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A Genome Wide Association Study on Age at First Calving Using High Density Single Nucleotide Polymorphism Chips in Hanwoo (*Bos taurus coreanae*)

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ABSTRACT: Age at first calving is an important trait for achieving earlier reproductive performance. To detect quantitative trait loci (QTL) for reproductive traits, a genome wide association study was conducted on the 96 Hanwoo cows that were born between 2008 and 2010 from 13 sires in a local farm (Juk-Am Hanwoo farm, Suncheon, Korea) and genotyped with the Illumina 50K bovine single nucleotide polymorphism (SNP) chips. Phenotypes were regressed on additive and dominance effects for each SNP using a simple linear regression model after the effects of birth-year-month and polygenes were considered. A forward regression procedure was applied to determine the best set of SNPs for age at first calving. A total of 15 QTL were detected at the comparison-wise 0.001 level. Two QTL with strong statistical evidence were found at 128.9 Mb and 111.1 Mb on bovine chromosomes (BTA) 2 and 7, respectively, each of which accounted for 22% of the phenotypic variance. Also, five significant SNPs were detected on BTAs 10, 16, 20, 26, and 29. Multiple QTL were found on BTAs 1, 2, 7, and 14. The significant QTLs may be applied via marker assisted selection to increase rate of genetic gain for the trait, after validation tests in other Hanwoo cow populations. (**Key Words:** First Calving, Hanwoo, Quantitative Trait Loci [QTL], Single Nucleotide Polymorphism [SNP], Whole Genome Association [WGA])

INTRODUCTION

Reproductive performance is a good determinant of productivity and sustainability in farm animals (Olsen et al., 2011). The period that a cow needs to reach its maturity and to reproduce for the first time is referred to as age at first calving (van Raden and Klaaskate, 1993). When the heifer reaches its puberty, it is inseminated at the onset and, after a gestation period, the heifer calves for the first time. Age at first calving (AFC) is an index of animal's entry into the production system for the next generation, and therefore, reducing the AFC is of crucial importance to enhance reproductive performance of animals (Sasaki et al., 2014).

Most of the agriculturally important traits are complex in genetic architecture due to their polygenic nature, i.e. many genes with small effects, which makes it difficult to identify and characterize individual quantitative trait loci (QTL) for genetic prediction (Datewyler et al., 2010; Hayes et al., 2010). However, due to rapid advancement of high throughput genotyping technologies, high density single nucleotide polymorphisms (SNPs) chips became available, which made it possible to perform whole genome association (WGA) studies in farm animals (Goddard and Hayes, 2009). The WGA analyses would significantly aid our understanding of genetic architecture for complex traits (Lee et al., 2013).

Due to low heritability of the reproduction traits, they are less responsive to genetic progress through selection. Also, the traits are sex-limited or measured only at maturity (Pryce et al., 1997; Dematawewa and Berger, 1998; Roxstrom et al., 2001). Therefore, for efficient genetic improvement of the reproductive traits, genome selection could be a good option, as it allows selection decisions at early stage (Goddard and Hayes, 2009) by performing WGA studies (Ball, 2005). Currently, several genome-wide SNP panels are available for cattle, enabling QTL mapping or prediction of animal's genetic merit for the traits of interest (Goddard and Hayes, 2009).

In cattle, there are some reports on WGA analyses about

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AFC (Olsen et al., 2011; Sasaki et al., 2014). Results of WGA study on AFC, date at first service, calving interval and some fertility traits were also reported in Japanese Black cattle (Minozzi et al., 2013; Sasaki et al., 2014). Olsen et al. (2011) reported WGA results for fertility traits in Norwegian cattle. Hawken et al. (2012) reported WGA results in Brahman and some tropical breeds to identity QTL regions for age at puberty. Age at first service was also investigated with WGA analyses in Holstein by Hoglund et al. (2009) and Daetwyler et al. (2008). However, there is no report about WGA results for reproductive traits in Hanwoo.

The aim of this study was to identify QTL for AFC in Korean native cattle, Hanwoo (*Bos taurus coreanae*), through the WGA analysis.

MATERIALS AND METHODS

Animals and phenotype data

Ninety six Hanwoo heifers were chosen in a local (Jook-Am Hanwoo) farm, Suncheon, Korea. The heifers were born between 2008 and 2010 from 13 sires. The heifers were raised in large pen with the capacity of about 100 individuals and fed *ad libitum* with a total mixed ration concentrates including 35% Italian ryegrass silage, 35% rice straws, 24% (yeast fermented) bran, and 6% brewers' grain. During the experimental period, estrus cycle of each heifer was checked mainly by one veterinarian, and the first artificial insemination was applied to the heifers at approximately 15 months of age. After pregnancy, the heifers were raised in the same large pens before pregnancy, sometimes driven out of the pens and pastured during days. Age at first calving was calculated between the birth days of a heifer and the first calf of the heifer.

Molecular data

The heifers were genotyped with the Illumina 50K bovine SNP chips according to the Infinium HD Assay Ultra Protocol (Matukumalli et al., 2009). Among the SNPs in the chip, some SNPs on autosomal chromosomes were screened and removed before WGA testing, which met the following three criteria; i) the number of genotype group with one or none (*e.g.* only AA genotypes and no AB or BB), ii) with a minor allele frequency less than 0.05, and iii) with the proportion of genotyped individuals less than 90%.

Statistical analysis

A SAS general linear model procedure (SAS version 9.1) was used to test pre-adjust the animal phenotypes before WGA testing. Then, the significant effect of birth year-season was fitted as a fixed effect in Animal model with a genome-relationship (G) matrix that was constructed using the R (version 2.15.0) subroutine (Van Raden, 2008). After the residuals of the phenotype were obtained using ASREML program (version 3.0), the residuals were regressed on additive and dominance effects of each SNP under a linear regression model (PLINK version 1.07). In the model, SNP genotypes with AA, AB, and BB were assigned as 1, 0, and –1 for the additive, and 0, 1, and 0 for the dominance effect, respectively. For significance threshold, 0.1% point-wise p value from *F* distributions was applied for each SNP test.

Among the significant SNPs, the best set of SNP markers were selected using the forward regression procedure (Neter et al., 1990), because some of the significant SNPs would yield redundant information due to linkage disequilibrium between closely linked SNPs. Inclusion of each SNP into the model was determined at α = 0.05 level. For significant SNPs, phenotypic variation explained by each (j^{th}) SNP (S^2_{snpj}) was calculated as $\sum_{i=1}^{3} \alpha_i^2 f_i - \mu^2$, where *i* denotes each genotype, α_i is the estimated additive or dominance effects, *e.g.* $-\bar{\alpha}$, $\bar{d}$, and $+\bar{\alpha}$ for BB, AB, and AA genotypes, respectively, for the SNP with both additive ($\bar{\alpha}$) and dominance ($\bar{d}$) effects, f_i is the frequency of i^{th} genotype, μ is the population mean that can be expressed as $+\bar{\alpha}(f_{AA}-f_{BB})+\bar{d}\, f_{AB}$ (Falconer and Mackay, 1996). Proportion of phenotypic variance due to the j^{th} SNP was then estimated as S^2_{snpj}/S^2_p, in which S^2_p was phenotype variance that was obtained from residual values of the trait after adjusting the fixed and polygenic effects. The proportion of phenotype variance due to all of the significant SNPs was estimated as $((RSS_{noSNP}-SS_{SNPs})/RSS_{noSNP})$, where *RSS* was residual sum of squares for the model with or without fitting the significant SNPs of the trait.

RESULTS AND DISCUSSION

Summary statistics of the AFC that were recorded on the 96 heifers are described in Table 1. The average AFC was 717.5±67.4, ranging from the minimum of 607 to the maximum of 898 days.

A total of 15 SNPs for AFC were detected (Table 2). The significant SNPs were located on ten bovine chromosomes (BTAs), among which 9 SNPs were located on BTAs 1, 2, 7, and 14. The two significant SNPs with strong statistical evidence were located at 128.9 Mb of

Table 1. Summary statistics for age at first calving in 96 Hanwoo heifers

Trait	Average	Std. Dev	Min	Max	CV
Age at first calving (d)	717.5	67.4	607	898	9.2

Std. Dev, standard deviation; Min, minimum; Max, maximum; CV, coefficient of variation (%).

Table 2. Identities, effects and positions of the significant SNPs for age at first calving in a population of Hanwoo heifers

SNP marker[1]	SNP[2]	BTA	kb[3]	Additive[4]	SE	Dominance[5]	SE	$-\log_{10}P$[6]	$\%\sigma_p^{2}$[7]
ARS-BFGL-NGS-98203	[C/A]	1	1,010	–7.22	6.09	32.78	8.21	3.50	0.15
ARS-BFGL-NGS-115015	[C/T]	1	27,181	–13.97	5.54	28.45	7.91	3.35	0.14
BTA-46908-no-rs	[C/A]	2	24,734	43.18	10.94	–33.72	12.55	3.48	0.21
UA-IFASA-7185	[T/G]	2	121,503	–2.03	5.78	35.27	7.89	4.73	0.19
ARS-BFGL-NGS-8433	[A/G]	2	128,973	36.44	9.65	–23.33	11.15	3.31	0.22
Hapmap57628-rs29027155	[A/G]	7	21,845	34.82	10.93	–50.81	12.85	3.40	0.15
Hapmap51497-BTA-80591	[A/G]	7	111,106	28.54	6.18	–34.50	8.24	5.76	0.22
Hapmap32423-BTA-92864	[T/C]	9	52,133	20.89	8.72	5.67	10.48	3.16	0.14
Hapmap60876-rs29013997	[A/C]	10	87,684	15.94	5.34	12.63	7.80	3.02	0.12
Hapmap27709-BTC-057052	[C/T]	14	5,925	7.22	11.03	–36.42	12.91	3.01	0.13
Hapmap51719-BTA-35195	[G/A]	14	62,751	5.27	5.68	26.89	7.68	3.02	0.13
ARS-BFGL-NGS-93176	[T/C]	16	55,239	–13.47	10.82	48.42	13.46	3.77	0.16
Hapmap48608-BTA-111028	[A/G]	20	49,810	1.59	5.31	–30.18	7.65	3.39	0.14
BTB-00932019	[C/A]	26	21,870	–18.95	6.26	30.31	8.48	3.35	0.14
UA-IFASA-9371	[G/T]	29	12,185	–26.15	8.64	2.89	11.22	3.14	0.12

SNP, single nucleotide polymorphism; BTA, bovine chromosomes; SE, standard error.
[1,3] SNP marker annotations and their positions. [2] Alternative nucleotides.
[4,5] Estimates of additive and dominance effects of the SNP. The estimates are allele substitution effects of the former against the latter SNP in the SNP column.
[6] Negative logarithm of base ten for the comparison-wise p-value of the test statistic against the null hypothesis of no SNP effect under the one SNP model.
[7] Proportion of phenotypic variance explained by each SNP or across all of the significant SNPs for the given trait.

BTA2 and at 111.1 Mb of BTA7, respectively, each of which accounted for 22% phenotypic variance (Table 2).

Among the 15 significant SNPs, ten SNPs had (over-) dominance mode of gene action, i.e. the estimates of dominance effect were greater than of additive effect (Table 2). This result indicates that dominance effect of the QTL is highly appreciable in reproductive traits in Hanwoo, which is in agreement with the previous reports about strong effects of heterosis on reproduction in cattle (Cundiff et al., 1974).

The number of heifers for WGA analysis in this study was very limited (n = 96), which did not enable detection of many QTL (SNP) for AFC with strong statistical evidence. As sample size is one of major factor in efficient genome wide association (GWA) analysis (Goddard and Hayes, 2009), more samples are needed to detect other QTL, and the detected QTL need to be validated by adding further samples. However, our results provide a good preliminary report regarding the first genome scan on reproductive traits in Hanwoo heifers.

Schulman et al. (2011) performed a GWA study in the Finish Ayrshire breed to identify QTL for non-return rate, time from first to last insemination, and time from calving to first insemination (in days) for cows. They identified significant SNPs on BTAs 1, 2, 4, 8, 12, 13, 20, 24, and 27. In this study, we detected significant SNPs on BTAs 1, 2, 7, 9, 10, 14, 16, 20, 26, and 29 (Table 2). Hawken et al. (2012) reported three QTL for age at puberty in Brahman and tropical breeds at 43.2 Mb, 81.3 Mb and 140.8 cM of BTA1, while one significant SNP for AFC SNP was detected at 27 Mb of BTA1 (Table 2).

Minozzi et al. (2013) reported one SNP for days to first service at 136.18 Mb of BTA 2 in Italian Holstein cattle. In the similar region, we found one AFC SNP (129 Mb). Sasaki et al. (2014) reported a significant SNP at 118.24 Mb of BTA 2 that was associated with AFC in Japanese Black Cattle, which is closely located at the region (121.5 Mb) where one AFC SNP was detected in this study. Datewyler et al. (2008) reported one QTL for calving age at first service at 24 cM of BTA2 in Holstein. We also detected an AFC SNP at 25 Mb of the same chromosome (Table 2).

In this study, we detected two AFC SNPs at 22 Mb and 111 Mb of BTA 7, respectively (Table 2). Holmberg and Anderson-Eklund (2006) reported QTL for still birth at 22 Mb of BTA7 in Swedish dairy cattle and Hoglund et al. (2009) found one QTL for number of inseminations per conception in Holstein cattle at 111.6 cM of BTA 7.

Sahana et al. (2010) reported a genomic region spanning 40.4 to 52.2 Mb of BTA 9 that was associated with the interval from first to last insemination in Holstein cattle, while Olsen et al. (2011) reported a QTL for retained placenta at 52.64 Mb of BTA 9 in Norwegian Red cattle. We also detected one AFC SNP at 52 Mb of BTA 9 in Hanwoo heifers (Table 2).

One significant SNP for AFC was detected at 88 Mb of BTA10 in this study. Kuhn et al. (2013) reported a QTL for dystocia and still birth at 80 to 87 cM of the same chromosome in Holstein.

On BTA 14, two significant AFC SNPs were detected at 6 Mb and 63 Mb, respectively (Table 2). Hawken et al. (2012) reported one QTL for age at puberty at 61.9 Mb of BTA14 in Brahman and Tropical Composite breed.

McClure et al. (2010) found one SNP for percent of unassisted birth in first calf heifers at 49.1 Mb of BTA 20 in Angus cattle. In the similar region (50 Mb), an AFC SNP was detected in this study (Table 2).

On BTA 26, McClure et al. (2010) detected a significant SNP for percent of unassisted birth in first calf heifers at 18.8 Mb in Angus cattle. We also found an AFC SNP at 22 Mb of the same chromosome. In a proximal chromosomal region (17.8 Mb), Peters et al. (2013) reported one SNP for the first service conception in Brangus cattle.

On BTA29, a significant AFC SNP was detected at 12 Mb, while Hawken et al. (2012) found a significant SNP for post-partum anestrus at 11.3 Mb of the same chromosome in Brahman and tropical composite breeds.

CONCLUSION

Early AFC results in short generation interval, which would improve lifetime productivity of cows. We identified 15 significant QTL for age at first caving in Hanwoo heifers, and in similar chromosomal regions where the QTL were detected, many QTL for the related reproductive traits were reported in other cattle breeds. The identified QTL would aid in performing marker assisted selection (MAS) for genetic improvement of AFC in Hanwoo cows. However, implementation of effective and efficient MAS requires validation of the detected QTL and characterization of the QTL e.g. causal mutations responsible for AFC.

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