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Copy number variation in bovine ß-defensin genes

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Introduction

It has become apparent, that copy number variation (CNV) is an important source of structural genomic variation. In human defensin genes CNV occurs to a large extent and contributes significantly to the susceptibility to infectious and inflammatory diseases. In this study we applied an quantitative Real time PCR (qRT-PCR) approach to determine, whether CNV does exist in bovine ß-defensins within and between breeds and whether it influences the resistance to mastitis.

Material & Methods

Animals: We selected two extreme groups of 100 German Holstein cows each with mean breeding values for mastitis susceptibility of -0.45 (s=0.12) and 0.76 (s= 0.28) from an experimental dairy farm. The breeding values are routinely estimated for this farm including records on clinical mastitis. In order to detect CNV between breeds, we additionally screened a panel of 42 animals from seven different breeds (Fig.2).

Determination of CNV: We performed qRT-PCR with genomic DNA and measured the relative amounts of template DNA for the two genes Lingual antimicrobial peptide (*LAP*) and Enteric ß-defensin (*EBD*, ß-defensin 1, *DEFB1*) in relation to the single copy glucagon gene using a modified delta-C_t method.

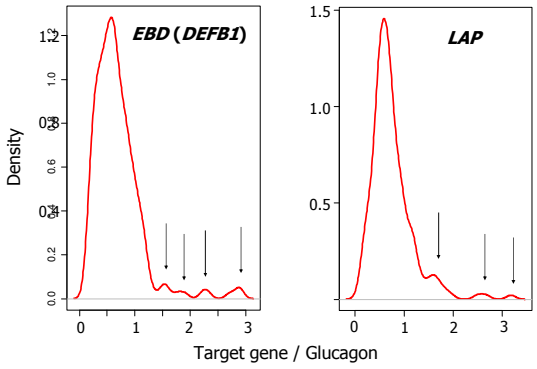

Figure 1. Distribution of the relative target gene copy number for all cows from the two groups (N=160). The arrows indicate local maxima possibly re-presenting groups of animals with differing copy numbers.

Results & Discussion

A t-test revealed no significant differences between the two extreme groups of German Holstein cows (p_{EBD}=0.1992, p_{LAP}=0.6466), but the results of the qRT-PCR for the two groups clearly indicate the existence of CNV for *LAP* and *EBD*. The distribution of the relative target gene copy number shows discrete local maxima possibly representing groups of animals with differing copy numbers (Fig. 1). The screening of different breeds revealed significant differences in relative copy number of *EBD* and *LAP* with F-test p-values of 1.05×10^{-7} and $2{,}29 \times 10^{-9}$, respectively. Especially the Angeln breed is significantly different from almost all other groups (Fig. 2).

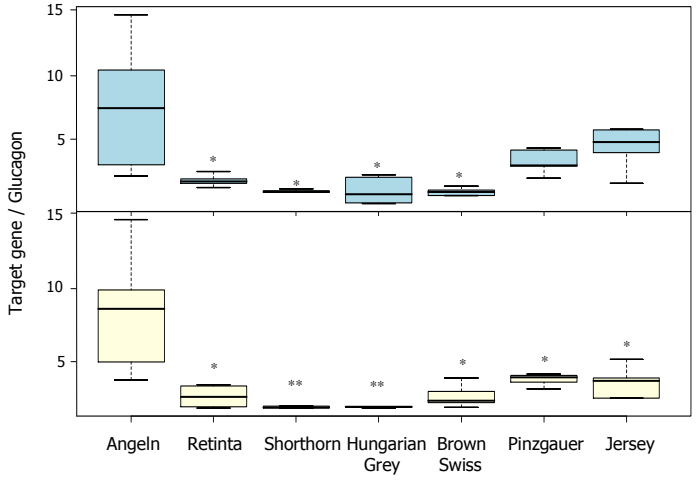

Figure 2. Differences in relative copy number between a panel of 7 breeds for *EBD* and *LAP*. The asterisks indicate the significance level of the difference between the Angeln breed and the other groups (*p<0.05, **p<0.01).

Conclusions

- Our results clearly indicate that detectable CNV of the bovine ß-defensin genes *EBD* and *LAP* exists within the German Holstein population as well as between different breeds.
- We did not find a relationship between the relative copy number of *EBD* and *LAP* and the estimated breeding value for mastitis resistance in German Holstein cows.

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