MICROSATELLITE DNA MARKER MAPPING OF BOVINE CHROMOSOME 1 FOR QTL AFFECTING BIRTH WEIGHT AND PREWEANING GROWTH IN JAPANESE BLACK CATTLE (WAGYU)

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SUMMARY

Interval mapping within 5 families of Japanese Black Cattle (Wagyu) to detect segregating QTL for birth weight and preweaning average daily gain on bovine chromosome 1 was performed by genotyping 132 half-sib progeny using 98 microsatellite DNA markers. A significant QTL for birth weight was detected at 114cM (95% confidence interval of 50-130.5cM) in one sire. Suggestive QTL for preweaning average daily gain were detected at 14cM and 18cM just below the chromosome-wide threshold in 2 sires. It was concluded that evidence of significant QTL in chromosomal regions of bovine chromosome one was compelling for birth weight hence enhancing the prospects of marker-assisted selection in this breed.

Keywords: Microsatellite DNA markers, QTL, Wagyu, preweaning growth.

INTRODUCTION

The use of molecular markers is now a technology with many applications, with the localisation of quantitative trait loci (QTL) being one of the most important (Ovilo *et al.* 2002). QTL mapping is the first step towards the identification of genes and causal polymorphisms for traits of economic importance (Seaton *et al.* 2002). Therefore, identifying QTL and closely associated markers has potential to significantly increase the rate of genetic improvement through the implementation of marker-assisted selection (MacNeil and Grosz, 2002). Marker-assisted selection may substantially increase the rate of response relative to selection based on estimated breeding value alone, especially for traits that are difficult or expensive to measure, are lowly heritable, occur late in life or are determined post-mortem (Davis and DeNise, 1998). Therefore, the objective of this study was to perform a genome scan for QTL on chromosome 1 affecting birth weight and preweaning average daily gain in Japanese Black (Wagyu) cattle.

MATERIALS AND METHODS

Animals. One hundred and thirty-two paternal half-sib progeny of five Japanese Black Cattle (Wagyu) families were produced by artificial insemination and genotyped at the Department of Livestock and Grassland Science, National Agricultural Research Centre for Western Region, Oda, Shimane Prefecture, Japan. The distribution of the number of progeny among the five sires was: 40, 36, 19, 17 and 20 respectively. The animals were weighed at birth (BWT) and every month until they were weaned at 6 months of age and preweaning average daily gain (PREADG) computed.

Total genomic DNA extraction. About 6-7 ml of blood was taken from the calves by jugular venipuncture, transferred into testubes containing EDTA-2Na and kept at 4ºC. A 0.15M NaCl buffer was added and the total volume brought up to 14–15 ml. The testubes were centrifuged at 2,800 rpm

for 7 mins at 4ºC. The middle layer containing the leucocytes was transferred into new testubes on ice. It was filled up to 2ml with 0.15M NaCl buffer and 6ml of distilled water added. The mixture was slowly shaken for 1min before adding 2ml of 0.6M NaCl in order to concentrate it to 0.15M. This was centrifuged at 1,500 rpm for 6 mins at 4ºC. The upper layer was discarded and 200 µl of 0.15M NaCl buffer added before gently dissolving with the tip of the pipette. 1ml of Lysis buffer containing Proteinase K and RNase A in the following proportions were added. About 1ml of Lysis solution (10mM Tris at pH 8, 10mM EDTA at pH 8, 0.6%SDS), 10 µl of RNase A (concentration of 10 mg/ml) and 25 µl of Proteinase K (concentration of 20 mg/ml) were mixed and incubated at 37ºC overnight for phenol extraction the following day.

Phenol extraction of genomic DNA. The same volume of phenol (pH8 equilibrated) was added to the incubated digest and sealed with thin parafilm. Then it was mixed in a rotor that was kept in a fridge to maintain the temperature at 4ºC for 30 mins to extract the long DNA fragments. This was followed by centrifugation at 2,800 rpm at 4ºC for 5 mins. The upper layer was transferred into new testubes and TE was used to make it up to 1ml if less. About 1ml of TE and chloroform in equal volumes (1:1) was added before taking off the lower layer. This was vortexed on a rotor mixer for 30 mins before centrifuging at 2,800 rpm for 5 mins at 4ºC. The upper layer was transferred into fresh testubes and TE was added to make it up to 1ml. Thereafter, 1 ml of chloroform was added. The mixture was gently vortexed for 30 mins before centrifuging at 2,800 rpm at 4ºC for 5 mins. The upper layer was transferred into fresh testubes. Ethanol (2.5 ml) was added to precipitate the white, thread-like genomic DNA. This was gently vortexed at room temperature. The precipitate was washed with 1ml of 70% ethanol and centrifuged at 10,000 rpm at 4ºC for 3 mins. The upper layer was completely discarded. The DNA sediment was evaporated for 2 mins in a centrifugal evaporator. TE (500µl) was added to the DNA and kept in the fridge at 4ºC before subjecting it to spectrophotometry and storage at 4ºC until ready for polymerase chain reaction (PCR).

Polymerase chain reaction (PCR). PCR pre-mix (13µl) that comprised of 10.55µl of distilled water, 1.04µl of 2.5mM dNTP Mixture (Takara, Shiga, Japan), 1.3µl of 10 x buffer containing 15mM $MgCl_2$ and 0.11 µl of 25mM of $MgCl_2$ was prepared. A primer (12.5 pmol/ µl) containing microsatellite DNA markers FAM (blue), HEX (yellow) and TET (Green) supplied by the Institute of Animal Genetics, Fukushima, Japan, was added to the PCR pre-mix. Genomic DNA (1µl) (concentration of 20ng/µl) was added followed by 0.5µl of Taq polymerase enzyme (concentration of 0.75 units/µl) containing 50% glycerol (Takara, Japan). The PCR plates were hotplate-sealed and subjected to PCR in a DNA thermal cycler. The annealing temp settings were 50ºC, 55ºC and 60ºC. The PCR products were then mixed with DNA size markers in different loading combinations containing 4µl of HEX, 1µl of FAM and 1µl of TET, properly labelled and stored for genotyping.

Genotyping. Prior to genotyping, the PCR products were first denatured by adding 4.5µl of DNA size marker to 0.8 µl of the PCR pre-mix, centrifuged for 1 minute at 1000 rpm before running the PCR machine at a denaturing temperature of 94ºC for 9 mins. The denatured products were subjected to electrophoresis and genotyping in a 377 DNA Sequencer. A total of 98 informative microsatellite DNA markers was utilised for the genotyping.

QTL analysis: Identity-By-Descent (IBD) probabilities at specific chromosomal locations from multiple marker data of chromosome 1 were determined and a linear model containing the fixed effects of sex, parity and season of birth as well as age as a covariate, was fitted to the IBD coefficients and phenotypic data. Data were analysed by generating an F-statistic by the regression of phenotype on the IBD probabilities of inheriting an allele from the sire. Permutation tests at chromosome-wide significance thresholds were carried out over 1, 000 iterations at 1cM intervals while the boostrap procedure was followed to estimate confidence intervals of the QTL locations. All these procedures were implemented in the QTL Express Computer programme with a web-based user interface developed by Seaton *et al* (2002) and available at http://qtl.cap.ed.ac.uk/

RESULTS AND DISCUSSION

Beef bulls are evaluated based on their own growth rate and yearling weight because of the positive correlation between growth and carcass traits (Baik *et al*. 2003). The growth of this breed as reflected by liveweight changes from birth to yearling age is important for the early attainment of slaughter weight. Table 1 shows that the BWT of Wagyu calves ranged from around 27 to 35 kg with a PREADG of 0.72 to 0.83 kg/day.

Table 1. Means and standard deviations (S.D.) of birth weight (BWT) and preweaning average daily gain (PREADG) of Wagyu calves

			Birth weight (kg)		PREADG (kg/day)	
Family	Informative Markers	Progeny	Mean	S.D.	Mean	S.D.
1	19	40	34.74	4.70	0.72	0.11
2	27	36	33.97	4.75	0.77	0.09
3	13	19	28.68	5.13	0.83	0.10
4	22	17	28.71	3.32	0.82	0.12
5	17	20	26.90	5.44	0.82	0.15
Total	98	132				

Table 2 shows the results of QTL effects and positions. A significant QTL for BWT was detected at 114cM (95% confidence interval of 50-130.5cM) in Sire 2. Suggestive QTL for PREADG were detected at 14cM and 18cM just below the chromosome-wide threshold in Sires 1 and 2. The detection and mapping of QTL for BWT, liveweight, growth and carcass traits in paternal half-sib families of Charolais x Brahman, Brahman, Piedmontese, Belgian Blue and Beefbooster cattle has been reported (Davis *et al*. 1998, Stone *et al*. 1999, Casas *et al*. 2000 and Li *et al* 2002) but not for Wagyu. Our findings are supported by Stone *et al*. (1999) who also reported a significant evidence for a BWT QTL on BTA1 located at 114cM in a Brahman x Hereford sire mated to *Hereford* cows with the Brahman allele increasing birth weight relative to the Hereford allele. Although evidence for preweaning growth related QTL was not compelling, it was supported by effects at or near the suggestive threshold. Our results demonstrate that there is a good prospect for marker-assisted selection for growth in Wagyu cattle. Davis *et al*. (1998) and Grosz and MacNeil (2001) detected QTL for BWT and other growth traits on BTA2 and

BTA5. Genotyping work in our laboratory on both BTA 2 and 5 is on-going and would shed more light on segregating QTL affecting pre- and post-weaning growth in the Wagyu.

Table 2. Sire QTL effects (ß±S.E.), estimated and average QTL positions and chromosome-wide F-statistics for preweaning growth of halfsib Japanese Black calves

	Birth weight				
Family	1	2	3	4	5
ß±S.E.	-3.57±1.96	3.70±1.58	3.10±2.43	-4.43±1.24	7.27±3.20
Estimated QTL (cM)	61	**114***	47	111	105
F-statistics	6.64	10.98	3.26	25.42	10.32
Likelihood Ratio	3.07	4.84	1.41	5.00	3.85
F-threshold (P<0.05)	9.10	10.22	11.69	43.04	15.66
95% Conf. Interv (cM)	40-119	50-130.5	40-125	40-126	48-117
	Preweaning average daily gain				
ß±S.E.	-0.09±0.05	0.07±0.04	-0.09±0.04	0.20±0.06	0.14±0.11
Estimated QTL (cM)	**14**	**18**	125	59	28
F-statistics	8.18	7.22	8.86	23.40	2.32
Likelihood Ratio	3.72	3.29	3.40	4.79	1.44
F-threshold (P<0.05)	9.25	9.43	12.94	79.62	14.75
95% Conf. Interv (cM)	13-122	17-132	40-125	5 0-130	20-120

* P chromosome-wide significance (P=0.05) and figures in bold are close to significance

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