Fat Cows - Selection Signatures in Korean Hanwoo and Australian Wagyu cattle

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ABSTRACT: Genotypic data of 700k markers for five beef cattle breeds were analyzed in regards to their allele frequencies based on their fixation index (F_{ST}). The two breeds of interest were the Korean Hanwoo and the Australian Wagyu which are known to have high intramuscular fat content. F_{ST} values were compared to Angus, Holstein, and Chinese Yeonbyun. We found 27 and 24 breed specific genome regions for the Hanwoo and Wagyu, respectively. These regions differed significantly between the five breeds, indicating selection pressure on these regions. We narrowed down 18 and 14 regions that were unique to the Hanwoo and Wagyu, respectively. Of these, six regions in the Hanwoo and three regions in the Wagyu harbor potential candidate genes involved either directly in fat metabolism and synthesis or indirectly in blood vessel development.

Keywords: beef cattle; intramuscular fat; selective sweep

Introduction

Admixture of domesticated cattle and selective sweeps have been the focus of many studies, some of which included as many as 48 cattle breeds from across the world (Decker et al. (2009)). Most of these studies focused on the general differences between groups of origin based on the concept that the different breeds not only physically diverged but also genetically accumulated different genome signatures due to natural selection. In this study, we focus on selected regions in the Korean Hanwoo and Australian Wagyu cattle (originally from Japan). These two breeds are renowned for their high intramuscular fat content (IMF) and good marbling qualities with low subcutaneous fat deposition even in comparison to other high meat quality beef breeds such as the Angus (Jo et al. (2012); Radunz et al. (2009)). Intramuscular fat is an important trait for meat quality and a good marbling structure is favored by consumers. However, IMF is a trait that currently can only be accurately measured after slaughter and is thus difficult to directly select for or even identify the underlying genomic regions.

A simple approach to identify potential regions under selection pressure is to compare differences between breeds in the fixation index based on allele frequencies (F_{ST} values). High F_{ST} values indicate regions that have a reduced genetic variability that might be a result of positive selection. Thus, comparing a genome of interest with a genome that has been under a different selection pressure should help to narrow down regions underlying unique phenotypes. Based on this concept, we compared the genome of Hanwoo and Wagyu cattle with the genomes of European beef and dairy cattle as well as with a related but unselected population of Chinese Yeonbyun cattle.

Materials and Methods

Animals. Genetic information of 892 Korean Hanwoo and 99 Australian Wagyu cattle were available. For comparison, 108 Australian Angus, 918 Holsteins, and 24 Chinese Yeonbyun cattle were used.

Genotypes. All animals were genotyped with the 700K BovineHD BeadChip (Illumina Inc., San Diego, CA, USA). In a breed-wise quality control, markers and animals with a call rate <95%, markers with a total or median gencall score under 0.6 in more than 10% of the samples, markers with a heterozygosity that deviated more than three standard deviations from other markers, markers out of Hardy-Weinberg equilibrium for a cut-off *P*-value of 1^{-15}, unmapped markers, and markers on the sex chromosomes were excluded from the analysis. Individual gencall scores under 0.6 were treated as missing. Finally, 309,541 markers that passed quality control in all five breeds were used in the study.

Data Analysis. (1) Based on the genomic relationship matrix, we explored the relationships among animals between and within the five populations. In order to assure an unbiased data for the comparison between breeds, we randomly selected 24 animals of each breed. **(2)** We calculated F_{ST} values based on allele frequencies according to Weir and Cockerham (1984). The F_{ST} values were smoothed to a point over 500 markers using the Haerdle-Steiger algorithm as implemented in the R function '*runmed*' (Haerdle and Steiger (1995)). An average F_{ST} value that differed more than three times the standard deviation from the population mean was considered as a genomic region putatively under selection pressure. Identified markers were combined into a single region if they were within 0.5 Mb of each other. **(3)** Lastly, we looked for potential candidate genes for meat traits, in particular IMF, within a 1 Mb window up- and downstream of each marker that was within the identified regions.

Results and Discussion

(1) All animals clearly clustered in their respective breeds. The two European breeds separated from the East-Asian breeds. As expected from previous reports, the Korean Hanwoo and Chinese Yeonbyun were more closely related to each other (showing some overlap) than to the Wagyu cattle (Figure 1). Besides the expected separation between the East-Asian and the European breeds, we also found that the Wagyu seemed to be closer to the Angus, and the Hanwoo/Yeonbyun closer to the Holstein (Figure 1).
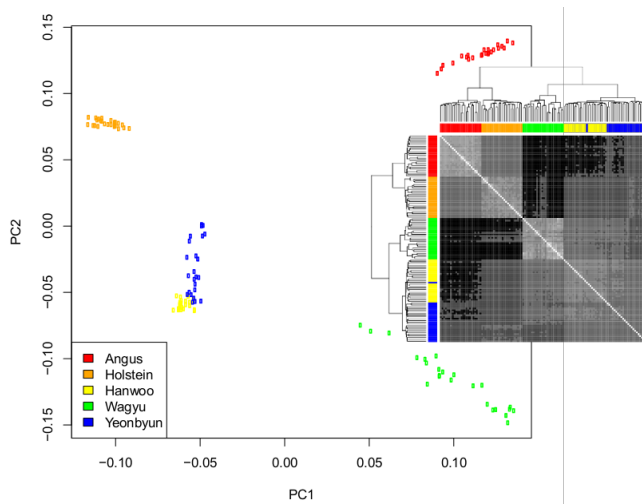
Figure 1. Singular value decomposition of five cattle breeds based on genome-wide SNPs

However, because the Hanwoo and Yeonbyun are so closely related, there is some bias in the estimation of the principal components due to the higher animal number in the combined Hanwoo/Yeonbyun group. Analysing all available data showed that the three East-Asian breeds clustered tightly together, equidistant from European beef and dairy cattle.

(2) Several regions with high F_{ST} values were found for all five cattle breeds. For the Hanwoo cattle, we identified 27 regions over 15 chromosomes, and for the Wagyu, we found 24 regions over 10 chromosomes that exceeded the three standard deviation threshold (Figure 2). Fifteen identified regions overlapped between at least two breeds. Overlapping regions between the Hanwoo and the Wagyu were found on chromosomes 11, 12, 20, and 22, between Hanwoo and Angus on chromosome 7, between Hanwoo and Holstein on chromosomes 12, 20, and 22, and between Hanwoo and Yeonbyun on chromosomes 5, 7, and

14 (Figure 2). Regions in close proximity to our areas on chromosome 2, 7, 20, and 22 were reported previously to be under positive selection in the Hanwoo (Lee et al. (2013); Lim et al. (2013); Porto-Neto et al. (2012)). Overlapping regions between Wagyu and Angus were found on chromosomes 1, and 16, between Wagyu and Holsteins on chromosomes 1, 12, and 20, and between Wagyu and Yeonbyun on chromosome 12, and 16 (Figure 2). Taking three standard deviations across the whole genome as a threshold showed that the F_{ST} values for the identified regions were significantly different (*P*<0.05) between all breeds apart for Wagyu and Angus (*P*=0.07), indicating a similar genome-wide selection pressure for these two breeds. However, comparing the identified regions on a chromosome-wide level showed that all regions identified for the Wagyu were significantly different from the regions in the Angus. Nevertheless, several identified regions in the Hanwoo were similar in their F_{ST} values to some other breeds: Hanwoo and Wagyu had similar values for a region on chromosome 2 (110-110.5 Mb; *P*=0.40) and chromosome 15 (0.7-1.3 Mb; *P*=0.73); Hanwoo and Holstein had similar F_{ST} values for a region on chromosome 2 (84.9-85 Mb; *P*=0.66). Therefore, some specific regions shared between Hanwoo, Holstein and Wagyu cattle seem to be under selection pressure, whether for the same reason (e.g. selection for the same trait) or not has to be further clarified.

To identify regions that affect high IMF, we focused on regions that were unique for the Hanwoo and the Wagyu population, and in particular those regions that overlapped in both breeds. This left 19 unique regions for the Hanwoo and 14 unique regions for the Wagyu (Figure 2). One region on chromosome 11 (40-41.3 Mb), and on chromosome 22 (23.4-26.4 Mb) overlapped between the Hanwoo and Wagyu population (Figure 2).

(3) Within the unique regions, five markers were found for Hanwoo and three markers for Wagyu that were located within protein-coding genes. Of these genes, the *fat*
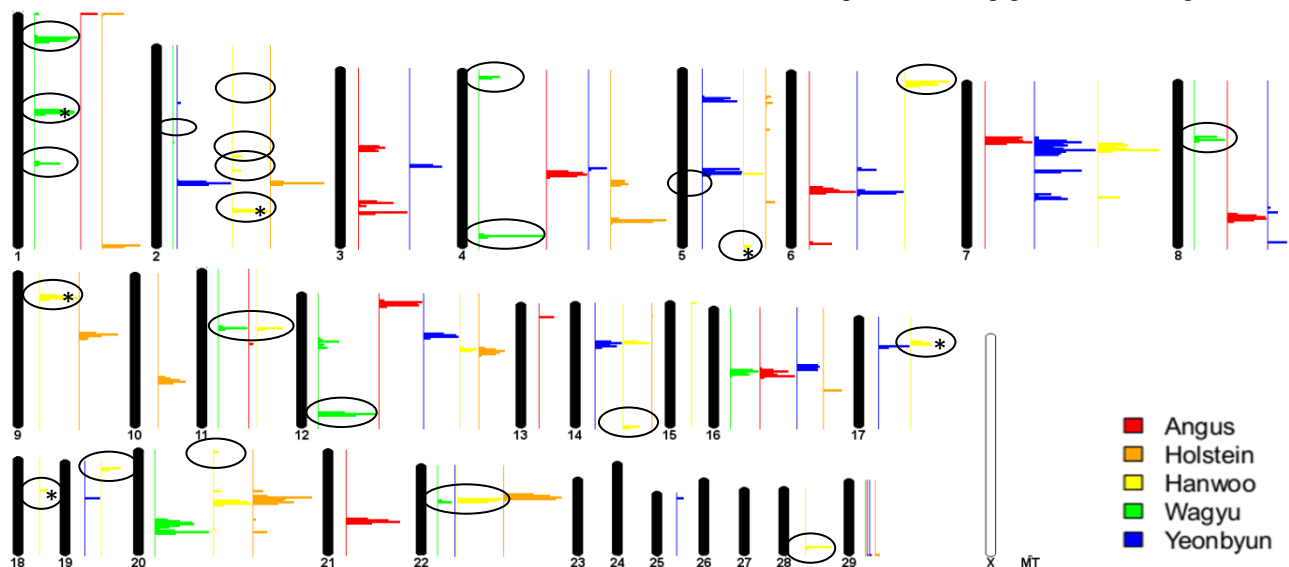

Figure 2. Genome-wide regions of F_{ST} -values across 29 bovine autosomes that were larger than three standard deviations within their breed with marker densities per region.
Circles: unique regions for the Hanwoo and Wagyu breed; * position of potential candidate genes for intramuscular fat.

mass and obesity associated (*FTO*) gene on chromosome 18 has been discussed in cattle genetics, and associated with milk fat in dairy cows (Zielke et al. (2013)). Other genes associated with fat synthesis but also with blood vessel size are listed in Table 1. The genes for blood vessel size have been added, despite the regional overlap with the Angus breed, because IMF is more likely to develop in red muscles (Hocquette et al. (2010)). Red muscles are characterized by a dense capillary network supporting their oxidative activity. Blood vessels infuse the muscle in-between the muscle fiber bundles which is also where IMF accumulates (Harper and Pethick (2004)). Thus, a well-developed capillary network and potentially blood vessel size might influence the development of IMF.

Table 1. Potential genes for intramuscular fat for unique regions identified in Hanwoo and Wagyu cattle.

Chr. (Mb)	Gene name	Function/Citation
Hanwoo		
2 (111.6)	*MOGAT1* (*monoacylglycerol O-acyltransferase 1*)	Winter et al. (2003) di- and triglyceride biosynthetic process
2 (111.7)	*ACSL3* (*acyl-CoA synthetase long-chain family member 3*)	adipocytokine signaling pathway (KEGG) fatty acid biosynthesis and degradation
9 (19.8)	*ELOVL4* (*ELOVL fatty acid elongase 4*)	fatty acid biosynthesis and elongation (KEGG)
17 (21.6)	*CHD9* (*chromodomain helicase DNA binding protein 9*)	fatty acid, triacyl-glycerol, and ketone body metabolism (REACTOME)
18 (21.8)	*RBL2* (*retinoblastoma-like 2*)	adipogenesis (WikiPathways)
18 (22.1)	*FTO* (*fat mass and obesity associated*)	Zielke et al. (2013)
Wagyu		
1 (69.9)	*HGD* (*homogenitsate 1,2-diocygenase*)	Zhou et al. (2010)
1 (68.4)	*PTPLB* (*protein tyrosine phosphatase-like, member b*)	fatty acid biosynthesis, elongation, metabolism (KEGG)
16 (42.7)	*NPPB* and *A* (*natriuretic peptide B*)	blood vessel size (GOA)

Conclusion

Results show that there are genome-wide regions with allele frequencies that indicate selection pressure. Whether or not this pressure is due to artificial or natural selection remains unclear, as the five cattle breeds were evolved in different environments. We were able to narrow down unique regions in the high marbling Hanwoo and Wagyu cattle that potentially harbor genes involved in IMF development by excluding regions that overlapped with other breeds. Including further beef breeds with low IMF should yield even better results, as we might be able to exclude further overlapping regions for meat and identify more regions for fat. Several genes were found that are known to be involved either directly in fat synthesis or indirectly in blood vessel development, which should be further investigated in regards to their sequence variations and associated trait effects.

Literature Cited

Decker, J. E., Pires, J. C., Conant, G. C., et al. (2009). Proc. Natl. Acad. Sci. USA. 106:18644-18649.

Haerdle, W., and Steiger, W. (1995). J. R. Stat. Soc. Ser. C. Appl. Stat. 44:258-268.

Harper, G. S., and Pethick, D. W. (2004). Aust. J. Exp. Agric. 44:653-662.

Hocquette, J. F., Gondret, F., Baeza, E., et al. (2010). Animal. 4:303-319.

Jo, C., Cho, S. H., Chang, J., et al. (2012). Anim. Front. 2: 32-38.

Lee, T., Cho, S., Seo, K. S., et al. (2013). BMB Rep. 46: 346-351.

Lim, D., Gondro, C., Park, H. S., et al. (2013). Asian-Aust. J. Anim. Sci. 26:603-608.

Porto-Neto, L. R., Lee, S.-H., Sonstegard, T., et al. (2014). Anim. Genet. doi:10.1111/age.12119.

Radunz, A. E., Loerch, S. C., Lowe, G. D., et al. (2009). J Anim. Sci. 87:2971-2976.

Weir, B. S., and Cockerham, C. C. (1984). Evolution. 38: 1358-1370.

Winter, A., van Eckeveld, M., Bininda-Emonds, O. R., et al. (2003). Cytogenet. Genome Res. 102:42-47.

Zhou, G. L., Cao, Y., Li, M., et al. (2010). Meat Sci. 85: 270-273.

Zielke, L. G., Bortfeldt, R. H., Reissmann, M., et al. (2013). PLoS One 8:e63406.