GENETIC MARKERS FOR POLLED CONDITION IN CATTLE – THE CURRENT STATUS AND THE FUTURE PLANS

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SUMMARY

Breeding polled cattle is a non-invasive alternative to the practice of dehorning. Horn status is presumably under the control of three genes i.e. polled, African horn, and scurs, especially in *Bos indicus* cattle. A research project was initiated with an aim to develop markers for determining the genetic status of genes underpinning the inheritance of horns. The results from an initial whole genome scan and a targeted fine scale mapping experiment led to the development of a validated molecular marker predicting the genetic status at the polled locus with a high degree of accuracy (>90%). Currently, accurate phenotypes of horn status are being recorded on the progeny generated on a collaborating Brahman breeding property. Future research plans involve whole genome scan of this resource population to understand scurs inheritance and to ascertain the existence of African horn gene.

INTRODUCTION

Horns in cattle increase the risk of injury to animal handlers. They also have an economic impact through carcass bruising and hide damage. Thus dehorning has become an accepted management practice to overcome the problems associated with horns. However, owing to the extensive nature of farming practices in northern Australia, dehorning occurs in older calves of 3.5 to 10 months of age (Prayaga 2007). This has animal welfare implications. Further, the practice of dehorning has economic costs to the beef enterprise such as the cost of labour of dehorning and the cost of secondary infection and mortality, albeit in rare instances. Therefore, breeding polled cattle is a non-invasive genetic selection option to replace the practice of dehorning.

The inheritance of horns is hypothesised to be relatively complex in *Bos indicus* animals with three interacting genes i.e. polled, African horn and scurs (small loose horns) genes. The polled locus has two alleles – an allele for poll (*P*) and an allele for horn (*p*). The gene for horn is recessive so that, in the absence of other interfering loci, homozygous individuals (*pp*) are horned and other genotypes (*PP*, *Pp*) are polled. The African horn gene (*Ha/ha*) is supposedly at a higher frequency in Zebu cattle and has a sex-influenced epistatic effect on the 'polled' locus so that male heterozygotes are horned and female heterozygotes are polled. A third gene called scurs controls the development of small and loosely attached horns. The scurs gene is also thought to be sex-influenced.

The polled gene has been mapped to the centromeric region of bovine chromosome 1 (BTA1) by several groups (Georges *et al.* 1993; Brenneman *et al.* 1996). Recently, Drögemüller *et al.* (2005) used twenty microsatellite markers generated from random sequencing of BAC subclones to positionally clone the *polled* locus in *Bos taurus* cattle and mapped the locus to a 1 Mb interval between microsatellite markers *RP42-218J17_MS1* and *BM6438* on BTA1. Asai *et al.* (2004) have mapped the scurs gene to BTA19; however, this has not been confirmed later. Importantly, the scurs phenotype can only be discerned in genetically polled animals (Prayaga 2007).

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A research project with an aim to develop genetic markers for polled, African horn and scurs genes was initiated to enable faster introgression of polled condition in tropical beef cattle. This paper reports the progress made in developing these markers and outlines future research.

MATERIALS AND METHODS

Whole genome scan. A group of unrelated Brahman (n=68) and Hereford (n=20) cattle were selected across various industry and research herds with a view to exploit linkage disequilibrium based association. The Hereford sample constituted 10 polled and 10 horned with equal number of males and females in each. However, sex was deliberately confounded with horn status in the Brahman sample to increase the power of detecting allele associations given the sex-influenced pattern of inheritance of scurs and African horn. Thus, the Brahman sample consisted of 33 polled males and 35 horned females. The DNA samples from this resource were genotyped with Affymetrix GeneChip® Bovine mapping 25K panel and Brahman specific 11.5K panel. In total, 34197 assays returned genotypes from both panels. SNP with minor allele frequencies less than 0.1 were omitted from analyses. Statistical analyses involved testing the chi-square statistic for significance given the observed and expected frequencies of three genotype classes for each SNP within each breed. Because of the prior knowledge, SNP located on BTA 1 only were included for analyses of Hereford data. Appropriate threshold p-values for significance testing for a false positive rate of 1% were derived to correct for the effect of multiple testing.

Fine mapping polled locus. A concurrent experiment to fine map the polled locus was also conducted on the same animals used for whole genome scan. This was conducted in two stages. Initially, 17 microsatellites described by Drögemüller *et al.* (2005) encompassing BTA1 region between 0.9 and 3.8 Mb were genotyped. With an additional designed marker, this yielded data from 18 microsatellites for the haplotype analysis. Based on the initial results, a further 15 highly polymorphic microsatellite markers were genotyped in the region in the second stage of the experiment. These markers were identified by feeding the identified genomic sequence into Sputnik sequence annotation pipeline (http://mips.gsf.de/proj/sputnik/) and embedded within the Bovine Genome Browser of CSIRO. Genotype scoring was performed using Genemapper™ ver4.0 from Applied Biosystems. Finally, genotype data from 39 SNP (from whole genome scan) and 33 microsatellites from the above-mentioned region were available for analyses. Haplotype reconstruction was carried out using PHASE ver2.1 (Stephens *et al.* 2001; Li and Stephens 2003) that uses Bayesian method to impute haplotypes. In addition to deriving hapolotype frequencies and recombination rates, a permutation test was performed to determine the significance of differences in haplotype frequencies between case (polled) and control (horned) groups.

RESULTS AND DISCUSSION

Whole genome scan. One SNP located on BTA1 (6.3Mb) was in complete agreement with the assumed Mendelian inheritance at polled locus in Herefords (P-value=0.0001), but not in Brahmans (P-value=0.05). However, this SNP is outside the region of interest on BTA 1 based on earlier reports. Further, this SNP was later found to be either tri allelic or an artefact. In Brahmans, 8 out of 12 most significant SNP (P-value<0.0001) were located on BTA1 between 1.1 and 20.8 Mb, with some of them outside the reported region of polled locus in *Bos taurus* animals. The other significant SNP were on chromosomes 9, 10 and 29. The inability to identify a number of significant SNP within the reported polled region could be due to the insufficient number of animals, especially in Herefords, and relatively lower density of markers in the region of interest.

Fine mapping polled locus. The aim of this study was to fine-map the polled locus in Brahman by increasing the power of detection through combining SNP information from the whole genome scan study with microsatellite genotyping. The differences in haplotype frequencies between polled and horned groups were significant (P-value=0.01) in breed specific datasets and in the combined data. This supports the alternate hypothesis that the imputed polled haplotypes were more similar to other polled haplotypes than to the imputed horned haplotypes.

The PHASE analyses generated 84 and 551 haplotypes for Hereford and Brahman respectively. These haplotypes represent those with the estimated frequency greater than 1% when merging segments of consecutive loci through its partition ligation method. In the Hereford sample, out of 84 haplotypes, 76% were unique to polled and 21% were unique to horned. In the Brahman sample, out of 551 haplotypes, the observed frequencies were complementary to those observed in Hereford: 25% were unique to polled and 73% were unique to horned. This distinct difference between polled and horned haplotype frequencies in both breeds further points to the presence of polled gene in this region. The background recombination rate (population parameter, r), determined as the median estimate over 100 runs in Hereford and Brahman data, was estimated to be 4.25×10^{-6} and 4.04×10^{-5} respectively. The breed specific estimates of factors by which the recombination rate between adjacent markers (i and i-1) exceeds r is presented in Figure 1 and this depicts the propensity of certain regions as recombination hotspots. The number of markers and the number of individuals in each dataset influence these estimates.

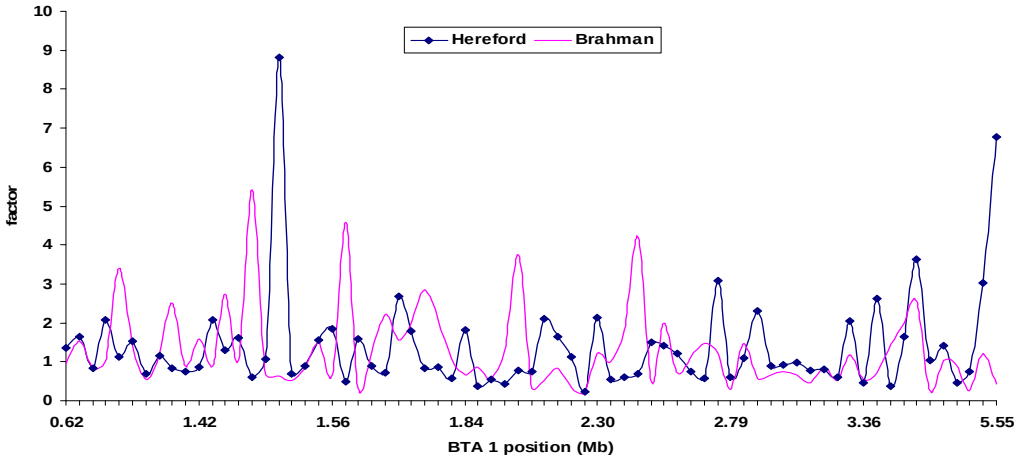

Fig 1. Estimates of variation from background recombination rate within each adjacent marker interval in Hereford and Brahman cattle

Further examination of the imputed haplotypes revealed that there is a complete association between an allele at *CSAFG29*, a newly designed microsatellite marker, and the polled condition. This particular allele is not observed in horned animals. The actual and the marker predicted horn status of the experimental population is presented in Table 1. This marker predicted all polled animals to be polled and with the exception of two animals, all horned animals to be horned. The two animals that deviated from expectation could be those with scurs and misclassified as horned at the time of dehorning. This misclassification could occur because of ambiguity associated with horn phenotype early in life.

Table 1. The actual horn status and *CSAFG29* predicted horn status of the experimental population

Breed	Actual	Predicted			Total
		Polled 'PP'	*Polled 'Pp'*	*Horned 'pp'*	
Brahman	Polled	17	16		33
	Horned		2*	33	35
Hereford	Polled	5	5		10
	Horned			10	10
Total		22	23	43	88

*deviation from expectation of zero, may be animals with scurs recorded as horned

CONCLUSIONS AND FUTURE PLANS

Breeding polled cattle could be achieved at a faster rate if the genetic status of the genes underpinning horn status is known. While the polled gene itself is elusive, markers in close association with the gene are identified. The current study proposes the use of a microsatellite marker for determining the genetic status at the polled locus even in *Bos indicus* cattle. Currently, around 1000 samples are being collected from the major Australian breeds with special focus on tropical breeds in a bid to validate this marker in the field. Efforts to develop markers for scurs and African horn are also underway. A Breeding program was conducted in 2007 at a collaborating property, Hillgrove Pastoral Company, to develop a Brahman resource population derived from mating 15 polled bulls with around 400 polled, horned and scurred heifers. Currently, around 230 calves are being regularly mustered for determining their accurate horn status phenotypes. In the coming months, this population will become the resource for the forthcoming whole genome association study. This research will enable us in identifying markers for scurs locus and in determining the presence or absence of African horn gene.

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