ORIGINAL ARTICLE

Association of swine vertnin (*VRTN*) gene with production traits in Duroc pigs improved using a closed nucleus breeding system

Kensuke HIROSE,[1] Satoshi MIKAWA,[2] Naohiko OKUMURA,[3] Go NOGUCHI,[4] Kazuo FUKAWA,[1] Naoe KANAYA,[3] Ayumi MIKAWA,[3] Aisaku ARAKAWA,[2] Tetsuya ITO,[1] Yoichi HAYASHI,[4] Fumio TACHIBANA[4] and Takashi AWATA[2]

[1]*Central Research Institute for Feed and Livestock ZEN-NOH (National Federation of Agricultural Cooperative Associations), Kamishihoro, Hokkaido,* [2]*National Institute of Agrobiological Sciences,* [3]*JATAFF Institute and* [4]*Central Research Institute for Feed and Livestock, ZEN-NOH (National Federation of Agricultural Co-operative Associations), Tsukuba, Ibaraki, Japan*

ABSTRACT

Vertnin (*VRTN*) is involved in the variation of vertebral number in pigs and it is located on *Sus scrofa* chromosome 7. Vertebral number is related to body size in pigs, and many reports have suggested presence of an association between body length (BL) and meat production traits. Therefore, we analyzed the relationship between the *VRTN* genotype and the production and body composition traits in purebred Duroc pigs. Intramuscular fat content (IMF) in the Longissimus muscle was significantly associated with the *VRTN* genotype. The mean IMF of individuals with the wild-type genotype (Wt/Wt) (5.22%) was greater than that of individuals with the Wt/Q (4.99%) and Q/Q genotypes (4.79%). In addition, a best linear unbiased predictor of multiple traits animal model showed that the Wt allele had a positive effect on the IMF breeding value. No associations were observed between the *VRTN* genotype and other production traits. The *VRTN* genotype was related to BL. The Q/Q genotype individuals (100.0 cm) were longer than individuals with the Wt/Q (99.5 cm) and Wt/Wt genotypes (98.9 cm). These results suggest that in addition to the maintenance of an appropriate backfat thickness value, *VRTN* has the potential to act as a genetic marker of IMF.

Key words: Duroc, polymorphism, vertebral number, vertnin.

INTRODUCTION

The total number of thoracic and lumbar vertebrae varies among pigs. Wild boars have 19 vertebrae, whereas European commercial breeds have 21–23 vertebrae. A quantitative trait locus (QTL) affecting vertebral number was initially detected on *Sus scrofa* chromosome 1 (SSC1) in an experimental F_2 family crossing of a Göttingen miniature male pig and two Meishan female pigs (Wada *et al*. 2000). A second QTL was identified in another F_2 family resulting from a cross between Asian and European breeds, where the F_2 family had both SSC7 and SSC1 QTLs (Mikawa *et al*. 2005). A gene encoding an orphan nuclear receptor (*NR6A1*) was identified as being responsible for the SSC1 locus (Mikawa *et al*. 2007). However, genetic variation in *NR6A1* was not detected in European commercial breed pigs until recently, when Mikawa *et al*. (2011) detected a 41-kb conserved region associated with the vertebrae number-increase allele (Q) of the

SSC7 QTL in European commercial breed pigs. A gene encoding a hypothetical protein responsible for controlling the vertebral number was found in that region and was named *vertnin* (*VRTN*). Three haplotypes of European *VRTN* consist of two major alleles (Q and wild-type allele, (Wt)) and one minor wild-type allele (Wt′) that has been detected only in one Landrace population. There are only nine candidate polymorphism sites, which makes genotyping of porcine *VRTN* feasible. *VRTN* has an additive effect on the vertebral number. The average vertebral numbers in the Wt/Wt,

Wt/Q and Q/Q genotypes in commercial meat pigs are 20.63, 21.18 and 21.65, respectively (Mikawa *et al.* 2011). The vertebral number in pigs is generally associated with body size, which may affect meat productivity and reproductive performance. The length of the loin muscle is negatively correlated with the loin eye muscle area (EMA) and backfat thickness (BF) (Bereskin & Steele 1988; Stewart & Schinckel 1989; Hicks *et al.* 1998). Therefore, variations in *VRTN* may affect phenotypic traits, such as the growth rate, fat deposition and body composition. However, correlations between the *VRTN* genotype and economic traits have yet to be investigated.

In this study, we determined the relationship between the *VRTN* genotype and economic and body composition traits in a Duroc population improved by a closed nucleus breeding system.

MATERIALS AND METHODS
Animals and data collection

The Duroc pig population used in this study was kept at the Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan) by following the Institute's guidelines for animal management. All animals were provided unlimited access to food and water during the test period.

The population formed a part of an improvement program using a closed nucleus breeding system. Boars and gilts were selected for breeding to produce the next generation on the basis of their breeding values (BVs) and the proportion of pigs and their pedigrees in relation to the desired improvements.

First, 28 boars (including three produced by artificial insemination) and 52 gilts were introduced as the base population. We divided the population into two further groups (the first and second groups) after the third generation (G3) to allow more effective improvements with more animals per generation. The first and second groups were produced from the first and second sets of offspring after the second generation, respectively. About 20 boars and 55 gilts were selected in the first group, and five boars and 30 gilts were selected in the second group. In addition, five boars were selected from the 20 boars in the first group after considering their BVs and pedigree. These were used in the second group to prevent separation of the blood relationship between the two groups. Therefore, 10 boars were used for crosses in the second group and these two groups were considered as one same line in each generation. The G6 population was the final generation of this closed nucleus population and it was created using boars and gilts selected from both the first and second groups of the G5 population.

The data collection method used was that described by Hirose *et al.* (2009, 2011). Population selection traits included average daily gain (ADG), BF, loin EMA, and intramuscular fat content (IMF). The objective was to increase the ADG, BF and IMF without changing loin EMA.

ADG was calculated during the test period (from 30 to 90 kg) as the weight gained divided by days elapsed. At approximately 90 kg live weight, BF and loin EMA were measured at a half-body-length position using a real-time B-mode ultrasound scanner (SSD-500; Aloka Co., Ltd, Tokyo, Japan). A computer program (SigmaScan Pro 5.0;

Systat, Inc., Richmond, CA, USA) was used to calculate the loin EMA. We detected high correlation coefficients between the intramuscular fat content sampled by needle biopsy method and content sampled from an approximately 100 g loin meat block at the seventh vertebrae in a previous study (r = 0.916, *n* = 30, *P* = 0.005, unpublished data). So we used intramuscular fat content sampled by needle biopsy method as an indicator for improving the IMF in the whole loin muscle. A biopsy sample was obtained from the loin muscle area at a position halfway along the body and at about 6.5 cm from the vertebral centerline. The crude fat content of the sample was used to determine the IMF, which was only measured in boars and gilts. Body length (BL) was measured as the length from the root of the tail to the root of the ears. Body height (BH) was measured at wither height. Chest circumference (CC) was measured around the chest, while the circumference of the foreleg cannon bone (CF) was measured around the cannon bone of the left front leg.

Selection method

Animals were selected to produce the next generation by considering their aggregate BVs and the proportion of pigs and their pedigrees in each generation. We used genetic and phenotypic parameters from our other Duroc line when predicting the BVs of the first generation, because we could not estimate accurate values for this population based on the limited numbers of animals in the first generation. From the second generation onwards, these parameters were obtained based on performance test data for this population. The BVs of each trait were calculated according to a best linear unbiased predictor (BLUP) of a multiple traits animal model using the PEST3.1 program (Groeneveld *et al.* 1992) after estimating genetic parameters using the VCE3.2 program (Groeneveld 1996). Generation, sex and lineage effects were used as fixed effects, while the additive genetic effect and error were included as random effects. Subsequently, the aggregate BVs were calculated by multiplying the relative economic weights by the predicted BV for each trait. The relative economic weights were obtained based on the genetic parameter of traits and the relative economic value of each trait using the method proposed by Hazel (1943). However, it was impossible to predict an accurate relative economic value for each trait, in which case we defined selection procedure to achieve our desired genetic gain by using the method of linear programming techniques rather than predicting the relative economic values. We calculated the relative selection index weights to maximize the genetic gains of IMF. Consequently, the aggregate BV was calculated from the following equation:

$$H = 0.518 \times BVADG + 29.799 \times BVBF + 6.592 \times BVEMA + 65.318 \times BVIMF.$$

Genotyping

Genomic DNA was extracted from tail tissue clippings of each pig using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Hilden, Germany) or the QuickGene DNA Tissue Kit (Fujifilm, Inc., Tokyo, Japan). All animals were genotyped for the previously identified haplotypes *NV107*, *NV123* and *NV149* (Mikawa *et al.* 2011) by PCR amplification along with sequence-specific primers. Primer sets were designed based on the AB554652 sequence, as shown in Table 1. The PCR reaction was performed using a reaction mix (15 µL total volume) containing 25 ng of genomic DNA, 7.5 µL of AmpliTaq Gold®360 Master Mix (Applied Biosystems, Foster City, CA,

Table 1 PCR primer sequence and size of allelic polymorphisms

Primer name	Primer sequence (5′-3′)	Final concentration rate (μmol/L)	PCR product length (base pairs)	
			Wt	Q
NV107	Forward; CGA CAG GAA CTC TGC ATC AA	0.30	295	
	Reverse; CAA ATA AAA TAG GTC TTT TTC C			
NV123	Forward; GAT CCT TGG TGA GCT CGA AT	0.15	213	242
	Reverse1; TCG TCA ACC CAC TGA GCA			
	Reverse2; CCT TCC TCC TCC TGG AGT CT			
NV149	Forward; GGA CAC CAG GCC TGA GAT TA	0.15		146
	Reverse; AAG AGG TTT CAA GGG CTT GA			

USA), and 0.15–0.3 μmol/L of each PCR primer. The PCR conditions were as follows: denaturation at 94°C for 9 min, 35 cycles of amplification at 94°C for 30 s, 57°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min.

Statistical analysis

Data were collected from 1414 Duroc pigs through four generations, that is, from the second to fifth generations. Associations between the *VRTN* genotype and traits were evaluated using the least squares method of the Minitab general linear model (Version 14.12.2; Minitab Inc., State College, PA, USA). The following linear model was used to analyze the data:

$$Y_{ijkl} = \mu + SE_i + GE_j + G_k + L_l + \beta W_{ijkl} + e_{ijkl} \text{ (Model A)}$$

where Y_{ijkl} is the phenotypic value of each trait, μ is the overall mean for each trait, SE_i is the effect of gender ($i = 0, 1, 2$), GE_j is the effect of the *VRTN* genotype ($j = 0, 1, 2$), G_k is the effect of generation ($k = 1, 2, 3, 4$), L_l is the effect of group ($l = 0.1$), β is the regression coefficient of the covariate weight measurement for each trait, W_{ijkl} is the covariate of the measurement weight, and e_{ijkl} is the random residual effect. BF and loin EMA were correlated with the measurement weight, and therefore these traits were analyzed using weight measurement as a covariate.

The BV predicted using a previous multiple-animal model (BLUP) for the trait was analyzed statistically when there was a significant association between the *VRTN* genotype and each trait. The BLUP model used in this program included the additive effect of polygene as a random effect, with gender, generation and group as fixed effects, as well as the covariates between measurement weight and each trait. ANOVA with genotype as the independent variable and BV as the dependent variable was used to analyze the association between the *VRTN* genotype and the BV. This ANOVA analysis was executed only for BVs of traits that had significant associations with the *VRTN* genotype.

Additive or dominant effects of *VRTN* were evaluated with the Qxpak program (Perez-Enciso & Misztal 2004) using the following Model B:

$$Y_{ijkl} = \mu + SE_i + GE_j + G_k + L_l + \beta W_{ijkl} + u_{ijkl} + e_{ijkl} \text{ (Model B)}$$

where GE_j represents the single locus of the *VRTN* genotypic effect, which is partitioned into additive (a) and dominance (d) effects. We conducted this analysis for the additive and dominance effects (a + d) and for only additive effects (a). u_{ijkl} is the infinitesimal genetic

effect of ijkl animals, which is distributed as $N (0, K\sigma_u^2)$ (K is the numerator relationship matrix).

Pedigrees of the base population of animals were traced back for the first generation in this population to produce the numerator relationship matrix. Thus, 1744 animals were used in this analysis, including animals that had not been genotyped. Likelihood ratio tests were performed by removing the *VRTN* genotypic effects from the model, while nominal *P*-values were obtained by assuming a chi-squared distribution for the likelihood ratio test. The proportion of additive genetic variance accounted for by the genotypic effect of *VRTN* gene was calculated as:

$$variance\ percentage = \left[2pq(a + d(p - q))^2 \right] / V_A$$

where p and q were allelic frequencies for allele Wt and allele Q, respectively, and V_A was the additive genetic variance of the trait obtained from animal model analysis ignoring *VRTN* genetic effects (Falconer 1989).

To select the most suitable model, Akaike's information criterion (AIC) values of the mathematical model were compared for the full Model A and the model where the *VRTN* genotypic effect was removed from Model A.

AIC value was calculated using GenStat (Version 8.1.0.152; VSN International Ltd, Hempstead, UK) with the restricted maximum likelihood (REML) method (Patterson & Thompson 1971). AIC value was defined as −2 log (maximum likelihood) + 2 (number of independently adjusted parameters within the model) (Akaike 1974, 1987), and the model with the minimum AIC value was considered the most suitable.

RESULTS
Selection

Selection to improve economic traits such as the average ADG, BF and IMF content was conducted using a multi-trait animal model BLUP in this Duroc population through five generations. The results of phenotypic and breeding values for each trait are shown in Table 2. Average phenotypic values of the ADG and BF at the fifth generation significantly increased by 25 g/day, 0.27 cm compared with the second generation, respectively. But phenotypic value of IMF decreased 0.12%. The BVADG, BVBF and BVIMF at the fifth generation significantly increased by 65 g/day, 0.28 cm and 0.36% compared with those

Table 2 The trend for phenotypic and breeding values in a Duroc population†

Sex	Generation	Phenotypic values‡					Breeding values‡			
		MW	ADG	BF	EMA	IMF	BVADG	BVBF	BVEMA	BVIMF
		(kg)	(kg/day)	(cm)	(cm²)	(%)	(kg/day)	(cm)	(cm²)	(%)
Boars	2	97.8 (6.2)	992 (79)	1.44 (0.29)	37.9 (3.8)	4.70 (2.00)	−25 (43)	0.01 (0.19)	0.4 (1.2)	0.23 (0.73)
	3	97.2 (6.2)	1042 (93)	1.52 (0.29)	38.2 (4.0)	4.51 (1.51)	−5 (52)	0.07 (0.19)	0.6 (1.3)	0.44 (0.53)
	4	95.4 (5.0)	1014 (95)	1.50 (0.27)	36.6 (3.6)	3.96 (1.08)	11 (56)	0.15 (0.20)	0.2 (1.3)	0.58 (0.54)
	5	94.8 (5.0)	1016 (101)	1.66 (0.32)	36.5 (3.0)	4.52 (1.43)	41 (59)	0.25 (0.21)	0.1 (1.0)	0.85 (0.57)
	P§	<0.001	0.002	<0.001	0.047	0.195	<0.001	<0.001	0.027	<0.001
Gilts	2	94.5 (4.6)	929 (83)	1.59 (0.32)	38.4 (3.6)	5.11 (1.95)	−27 (47)	−0.02 (0.23)	0.5 (1.2)	0.18 (0.74)
	3	95.5 (5.3)	975 (88)	1.78 (0.34)	38.5 (3.7)	4.71 (1.25)	−8 (46)	0.09 (0.22)	0.6 (1.2)	0.39 (0.53)
	4	94.7 (4.2)	970 (94)	1.73 (0.31)	37.0 (3.2)	4.74 (1.50)	15 (55)	0.15 (0.21)	0.3 (1.3)	0.65 (0.59)
	5	93.0 (3.6)	956 (95)	1.93 (0.35)	36.1 (3.3)	5.06 (1.48)	37 (57)	0.27 (0.22)	−0.1 (1.0)	0.80 (0.65)
	P§	<0.001	0.011	<0.001	<0.001	0.091	<0.001	<0.001	<0.001	<0.001
Barrows	2	96.6 (6.4)	1010 (92)	1.70 (0.35)	37.8 (4.1)	–	−23 (51)	−0.04 (0.24)	0.4 (1.3)	0.23 (0.49)
	3	96.8 (7.2)	1068 (105)	1.96 (0.35)	39.2 (4.4)	–	−2 (57)	0.13 (0.22)	0.7 (1.1)	0.40 (0.46)
	4	94.0 (3.8)	1049 (107)	1.86 (0.38)	36.3 (3.4)	–	20 (64)	0.17 (0.24)	0.2 (1.4)	0.67 (0.38)
	5	93.4 (4.1)	1014 (90)	2.01 (0.33)	35.7 (3.7)	–	34 (52)	0.29 (0.23)	0.0 (1.0)	0.84 (0.43)
	P§	0.001	0.821	<0.001	0.14	–	<0.001	<0.001	0.065	<0.001
Total	2	96.1 (5.7)	966 (89)	1.55 (0.33)	38.2 (3.7)	4.94 (1.97)	−25 (46)	−0.01 (0.22)	0.4 (1.2)	0.20 (0.70)
	3	96.3 (6)	1014 (100)	1.70 (0.36)	38.5 (3.9)	4.62 (1.37)	−6 (50)	0.09 (0.21)	0.6 (1.2)	0.41 (0.52)
	4	94.9 (4.5)	999 (100)	1.65 (0.33)	36.7 (3.4)	4.36 (1.37)	14 (57)	0.15 (0.21)	0.2 (1.3)	0.62 (0.55)
	5	93.8 (4.4)	991 (101)	1.82 (0.37)	36.2 (3.2)	4.82 (1.48)	39 (57)	0.27 (0.22)	0.0 (1.0)	0.83 (0.59)
	P§	<0.001	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	<0.001	<0.001

†Mean values (SD) ‡MW, mesurement weight; ADG, average daily gain; BF, backfat thickness; EMA, eye muscle area; IMF, intermuscular fat content; BVDG, breeding value of ADG; BVBF, breeding value of BF; BVEMA, breeding value of EMA; BVIMF, breeding value of IMF. §P-values were estimated by GLM to compare between second and fifth generations.

of the second generation, respectively. Therefore, these values showed that improved gains had been established according to improvement goal. In this population, the trend of breeding value for IMF did not conform to that of the phenotypic value through four generations. However, the reason for this was not clear in this study.

The BVEMA decreased slightly by 0.44 cm² compared with those of the second generation. The improvement goal of loin EMA was to maintain the size of the first generation. Therefore, this slight change in BVEMA indicates that the loin EMA improvement was fairly successful.

VRTN allele frequencies

Table 3 shows the allelic and genotypic frequencies for the Wt and Q *VRTN* polymorphisms. The allelic and genotypic frequencies of *VRTN* changed from the second generation to the fifth, while the Wt allele and the Wt/Wt genotype increased significantly according to Pearson's chi-square test ($\chi^2 = 6.163$, df = 1, $P = 0.013$; $\chi^2 = 7.962$, df = 2, $P = 0.019$; respectively).

Association of *VRTN* genotype and economic traits

Table 4 shows the phenotypic values of the measured traits for each *VRTN* genotype. The *VRTN* genotype was significantly associated with the IMF content ($P = 0.003$). Pigs with the Wt/Wt genotype had a significantly higher mean IMF ($5.22 \pm 0.16\%$) than those with the Q/Q genotype ($4.79 \pm 0.13\%$, $P = 0.013$). This effect was observed only in boars (Wt/Wt: $5.06 \pm 0.19\%$; Q/Q: $4.38 \pm 0.14\%$, $P = 0.008$), whereas the differences in gilts were not statistically significant (Wt/Wt: $5.22 \pm 0.18\%$; Q/Q: $5.02 \pm 0.12\%$, $P = 0.543$). There was no evidence of any effects of the *VRTN* genotype on other traits such as ADG, BF or loin EMA.

We evaluated the association only between the BVIMF and *VRTN* genotypes because there was a significant association only in the phenotypic IMF value. There was a highly significant difference ($P = 0.005$) among *VRTN* genotypes with respect to the BVIMF. The BVIMF for the Wt/Wt genotype was larger than that for the Q/Q genotype.

Table 3 Genotypic and allelic frequencies of the Wt and Q gene polymorphisms

Generation	Number of pigs				Genotypic frequency†			Allelic frequency‡	
	Total	Boar	Gilt	Barrow	Wt/Wt	Wt/Q	Q/Q	Wt	Q
Total	1414	588	630	196	14.9 (210)	51.1 (722)	34.0 (481)	40.4 (1142)	59.6 (1684)
G2	283	113	129	41	12.7 (36)	46.6 (132)	40.6 (115)	36.0 (204)	64.0 (362)
G3	344	131	165	48	16.6 (57)	47.4 (163)	36.0 (124)	40.3 (277)	59.7 (411)
G4	366	154	163	49	13.9 (50)	54.9 (194)	31.1 (115)	41.4 (294)	58.6 (424)
G5	421	190	173	58	15.9 (67)	53.7 (226)	30.4 (128)	42.8 (355)	57.2 (477)

†Percentage of each genotype. Number of pigs in parentheses. ‡Percentage of each allele. Number of alleles in parentheses.

Table 4 Association between porcine polymorphisms and economic traits in Duroc pigs

Traits†	Sex	Total‡	Genotype§			*P*-value
			W/W	W/Q	Q/Q	
ADG (g/day)	Boar	1017 ± 4 (588)	1008 ± 10 (95)	1006 ± 6 (300)	1012 ± 7 (193)	0.823
	Gilt	959 ± 4 (630)	951 ± 9 (96)	960 ± 6 (321)	955 ± 7 (213)	0.647
	Barrow	1035 ± 7 (196)	1068 ± 23 (20)	1023 ± 10 (101)	1034 ± 12 (75)	0.171
	Total	994 ± 3 (1414)	1000 ± 7 (211)	998 ± 4 (722)	1000 ± 5 (481)	0.941
BF (cm)	Boar	1.54 ± 0.01 (588)	1.51 ± 0.03 (95)	1.52 ± 0.02 (300)	1.52 ± 0.02 (193)	0.926
	Gilt	1.77 ± 0.01 (630)	1.83 ± 0.03 (96)	1.76 ± 0.02 (321)	1.75 ± 0.02 (213)	0.105
	Barrow	1.90 ± 0.03 (196)	1.92 ± 0.08 (20)	1.85 ± 0.04 (101)	1.90 ± 0.04 (75)	0.545
	Total	1.70 ± 0.01 (1414)	1.75 ± 0.02 (210)	1.72 ± 0.01 (722)	1.72 ± 0.02 (481)	0.326
EMA (cm²)	Boar	37.2 ± 0.2 (587)	37.4 ± 0.4 (95)	37.5 ± 0.2 (300)	37.1 ± 0.3 (192)	0.523
	Gilt	37.4 ± 0.1 (628)	37.8 ± 0.4 (96)	37.6 ± 0.2 (321)	37.3 ± 0.3 (211)	0.346
	Barrow	37.1 ± 0.3 (195)	38.6 ± 0.9 (20)	36.7 ± 0.4 (101)	37.7 ± 0.5 (74)	0.058
	Total	37.3 ± 0.1 (1410)	37.7 ± 0.3 (210)	37.4 ± 0.2 (722)	37.3 ± 0.2 (481)	0.317
IMF (%)	Boar	4.35 ± 0.07 (397)	5.06 ± 0.19[a] (61)	4.61 ± 0.11[ab] (216)	4.38 ± 0.14[b] (120)	0.008
	Gilt	4.88 ± 0.07 (486)	5.22 ± 0.18 (72)	5.15 ± 0.10 (252)	5.02 ± 0.12 (162)	0.543
	Total	4.60 ± 0.05 (883)	5.22 ± 0.16[a] (133)	4.99 ± 0.12[ab] (468)	4.79 ± 0.13[b] (282)	0.013
BVIMF (%)	Total	0.43 ± 0.98 (1414)	0.54 ± 1.10[b] (211)	0.48 ± 0.99[ab] (722)	0.32 ± 0.91[a] (481)	0.005

a-b: Means within a row with no common superscript differ significantly ($P < 0.05$). †ADG, average daily gain; BF, backfat thickness; EMA, eye muscle area; IMF, intramuscular fat content; BVIMF, breeding value of intramuscular fat content. ‡Mean values (±SE) of all pigs in each sex. §Least square mean values (± SE). Different letters denote significant differences between genotypes. Number of pigs is given in parentheses.

Association of *VRTN* genotype and body composition traits

Table 5 shows the phenotypic values of the body composition traits for each *VRTN* genotype. The *VRTN* genotype was significantly associated with BL in boars, gilts, barrows and the total population (P = 0.021, P = 0.015, P = 0.001, P < 0.001, respectively). Significant differences between the *VRTN* genotype and other traits (e.g., BH and CC) were detected in

some cases, but in one gender only, while the differences were not statistically significant at the overall population level.

Additive and dominant effects of *VRTN* on each trait

Table 6 shows the additive and dominant effects of *VRTN* on economic traits and body composition traits. The *VRTN* genotype did not significantly affect IMF in

Table 5 Association between VRTN genotypes and body composition traits in Duroc pigs

Traits†	Sex	Total‡	Genotype§ W/W	Genotype§ W/Q	Genotype§ Q/Q	*P*-value
BL (cm)	Boar	100.3 ± 0.2 (588)	99.3 ± 0.4 (95)	99.7 ± 0.2 (294)	100.2 ± 0.3 (194)	0.072
	Gilt	100.0 ± 0.1 (630)	99.0 ± 0.3ᵃ (96)	99.6 ± 0.2ᵃᵇ (317)	99.9 ± 0.2ᵇ (214)	0.041
	Barrow	99.6 ± 0.3 (196)	97.6 ± 0.7ᵃ (19)	99.1 ± 0.3ᵃᵇ (99)	100.1 ± 0.4ᵇ (74)	0.005
	Total	100.1 ± 0.1 (1414)	98.9 ± 0.2ᵃ (210)	99.5 ± 0.1ᵇ (710)	100.0 ± 0.2ᶜ (482)	<0.001
CC (cm)	Boar	105.5 ± 0.1 (588)	105.5 ± 0.3 (95)	105.6 ± 0.2 (294)	105.7 ± 0.2 (194)	0.789
	Gilt	106.1 ± 0.1 (630)	106.9 ± 0.3ᵇ (96)	106.1 ± 0.2ᵃ (317)	106.3 ± 0.2ᵃᵇ (214)	0.039
	Barrow	107.4 ± 0.3 (196)	107.3 ± 0.6 (19)	107.3 ± 0.3 (99)	107.4 ± 0.3 (74)	0.970
	Total	106.0 ± 0.1 (1414)	106.7 ± 0.2 (210)	106.4 ± 0.1 (710)	106.4 ± 0.1 (482)	0.470
BH (cm)	Boar	62.2 ± 0.1 (588)	62.6 ± 0.2 (95)	62.3 ± 0.1 (294)	62.3 ± 0.1 (194)	0.312
	Gilt	61.5 ± 0.1 (630)	61.8 ± 0.2ᵇ (96)	61.7 ± 0.1ᵇ (317)	61.2 ± 0.1ᵃ (214)	0.016
	Barrow	61.4 ± 0.2 (196)	61.3 ± 0.5 (19)	61.6 ± 0.2 (99)	61.5 ± 0.2 (74)	0.833
	Total	61.8 ± 0.1 (1414)	62.0 ± 0.1 (210)	61.9 ± 0.1 (710)	61.7 ± 0.1 (482)	0.062
CF (cm)	Boar	18.5 ± 0.0 (588)	18.6 ± 0.1 (95)	18.7 ± 0.1 (294)	18.5 ± 0.1 (194)	0.131
	Gilt	17.7 ± 0.0 (630)	17.8 ± 0.1 (96)	17.8 ± 0.0 (317)	17.9 ± 0.1 (214)	0.732
	Barrow	17.9 ± 0.1 (196)	18.1 ± 0.2 (19)	18.0 ± 0.1 (99)	17.8 ± 0.1 (74)	0.147
	Total	18.1 ± 0.0 (1413)	18.1 ± 0.1ᵃᵇ (210)	18.2 ± 0.0ᵇ (710)	18.1 ± 0.0ᵃ (482)	0.044

a-b: Means within a row with no common superscript differ significantly (P < 0.05). †BL, body length; CC, chest circumference; BH, body height; CF, cannon circumference of foreleg. ‡Mean values (± SE) of all pigs in each sex. §Least square mean values (± SE). Different letters denote significant differences between genotypes. Number of pigs is given in parentheses.

Table 6 Additive and dominance effects of VRTN on economic traits and body composition traits†

Traits‡	Sex	Model§	LRT¶	*P*	a ± SE¶	d ± SE¶	Variance (%)¶
EM	Barrow	a + d	8.020	0.018	0.78 ± 0.54	−1.8 ± 0.63	3.40
IMF	Boar	a + d	6.581	0.037	0.31 ± 0.12	−0.11 ± 0.15	5.16
		a	6.050	0.014	0.29 ± 0.12	–	5.04
	Total	a	3.980	0.046	0.17 ± 0.08	–	1.49
BL	Boar	a + d	8.358	0.015	−0.55 ± 0.22	−0.23 ± 0.27	3.35
		a	7.611	0.006	−0.59 ± 0.21	–	3.44
	Gilt	a + d	11.905	0.003	−0.65 ± 0.20	−0.08 ± 0.24	5.03
		a	11.800	0.001	−0.67 ± 0.19	–	5.03
	Barrow	a + d	13.431	0.001	−1.52 ± 0.42	0.35 ± 0.49	21.45
		a	12.932	<0.001	−1.4 ± 0.38	–	20.75
	Total	a + d	25.590	<0.001	−0.71 ± 0.15	−0.03 ± 0.17	5.36
		a	25.547	<0.001	−0.72 ± 0.14	–	5.46
CC	Gilt	a + d	7.035	0.030	0.29 ± 0.17	−0.46 ± 0.2	0.55
CF	Boar	a + d	11.739	0.003	0.11 ± 0.04	0.10 ± 0.54	3.84
		a	8.644	0.003	0.13 ± 0.04	–	4.03
	Barrow	a	4.105	0.043	0.16 ± 0.19	–	5.80
	Total	a + d	8.887	0.012	0.04 ± 0.03	0.08 ± 0.03	0.52

†Only those for which statistically significant (P < 0.05) gene effects were detected are listed for each trait. ‡EM, eye muscle; IMF, intermuscular fat content; BL, body length; CC, chest circumference; CF, cannon circumference of the foreleg. §a + d: model includes both additive and dominance effects as VRTN effect; a: model includes only additive effect as VRTN effect. ¶Additive and dominance effects were genotypic values of (TT-CC).2 and TC-(TT+CC)/2, respectively. LRT, likelihood ratio test. Variance (%) = the proportion of additive genetic variance accounted for by the VRTN genotypic effect.

the additive and dominance models ($P = 0.117$), but it had a significant association in the additive model ($P = 0.046$). There was a highly significant association of BL in both the additive and dominance models ($P < 0.001$) and the additive model only ($P < 0.001$). For CF, there was a significant association between the additive and dominance models ($P = 0.012$) in all animals. However, for the traits which were related with *VRTN* genotype, the proportion of additive genetic variance accounted for by *VRTN* genotypes were not high (Table 5).

Comparison of the statistical model fitness

The AIC values estimated when using the *VRTN* genotype for IMF were smaller than those estimated when not using the *VRTN* genotype (AIC = 1618.8 and 1624.5, respectively). The *VRTN* genotype had a highly significant effect on IMF ($P = 0.045$) in the Wald test results using the *VRTN* genotype as a fixed effect, in the REML variance components analysis.

DISCUSSION

The association analysis indicates that the swine *VRTN* genotype had a significant effect on the phenotypic value of IMF and BL in Duroc swine. Wt/Wt pigs had a higher IMF content and a shorter BL compared with Q/Q pigs. Moreover, the IMF breeding was significantly greater in Wt/Wt pigs compared with Q/Q pigs. Stewart and Schinckel (1989) reported that the swine carcass length was positively correlated with the total lean content and negatively correlated with the BF. The *VRTN* genotype affected the IMF, but it did not affect the BF in this study. Some studies have reported that genes are involved in the regulation of fat deposition in muscle without affecting fat deposition elsewhere. For example, the *H-FABP* (Gerbens *et al.* 2000) and *SREBF1* (Chen *et al.* 2008) genotypes are associated with IMF without affecting BF in pigs. The functional effect of *VRTN* remains unclear, but our results indicate that *VRTN* may be involved in the regulation of fat deposition in muscles.

Furthermore, the Wt allele frequency increased, suggesting that it was probably synchronized with an increase in the average BV for the IMF content through four generations (from the second to the fifth). This suggests that *VRTN* may affect intramuscular fat deposition.

There was a significant difference between the *VRTN* genotype and the phenotypic value of CF. The role of *VRTN* was not clear in our study, because components such as the size of cannon bone or the muscle content around cannon bone can have an effect on the circumference of the foreleg. There is a need for more research on the relationship between the *VRTN* genotype and the circumference of the foreleg.

IMF is related to meat quality, and numerous taste panel studies have demonstrated that IMF is positively associated with juiciness, flavor and tenderness (De Vol *et al.* 1988; Wood *et al.* 1988; Fernandez *et al.* 1999; Lonergan *et al.* 2002). It is also known that IMF has a positive correlation with BF. In Japan, pigs without an appropriate BF are less valuable according to the Japanese carcass grading regulations. Therefore, it is important that BF is maintained at an appropriate value when the aim is to increase IMF. The present study suggests that *VRTN* could be a useful genetic marker for improving IMF, because the variation in *VRTN* was not related to BF in the Duroc population.

Although the proportion of additive genetic variance for IMF accounted for by *VRTN* genotypes were not high, the AIC value which includes the *VRTN* genotype effect showed smaller than that without considering the *VRTN* genotype effect. The model with the minimum AIC value was considered the suitable model. This result suggests that it is useful to consider the *VRTN* genotype in a mathematical model for predicting a more accurate breeding value of IMF in this Duroc population.

Several studies have detected QTLs related to IMF on SSC7, which is the chromosomal locus where *VRTN* is located in crossbred populations. Sato *et al.* (2003, 2006) detected a significant QTL affecting IMF on SSC7 in a Meishan × Duroc F_2 resource population, while Bidanel *et al.* (1998) also detected a significant QTL affecting IMF in a Meishan × Large White crossbred pig population. However, the positions of these QTL do not overlap with that of *VRTN*. Uemoto *et al.* (2008) detected no significant QTLs for IMF on SSC7 in a pure Duroc population, while Sanchez *et al.* (2007) detected no QTLs for IMF in a Duroc × Landrace cross population. The difference between the current results and those of previous studies may be attributable to the differences in the genetic background of the populations used in the different investigations. We did not perform a QTL analysis for this population, but we are now executing a genome-wide association study for this Duroc population. In this ongoing analysis, we have detected an area in SSC7 that is significantly correlated with IMF (data not shown). The association between that area and the *VRTN* genotype remains unclear, but the processing of this genome-wide association study might detect a genetic mutation on SSC7 that is related to IMF content.

Our results suggest that one *VRTN* allele might produce an increase of 0.54 cm in terms of BL in a 90-kg live weight animal. Mikawa *et al.* (2011) reported that the Q allele of *VRTN* increased the vertebral number with an additive effect of 0.51 in a meat-pig population. The average length of each vertebra is generally about 3–4 cm in 90-kg live weight

Duroc pigs; thus, the Q allele may increase the BL by approximately 1.5–2.0 cm, which is very different from our result. Therefore, the *VRTN* genotype may affect the length of each vertebra. Moreover, BL was defined in this investigation by measuring the distance between the base of the tail to the top of the head, which included thoracic, lumbar, cervical, and sacral vertebrae. Therefore, the *VRTN* genotype may simultaneously affect the lengths of cervical and sacral vertebrae. Moreover, Uemoto *et al.* (2008) detected significant QTLs on SSC7 that affected the thoracic vertebrae number or carcass length. However, there were differences in the QTL genotypic heritability and the residual polygenic heritability for each QTL. This shows that vertebrae number is not always consistent with the BL. Further investigations involving measurements of swine carcasses are needed to confirm the relationship between the *VRTN* genotype, carcass length and vertebrae number. We performed our analysis using only one Duroc population. In future, other breeds and populations should be studied to clarify the effects of *VRTN* on porcine productive traits, particularly fat deposition.

ACKNOWLEDGMENTS

REFERENCES

Akaike H. 1974. A new look at the statistical identification model. *IEEE Transactions on Automatic Control* **19,** 716–723.

Akaike H. 1987. Factor analysis and AIC. *Psychometrika* **52,** 317–332.

Bereskin B, Steele NC. 1988. Estimates of genetic parameters for carcass measures of body composition and growth in swine. *Journal of Animal Science* **66,** 2498–2507.

Bidanel JP, Milan D, Woloszyn N. 1998. Mapping of QTLs in F2 crosses between Meishan and Large White pig breeds. In: *Proceedings of the XXVIth International Conference on Animal Genetics.* p. 105. International Society for Animal Genetics, Auckland.

Chen J, Yang XJ, Xia D, Chen J, Wegner J, Jiang Z, Zhao RQ. 2008. SREBF1 expression and genetic polymorphism significantly affect intramuscular fat deposition in the longissimus muscle of Erhualian and Sutai pigs. *Journal of Animal Science* **86,** 57–63.

De Vol DL, McKeith FK, Bechtel PJ, Novakofski J, Shanks RD, Carr TR. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *Journal of Animal Science* **66,** 385–395.

Falconer DS. 1989. *Introduction to Quantitative Genetics*, 3rd edn. Longman scientific & Technical, Harlow.

Fernandez X, Monin G, Talmant A, Mourot J, Lebret B. 1999. Influence of intramuscular fat content on the quality of pig meat – 1. Composition of the lipid fraction and sensory characteristics of *m. longissimus lumborum*. *Meat Science* **53,** 59–65.

Gerbens F, de Koning DJ, Harders FL, Meuwissen TH, Janss LL, Groenen MA, Veerkamp JH, Van Arendonk JA, te Pas MF. 2000. The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. *Jounal of Animal Science* **78,** 552–559.

Groeneveld E. 1996. *REML VCE: A Multivariate Multi-Model Restricted Maximum Likelihood (Co)Variance Component Estimation Package Version 3.2 User's Guide*. Institute of Animal Husbandry and Animal Behavior, Federal Research Center of Agriculture, Mariensee.

Groeneveld E, Kovac M, Wang T, Fernando RL. 1992. A generalized computing procedure for setting up and general purpose BLUP package for multivariate prediction and estimation. *Archiv für Tierzucht* **35,** 399–412.

Hazel LN. 1943. The Genetics basis for constructing selection indexes. *Genetics* **2,** 476–490.

Hicks C, Satoh M, Ishi K, Kuroki S, Fujiwara T. 1998. Estimates of genetic parameter for daily gain and carcass traits in swine. *Animal Science Technology* **69,** 1094–1098.

Hirose K, Nakamura M, Takizawa T, Fukawa K, Ito T, Ueda M, Sasaki T, Tanaka K. 2009. An insertion/deletion variant of a thymine base in exon 2 of the porcine beta 3-adrenergic receptor gene associated with loin eye muscle area. *Animal Science Journal* **80,** 624–630.

Hirose K, Takizawa T, Fukawa K, Ito T, Ueda M, Hayashi Y, Tanaka K. 2011. Association of an SNP marker in exon 24 of a class 3 phosphoinositide-3-kinase (*PIK3C3*) gene with production traits in Duroc pigs. *Animal Science Journal* **82,** 46–51.

Lonergan EH, Baas TJ, Malek M, Dekkers JCM, Prusa K, Rothschild MF. 2002. Correlations among selected pork quality traits. *Journal of Animal Science* **80,** 617–627.

Mikawa S, Hayashi T, Nii M, Shimanuki S, Morozumi T, Awata T. 2005. Two quantitative trait loci on *Sus scrofa* chromosome 1 and 7 affecting number of vertebrae. *Journal of Animal Science* **83,** 2247–2254.

Mikawa S, Morozumi T, Shimanuki S, Hayashi T, Uenishi H, Domukai M, Okumura N, Awata T. 2007. Fine mapping of a swine quantitative trait locus for number of vertebrae and analysis of an orphan nuclear receptor, germ cell number factor (NR6A1). *Genome Research* **17,** 586–593.

Mikawa S, Sato S, Nii M, Morozumi T, Yoshioka G, Imaeda N, Yamaguchi T, Hayashi T, Awata T. 2011. Identification of a second gene associated with variation in vertebral number in domestic pigs. *BMC Genetics* **12,** 5.

Patterson HD, Thompson R. 1971. Recovery of inter-block information when block sizes are unequal. *Biometrika* **58,** 545–554.

Perez-Enciso M, Misztal I. 2004. Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. *Bioinformatics* **20,** 2792–2798.

Sanchez MP, Iannuccelli N, Basso B, Bidanel JP, Billon Y, Gandemer G, Gilbert H, Larzul C, Legault C, Riquet J, Milan D, Roy PL. 2007. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. *BMC Genetics* **8,** 55.

Sato S, Hasebe H, Sato S, Asahi Y, Hayashi T, Kobayashi E, Sugimoto Y. 2006. High-resolution physical mapping and construction of a porcine contig spanning the intramuscular fat content QTL. *Animal Genetics* **37,** 113–120.

Sato S, Oyamada Y, Atsuji K, Nade T, Sato S, Kobayashi E, Mitsuhashi T, Nirasawa K, Komatsuda A, Saito Y, Terai S, Hayashi T, Sugimoto Y. 2003. Quantitative trait loci analysis for growth and carcass trait in a Meishan x Duroc F₂ resource population. *Journal of Animal Science* **81,** 2938–2949.

Stewart TS, Schinckel AP. 1989. Genetic parameters for swine growth and carcass traits. In: Young LD (ed.), *Genetics of Swine*, pp. 77–79. USDA-ARS, Nebraska.

Uemoto Y, Nagamine Y, Kobayashi E, Sato S, Tayama T, Suda Y, Shibata T, Suzuki K. 2008. Quantitative trait loci analysis on *Sus scrofa* chromosome 7 for meat production, meat quality, and carcass traits within a Duroc purebred population. *Journal of Animal Science* **86,** 2833–2839.

Wada Y, Akita T, Awata T, Furukawa T, Sugai N, Inage Y, Ishii K, Ito Y, Kobayashi E, Kusumoto H. 2000. Quantitative trait loci (QTL) analysis in a Meishan × Göttingen cross population. *Animal Genetics* **31,** 376–384.

Wood JD, Enser M, Moncrieff CB, Kempster AJ. 1988. Effects of carcass fatness and sex on the composition and quality of pig meat. *Proceeding 34th International Congress of Meat Science and Technology*, pp.562-564. Brisbane, Australia.