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PLEIOTROPIC AND EPISTATIC INTERACTIONS BETWEEN STILLBIRTH AND CALVING EASE IN CATTLE

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ABSTRACT

Pleiotropic and epistatic interactions were analyzed for stillbirth and calving ease in Fleckvieh cattle, with deregressed breeding values from 7384 bulls used as phenotypes. The genotype data consisted of 41,884 SNPs after quality control. A multivariate linear regression approach was used to obtain pleiotropic and epistatic effects using the CAPE package in R. Total of five regions on chromosomes 6, 14, 17 and 21 showed evidence of pleiotropic interaction. The genes in these significant regions were also relevant to the two phenotypes of interest, such as PLAG1 affecting growth and height, XKR4 affecting rump thickness and insulin levels, ELMOD2 connected to immune response, UCP1 connected to thermoregulation and UBE3A connected to the Prader-Willi syndrome. The results for epistatic effects were less convincing, although they identified connections to possible regulatory genes within chromosome 21.

Key words: cattle, breeds, Fleckvieh cattle, functional traits, genetics, genetic interactions, SNP, genes

1 INTRODUCTION

With the availability of dense molecular markers at a relatively cheap price we have now the possibility to analyze the genomic background of any qualitative or quantitative trait. Genome wide association studies can identify genomic regions and genes influencing a trait of interest. There are cases however, when this single gene single trait restriction is not descriptive enough. In some cases a single gene could affect multiple traits, a phenomenon called pleiotropy, in other cases at least two genes need to interact for a single phenotype to show, also known as epistasis.

Functional traits are good candidates for research on genetic interactions both between and within traits. Although absence of extremely influential genes on the phenotypes is suggested by their low heriatability, it is likely that an unseen network of interactions is present between the genes of small effect. Uncovering such relationships and interactions was also the motivation for our research.

Therefore, the aim of this paper was to identify pleiotropic and epistatic effects for stillbirth and calving ease in cattle.

2 MATERIALS AND METHODS

Single nucleotide polymorphism (SNP) data from 7384 Fleckvieh bulls genotyped with both versions of Illumina BovineSNP50 BeadChips and the Illumina HD chip were used in the study. For analysis only autosomal SNPs present on all chips were used, with mapped to the UMD3 bovine genome assembly. Standard quality control procedures were applied to remove genotyping errors using PLINK1.9 (Chang *et al.*, 2015). In this step

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SNPs with more than 10 % missingness per SNP, less than 0.01 minor allele frequencies, with *p*-value less than 1E-9 for Hardy-Weinberg equilibrium threshold, and animals with more than 10 % missing SNPs were removed from the analysis. After all quality control steps a total of 7384 animals and 41,884 SNPs remained for analysis.

Deregressed breeding values for stillbirth rate and direct calving ease with minimum reliability of 0.3 provided by ZuchtData EDV-Dienstleistungen GbmH were used as phenotypes.

The CAPE package (Tyler *et al.*, 2013) in R was used for combined detection of pleiotropy and epistasis. This software maximizes the linear independence of phenotypes by performing singular value decomposition on the traits of interest, deriving so called eigentraits (ET). Commonly the two ET accounting for the most variance explained i.e. with the largest contributions to the phenotypes were selected for further analysis. A genome wide single variant scan was performed to identify the 100 most influential loci for follow up analysis. To avoid effects driven purely by LD, marker pairs with Pearson correlations above 0.6 were skipped before marker selection. Pair-scans were performed for each ET by multivariate linear regression, with an intercept, main effects and interaction term for each pair of markers. The significant main effect coefficients for variant-to-phenotype influences denote pleiotropy, while interaction coefficients denote epistatic effects. The Bonferroni-Holm approach (Holm, 1979) was used to correct for multiple testing.

In CAPE the epistatic effects are interpreted as the degree to which the alternate allele at the source locus changes the phenotypic effects of the alternate allele at the target locus. For example, there is a cross between two strains "A" (reference allele) and "B" (alternate allele). Let allele "B" at locus 1 have a positive main effect on the phenotype. Let there be epistasis between locus 1 and locus 2. According to CAPE when allele "B" is present at locus 2, the allele suppresses the positive effect of the "B" allele at locus 1, such that it now the allele at locus 1 has a negative effect on the phenotype. So when only locus 1 has the "B" allele, that locus has a positive main effect on the phenotype, but when locus 2 also has a "B" allele, the "B" allele at locus 1 is changed through epistasis to have a negative effect on the phenotype.

According to this approach to epistasis the direction of such effect can be derived. The coefficient shows the extent of enhancement or suppression of the phenotypic effect of the target locus, given the presence of the alternate allele at the source locus. The derived model fits all phenotypes. So if locus 2 suppresses the effect of locus 1, it will suppress its effect across all phenotypes for which locus 1 has a main effect.

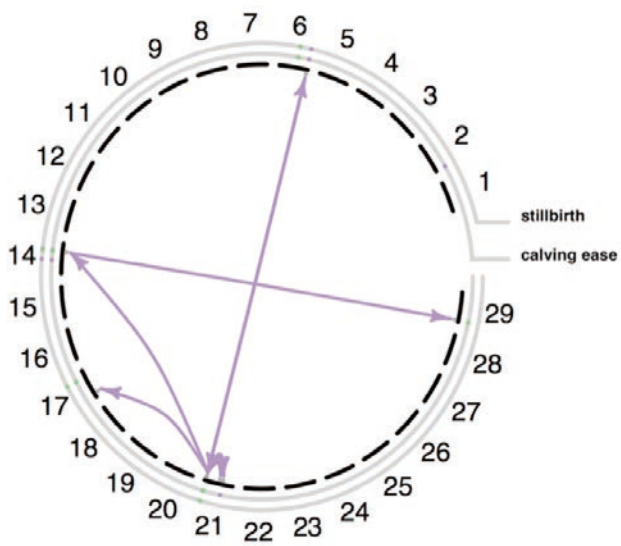

Figure 1: *Network graph showing the combination of the traits stillbirth and calving ease*

3 RESULTS AND DISCUSSION

The correlations between calving ease and stillbirth deregressed breeding values used as phenotypes were in the range of 0.8, which is the upper boundary for analysis of pleiotropic effects. Similarly high correlations from 0.78 to 0.84 between the two calving traits were found by (Steinbock *et al.*, 2003). Yet it has been reported that only 43 % of the stillbirths were caused by difficult calving and 32 % were born without any signs of difficulties during the birth process (Berglund *et al.*, 2003). This emphasizes that calving difficulties were not the only reason for stillbirth, thus supporting our efforts to find epistatic regions influencing both traits.

Five genomic regions on four chromosomes showed significant pleiotropic effects for the stillbirth and calving ease trait pair, with their center around 4.3 Mb on BTA6, 47.0 Mb on BTA6, 17.5 Mb on BTA17, and 2.1 Mb on BTA21 and a wider region around 23–24 Mb on BTA14 (Fig. 1).

QTL for direct still birth on BTA6 with a pleiotropic effect on the trait combination stillbirth and calving ease was reported by (Kühn *et al.*, 2003). These results are supported by (Schrooten *et al.*, 2000), who found indication for a QTL for the trait calving ease at the same chromosomal region. Furthermore, Schrooten *et al.* (2000) detected a QTL for size of the animals and the dairy character in this region. Both of these traits might influence the delivery

Table 1: *Epistatic interactions for stillbirth and calving ease*

	Source SNP[2]	BTA Source	Position Source [1]	Target SNP [2]	BTA Target	Position Target [1]
1	BTB.01281052	6	4.22	ARS.BFGL.NGS.53975	21	2.15
2	BTB.01281052	6	4.22	ARS.BFGL.NGS.108925	21	2.46
3	BTB.00557532	14	24.64	BTB.00998633	29	2.93
4	ARS.BFGL.NGS.53975	21	2.15	Hapmap39377.BTA.113470	21	36.62
5	ARS.BFGL.NGS.53975	21	2.15	BTB.00820789	21	44.72
6	ARS.BFGL.NGS.108925	21	2.46	BTB.01281052	6	4.22
7	ARS.BFGL.NGS.108925	21	2.46	ARS.BFGL.BAC.36626	17	64.84
8	ARS.BFGL.NGS.108925	21	2.46	UA.IFASA.7382	14	23.48
9	Hapmap39377.BTA.113470	21	36.62	ARS.BFGL.NGS.53975	21	2.15
10	BTB.00820789	21	44.72	ARS.BFGL.NGS.53975	21	2.15
11	BTB.00820789	21	44.72	ARS.BFGL.NGS.108925	21	2.46

[1] Positions shown in megabases; [2] All significant interactions are shown, one in each direction in case both of them were significant.

of the calf. A QTL for birth weight was also identified in the same chromosomal region of BTA6 by (Casas *et al.*, 2000).

Similarly to our results, the genomic regions on BTA 14 and BTA 21 were also highlighted in a genome wide association study of calving ease and growth related traits in Fleckvieh cattle (Pausch *et al.*, 2011). The region on BTA14 was associated with growth related traits, influencing the body size of the calf and eventually the calving ease. The region at BTA21 contained genes whose lack of expression leads to Prader–Willi syndrome. One of the sympthomatic effects of this disorder are fetal growth anomalies, which influence the stillbirth rate.

The presence and function of genes was also investigated within the ± 0.5 Mb region from the pleiotropic SNP. There were several genes in each identified region, although their connectedness to stillbirth or calving ease was not always clear. The most important genes were PLAG1 on BTA14 affecting growth and height (Utsunomiya *et al.*, 2013), XKR4 on BTA14 affecting rump thickness and insulin levels (Bolormaa *et al.*, 2011), ELMOD2 on BTA17 connected to immune response (Thompson-Crispi *et al.*, 2014), UCP1 on BTA17 connected to thermoregulation (Sonstegard and Kappes, 1999) and UBE3A on BTA21 connected to the Prader-Willi syndrome (Horsthemke and Wagstaff, 2008).

Table 1 shows the epistatic interactions between genomic regions as interpreted by the CAPE software. They seem to be centered on BTA21 for most SNP pairs. Although all of these SNP pairs were shown to have a highly significant interaction, but the standard errors of effect sizes were also very high (results not shown). For this reason caution has to be exercised when interpreting the results.

The 2.1–2.5 Mb region of BTA 21 contains a large number of uncharacterized, possibly protein coding genes. The two known genes in the region are UBE3A and ATP10A connected to Angelman and Prader-Willi syndromes. The epistatic interactions from these regions are pointing to different directions, such as BTA6 (4.22 Mb), BTA14 (23.48 Mb), BTA17 (64.84 Mb), BTA21 (36.62 Mb) and BTA21 (44.72 Mb).

The region in BTA6 contains no obvious candidates, although the NDNF might be connected to craniofacial development (Melvin *et al.*, 2013). The 23–24 Mb region of BTA14 contains growth and size related genes as mentioned above. The identified region on BTA17 is extremely rich in genes, thus we have no clear indication about the true target.

The 36.62Mb region of BTA21 contains multiple uncharacterized genes and the MIR2888-1, a non-coding RNA gene with regulatory function. It was shown to be expressed in bovine ovaries (Hossain *et al.*, 2009). Interestingly the 44.72 Mb region of BTA21 also contains a similar regulatory gene MIR6522, which was also found to be expressed in testicular and ovarian tissues of cattle (Huang *et al.*, 2011). Although there is no clear connection of gonadal functions to either of our traits, the authors suggest that it might be involved in different biological pathways, which encourages further research.

An additional epistatic interaction was identified between the 24 Mb region of BTA14 and 2.93 Mb region of BTA29. The only gene close to the identified SNP on BTA29 is FAT3, that was shown to participate in morphogenetic processes of animal development (Nagae *et al.*, 2007).

Figure 2 shows an example of a previously identified epistatic interaction from Table 1. The alternate allele in

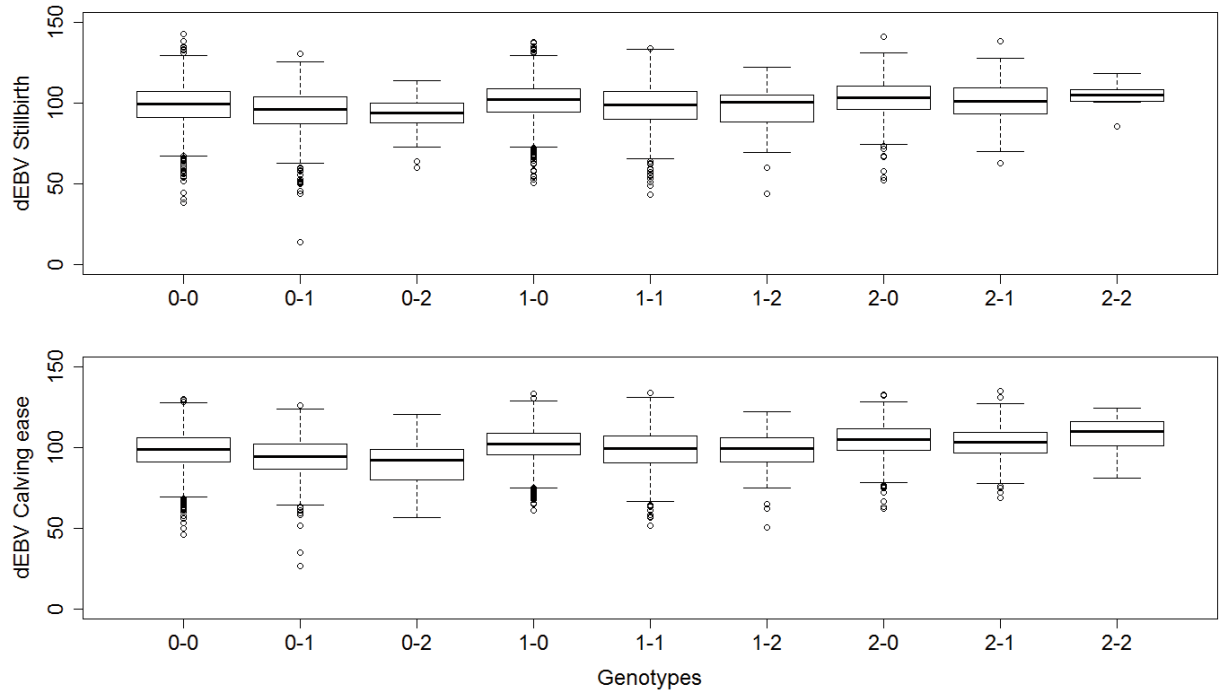

Figure 2: *Deregressed breeding values (dEBV) for stillbirth and calving ease according to genotype combinations (0 – homozygous for the reference allele, 1 – heterozygous, 2 – homozygous for the alternate allele). The values on the X axis denote genotype combinations for SNPs ARS.BFGL.NGS.108925 (BTA21, before dash) and UA.IFASA.7382 (BTA14, after dash)*

locus 2 (UA.IFASA.7382, BTA14) decreases the phenotype value if the reference allele is homozygous in locus 1 (ARS.BFGL.NGS.108925, BTA21), but seems to have the opposite effect when locus 1 is homozygous for its alternate allele.

4 CONCLUSIONS

Pleiotropic and epistatic regions influencing stillbirth and calving ease were identified in our study. Pleiotropic effects were shown on BTA6, 14, 17 and 21, containing genes related to body size, growth, immune response and thermoregulation. The epistatic interactions were also found on BTA29 in addition to the chromosomes involved in pleiotropy. Two of these pleiotropic interactions within BTA21 were involving regulatory genes involved in molecular pathways of gonadal functions, which also merits further research.

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