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Mapping of quantitative trait loci for carcass traits in a Japanese Black (Wagyu) cattle population

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Summary

To detect quantitative trait loci (QTL) that influence economically important traits in a purebred Japanese Black cattle population, we performed a preliminary genome-wide scan using 187 microsatellite markers across a paternal half-sib family composed of 258 offspring. We located six QTL at the 1% chromosome-wise level on bovine chromosomes (BTA) 4, 6, 13, 14 and 21. A second screen of these six QTL regions using 138 additional paternal offspring half-sib from the same sire, provided further support for five QTL: carcass weight on BTA14 (22–39 cM), one for rib thickness on BTA6 (27–58 cM) and three for beef marbling score (BMS) on BTA4 (59–67 cM), BTA6 (68–89 cM) and BTA21 (75–84 cM). The location of QTL for subcutaneous fat thickness on BTA13 was not supported by the second screen ($P > 0.05$). We determined that the combined contribution of the three QTLs for BMS was 10.1% of the total variance. The combined phenotypic average of these three Q was significantly different ($P < 0.001$) from those of other allele combinations. Analysis of additional half-sib families will be necessary to confirm these QTL.

Keywords carcass traits, cattle, half-sib family, Japanese Black, quantitative trait loci.

We recently located quantitative trait loci (QTL) for growth and carcass traits using a purebred Japanese Black (Wagyu) paternal half-sib family (Mizoshita *et al.* 2004). However, QTL mapping based on a single sire will uncover only a fraction of the segregating QTL present in a population or breed. To discover more segregating QTL in Japanese Black cattle, DNA and carcass data were collected from several paternal half-sib families at slaughterhouses. Here, we report QTL mapping results using one of these purebred Wagyu half-sib families.

A paternal half-sib family composed of 258 offspring (collected between 29 and 32 months of age) was used in a preliminary screen. In a second screen, 138 additional offspring were included in the analysis. Carcass data were cold carcass weight (CW), beef marbling score (BMS), rib eye area (REA), rib thickness (RT), subcutaneous fat thickness (SFT) and carcass yield estimate (YE) as described previously (Mizoshita *et al.* 2004). Briefly, BMS was scored from 1 to 12 with a standard model panel, in which higher scores correspond to more marbling (JMGA 1998). Phenotypic values were corrected with best linear unbiased estimates of fixed effects estimated by MTDFREML (Boldman *et al.* 1995). Supplementary Table S1 presents the mean values and SD of these six traits. Samples of DNA were prepared from adipose tissue and semen using a standard protocol. The polymerase chain reaction (PCR) conditions for microsatellite markers were optimized (Kappes *et al.* 1997; Ihara *et al.* 2004). Microsatellites were amplified by PCR and then analysed by gel electrophoresis on an ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA). Genotype data were captured using GENESCAN and GENOTYPER software (Applied Biosystems). Marker locations were obtained from the Shirakawa-USDA bovine linkage map (Ihara *et al.* 2004). Phases of the sire's chromosomes were determined at each pair of two consecutive heterozygous markers using allele transmission to offspring so that recombination between two markers was minimized. For detection of multiple QTL, we adopted the methods of Haley *et al.* (1994) and Seaton *et al.* (2002) based on a least-squares simple regression model within a paternal half-sib family as described previously (Mizoshita *et al.* 2004). Linear regression analysis was performed using the following model:

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$$y_i = \mu + \mathrm{Prob}(Q)_i a + e_i$$

where, μ is the fixed effect, a is the allele substitution effect of q to Q, and y_i, $\mathrm{Prob}(Q)_i$ and e_i are the phenotypic value, the probability of Q genotype at a given location and the residual error for individual i respectively. An allele substitution effect for q and Q was calculated as an estimator of a. Another QTL mapping method was performed using a non-parametric, sum of rank-based multipoint approach (Coppieters *et al.* 1998). The LOD drop-off method was used to calculate the support interval for each putative QTL (Ott 1992). Thresholds for significance of the F-statistic value were obtained by 10 000 random permutations of the phenotypic data (Churchill & Doerge 1994). To control the thresholds for the error rate of multiple trait analysis, we applied the false discovery rate (FDR) suggested by Weller *et al.* (1998).

We genotyped 187 microsatellite markers (Supplementary Table S2) on 29 autosomes across the 258 half-sib offspring as well as their sire, with an average information content of 0.64. Several mapping methods have been applied to the half-sib design, including interval mapping using regression (Haley *et al.* 1994), a maximum likelihood method (Georges *et al.* 1995) and a rank-based non-parametric method (Coppieters *et al.* 1998). In the present study, to locate as many QTL as possible, both non-parametric and parametric methods were used for QTL mapping. We mapped six QTL for four traits (CW, RT, SFT and BMS) at the 1% chromosome-wise level (Table 1). Significant QTL (chromosome-wise level: $P < 0.01$) for BMS on BTA4 was obtained by the non-parametric test, but not the parametric test ($P > 0.01$). To further investigate these six QTL, 138 additional microsatellites were genotyped on 396 offspring (Table S2). Table 2 summarizes the mapping results with QTL positions, log(1/P), F-statistic values, Q to q allele substitution effects and QTL contributions (variance explained by the regression model), calculated at the positions with the highest recorded F-statistic values. Five of the six QTL were supported by both non-parametric and parametric tests: one QTL for CW on BTA14 (22–39 cM; Fig. 1c), one QTL for RT on BTA6 (27–58 cM; Fig. 1b) and three QTL for BMS on BTA4 (59–67 cM; Fig. 1a), BTA6 (68–89 cM; Fig. 1b) and BTA21 (75–84 cM; Fig. 1d). The QTL for SFT on BTA13 was not significant in the second screen ($P > 0.05$). Mizoshita *et al.* (2004) located QTL for CW on BTA14 (48–51 cM) and BMS on BTA4 (52–62 cM). Allele substitution effects for q and Q alleles on BTA4, BTA6, and BTA21 for BMS scores were 0.8, 0.9 and 0.7 respectively (Table 2). These three regions explained 4.3%, 4.0% and 2.6% of the total variance for BMS respectively (Table 2). To estimate the combined contribution of these three QTL to the total variance, the contribution ratio (R^2) calculated by ANOVA was 10.1%, which is close to the sum of the three individual variances (10.9%), suggesting that these three QTL are additive. To further investigate the additive effect of the three QTL, we estimated phases of the QTL in 337 of the 396 offspring at probabilities of more than 90% (Supplementary Table S3). Although the addition of a second Q allele did not increase the BMS trait significantly, 44 animals harbouring all three Q alleles had

Table 1 Analysis of QTL for carcass traits in a Japanese Black cattle paternal half-sib family (preliminary screen).

Trait	Chromosome	cM	Log(1/*P*)[1]	*F*-statistic value[2]	FDR[3]
CW (kg)	14	26	2.47†	12.0†	0.062
RT (cm)	6	52	2.07**	11.0**	0.098
SFT (cm)	13	28	2.54†	13.5†	0.041
BMS	4	51	2.12**	7.1*	0.351
BMS	6	51	2.51**	11.1**	0.105
BMS	21	77	4.00‡	23.4‡	<0.0001

*, **5 and 1% chromosome-wise significance levels, respectively.
†, ‡5 and 1% experiment-wise significance levels, respectively.
CW, carcass trait; RT, rib thickness; SFT, subcutaneous fat thickness; BMS, beef marbling score; QTL, quantitative trait loci; FDR, false discovery rate.
[1]Non-parametric test (Coppieters *et al.* 1998).
[2]Parametric test (Mizoshita *et al.* 2004).
[3]FDR (Weller *et al.* 1998).

Table 2 QTL for carcass traits in a Japanese Black cattle paternal half-sib family (secondary screen).

Trait	Chromosome	Log(1/*P*)[1]	*F*-statistic value[2]	cM[3]	*Q* to *q* allele substitution effect	QTL contribution (%)[4]
CW (kg)	14	5.00**	28.1**	26 (22–39)	18.9	6.4
RT (cm)	6	3.30**	12.1*	49 (27–58)	0.2	2.7
BMS	4	3.70**	18.8**	63 (59–67)	0.8	4.3
BMS	6	3.70**	17.6**	77 (68–89)	0.9	4
BMS	21	2.75*	15.3*	77 (75–84)	0.7	2.6

*, **1 and 0.1% chromosome-wise significance levels, respectively.
CW, carcass trait; RT, rib thickness; SFT, subcutaneous fat thickness; BMS, beef marbling score; QTL, quantitative trait loci.
[1]Non-parametric test (Coppieters *et al.* 1998).
[2]Parametric test (Mizoshita *et al.* 2004).
[3]Support interval in parentheses
[4]A QTL contribution (%) in the family was calculated as (variance explained by the linear regression model/total variance) × 100.

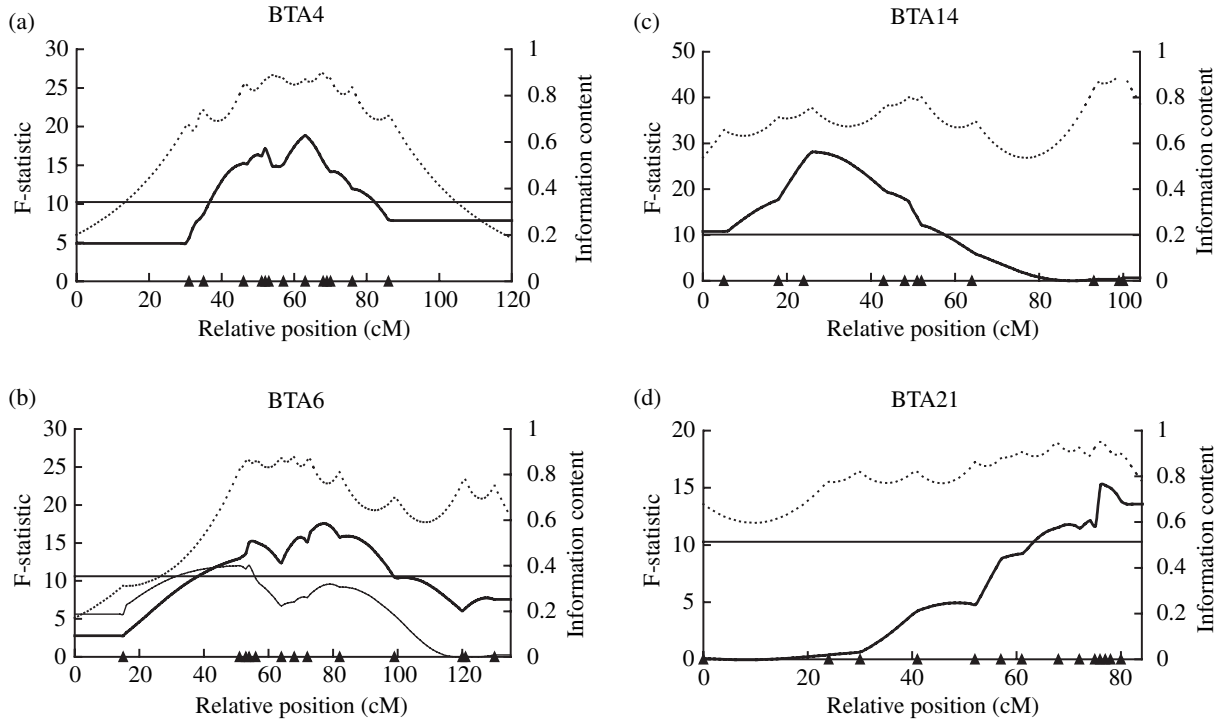

Figure 1 *F*-statistic profiles for carcass traits. Horizontal lines indicate the threshold for the chromosome-wise 1% levels (solid line). Dashed lines indicate information content (right *y*-axis). Marker positions are identified as triangles above the *x*-axis. (a) BMS (thick line) on BTA4. Markers were *BMS1172*, *BMS1237*, *BMS1840*, *BMS1840*, *MAF50*, *DIK008*, *BMS2172*, *BM6437*, *BMS1237*, *BMS1840*, *BMS779*, *BMS2571*, *OARCP26* and *DIK026*. (b) BMS (thick line) and RT (thin line) on BTA6. Markers were *ILSTS090*, *BMS382*, *BM3026*, *BM143*, *BMS690*, *BM4322*, *BMS483*, *ILSTS097*, *BMS360*, *BM415*, *BMS511*, *BMS739* and *BL1038*. (c) CW (thick line) on BTA14. Markers were *BM1508*, *ILSTS011*, *RM011*, *BL1009*, *DIK062*, *BMC1207*, *BM302*, *RM192*, *BMS2055*, *INRA092* and *BL1036*. (d) BMS (thick line) on BTA21. Markers were *BM8115*, *ILSTS095*, *BM103*, *UWCA4*, *TGLA337*, *DIK2913*, *BM846*, *TALA122*, *AFZ1*, *NLBCMK1*, *NLBCMK14*, *BMS743*, *BMS670*, *DIK3000*, *IDVGA-30* and *BMS2382*.

a significantly higher average BMS ($P < 0.001$; Student's *t*-test) than animals with other allele combinations.

In the present study, we located two QTL in similar regions as previously reported in Japanese Black cattle: CW on BTA14 and BMS on BTA4 (Mizoshita *et al.* 2004). However, three QTL (RT and BMS on BTA6; BMS on BTA21) are newly reported in this study. Analysis of additional paternal half-sib families will provide more information on these QTL.

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Supplementary Material

The following material is available for this article online at http://www.blackwell-synergy.com

Table S1 Phenotypic values for carcass traits in paternal half-sib Japanese Black cattle family.

Table S2 Genetic markers used in QTL mapping.

Table S3 BMS trait averages of animals that inherited different combination of haplotypes.