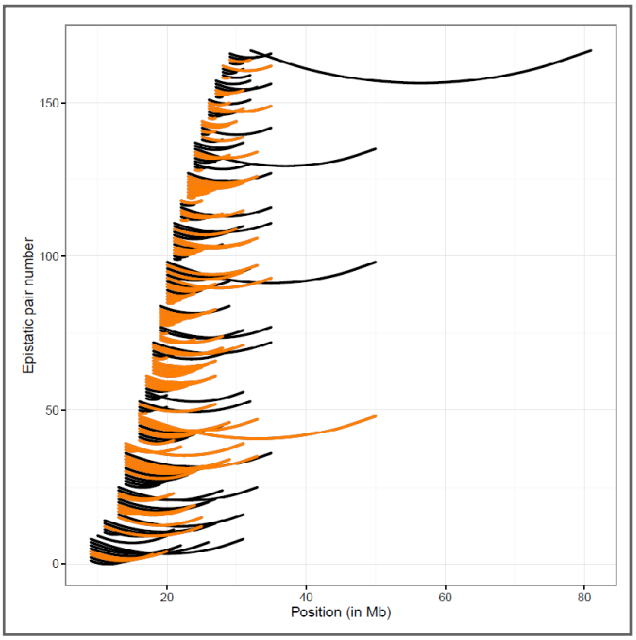

Figure 1 Significant epistatic effect for stillbirth on chromosome 14
P ≤0.05 in black, *P* ≤0.001 in gold

Another interesting gene is XKR4, explaining 2.45% of the phenotypic variation for prolactin levels in several cattle breeds, including Simmental (Bastin et al., 2014). Prolaktin in turn has a confirmed effect on miscarriages in humans (Hirahara et al., 1998). It should be noted that the miscarriages influenced by prolactin levels occurred in the first trimester of pregnancy. Although early miscarriages were different from our definition of stillbirth, the potential effect of this gene should be considered when analyzing calving or fertility related traits.

In addition to the relatively short distance epistatic effects there are three SNPs showing significant interactions involving the 50.4 Mb region of BTA14. While there is no known gene at this exact location, the TRPS1 gene was in the immediate vicinity (50.8–51.1 Mb). This gene was connected to growth and meat production traits in cattle and sheep, but more importantly to skeletal abnormalities in humans (Lüdecke et al. 1999). While calves with bone problems could survive the whole gestation, the consequences of the disorder would likely appear during or right after birth. Severe abnormalities would lead to the death of the calf.

The highest distance epistatic interaction was between a highly significant region of BTA14 (around 30Mb) and a SNP in the middle of RALYL gene on 80.5Mb. This gene was identified, but not well studied in any species, including humans and mice.

To conclude, genes on BTA14 seem to have an exceptional importance for stillbirth in cattle. The GWAS results peak at 24–25 Mb, while the majority of the epistatic SNP pairs are within the 9–31 Mb region of BTA14. Epistatic interactions with other regions of BTA14 were also detected. Genes residing in these regions influence height and body size, but also bone malformations, which in turn influence the stillbirth rate in cattle.

4 Conclusions

To conclude, genes on BTA14 seem to have an exceptional importance for stillbirth in cattle. The GWAS results peak at 24–25 Mb, while the majority of the epistatic SNP pairs are within the 9–31 Mb region of BTA14. Epistatic interactions with other regions of BTA14 were also detected. Genes residing in these regions influence height and body size, but also bone malformations, which in turn influence the stillbirth rate in cattle

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