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Identification of genetic polymorphisms in *FABP3* and *FABP4* and putative association with back fat thickness in Korean native cattle

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The aim of this study was to determine whether single nucleotide polymorphisms (SNP) in the beef cattle adipocyte fatty-acid binding protein 3 and 4 (FABP3 and FABP4) genes are associated with carcass weight (CW) and back fat thickness (BF) of beef cattle. By direct DNA sequencing in 24 unrelated Korean native cattle, we identified 20 SNPs in *FABP3* and *FABP4*. Among them, 10 polymorphic sites were selected for genotyping in our beef cattle. We performed SNP, haplotype and linkage disequilibrium studies on 419 Korean native cattle with the 10 SNPs in the FABP genes. Statistical analysis revealed that *220A>G* (*I74V*) and *348+303T>C* polymorphisms in *FABP4* showed putative associations with BF traits (P = 0.02 and 0.01, respectively). Our findings suggest that the polymorphisms in *FABP4* may play a role in determining one of the important genetic factors that influence BF in beef cattle. [BMB reports 2008; 41(1): 29-34]

INTRODUCTION

Carcass weight (CW) and back fat (BF) thickness are economically important traits in cattle. The FABPs are members of a family of conserved intracellular lipid-binding proteins (1) and so far 9 tissue-specific cytoplasmic FABPs have been identified (2). Among these, FABP3 and FABP4 have been known as Heart FABP (H-FABP) and Adipocyte FABP (A-FABP), respectively. The FABP3 is involved in fatty acid transport from cell membrane to the intracellular sites of fatty acid utilization and is mainly expressed in cardiac and skeletal muscle (3). In pigs, the FABP3 gene was mapped to chromosome 6 (4), and the chromosomal region of the gene was speculated as a quantitative trait locus (QTL) region for fatness traits (5-7). The FABP4 gene was mapped to chromosome 6 in the pigs, and the chromosomal region of the gene was suggested as a QTL for intramuscular fat (IMF) (8, 9). Thus, these two members of the FABP family have been considered as candidate genes for pig fatness traits.

In this study, we have examined genetic association of the FABP3 and FABP4 polymorphisms with CW and BF thickness in Korean Native Cattle (KNC). In the previous studies, bovine FABP3 gene was mapped to bovine chromosome 2 by fluorescence in situ hybridization and radiation hybrid mapping (10) and the bovine FABP3 gene has not been suggested as a QTL region for fatness traits. Recently, the FABP4 gene in bovine was suggested to be significantly associated with marbling and subcutaneous fat depth by analyzing two SNP polymorphisms (11).

Based on the biological properties and previous association studies of fatness traits as above, it is hypothesized that FABP3 and FABP4 play an important role in the carcass traits in cattle. We performed extensive screening of FABP3 and FABP4 by direct sequencing to detect polymorphisms and examined genetic association with CW and BF. Here, we present 20 genetic polymorphisms found in FABP3 and FABP4, and the results of an association study in KNC.

RESULTS AND DISCUSSION

By direct DNA sequencing of 24 unrelated KNC, 20 polymorphisms were identified in *FABP3* and *FABP4*. Locations and allele frequencies of polymorphism are shown in Table 1. Hardy-Weinberg equilibrium (HWE) of SNPs is also shown and the distributions of genotypes were in agreement with HWE within all SNPs. Selecting an informative subset of SNPs to maximize the ability to detect SNPs-traits association is of great interest and importance. We selected SNPs that met the following criteria for the following large scale association study: 1) location (non synonymous polymorphisms were preferred), 2) frequency of minor allele (freq. > 0.05), and 3) LD

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Table 1. Genotypes and minor allele frequencies of 20 polymorphisms in FABP3 and FABP4 discovered in 24 unrelated Korean native cattle

Gene	SNP name	Position	AA change	Genotype (N. of cattle)			Total N. of cattle	Minor allele frequency	Heterozygosity	HWE
FABP3	*c.-46-1511C>A*	Promoter	·	C(17)	AC(2)	A(3)	N(22)	0.182	0.298	0.005
	c.-46-787G>A	Promoter	·	G(15)	AG(9)	A(0)	N(24)	0.188	0.305	0.528
	c.-46-295AACinsdel	Promoter	·	ins(10)	insdel(12)	del(0)	N(22)	0.273	0.397	0.213
	c.21T>C (G7G)	Exon1	G7G	T(16)	CT(8)	C(0)	N(24)	0.167	0.278	0.619
	c.73+67G>C	Intron1	·	C(5)	CG(16)	G(3)	N(24)	0.458	0.497	0.244
FABP4	*c.-60-1682C>T*	Promoter	·	C(5)	CT(13)	T(5)	N(23)	0.500	0.500	0.822
	c.-60-1643C>T	Promoter	·	C(5)	CT(13)	T(5)	N(23)	0.500	0.500	0.822
	c.-60-1484G>C	Promoter	·	G(21)	CG(2)	C(0)	N(23)	0.043	0.083	0.977
	c.-60-235A>G	Promoter	·	G(8)	AG(5)	A(6)	N(19)	0.447	0.494	0.125
	c.-60-227A>G	Promoter	·	G(8)	AG(6)	A(5)	N(19)	0.421	0.488	0.308
	c.-24G>A	5'UTR	·	G(19)	AG(1)	A(0)	N(20)	0.025	0.049	0.993
	c.74-157A>T	Intron1	·	A(18)	AT(4)	T(0)	N(22)	0.091	0.165	0.896
	c.74-9C>G	Intron1	·	C(5)	CG(12)	G(5)	N(22)	0.500	0.500	0.913
	c.220A>G (I74V)	Exon2	I74V	A(5)	AG(12)	G(5)	N(22)	0.500	0.500	0.913
	c.246+33Ainsdel	Intron2	·	ins(5)	insdel(12)	del(5)	N(22)	0.500	0.500	0.913
	c.328G>A (V110M)	Exon3	V110M	G(9)	AG(10)	A(1)	N(20)	0.300	0.420	0.696
	c.348C>G (L116L)	Exon3	L116L	C(4)	CG(13)	G(3)	N(20)	0.475	0.499	0.399
	c.348+34C>T	Intron3	·	C(4)	CT(13)	T(3)	N(20)	0.475	0.499	0.399
	c.348+56C>T	Intron3	·	C(4)	CT(13)	T(3)	N(20)	0.475	0.499	0.399
	c.348+303T>C	Intron3	·	C(5)	CT(12)	T(3)	N(20)	0.450	0.495	0.638

Table 2. Genotypes and minor allele frequencies of 10 polymorphisms in *FABP3* and *FABP4* genotyped in a larger Korean native cattle (n = 419)

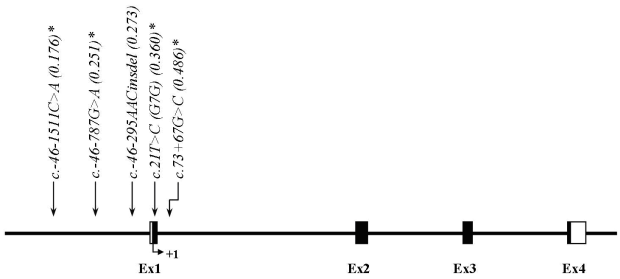
Gene	SNP name	Position	AA change	Genotype (N. of cattle)			Total N. of cattle	Minor allele frequency	Heterozygosity	HWE
FABP3	*c.-46-1511C>A*	Promoter	·	C(284)	AC(111)	A(17)	N(412)	0.176	0.290	0.354
	c.-46-787G>A	Promoter	·	G(230)	AG(159)	A(24)	N(413)	0.251	0.376	0.879
	c.21T>C (G7G)	Exon1	G7G	T(162)	CT(166)	C(55)	N(383)	0.360	0.461	0.504
	c.73+67G>C	Intron1	·	G(114)	CG(179)	C(103)	N(396)	0.486	0.500	0.166
FABP4	*c.-60-235A>G*	Promoter	·	A(179)	AG(193)	G(47)	N(419)	0.342	0.450	0.897
	c.-60-227A>G	Promoter	·	A(122)	AG(127)	G(38)	N(287)	0.354	0.457	0.863
	c.74-157A>T	Intron1	·	A(291)	AT(118)	T(7)	N(416)	0.159	0.267	0.444
	c.220A>G (I74V)	Exon2	I74V	A(159)	AG(201)	G(55)	N(415)	0.375	0.469	0.791
	c.328G>A (V110M)	Exon3	V110M	G(206)	AG(161)	A(37)	N(404)	0.291	0.413	0.793
	c.348+303T>C	Intron3	·	T(147)	CT(179)	C(44)	N(370)	0.361	0.461	0.643

(only one polymorphism was chosen if it was in absolute LD ($r^2=1$) with one or more other polymorphisms). By pair-wise linkage analysis with DNA from the 24 unrelated KNC (Fig. 1), which were used for direct sequencing, we have found that a set of polymorphisms were in absolute LDs (|D'| = 1 and r^2 = 1) in *FABP4* (Fig. 1, B-1). As a result, 10 polymorphisms, four SNPs (*-46-1511C>A, -46-787G>A, 21T>C (G7G), 73+67G>C*) in *FABP3* and six SNPs (*-60-235A>G, -60-227A>G, 74-157A>T, 220A>G (I74V), 328G>A (V110M), 348+ 303T>C*) in *FABP4* were genotyped in larger scale KNC (n = 419) (Table 2).

Associations of *FABP3* and *FABP4* polymorphisms with CW and BF were analyzed using the mixed effect model with sire and age as covariates. Sire was treated as a random effect and age a fixed effect. The P value of 0.05 was considered significant. Statistical analyses revealed that the *220A>G (I74V)*, *348+303T>C* polymorphisms in *FABP4* were positively associated with BF in *FABP4* (P = 0.02 and P = 0.01, respectively), whereas these are not associated with CW. BF of *220A>G (I74V)* and *348+303T>C* was high in major allele homozygotes (0.74 ± 0.28 and 0.75 ± 0.29) and intermediate or low in heterozygotes and minor allele homozygotes (heterozygotes: 0.68 ± 0.27 and 0.69 ± 0.28, and minor allele homozygotes: 0.69 ± 0.30 and 0.66 ± 0.27) (Table 3). But when Bonferroni corrections were strictly adopted, associated P-value could not retain the significance (the threshold of significance would be 0.0025 [10 polymorphisms and 2 phenotypes analyzed]).

The pair-wise D' values of the SNPs were generally above 0.80, with some differences noted between *FABP3* and *FABP4* (Fig. 1, A-3 and B-3). The pair-wise D' values in *FABP4* were nearly 1 among almost all SNP pairs, indicating that the SNPs

A-1. Map of *FABP3* on chromosome 2q45 (6.9 kb)



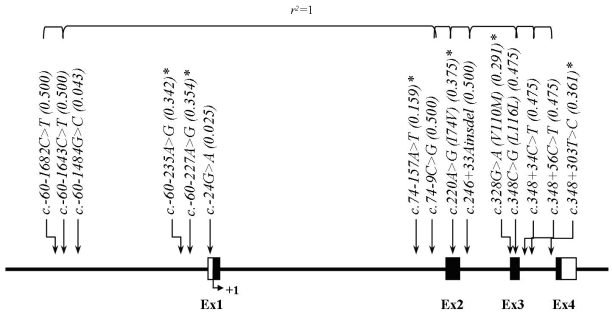
A-2. Haplotypes in *FABP3*

Hap.	-46-1511C>A	-46-787G>A	21T>C	73+67G>C	Freq.
ht1	C	G	C	G	0.366
ht2	C	A	T	C	0.241
ht3	A	G	T	C	0.169
ht4	C	G	T	G	0.139
ht5	C	G	T	C	0.069
Others(1)	.	.	.	.	0.015

A-3. LDs among *FABP3* polymorphisms

		| D' |			
	Polymorphisms	-46-1511C>A	-46-787G>A	21T>C	73+67G>C
r^2	-46-1511C>A	-	0.86	0.97	0.96
	-46-787G>A	0.05	-	0.98	0.94
	21T>C	0.12	0.18	-	0.99
	73+67G>C	0.21	0.31	0.55	-

B-1. Map of *FABP4* on chromosome 14 (4.4 kb)



B-2. Haplotypes in *FABP4*

Hap.	-60-235A>G	-60-227A>G	74-157A>T	220A>G	328G>A	348+303T>C	Freq.
ht1	G	G	A	G	G	C	0.325
ht2	A	A	A	A	A	T	0.274
ht3	A	A	A	A	G	T	0.179
ht4	A	A	T	A	G	T	0.156
ht5	A	A	A	G	G	C	0.026
ht6	G	A	A	G	G	C	0.016
Others(2)	.	.	.	.	.	.	0.024

B-3. LDs among *FABP4* polymorphisms

		| D' |					
	Polymorphisms	-60-235A>G	-60-227A>G	74-157A>T	220A>G	328G>A	348+303T>C
r^2	-60-235A>G	-	0.92	1	0.98	0.98	0.99
	-60-227A>G	0.85	-	0.91	0.91	0.83	0.92
	74-157A>T	0.10	0.1	-	1.00	1	0.96
	220A>G	0.84	0.75	0.12	-	0.97	0.99
	328G>A	0.20	0.15	0.08	0.23	-	1
	348+303T>C	0.89	0.77	0.11	0.98	0.23	-

Fig. 1. Gene maps, haplotypes, and LD coefficients among *FABP3* and *FABP4* polymorphisms. A-1. Map of polymorphisms in *FABP3* on chromosome 2. A-2. Haplotypes in *FABP3*. [(1)]Others contain rare haplotypes: CATG, AGCG, CACG and AATC. A-3. LDs among *FABP3* polymorphisms. B-1. Map of polymorphisms in *FABP4* on chromosome 14. B-2. Haplotypes in *FABP4*. [(2)]Others contain rare haplotypes: AGAAAT, AGAGGC, AAAGAC and GAAAAT. B-3. LDs among *FABP4* polymorphisms. Coding exons are marked by black blocks, and 5' and 3' UTRs by white blocks. First base of the translation site is denoted as nucleotide +1. Asterisks (*) indicate polymorphisms genotyped in a larger Korean native cattle (n = 419). Minor allele frequencies of other polymorphisms are based on 24 sequencing samples only.

were highly associated with each other. According to the criteria of (12), one haplotype blocks across were revealed from the linkage disequilibrium (LD) data for four SNPs in *FABP3* and six SNPs in *FABP4* (Fig. 1).

In order to perform haplotype-based association analysis, nine different major haplotypes (freq. > 0.05) were constructed in *FABP3* and *FABP4* (Fig. 1, A-2 and B-2). The five most frequent haplotypes in *FABP3* had a summed probability of 0.984 and four most frequent haplotypes had a summed probability of 0.934 in *FABP4*. Other hapotypes were rare haplotypes that we did not analyze. Haplotype analysis based on the nine common haplotypes revealed that haplotypes in *FABP3* and *FABP4* were not associated with CW and BF (Table 3). Haplotype analysis is generally more powerful than single SNP analysis: haplotype is a combination of several SNPs that show a stronger association with disease than any single SNP and reflects evolutionary history/linkage disequilibrium pattern more accurately. Nevertheless, haplotype analysis is difficult because there is often only partial information available about the genomes even if more informative than individual SNPs.

In the present study, four SNPs (*-46-1511C>A, -46-787G>A, 21T>C (G7G), 73+67G>C*) in *FABP3* and six SNPs (*-60-235A>G, -60-227A>G, 74-157A>T, 220A>G (I74V), 328G>A (V110M), 348+303T>C*) in *FABP4* showed no strong associations with CW and BF in KNC in exception of a putative association of *220A>G* and *348+303T>C* polymorphisms in *FABP4* with BF. It might be argued that the statistical power to detect significant association in this study could be low. However, on the basis of Hirschhorn and Altshuler's review (13), in the case of putative results such as in this study, replication is paramount, because prior probabilities of true association are low and thus, all genetic associations demand a high level of proof. Recently several research groups reported association of *FABP4* polymorphisms with back fat traits in pigs (14, 15). Our result also indicated the BF association with bovine *FABP4* polymorphisms. Replication of our finding in an independent dataset and/or functional validation of polymorphisms should be performed in the future.

Table 3. Association analyses of *FABP3* and *FABP4* polymorphisms with carcass traits (CW and BF) among Korean native cattle

Traits	Gene	SNP	Position	C/C	C/R	R/R	P
CW	FABP3	*c.-46-1511C>A*	Promoter	284 (313.36 ± 34.09)	111 (306.59 ± 34.34)	17 (304.94 ± 30.10)	0.33
		c.-46-787G>A	Promoter	230 (312.51 ± 34.82)	159 (309.16 ± 32.07)	24 (306.88 ± 35.44)	0.76
		c.21T>C (G7G)	Exon1	162 (307.27 ± 34.77)	166 (313.70 ± 31.00)	55 (307.58 ± 39.79)	0.21
		c.73+67G>C	Intron1	114 (314.60 ± 38.40)	179 (313.89 ± 31.26)	103 (302.31 ± 33.77)	0.04
		FABP3_ht1	·	178 (308.48 ± 34.43)	194 (314.44 ± 30.89)	62 (310.23 ± 37.81)	0.36
		FABP3_ht2	·	249 (312.99 ± 34.46)	161 (309.55 ± 31.50)	24 (307.25 ± 35.94)	0.74
		FABP3_ht3	·	302 (313.75 ± 33.44)	117 (306.88 ± 33.67)	15 (299.20 ± 27.10)	0.18
		FABP3_ht4	·	320 (309.18 ± 33.23)	107 (317.74 ± 31.62)	7 (315.57 ± 58.15)	0.13
		FABP3_ht5	·	375 (311.22 ± 33.52)	58 (312.60 ± 33.55)	1 (305.00)	0.99
	FABP4	*c.-60-235A>G*	Promoter	179 (313.26 ± 32.55)	193 (309.16 ± 34.84)	47 (309.77 ± 33.00)	0.19
		c.-60-227A>G	Promoter	122 (309.28 ± 32.32)	127 (302.83 ± 32.39)	38 (306.13 ± 31.18)	0.23
		c.74-157A>T	Intron1	291 (310.75 ± 33.79)	118 (311.59 ± 34.42)	7 (319.14 ± 21.04)	0.84
		c.220A>G (I74V)	Exon2	159 (313.33 ± 33.66)	201 (308.75 ± 33.19)	55 (313.22 ± 36.22)	0.21
		c.328G>A (V110M)	Exon3	206 (311.50 ± 34.47)	161 (308.99 ± 33.03)	37 (312.00 ± 27.41)	0.85
		c.348+303T>C	Intron3	147 (312.82 ± 34.35)	179 (306.73 ± 33.06)	44 (311.68 ± 35.34)	0.10
		FABP4_ht1	·	193 (313.70 ± 32.85)	200 (309.22 ± 34.41)	41 (311.15 ± 31.56)	0.14
		FABP4_ht2	·	234 (311.86 ± 34.33)	162 (310.18 ± 33.34)	38 (313.68 ± 28.74)	0.85
		FABP4_ht3	·	287 (311.37 ± 32.07)	139 (310.41 ± 36.62)	8 (329.25 ± 19.34)	0.22
		FABP4_ht4	·	306 (310.88 ± 33.26)	121 (312.24 ± 34.64)	7 (319.14 ± 21.04)	0.64
BF	FABP3	*c.-46-1511C>A*	Promoter	284 (0.72 ± 0.29)	111 (0.68 ± 0.26)	17 (0.62 ± 0.27)	0.10
		c.-46-787G>A	Promoter	230 (0.69 ± 0.27)	159 (0.72 ± 0.30)	24 (0.68 ± 0.33)	0.69
		c.21T>C (G7G)	Exon1	162 (0.70 ± 0.29)	166 (0.72 ± 0.28)	55 (0.69 ± 0.28)	0.57
		c.73+67G>C	Intron1	114 (0.71 ± 0.27)	179 (0.73 ± 0.29)	103 (0.69 ± 0.28)	0.24
		FABP3_ht1	·	178 (0.70 ± 0.28)	194 (0.71 ± 0.28)	62 (0.68 ± 0.26)	0.60
		FABP3_ht2	·	249 (0.70 ± 0.26)	161 (0.71 ± 0.29)	24 (0.72 ± 0.35)	0.96
		FABP3_ht3	·	302 (0.72 ± 0.29)	117 (0.67 ± 0.26)	15 (0.60 ± 0.27)	0.08
		FABP3_ht4	·	320 (0.70 ± 0.28)	107 (0.70 ± 0.27)	7 (0.89 ± 0.31)	0.20
		FABP3_ht5	·	375 (0.70 ± 0.28)	58 (0.74 ± 0.26)	1 (0.80)	0.72
	FABP4	*c.-60-235A>G*	Promoter	179 (0.72 ± 0.28)	193 (0.70 ± 0.29)	47 (0.69 ± 0.28)	0.28
		c.-60-227A>G	Promoter	122 (0.73 ± 0.28)	127 (0.69 ± 0.25)	38 (0.64 ± 0.28)	0.10
		c.74-157A>T	Intron1	291 (0.71 ± 0.28)	118 (0.68 ± 0.27)	7 (0.73 ± 0.28)	0.80
		c.220A>G (I74V)	Exon2	159 (0.74 ± 0.28)	201 (0.68 ± 0.27)	55 (0.69 ± 0.30)	0.02
		c.328G>A (V110M)	Exon3	206 (0.70 ± 0.29)	161 (0.70 ± 0.26)	37 (0.77 ± 0.31)	0.23
		c.348+303T>C	Intron3	147 (0.75 ± 0.29)	179 (0.69 ± 0.28)	44 (0.66 ± 0.27)	0.01
		FABP4_ht1	·	193 (0.72 ± 0.28)	200 (0.69 ± 0.28)	41 (0.66 ± 0.27)	0.16
		FABP4_ht2	·	234 (0.70 ± 0.29)	162 (0.69 ± 0.25)	38 (0.77 ± 0.31)	0.15
		FABP4_ht3	·	287 (0.69 ± 0.27)	139 (0.73 ± 0.29)	8 (0.83 ± 0.37)	0.18
		FABP4_ht4	·	306 (0.71 ± 0.28)	121 (0.68 ± 0.27)	7 (0.73 ± 0.28)	0.68

Genotype and haplotype distributions, means, standard deviations (SD), P values controlling for sire and age at slaughter as covariates was shown.
*C/C, C/R, and R/R represent the common allele, heterozygotes and homozygotes for the rare allele, respectively.

MATERIALS AND METHODS

Animals and phenotypic data

The KNC genomic DNA samples were obtained from 419 steers produced from 76 sires used in progeny testing program of National Livestock Research Institute (NLRI) of Korea. The dams were inseminated randomly with young sires. All steers were fed for 731.39 ± 16.53 days period under tightly controlled feeding program in Daekwanryeong and Namwon branch of NLRI. They were weaned at a mean age of 3 months and fed until they were 6 months old with 30% concentrates and 70% roughage. After 6 months of age, they were fed with concentrates consisting of 15% crude protein (CP)/71% totally digestible nutrients (TDN) until they were 14 months old; 13% CP/72% TDN until 20 months; and 11% CP/73% TDN until 24 months of age, respectively. The roughage was offered *ad libitum*, and steers had free access to fresh water during the whole period. Live weights were determined before slaughter. Mean of live weights was 539.93 ± 51.96 kg. Yield grades for carcasses were determined by cold carcass weight (CW) and back fat thickness (BF). After a 24-h chill, CW was measured, and then the left side of each carcass was cut between the last rib and the first lumbar vertebrae to determine BF (16). Means of carcass traits were 311.47 ± 33.39 kg for CW and 0.70 ± 0.28 cm for BF.

Sequencing analysis of *FABP3* and *FABP4*

We have sequenced all exons of *FABP3* and *FABP4* and their flanking regions, including promoter regions (1.5 kb), to discover variants in 24 unrelated KNC using the ABI PRISM 3730

DNA analyzer (Applied Biosystems, Foster City, CA). Fourteen primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (Ref. Genome seq.; *FABP3*: NC_007300 and *FABP4*: NC_007312). Primer information is available in the Supplementary Table 1 and on website (http://www.snp-genetics.com/user/additional_list.asp). Sequence variants were verified by chromatograms (Supplementary Fig. 1 and 2).

Genotyping by single-base extension (SBE) and electrophoresis

For genotyping of polymorphic sites, amplifying and extension primers were designed for single-base extension (SBE) (17). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems). To clean up the primer extension reaction, one unit of SAP (shrimp alkaline phosphatase) was added to the reaction mixture, and the mixture was incubated at 37°C for 1 hour, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products, and Genescan 120 Liz size standard solution was added to Hi-Di formamide (Applied Biosystems) according to the recommendations of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program of ABI Prism GeneScan and Genotyper (Applied Biosystems). Probe information is available in the Supplementary Table 2 and on website (http://www.snp-genetics.com/user/additional_list.asp).

Statistics

A goodness of fit chi-square test was used to test for Hardy-Weinberg equilibrium (HWE) by comparing the observed number of subjects for each genotype with expected number of subjects assuming HWE and so genotype distributions were tested at each polymorphic locus for departure from HWE. Associations between individual SNPs and traits measures were determined by the mixed random effect model using library (nlme) in R statistical package (http://www.r-project.org). Association analysis with CW were performed using a mixed effect model treating "sire" as a random effect and "age" at slaughter was also included in the model as a fixed effect. This mixed effect model used to analyze association with CW was the same as the one BF trait include that age and sire were used as a covariate in the model. Other covariates were not available for this analysis.

We examined a widely used measure of linkage disequilibrium (LD) between all pairs of biallelic loci, Lewontin's D' (18) and r^2. Lewontin's D (the correlation coefficient [Delta, |D'|]) was used to determine whether the pairs of sites were in LD. Haplotypes and their frequencies were inferred using the algorithm developed by Stephens *et al.* (19). LD blocks were determined according to the confidence interval (CI) method using the Gabriel criteria (12). Phase probabilities of each site were calculated for each individual using Haploview software package (20). For the haplotype analyses, we fit the model with the same covariates in a similar manner of SNP association test. P-values less than 0.05 were considered significant.

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