Contributed paper

GENOME-WIDE ANALYSES OF SCUR GENETICS IN CATTLE

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SUMMARY

This research has investigated genomic data (770K SNP genotypes) on 197 animals of Brahman, Droughtmaster and Hereford. Analyses of genome-wise association (GWAS) and composite selection signals (CSS) were conducted to find genomic regions underlying polledness (test) and scurs (discovery) in individual and combined (multi-breed) cohorts of horned, polled and scurred animals. Both GWAS and CSS successfully detected the poll-locus, whereas the GWAS results failed to localize any novel or previously proposed scur regions. CSS results coincided with 4 out of 7 previously detected regions as well as found several novel genomic regions. However, none of the significant regions harbour genes of profound effect on scur development. Overall, the results suggest that scur genetics has complex inheritance patterns and we discuss that many genetic factors and non-genetic effects interact variably to control development of scurs in cattle.

INTRODUCTION

Scurs are horn-like-structures, grow slower than horns and remain unattached from the cranium in cattle. Scurs appear in genetically heterozygous (HP) animals at the POLL locus – genomic region on bovine autosome (BTA) 1 associated with complete absence of horns and scurs called polledness (PP), which is caused by either Celtic (Pc) or Friesian (Pf) types of insertion-deletions (mutations). However, scurs are seen in a relatively smaller proportion of a cattle population, given that many heterozygous animals remain polled due to conditional factors, such as sex of the animal (Aldersey *et al.* 2020). Eradication of scurs in cattle is as vital as horns due to the related economic and welfare impact because scurred animals often undergo dehorning and certainly transmit a horn (H) allele to their offspring. Inheritance of scurs has been alleged to be under single gene control (White and Ibsen 1936). However, the "scur gene" in cattle remains to be discovered. To date, investigations to identify a causal gene have pointed to different genomic regions across different cattle breeds. A well-known region on BTA19 (26-29 Mbp of ARS-UCD1.2 bovine assembly location) was initially discovered in Canadian beef cattle and recently affirmed an epistatic interaction with the POLL locus (Asai *et al*. 2004; Ketel and Asai-Coakwell 2020). Those findings were not sustained in Hereford, Angus and French Charolais cattle (Capitan *et al*. 2009). A dominant inheritance pattern was later reported in the French Charolais and their proposed type-2 scurs development has been linked to *TWIST1* gene on BTA4 (Capitan *et al.* 2011). Notably, appearance of type-2 scurs was specific to heterozygous French Charolais and the underlying homozygous frameshift mutation has embryonic lethality. The scurs locus in Simmental cattle was mapped on BTA19 (48-49 Mbp), which is a different location from those reported earlier (Tetens *et al.* 2015). The study also pointed to a multi-locus involvement regarding the development of scurs. Recently, genic and non-genic regions on BTA5 (44-45 Mbp), BTA12 (7.5-8.5 Mbp), BTA16 (40-41 Mbp, *SUCO* gene) and BTA18 (46-47.5 Mbp, *ARHGAP33* gene) were found in a multi-locus association in Holstein (Gehrke *et al.* 2020). As yet, the reported studies have not converged and elucidation for inheritance patterns and genetic control of scur development is an ongoing task. Investigation of scurs in other breeds and by using high-density genotyping dataset can be valuable in understanding the scurs genetics. This study has performed the analyses of genome-wide association and signatures of selection to localize scur genomic regions within Brahman, Droughtmaster and Hereford breeds and in a multi-breed framework.

MATERIALS AND METHODS

A total of 197 animals of 3 breeds (Table 1) were sampled (tail-hair or blood), phenotyped (horned, polled and scurred), and diagnosed for the POLL genotype by the optimized poll testing (OPT) assay (Randhawa *et al.* 2020). Genomic DNA were used for Illumina BovineHD Genotyping BeedChip array. The 770K genotypes were quality control filtered to remove SNPs with MAF < 5% and call rate < 90%, and 657,543 SNPs were retained. Imputation of missing genotypes and haplotype phasing was performed with BEAGLE 3.3 (Browning and Browning 2009). The genome-wise association (GWAS) analyses were performed using the *qtscore* function (trait = "binomial") in R-package: GenABEL (Aulchenko *et al.* 2007). A significance threshold of $p < 1.0^{-7}$ was used to detect putative SNPs underlying the phenotypes. The composite selection signal (CSS) analyses were performed in R program for pairwise contrasting phenotypes (Randhawa et al. 2014). The smoothed CSS scores were used to capture the putative genomic regions using the top 0.1% threshold. Analyses of GWAS and CSS were conducted to find genomic regions underlying polledness (control: HH-horned vs PP-polled) and scurs (HP-scurred vs HP-polled) in individual and combined (multi-breed) cohorts (Table 1).

Table 1. Phenotyped and genotyped animals for genome-wide analyses

Breeds	Control (Horn vs Poll)		Discovery (Poll vs Scur)		Total
	Horned (HH)	**Polled** (PcPc)	**Polled** (HPc)	**Scurred** (HPc)	
Brahman	8	9	25	24	66
Droughtmaster	8	8	25	25	66
Hereford	8	8	24	25	65
Combined	**24**	**25**	**74**	**74**	**197**

HH: homozygous horned animals, PcPc: homozygous polled animals, HPc: heterozygous animals

RESULTS AND DISCUSSION

Analyses to localize POLL region (control) were successful by CSS for all datasets. However, GWAS showed sensitivity to sample size with significant peak for only combined data (Figure 1).

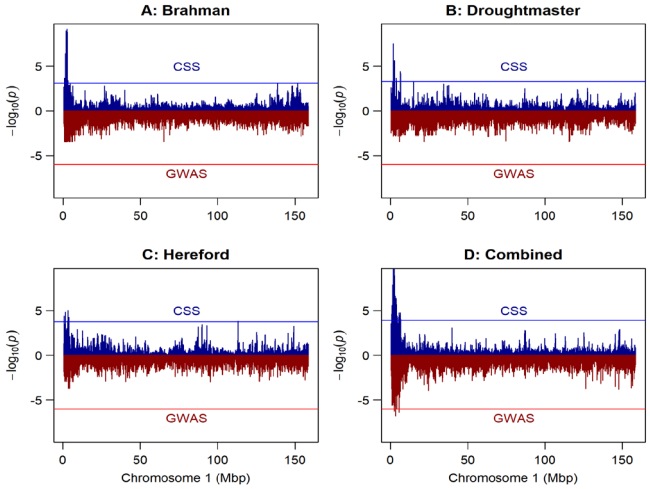

Figure 1. CSS (blue, positive) and GWAS (red, negative) results to detect the POLL region on chromosome 1 in A) Brahman, B) Droughtmaster, C) Hereford and D) Combined data. Red and blue lines show genome-wide significance at top 0.1% (CSS) and p=10^{-7} (GWAS), respectively

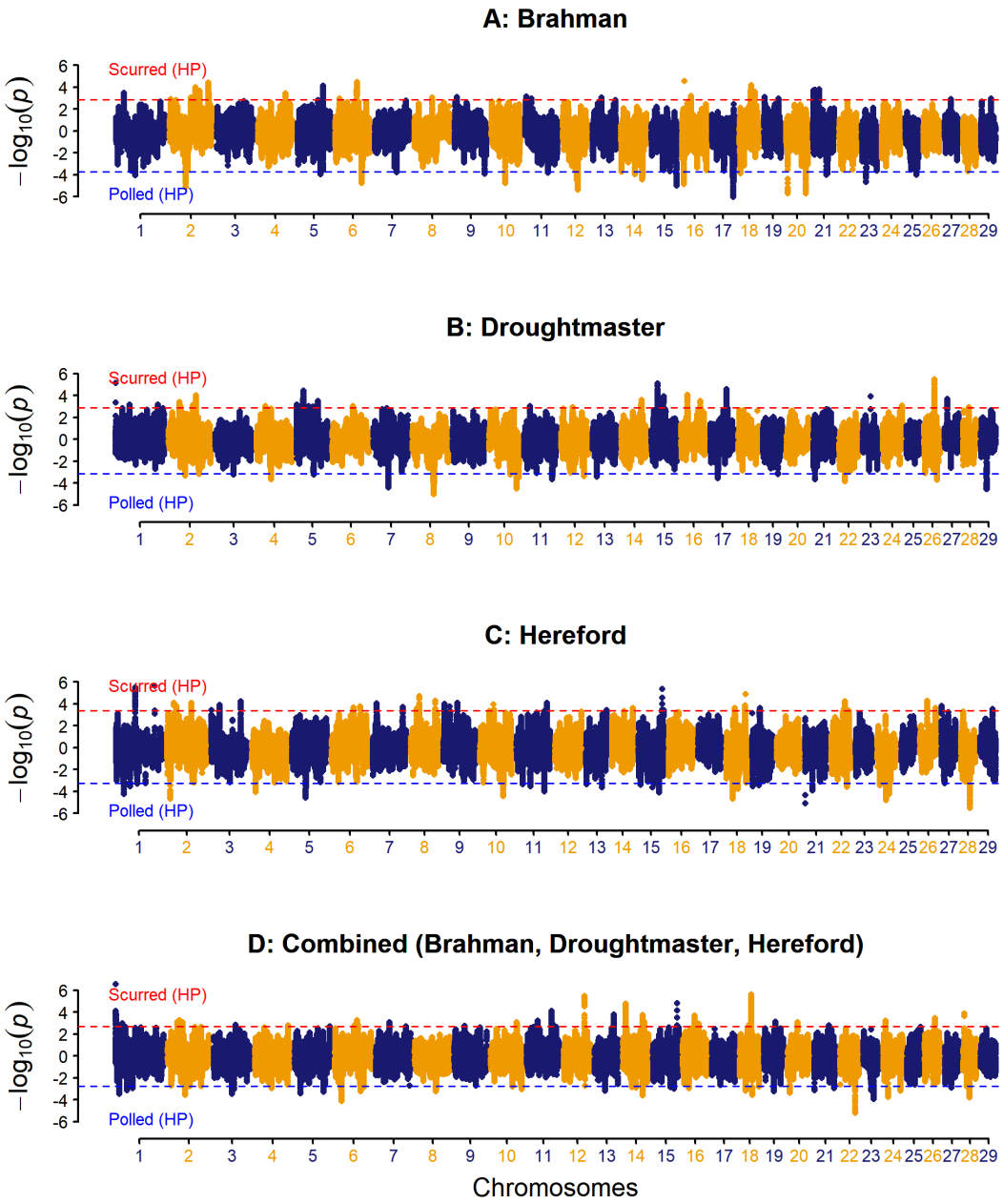

Figure 2. Manhattan plots of CSS results in breed-wise and combined data using 657,543 SNPs. Peaks above the red and below the blue dashed lines capture candidate regions for scurs and polledness, respectively at the significance thresholds (0.1%)

In the discovery analyses, CSS detected multiple regions within Brahman, Droughtmaster, Hereford and the combined dataset (Figure 2). However, GWAS analyses failed to reach above the significance thresholds, although many regions were found above a suggestive threshold of $-\log_{10}$

(p)10^{-5} (results not shown). Note that CSS found some genes – *SUCO* (BTA16) and *ARHGAP33* (BTA18) – and non-genic regions (BTA5, BTA19) that overlapped with previous research (Tetens *et al.* 2015; Gehrke *et al.* 2020). Multiple genomic regions identified by CSS (Figure 2) suggest that many genes interacting through complex polygenic networks may control scurs development. Hence, the finding does not support a simple mono-genic inheritance model as initially proposed by White and Ibsen (1936). However, the results are non-conclusive to identify regions or genes with strong association with the scurs phenotype. The GWAS analyses might have limited power for successful association mapping for the complex trait of scurs due to lower sample size. Therefore, further investigations by including higher sample sizes and improved phenotyping accuracy will increase the power of genomic association mapping of scur controlling variants. Nonetheless, other factors, such as POLL locus heterogeneity (Pc, Pf) and sex of the animal further complicate the understanding of scurs genetics (Gehrke *et al.* 2020), thus extensive research designs are suggested. Although scurs are substantially less common than horns (Randhawa *et al.* 2020), and are less damaging and easily manageable, they will continue to appear as the beef breeds transition from horned to polled cattle. With increased frequencies of polled alleles (Pc and Pf), and as more homozygous polled breeding stock become available, the incidence of scurs is expected to decrease.

CONCLUSIONS

Generally scurs develop in genetically heterozygous cattle, which carry an allele of either Celtic or Friesian mutations at the POLL locus. However, understanding of scurs genetics remains limited because it is unclear why some heterozygous animals remain polled. Interestingly, previous studies have associated 7 genomic regions on 6 chromosomes and none of them coincided in independent populations. This study has found 4 out of 7 previously detected regions as well as identifying several novel genomic regions, suggesting a polygenic inheritance model. None of the significant regions harbour genes of profound effect on scur development. Several factors including sex and mutation type have also been found to effect scur development, but the discovery of "scur genes" remains a challenge.

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