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GENOMIC BACKGROUND OF ENTROPION IN FLECKVIEH CATTLE

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SUMMARY

Runs of homozygosity (ROH) and genome wide association study (GWAS) were used to identify genomic regions of entropion in Austrian Fleckvieh cattle. Entropion is an eye disorder where the eye lids turn inward, causing inflammations, cornea damage or even blindness if left untreated. A total of 196 bulls genotyped by BovineSNP50 BeadChip were analysed, nine of which had the disorder confirmed by a veterinarian, ten were unconfirmed and 177 were healthy control animals. Runs of homozygosity analysis highlighted regions on seven chromosomes where the proportion of animals in ROH significantly ($p<0.001$) differed between cases and controls. None of the 19 genes identified in these regions showed any connection to entropion or eye development. The GWAS study using only cases and controls had a mild peak directly on top of the ITGA9 gene. This gene was previously identified in mice affecting cornea and eye lid development, therefore we consider it being a candidate to influence entropion also in cattle.

Key-words: cattle, SNP, GWAS, runs of homozygosity, eye disorder

INTRODUCTION

Entropion is a non-lethal medical condition characterized by the lower or upper eye lid turning inwards. Subsequently the eyelashes irritate the eyeball and the cornea, causing inflammation and damage to the eye, or even loss of vision when untreated. It appears almost in all species, such as cats (Williams and Kim, 2009), dogs (Willis et al., 1999), pigs (Allbaugh, 2009), sheep (Basrur and Yadav, 1990), goats (Donnelly et al., 2014) and horses (Labelle et al., 2011).

The genetic background of entropion is complex, suggesting involvement of multiple genes, but no consensus on the mode of inheritance. The research focus in farm animals appears to be on sheep, due to high occurrence of entropion and relatively high heritability of 0.08-0.21 (Sakul and Kellom, 1997). Six regions influencing entropion in sheep were identified in a GWAS study by Mousel et al. (2014). To our knowledge there is no similar information available in cattle.

While the frequency of the disorder is likely similar to that of other cattle breeds, breeders of the Austrian Fleckvieh raised concerns about its occurrence. The aim of our study was thus to respond to this request and to search for genomic region(s) affecting the occurrence of entropion in Austrian Fleckvieh cattle.

MATERIAL AND METHODS

The data consisted of 196 genotyped Fleckvieh bulls, managed in two near-by locations by a breeders association, with Illumina BovineSNP50 BeadChip. Nine of those animals had entropion diagnosed by a veterinarian. Additional 10 bulls had changes on the eyelids, indicating possible abnormal condition, but without clear identification of entropion. Therefore we denoted these bulls as "uncertain". The remaining 177 bulls showed no sign of entropion or any other eye disorder and were assigned to the control group. Two evaluation scenarios were considered, given the uncertainty of diagnosis for some bulls: scenario 1 using affected, uncertain and control groups analysed together; scenario 2 using only affected and control bulls, i.e. the uncertain bulls deleted.

No prior checks were performed to compare the relatedness of the affected versus non-affected animals, assuming that inclusion of population structure in the GWAS analyses accounts for relatedness.

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Two methodologies were used to determine the genetic background of entropion: 1. comparison of runs of homozygosity (ROH) patterns between the affected and control groups (scenario 1 only); 2. a genome wide association study (GWAS) considering both scenarios. Two separate quality control approaches for ROH and GWAS followed, similarly to Mészáros et al. (2015).

For the ROH analyses we included only SNPs with GenCall ≥0.7 and GenTrain ≥0.4, following Ferenčaković et al. (2013). No other quality checks were done. The minimal length of ROH was set to 1Mb with minimum of 30 SNPs, without missing or heterozygous SNPs in the run. The analysis was done separately for cases and controls. Regions of interest were identified as those significantly differing ($p<10^{-3}$) in their ROH status between cases and controls. The uncertain cases were not considered for ROH analyses.

The quality control criteria for the genome wide association study included limitation of the minor allele frequency (min. 1%), deviation from Hardy-Weinberg ($p<10^{-6}$) and individual call rate (min. 90%). The number of SNPs remaining after quality control was 36,624 in scenario 1 and 36,682 in scenario 2. The average distance between SNPs was 72.2 kb after quality control in both cases. The population structure was considered using eigenvectors computed with the GemTools R package (Klei et al., 2011). Single SNP regression was used to find significant regions within the genome using R (R Core Team, 2012), using the Bonferroni correction as the significance threshold.

In the follow up analysis the regions of interest were studied in more details. Genes with possible connections to eye development in these regions were identified using the data base of National Center for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION

The ROH analyses were conducted in the first step, to see if the causal regions for the entropion were in homozygous segments. If a certain genomic region would be in a ROH in all cases but only in some of the controls, it could be identified as a very strong candidate region. A similar approach was applied in Drögemüller et al. (2011).

In our case however, there was no region suggesting that entropion in cattle would be caused by recessive homozygotes, which confirmed results of Sakul and Kellom (1997). Several genomic regions showed very different patterns of ROH in cases and controls. The ROH results from chromosome 2 are shown as an example in Figure 1. The proportion of the animals in ROH is shown at the bottom, with apparent differences in some regions. The p values were calculated for each SNP to denote significance of the difference (Figure 1, top). An arbitrary value of $p<10^{-3}$ was used as a threshold to identify regions for further investigation.

Regions on chromosomes 2, 6, 11, 12, 13, 19, and 21 showed significantly different patterns in ROH, harbouring 19 genes in total. None of these 19 genes had a previously identified connection to eye development or disorders however. There was a complete lack of genes in multiple regions.

The genome wide association study was done considering two scenarios. In both cases the population structure was considered via eigenvectors, included as fixed effects into the model.

In the first step the uncertain cases were included into the evaluation, considering them as truly affected cases. After the analysis had been conducted there was only a single significant SNP over the Bonferroni line in the 42 Mb region of chromosome 6 (not shown). There were several genes in the region, but without apparent links to entropion.

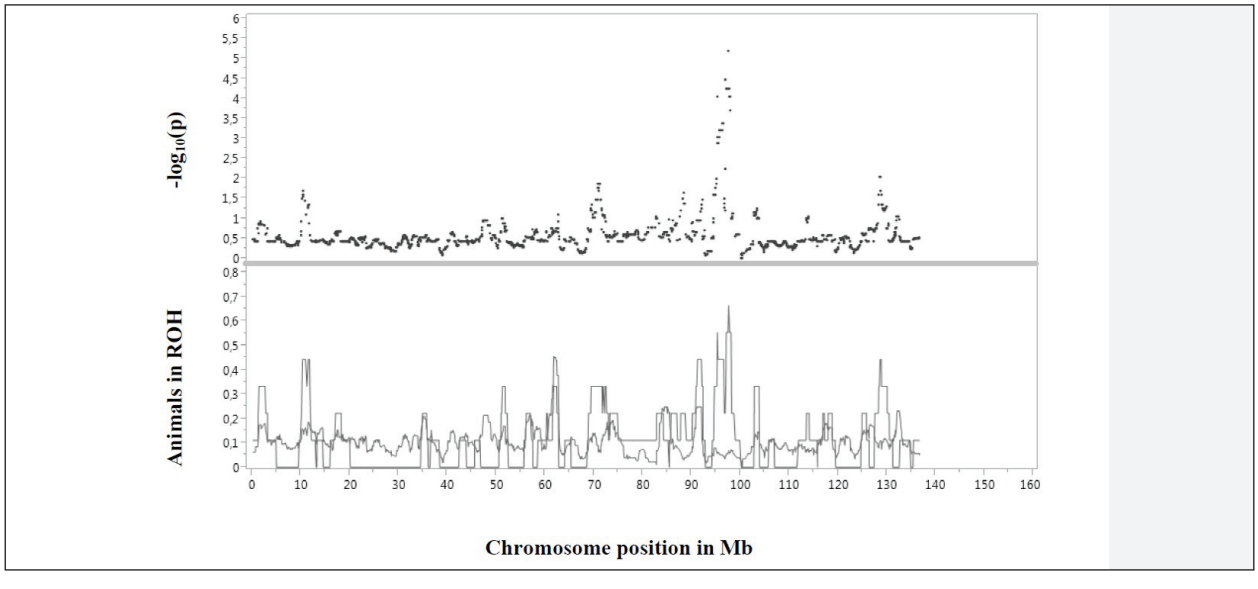

Figure 1. Comparison of segments in run of homozygosity in affected and control animals (chromosome 2)

In the second scenario only the confirmed cases were used in the GWAS run (Figure 2). Several regions were suggestive (i.e. close to the Bonferroni line), thus closely checked for genes. The most important finding was from chromosome 22 at 11.09 Mb, directly on the top of the integrin alpha 9 gene (ITGA9; 10.95-11.31Mb). The gene was previously identified affecting the cornea (Stepp et al., 1995) and eye lid development (Stepp, 1999; Banks, 2000) in mice. The ITGA9 gene has also strong connections to the lymphatic system (Bazigou et al., 2009), and it has been shown to be up regulated during corneal lymphangiogenesis and lymphatic valve formation (Truong et al., 2011).

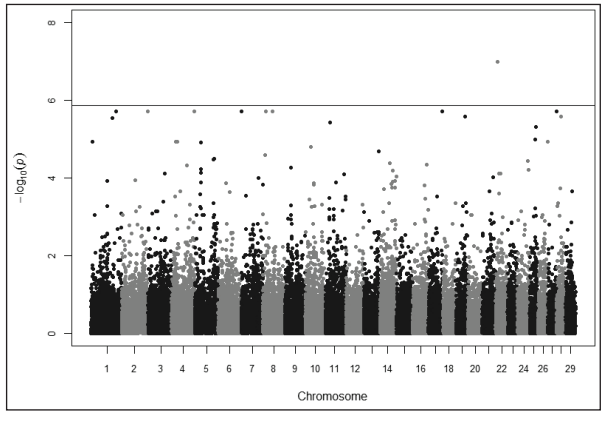

Figure 2. Genome wide association study comparing affected and control individuals. Uncertain cases were not considered here

From our analysis it was apparent that caution should be exercised when including animals with uncertain disease occurrence into genome wide association studies. Our analyses identified different regions of interest, when uncertain cases were considered, as opposed to the analysis with only the certain cases and controls. Possibly some of the uncertain cases had the symptoms of entropion, without being truly affected and therefore introducing a bias.

The most interesting region on chromosome 22 was identified by a single SNP. After critical review of the results we have to acknowledge that the result was not extremely convincing, even though there was an underlying gene with strong connection to the development of eyelids. Typically in a single SNP analysis a strong GWAS signal would involve "tower like" structures in the Manhattan plot, with multiple highly significant SNPs very near to each other. This was not our case. The reason could be that the low number cases did not allow to precisely identify the possibly complex genomic background of entropion. Therefore a follow up analysis with more cases and higher density genotype data would be of interest.

CONCLUSION

Two methods were used to identify possible causes of entropion in Austrian Fleckvieh cattle. The ROH analyses identified regions of interest on seven chromosomes, harbouring 19 genes. None of these genes was associated with eye development or disorders however, by the literature research. The genome wide association study considering affected and control individuals identified the gene ITGA9 on chromosome 22 as a possible candidate influencing entropion in cattle.

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