

SHORT COMMUNICATION

Detection of selection signatures based on the integrated haplotype score in Chinese Jinnan cattle

Yuanqing Zhang, Dong Chang He, Jing Cheng, Fang Xu, Bo Li, Guang Jin, Xi Zhong Zhang

Institute of Animal Husbandry and Veterinary Science, Shanxi Academy of Agricultural Science, Taiyuan 030032, P. R. China

ABSTRACT

Jinnan cattle, one of the five major breeds of Chinese yellow cattle, are the traditional draft and beef breed in southern Shanxi province, China. Our study aimed to search genes that will allow the selection of important economic traits through selection signature detection, which can help in discovering the action mechanism of positive selection in the variety breeding process of Jinnan cattle.

Keywords: Breeding; Integrated haplotype score; Jinnan cattle; Selection signature

BACKGROUND

Both natural and artificial selection have resulted in cattle breeds that are specialized for particular uses. Several specific genomic regions have been under selection pressure, and it is widely accepted that these genomic sequences, or selection signatures, control or affect key phenotypes that are involved in some traits. Positive selection pressure can reduce or even eliminate the frequency of negative alleles in the offspring's genome (Biswas and Akey, 2006). Thus, continuous selection pressure over a few generations may result in linkage disequilibrium (LD) patterns between mutations and neighboring loci (Sabeti et al., 2002). Therefore, selection signatures in a population could provide genomic information to facilitate selection and provide insights into evolutionary history (Akey et al., 2002; Willing et al., 2010).

The selection signatures are detected by different methods, such as McDonald and Kreitman test (McDonald and Kreitman, 1991), H-statistic (Fay and Wu, 2000), integrated haplotype score (iHS) (Voight et al., 2006a), and extended haplotype homozygosity (EHH) test (Sabeti et al., 2002). The study found that the EHH test can detect signatures of positive selection in a population between the allele frequency and LD patterns with neighboring alleles (Barendse et al., 2009). EHH is defined as the probability that two randomly chosen chromosomes carrying the

aim core haplotype are identical by descent for the entire interval between a certain locus and the core region. As an extension of the EHH, iHS compares EHH between ancestral and derived alleles within a population. Thus, iHS test can avoid the influence of heterogeneous recombination rates across the genome, and the iHS method performs best when a selected allele segregates at intermediate frequencies in the population.

Recently, several selection signatures have been detected in various independent cattle populations or breeds (Pan et al., 2013; Qanbari et al., 2011; Qanbari et al., 2010; Xu et al., 2015). Chinese Jinnan cattle, an independent population of the major Chinese yellow cattle, is a traditional draft and beef breed in southern Shanxi province, China (An et al., 2005). In this study, we detected iHS selection signatures and explored their potential functional genes in Chinese Jinnan breeds using high-density single nucleotide polymorphism (SNP) genotyping data.

Jinnan is one of the five excellent local cattle breeds in China. Jinnan cattle show the better characteristics such as stronger physique, muscular, heat resistance, and crude feed tolerance. With the transformation of the agricultural industry structure and the introduction of foreign cattle breed, Jinnan cattle faced a severe challenge. The number of Jinnan cattle sharply declined, and the frequency of

*Corresponding author:

Jing Cheng, Institute of Animal Husbandry and Veterinary Science, Shanxi Academy of Agricultural Science, Taiyuan 030032, P. R. China.
Phone/Fax: (+86 351) 7561076, E-mail: chengjing1104@163.com

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purebreds decreased. This study elucidated the selection mechanisms and breeding value of the Jinnan breed.

MATERIALS AND METHODS

Animals and genotypes

All cattle used in this study were healthy juvenile Jinnan cattle weighted 300 ± 30 kg, obtained from Jinnan cattle elite reservation farm. The animals were raised in compliance with the International Guiding Principles for Biomedical Research Involving Animals (<http://www.cioms.ch/frame.1985.texts.of.guidelines.htm>), housed under room temperature with good ventilation hygienic conditions, and fed ad libitum.

The study population comprised 65 Chinese Jinnan cattle with no genetic relationships. Ear tissues were sampled and snapped frozen in liquid nitrogen for further use. Total DNA was isolated from ear tissues using DNAiso Reagent (Japan, Dalian, China) according to the manufacturer's protocol.

An Illumina Bovine SNP50 bead chip assay (San Diego, CA, USA) and Genome Studio were used for genotyping. PLINK was employed for quality control, where only SNPs located on autosomal chromosomes in the UMD3.1 genome were selected for autozygosity determination, and marker scores with a minor allele frequency of $<5\%$ were analyzed. After quality control, 39,814 SNPs from 48 individuals had a genotype call rate of $\geq 95\%$.

iHS test

The iHS method depends on information regarding the status of the ancestral and derived alleles for each SNP. The ancestral alleles of all bovine SNPs were established before computing iHS (Rocha *et al.*, 2014) (http://genome.jouy.inra.fr/downloads/Bovine_Ancestral_Allele/). The R package 'rehh' was used to compute iHS for each autosomal SNP (Gautier and Vitalis, 2012). Single-site iHS values were computed across the genome for each breed and averaged within nonoverlapping windows of 500 kb. The window size employed was based on the extent of LD, as described previously (Gautier and Naves, 2011). Standardized iHS was calculated as follows:

$$iHS = 1n \left(\frac{iHHA}{iHHD} \right)$$

where iHHA and iHHD represent the integrated EHH scores for the ancestral and derived core alleles, respectively. The iHS values were standardized to ensure that they followed a standard normal distribution (Voight *et al.*, 2006b).

Bioinformatics analyses

Gene annotation was performed by exploring the UMD3.1 locations of genes via NCBI database (ftp://ftp.ncbi.nih.gov/genomes/Bos_taurus/mapview/seq_gene.md.gz). BioMart (www.ensembl.org/biomart) was used to determine the orthologous human gene ID of each gene detected. Enrichment analysis of these genes was performed using DAVID 6.7 by aligning the detected genes with human genes (Huang *et al.*, 2009). Functional annotations, i.e., Gene Ontology (GO) biological process, GO cellular component, and GO molecular function, were assigned to genes using the functional annotation tool.

RESULTS

iHS tests

The selection signatures in Jinnan cattle were detected on the basis of 39,814 SNPs that covered the bovine UMD3.1 genome, with a mean distance of 6.28 kb between adjacent SNPs. The mean distance between adjacent SNPs per chromosome ranged from 5.74 kb on chromosome 25 to 7.36 kb on chromosome 5. The iHS method identified 19 significant regions in the genome of Chinese Jinnan cattle (Table 1). Genome-wide distribution of the iHS values was used to visualize the chromosomal distribution of selection signatures (Fig. 1). Throughout the entire genome, the lowest iHS, -5.88 , was in chromosome 13 and the highest, 4.84, was in chromosome 4.

Annotation of candidate regions

Annotation of genomic regions containing selection signatures detected several candidate genes, and those in

Table 1: Genomic regions with significant integrated haplotype scores (iHS) in Chinese Jinnan breeds

Genomic region (kb)	Mean iHS value
Chr1: 73500001-74000000	2.24
Chr1: 9700001-10100000	2.04
Chr5: 21500001-22000000	2.01
Chr5: 56000001-56500000	2.56
Chr7: 56500001-57000000	2.01
Chr8: 31500001-32000000	2.05
Chr8: 37000001-37500000	2.13
Chr8: 40500001-41000000	2.14
Chr13: 48500001-49000000	2.11
Chr14: 20000001-20500000	2.06
Chr14: 21000001-21500000	2.33
Chr14: 21500001-22000000	2.12
Chr14: 22500001-23000000	2.83
Chr14: 23000001-23500000	2.31
Chr14: 26000001-26500000	2.07
Chr14: 27500001-28000000	2.16
Chr14: 28000001-28500000	2.11
Chr14: 29500001-30000000	2.28
Chr27: 1000001-1500000	2.03

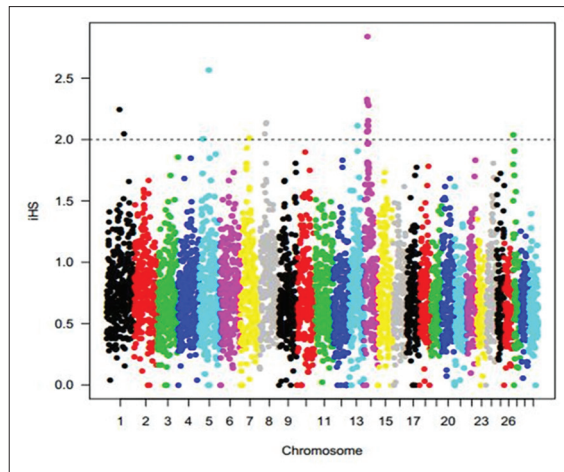


Fig 1. Average integrated haplotype score for each 500-KB window.

significant iHS genomic regions in Jinnan cattle comprised 61 candidate genes (Additional file 1). Moreover, some of our candidate genes were previously detected on the basis of quantitative trait loci (QTL) and genome-wide association study (GWAS) analyses of cattle populations.

Bioinformatics analysis

To clarify the molecular functions of these genes, we examined their GO classifications. The candidate genes detected in Jinnan cattle were enriched in several significant GO terms (Additional file 2), particularly the HES1, CDK4, and GLI1 genes.

DISCUSSION

iHS tests for several commercial dairy and beef breeds, including Angus, Belgian Blue, Charolais, Hereford, Holstein–Friesian, Limousin, and Simmental cattle, have identified more than 80 significant selection signature regions (Zhao *et al.*, 2015). The iHS tests for Pinzgau cattle, which is a local breed of domestic cattle from the Pinzgau region of the federal state of Salzburg in Austria, showed that only seven regions had an iHS of >1.7 (Kasarda *et al.*, 2015). This suggests that these commercial breeds have undergone stronger and more intensive selection pressure than Chinese Jinnan breed.

Several genes have also been reported on the basis of iHS tests of other commercial dairy and beef breeds (Qanbari *et al.*, 2011; Zhao *et al.*, 2015), such as carbonic anhydrase VIII (CA8) and Ras-related protein Rab-2A (RAB2A) in both Limousin and Charolais; protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 1 (PCMTD1), family with sequence similarity 110: member B, UBX domain protein 2B, cytochrome P450: family 7: subfamily A: polypeptide 1, and syndecan binding protein in Limousin; and minichromosome maintenance complex

component 8, cardiolipin synthase 1, and ferritin family member 1 in Holstein–Friesian, which is a dairy breed. None of these genes overlapped with the candidate genes identified in the Angus, Belgian Blue, Hereford or Simmental breeds using iHS tests.

In previous studies, several of the genes identified by the iHS tests were suggested to be associated with health, meat, milk production, and reproductive traits (Additional file 3). Interestingly, the results showed that 47 of 61 candidate genes located in QTL regions were associated with meat and carcass traits, and 11 of 61 candidate genes were reported to be associated with meat and carcass traits on the basis of GWAS data. All these 11 genes were present in the 47 candidate genes in QTL regions associated with meat and carcass traits, including chromosome 8 open reading frame 22, CA8, gamma-glutamyl hydrolase, neuropeptides B/W receptor 1, opioid receptor, kappa 1, PCMTD1, RAB2A, RB1-inducible coiled-coil 1, tocopherol (alpha) transfer protein, leucine rich adaptor protein 1-like, and tyrosinase-related protein-1 (Doran *et al.*, 2014; McClure *et al.*, 2012; Nalaila *et al.*, 2012; Nishimura *et al.*, 2012; Saatchi *et al.*, 2014; Veneroni-Gouveia *et al.*, 2012). These results suggested that the Jinnan breed has high genetic potential for beef cattle breeding.

People have documented the association of these genes with the regulation of muscle cell proliferation on the basis of *in vitro* experiments (Korzelius *et al.*, 2011; Lazaro *et al.*, 2002; Liu *et al.*, 2014; von Baer *et al.*, 2014; von Grabowiecki *et al.*, 2015). Thus, these results provide further evidence that the Jinnan breed is an important resource with unique features for beef cattle breeding.

CONCLUSIONS

A genome-wide scan based on iHS statistics identified several regions that have been affected by selection in Chinese Jinnan cattle. Among 39,814 SNPs, we estimated positive selection signatures using the iHS test, which showed that the breeding history of Chinese Jinnan cattle had been subject to low positive selection compared with that undergone by commercial dairy and beef breeds. Annotation of the genome regions with selection signatures detected 61 candidate genes, including 11 interesting genes annotated in significant regions that overlapped with both QTL and GWAS data associated with meat traits in cattle populations. GO analysis found that the selection signatures were related to the positive regulation of muscle cell proliferation. These results confirm the importance of Chinese Jinnan cattle as a genetic resource for beef cattle breeding and the need to preserve populations.

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Authors' contributions

YZ, DH and JC conceived of the study, carried out the molecular biological studies and drafted the manuscript. BL and GJ participated in the sequence analysis. JC and FX performed the statistical analysis. YZ, DH and XZ coordinated and helped to complete the experience. All authors read and approved the final manuscript.

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Additional files

Additional file 1: Table S1. Genes associated with significant integrated haplotype scores in the Chinese Jinnan breed.

Additional file 2: Table S2. Enriched Gene Ontology terms for genes in regions under selection in Chinese Jinnan cattle.

Additional file 3: Table S3. Regions under selection in Chinese Jinnan cattle compared with quantitative trait loci and genome-wide association study data.

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