Genetic studies on usefulness of gene markers in Duroc pig population improved by closed nucleus breeding system

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Contents

General Introduction

In recent years, business performance in Japanese pig industry have been deteriorated by increasing production costs due to higher feed prices and imports of low price pork from abroad, and pig herd size in Japan which recorded 12 million heads in 1989 is a steadily decreasing every year. It is required the efficiency of pig productivity by improving of feeding and management technology and breeding pigs that have superior genetic talent in order to stabilize the domestic pig production management.

The methods of pig breeding scheme conducted in Japan are mainly distinguished between open nucleus breeding system and closed nucleus breeding system. Open nucleus breeding system is the method conducted by the improvement of desired traits of the population with introducing genetic resources continuously from other populations, which is mainly performed in private breeders in Japan. On the other hands, closed nucleus breeding systems is uniquely developed in Japan, which is mainly performed in public breeders such as local governmental institute or National Agricultural Co-operative (ZEN-NOH). The closed nucleus breeding scheme is performed by repeating the selection superior pigs through about five generations in the isolated population, in which new genetic resources are not introduced from other populations. “Strain development” is one of nucleus breeding system, the pig lines produced by this method is named “Strain pigs” which have highly genetic identity.

In any case, the traits for improvement are mainly production traits (ex. growth rate, backfat thickness, loin eye muscle area, or intramuscular fat content), fertility traits (e.g. total number born, number of born alive),

body composition (e.g. leg soundness), meat quality (e.g. softness, tenderness), or anti-disease traits. These traits are quantitative traits influenced by many genes that have complicated relationship, therefore, it is usual for improvement of these traits that the selection of individuals is performed on base of the values called an estimated breeding value (EBV) which is calculated by average of all additive genetic effects relate with the traits.

In modern breeding works, most genetic progress for quantitative traits in livestock has been performed by predicting the EBVs derived from phenotype without knowledge of each gene that affects the trait. EBVs are calculated by means of statistical model such as BLUP (Best linear unbiased prediction) method. BLUP is the method that predicts individual breeding values from only phenotypic value, environmental effects and pedigree information, which treat the genetic architecture of each genetic loci relate with quantitative traits of interest as a "black box". Breeding method like this has been implemented to genetic improvement in main livestock species, and genetic progress of various traits was actually succeeded.

Recently, many approach that implicate genetic evaluation of individuals by not only phenotype-based method, but by gaining insight into the "black box" of quantitative traits with using molecular genetic information have been investigated in order to increase selection accuracy and decreasing generation intervals.

To date, these techniques for finding genes and QTL, in particular the candidate gene approach, have resulted in revealing partially inside of the black box and discovered several genes or markers that are available

in the pig industry, which have been well summarized by Dekkers (2004), Dekkers *et al.*(2010), Eveline *et al.* (2008).

Prime examples are *RYR1*(ryanodine receptor 1) for meat quality Fujii et al., 1991), *PRKAG3* (protein kinase AMP-activated gamma 3, SSC15) for the pH value and water holding capacity rate of meat (Milan *et al.*, 2000), *IGF2* (Insulin like growth factor 2, SSC2) for muscle mass, and *MC4R*(melanocortin 4 receptor) for the backfat thickness and feed intake (Kim *et al.*, 2000), which are used in pig breeding organizations.

However, though many candidate genetic markers have been detected, the genetic makers that are available in the industry were very limited, because there are still some barriers to use such markers in actual pig breeding programs.

First, many study reported the association between genes and traits used the experimental population composed by one generation. There are not many study which was researched about how the genotypic transition affect the traits in the process of the selection to achieve breeding goal though several generation in such as the closed nucleus breeding system. The frequencies of genes in the closed population are significantly affected by some factors such as random genetic drift, founder effect, or bottleneck effect. It is possible to verify that how the traditional closed nucleus breeding systems extract the available genotype that was in the founder population.

Second, many reports detected the polymorphism that has related with pig production trait, however, most of those reports indicate only an independent single polymorphism effect for traits. It is simple to adjust genetic information for improving Menderian inherit traits such as PSE

which was induced by *RYR1* gene by excluding or fixing a specific allele at single loci. In contrast, production traits such as backfat thickness or growth rate are quantitative traits were controlled by many genes and highly influenced by environment. Therefore, using multiple candidate markers simultaneously enable to establish more effective breeding methods. Although many genetic markers related to one trait were detected, each marker was detected in different population (breeds, strains) that was genetically separated, and there were few reports that evaluated multiple genetic effect of marker in one population.

Thirdly, most association studies performed by using some genes involve additive and dominance effect independently, but there are a few investigations that consider interaction effect between the genes. Große-Brinkhaus *et al.* (2010) showed that the significance of epistatic QTL pairs associated with various traits such as meat quality, carcass composition in Duroc × Pietrain population. Fixing specific alleles does not always lead to establish efficient breeding program, because quite a few negative interaction effects between the loci that related quantitative traits was reported in the studies using various animals (Carlborg *et al.* 2003; Duthie *et al.* 2010; Hager *et al.* 2012). Therefore, it is very important to choose more appropriately model to predict individual genetic value. However, there are few reports regarding the combination effect and interaction effect of multiple candidate genes.

The aims of this study were to evaluate genetic effect of genes that might affect productive traits in the Duroc population improved by closed nucleus breeding system, and to establish the breeding scheme by adding genotype information. In Chapter 2, we analyzed the association of

ADRB3 that is reported to affect energy metabolisms in human or mouse between pig production traits. In Chapter 3, we investigated the relationship between *PIK3C3* gene polymorphisms and economic traits in a Duroc population. We also tested the usefulness of *PIK3C3* genotyping for estimating the breeding values of porcine productive traits in statistical models. In Chapter 4, we investigated the relationship between the *VRTN* genotype and economic and body composition traits in a Duroc population. In Chapter 5, we evaluate combination and interaction effect between five genes (*LEP*, *LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) that might affect productive traits in Duroc population, and compared the mathematical models that include the multiple gene effects.

Duroc pigs used entirely in this study were from a line selected through five generations at Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan) from 2004 to 2010. Whole improvement scheme and selection criteria of this population were described in Chapter 1.

CHAPTER 1. Breeding experiment on the development strain in Duroc pigs

1-1. Introduction

In Japanese commercial pig farm, it is commonly used three way cross method as pig producing system in order to take advantage of hybrid vigour effect (heterosis) obtained from genetic differences that exist within breeds.

Three way cross system utilize three strains generally based on pure breeds, and these strains were classified into dam line and sire line depending on application. Dam line has been developed by selecting for fertility traits such as prolificacy (Number of total piglets or piglets born alive per litter) and mothering ability (Number of piglets weaned per sow per year). On the other hand, sire line has been developed by selecting primarily for growth rate and meat quality.

Two breeds (Landrace and Large White) that have excellent fertility traits are generally used as sire line, and Duroc breed that have excellent meat quality traits is used as sire line in Japan. Thus, it is usually practiced by crossing Duroc as terminal boar F1 hybrid sows obtained by multiplying Landrace and Large White to produce commercial pig in the Japanese three way cross system, because this combination can capitalize maximally advantage of heterosis, balance the fertility traits and the production traits.

And we encourage utilizing the pig strain improved by closed nucleus breeding system as terminal boar, because that have high genetic uniformity and can stably supply the high quality meat that market

demands. Recently, terminal boars that have more superior productive traits such as high growth rate or high meat quality than previous strain are quite required. The aim of this development strain experiment is to produce a Duroc strain to be used as terminal sire that have excellent talent to contribute efficient meat production and superior meat quality. Therefore, we configured improvement goal to increase average daily gain (ADG) in test period from 30kg live weight to 90kg live weight, backfat thickness (BFT), and intramuscular fat content in the loin muscle (IMF) at 90kg live weight, without changing the size of loin eye muscle area (EMA) comparing our previous Duroc strain.

1-2. Materials and Methods

1-2-1.Animals

The number of animals at each selection stage in each generation was shown in Table 1. First, 28 boars (three boars were introduced by semen) and 52 gilts were introduced as a base population in 2004. These resources were derived from Japanese breeding company, foreign breeding farms, and local government experimental stations. This strain was improved by closed nucleus breeding program, thereby new resources have not been introduced from other population after being introduced as base population.

The piglets at first generation were introduced by cesarean delivery under SPF (specific pathogen free) condition. After second generation, piglets were produced by natural farrowing from selected parents in previous generation.

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. Average 15 boars and 55 gilts were selected in the first group, and average 6 boars and 26 gilts were selected in the second group at each generation. In addition, five boars were selected from the 20 boars in the first group after considering their genetic performance and pedigree for crossing in the second group at third and fourth generation. These were used in the second group to prevent separation of the genetic relationship between the two groups. As half of boars used for crosses in the second group were consistent in first group, these two groups were considered as one same line in each generation. The 6^{th} generation 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 fifth generation.

1-2-2. Selection methods

All sows fallowed within a three-month period, thereby average of 500 piglets in first group and average of 200 piglets in second group were obtained in each generation. In the first selection test, at seven weeks of age, piglets that showed leg weakness and/or slow growth were removed from the population. In each generation, average of 230 piglets (first group) and 100 piglets (second group) were passed the first selection, and they were supplied for performance test. Performance tests began when body weight reached 30 kg and ended at approximately 90 kg. ADG over the test period was calculated as weight gained divided by days elapsed.

At approximately 90 kg live weight, BFT and EMA were measured at a half-body-length position using a real time B-mode ultrasound scanner (SSD-500 ALOKA Co., Ltd. Tokyo, Japan). Computer software (SigmaScan Pro 5.0, Systat, Inc., Richmond, CA, USA) was used to determine the EMA. Subsequently, a biopsy was taken from loin muscle at a position half way along the body and about 6.5 cm from the vertebral centerline. Crude fat content in sampled loin tissue was used as a measured of IMF. We detected high correlation coefficient between the intramuscular fat content sampled by needle biopsy method and that content sampled from the approximately 100g loin meat block at the 7th vertebrae in 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 intramuscular fat content in the whole loin muscle. Only boars and gilts were measured these traits, but barrows (about one barrow per litter) were also measured these traits except IMF.

All animals were provided unlimited access to food and water during the test period by following our Institute's guidelines for animal management, and they were reared in performance testing pens with group feeding in a concrete-floored building (Figure1). All pens were set in a windless building and room temperature was kept approximately 18℃ by air condition system except summer.

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 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 ADG and IMF with keeping the size of EMA at first generation. Consequently, the aggregate BV (H) was calculated from the following equation:

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

Animals that produce the next generation were selected by considering their aggregate BV, the proportion of pigs, and their pedigree in each generation.

1-2-3. Mating methods

The coefficients of inbreeding and relationship in this population

were calculated by using pedigree from the base population with 'CoeFR' software (Satoh., 2000).

In order to make the all selected pigs have relationship each other at fifth generation and to prevent consanguine mating such as full-sib or half-sib mating, the mating in each generation was performed under considering the relationship coefficients between couples.

1-3. Results and Discussion

1-3-1. Change of selection traits

Selection to improve economic traits such as the average ADG, BFT, and IMF content in this Duroc population was conducted by our breeding program through five generations, finally, 20 boars and 61 gilts were selected at fifth generation. The results of phenotypic and breeding values for each trait are shown in Table 2. Average phenotypic values of the ADG and BFT of boars at the fifth generation significant increased by 44g/day, 0.21cm compared with first generation, respectively. But phenotypic value of IMF decreased 0.31%. The each breeding value of ADG, BFT, and IMF at the fifth generation significantly increased by 82 g/day, 0.32cm, and 0.83% compared with those of the first generation, respectively. Therefore, these values showed that improved gains had been established according to improvement goal. In this experiment, the trend of breeding value for IMF did not conformed to that of the phenotypic value through generations. However, it was not clear the reason in this study.

Average phenotypic value of EMA of boars at fifth generation was about the same as fifth generation's one. The BVEMA decreased slightly by 0.3 cm^2 compared with that of the first generation. The improvement

goal of loin EMA was to maintain the size of the first generation. Although there was statistically significant difference in BVEMA between first and fifth generation, this change in BVEMA did not affect in actual meat production. Therefore, the loin EMA improvement was fairly successful in this experiment.

1-3-2. Genetic parameter of selected traits

Table 3 shows that genetic parameters in this population estimated by using all phenotypic and pedigree data at fifth generation. All heritability estimates for trait and genetic correlations between traits showed highly significant difference in Wald tests (Table 3). Heritability estimates for ADG, BFT, and EMA were mostly moderate (0.43, 0.65, and 0.24, respectively), but that value for IMF was low (0.13). Estimates of heritability for ADG and BFT in our Duroc line were in range of reports (ADG; 0.03 - 0.49, BFT; 0.12 - 0.74) listed by Clutter. (2010). Present estimate of heritability for EMA was lower than other Japanese Duroc line (0.45) reported by Suzuki *et al.* (2005), but that was equivalent to other Duroc population (0.25) reported by Salces *et al.* (2006). We analyzed IMF of samples collected by biopsy method in this experiment, but there has been no describes about porcine IMF collected by our method. Heritability estimates depend on the methods of measuring traits, therefore we could not directly compare between present heritability estimate for IMF and that value in other populations. Ciobanu *et al.* (2010) reported the average heritability of IMF was moderate (0.50), and Suzuki *et al.* (2005) also estimated that the heritability for IMF was 0.39 in Duroc population. Those estimates were

analyzed by the phenotypic values collected from carcass. Our heritability estimate for IMF was lower than those values. It may be due to difference of sampling methods.

Estimates of genetic correlation of ADG with BFT and IMF were positive and moderate (0.25 and 0.37). Meanwhile genetic correlation estimate between ADG and EMA was negative (-0.12). Present result suggests that improvement ADG increase fat deposition such as BFT and IMF in this population. There were quite variable in the reports of genetic correlation of ADG with BFT and EMA, ranging from negative and moderate positive (Lo *et al.*, 1992; Kuhlers *et al.*, 2001; Suzuki *et al.*, 2005; Clutter., 2010). It appears that its variety may be due to pig breeds or strain. Lo *et al.* (1992) and Suzuki *et al.* (2005) estimated that the genetic correlation between ADG and BFT in Duroc line was 0.21 and 0.34, respectively, which are similar to the results from present study. For the genetic correlation between ADG and IMF in Duroc, Suzuki *et al.* (2005) estimated that it was 0.23, which was comparatively lower than our present results. Furthermore, the genetic correlation between BFT and IMF in the present experiment was 0.63, which was higher than previous reports. For example, Newcom *et al.* (2005) and Suzuki *et al.* (2005) reported that the estimates of genetic correlation between BFT and IMF were 0.24 and 0.36, respectively. Those estimates were calculated by using phenotypic IMF value obtained from carcass. Therefore, the difference between our results and theirs may be due to measuring sample methods.

The estimate of genetic correlation between ADG and EMA in present experiment (-0.12) was very similar to the result (-0.09) in

Japanese Duroc line by Suzuki *et al.* (2005), but not to the result (0.24) in Duroc line of United States by Lo *et al.* (1992). The genetic correlation estimates between BFT and EMA has generally shown negative, for example, -0.56 (Lo *et al.*, 1992), -0.45(Suzuki *et al.*, 2005), -0.31(Kuhlers *et al.*, 2001), and -0.31 (Salces *et al.*, 2006). Similar to these studies, our present result also showed negative (-0.24).

1-3-3. The relationship and inbreeding coefficient

Table 4 shows that the trend of average coefficients of inbreeding and relationship from first to fifth generation. Those values in this population increased through the generation by conducting organized mating. An average inbreeding coefficient of selected pigs at fifth generation was 3.02%, and average relationship coefficient among those pigs was 10.09%. All selected pig at fifth generation had relationship each other.

1-4. Implications

We performed phenotype-based BLUP method for genetic evaluation as selection criteria in this breeding program, and achieved improving for growth trait and meat quality trait in this Duroc population through five generations. To make genetic progress efficiently, it needs considering the genetic evaluation methods such as the methods that include gene marker information.

Table 1 The number of animals at each selection stage by generation

Generation	Sex	Born	First Selection	Second selection	selection	Farrow
G0	Boar					28
	Gilt					52
G1	Boar	254	125	25	16	16
	Gilt	229	127	74	60	54
G2	Boar	207	115	25	20	17
	Gilt	220	141	78	64	45
G3_1st group	Boar	128	88	15	15	13
	Gilt	164	110	65	65	53
G3_2nd group	Boar	96	47	8	13(5)	12(4)
	Gilt	123	52	33	30	25
G4_1st group	Boar	211	104	20	15	15
	Gilt	187	109	60	58	56
G4_2nd group	Boar	86	46	8	11(5)	9(4)
	Gilt	88	59	33	30	25
G5_1st group	Boar	235	131	25	15	16
	Gilt	199	113	61	42	42
G5_2nd group	Boar	99	60	8	5	5
	Gilt	103	62	30	19	16
Total	Boar	1316	716	134	110 (10)	103 (8)
	Gilt	1313	773	434	368	316

Given in parentheses are number of boars of first group used for mating in second group.

(A)

(B)

Figure1. Equipment for management of experimental pigs

(A) Pigs under the performance test. All pigs were reared in performance testing pens with group feeding in a concrete-floored building

(B) All testing pens are built in the windless building

Table 2 The trend for phenotypic and breeding values in Duroc population[1]

Sex	generation	Phenotypic values[2] MW (kg)	ADG (kg/day)	BF (cm)	EMA (cm^2)	IMF (%)	Breeding values[3] BVADG (kg/day)	BVBFT (cm)	BVEMA (cm^2)	BVIMF (%)
Boars	1	95.0 (5.1)	972 (114)	1.45 (0.31)	36.5 (3.8)	4.83 (1.34)	-41 (70)	-0.07 (0.22)	0.4 (1.1)	0.02 (0.57)
	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[3]	<0.001	<0.001	<0.001	0.108	0.097	<0.001	<0.001	0.012	<0.001
Gilts	1	93.9(4.4)	906 (98)	1.67 (0.39)	37.9 (3.9)	5.30 (1.37)	-44 (62)	-0.09 (0.26)	0.63 (1.1)	-0.06 (0.58)
	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[3]	<0.001	<0.001	<0.001	<0.001	0.176	<0.001	<0.001	<0.001	<0.001
Barrows	1	-	-	-	-	-	-	-	-	-
	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[3]	0.001	0.821	<0.001	0.14	-	<0.001	<0.001	<0.001	<0.001
Total	1	94.4 (4.8)	939 (111)	1.56 (0.37)	37.2 (3.9)	5.24 (1.60)	-43 (66)	-0.08 (0.24)	0.5 (1.1)	-0.02 (0.58)
	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 (6.0)	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 (G2-G5)[4]	<0.001	<0.001	<0.001	<0.001	0.030	<0.001	<0.001	<0.001	<0.001

[1] Mean values. Given in parentheses are S.D.

[2] MW:mesurement weight, ADG: Average daily gain; BFT: Backfat thickness; EMA: Eye muscle area; IMF: Intramuscular fat content, BVADG:Breeding value of ADG, BVBFT:Breeding value of BFT, BVEMA: Breeding value of EMA, BVIMF:Breeding value of IMF

[3] *P* values were estimated by GLM to compare between 1st generation and 5th generation.

[4] *P* (G2-G5) values were estimated by GLM to compare between 2nd generation and 5th generation, bacause there were no data for barrows at 1st generation

Table3.Restricted maximum liklihood estimates of genetic parameter between selected traits[1]

	ADG[2]	BFT[2]	EMA[2]	IMF[2]
ADG	0.43 ± 0.03	0.25 ± 0.03	-0.12 ± 0.04	0.37 ± 0.10
P^3	0.14×10^{-47}	0.79×10^{-18}	0.27×10^{-2}	0.22×10^{-3}
BFT		0.65 ± 0.03	-0.23 ± 0.04	0.63 ± 0.08
P^3		0.42×10^{-5}	0.89×10^{-10}	0.34×10^{-16}
EMA			0.24 ± 0.03	-0.23 ± 0.07
P^3			0.12×10^{-16}	0.10×10^{-2}
IMF				0.13 ± 0.02
P^3				0.80×10^{-12}

[1] Diagonal: heritability ± standard error; Above daigonal: genetic correlation ± standard error

[2] ADG: Average daily gain; BFT: Backfat thickness; EMA: Eye muscle area; IMF: Intramuscular fat content

[3]Wald test results for each maximum likelihood estimates of genetic parameter

Table 4. Trend of average coefficient of inbreeding and relationship in Duroc population

	Generation				
	G1	G2	G3	G4	G5
Inbreeding coefficient (%)[1]	0	0	0.12	1.68	3.15
Relationship coefficient (%)[1]	1.91	4.48	6.74	8.53	10.09

[1] Average values for coefficient of inbreeding and ralationship at each generation

CHAPTER 2. Association of porcine beta 3-adrenergic receptor (*ADRB3*) gene with production traits in Duroc pigs

2-1. Introduction

Genetic association studies test is analyzing correlation between genetic variation and traits in order to identify candidate genes or genome regions that contribute to a specific trait. Implicating this method in livestock, it is generally used the genes which had been revealed those physiological function in another species such as mouse or human. Single-nucleotide polymorphisms (SNPs) are the most widely tested markers in association studies, but microsatellite markers, insertion/deletions, variable-number tandem repeats (VNTRs), and copy-number variants (CNVs) are also used. Many associations with polymorphisms in candidate genes have been confirmed in various livestock.

Growth and energy metabolism are important characteristics in animal production. Adipose tissue has been the focus of recent efforts to identify candidate genes involved in energy metabolism, especially in studies concerning human obesity, because adipose tissue plays a crucial part in regulating the storage and mobilization of energy (Perusse *et al.*, 2005). The β3-adrenergic receptors (ADRB3s) are guanine nucleotide-binding protein-coupled receptors predominantly found on the surface of adipocytes, and are major mediators of lipolytic and thermogenic effects in brown and white adipose tissue (Nahmias *et al.,* 1991; Arch & Kaumann., 1993).

An amino acid substitution [Trp 64→Arg] in human *ADRB3* has

been examined by various authors (reviewed in Strosberg, 2000). Some of those studies suggested that people with the Trp64→Arg substitution may have an increased capacity to gain weight and may tend to have a lower resting metabolic rate (Kadowaki *et al.,* 1995). Others have reported that this mutant allele may accelerate the onset of non-insulin-dependent diabetes mellitus by altering the balance of energy metabolism in visceral adipose tissue (Silver *et al.,* 1997; Masuo *et al.,* 2005). In domestic animals, Forrest *et al* (2003, 2006, 2007) identified that variation in the ovine *ADRB3* locus was associated with the cold-related mortality rate in lambs. Such findings suggest that variation in domestic animals *ADRB3* may affect economically important traits, such as fat deposition and thermogenesis, thereby influencing growth and meat quality.

The porcine *ADRB3* gene has two exons (Figure 2), and five polymorphic haplotypes have been identified (Tanaka *et al.,* 2007). Among these, insertion or deletion polymorphisms of thymine in exon 2 (c.1211 T(5_6)) were interesting because this variation resulted in a frameshift. The allele with a direct repeat of five thymine bases (T5) coded 407 amino acids compared with 405 amino acids for the allele with a direct repeat of six thymine bases (T6). In this study, we assessed T5 and T6 variations in *ADRB3* in Duroc pigs and analyzed the association of these variations with several animal production traits.

2-2. Materials and Methods

2-2-1. Animals and data collection

The pig population used in this study was a pure Duroc strain that was part of an improvement program by nucleus breeding system at the

Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan). Data in this part were collected from 735 Duroc pigs in first group of three generations from first to third.

The details of selection criteria, selection methods, and data collection methods were described in Chapter 1. In this part, we analyzed the phenotypic values and breeding values of four production traits (ADG, BFT, EMA, and IMF). All animals were provided unlimited access to food and water during the experimental period, and all experiments were performed in accordance with our institutional guidelines for animal management.

2-2-2. Genotyping

Genomic DNA was extracted from tail tissue clips from each individual using a DNeasy Blood and Tissue Kit (Qiagen Inc, Hilden, Germany). All animals were genotyped for the T5-T6 *ADRB3* polymorphisms the using PCR-restriction fragment-length polymorphism (PCR-RFLP) method described by Tanaka *et al.* (2007). Mismatch primer sets (forward: 5′-CCATTTTCAGGGCTTCCTGGGGCCTT-3′, reverse: 5′-GCCACTTGGTAAGGAATTCCCCCTT-3′) were used for PCR detection. The PCR conditions were as follows: denaturation at 94°C for 5 min, 33 cycles of amplification at 94°C for 1 min, 54°C for 1 min, 72°C for 45 s, and a final extension step at 72°C for 10 min. For the PCR-RFLP assays, 2 μL of the PCR products was used for restriction digestion with 5 U of *Xag*I (Fermentas Inc., Glen Burnie, MD, USA) in 10x digestion buffer added to a total volume of 10 μL. (Figure 3).

2-2-3. Statistical analyses

The GLM procedure of MINITAB (Version14.12.2, Minitab Inc., State College, PA, USA) was used to obtain least squares means of ADG, BF, EM, and IMF to account for the fixed effects of *ADRB3*genotype at each sex, and to test significance of the results. Both additive and dominance effects of the *ADRB3* alleles were estimated with MINITAB using its REG procedure. The additive effect of alleles was defined as −1, 0, and 1 for homozygous (T5/T5, T6/T6) and heterozygous (T5/T6) genotypes. The dominant model was defined as −1, 1, and 1 for T5/T5, T5/T6, T6/T6, while the recessive model was defined as 1, 1, and −1 for T5/T5, T5/T6, T6/T6, respectively. The linear model used to analyze the data was as follows:

$$Y_{ijk} = \mu + Genotype_j + Generation_k + \beta Weight_{ijk} + e_{ijk}$$

where, Y_{ijk} is the phenotypic value of each trait, μ is the overall mean for each trait, $Genotype_j$ is the effect of *ADRB3* genotype, $Generation_k$ is the effect of generation, $Weight_{ijk}$ is the weight measurement , β is the covariate of the weight measurement and e_{ijk} is the random residual effect.

For analysis of breeding values of each trait, both the fixed effects of sex and generations and the covariates between measurement weight and each trait were included in the BLUP model, An ANOVA with genotype as the independent variable was used to analyze the association of genotype with breeding value of each trait. The relative contributions of the ADRB3 genotypes to the variance of the traits' breeding values were estimated by

PEST3.1 program and used the *ADRB3* genotypes as the fixed effect.

2-3. Results

2-3-1. *ADRB3* gene allelic frequencies

The genotypic and allelic frequencies for the T5 and T6 *ADRB3* gene polymorphisms are presented in Table 5. There was no significant difference in genotype distribution between each sex ($\chi^2 = 6.882$, df = 4, $p = 0.142$).

2-3-2. Association of genotypes with economic traits

Table 6 shows the phenotypic values of the measured traits for the *ADRB3* genotypes. There was no evidence of an effect of these polymorphisms on ADG, BFT, and IMF in this study. However, the *ADRB3* genotype was significantly associated with EMA in gilts (Table 6). T6-homozygote gilts had a significantly higher mean EMA (40.6 ± 0.6 cm^2) than the T5-homozygote (38.1 ± 0.4 cm^2, $p = 0.002$) and the heterozygotes (38.8 ± 0.3 cm^2, $p = 0.034$). Although the differences did not reach statistical significance, T6-homozygous boars and barrows had tendencies toward larger EMA than that of T5-homozygous boars and barrows (Table 6). Therefore, as analyzing in total pigs, the *ADRB3* genotype was significantly associated with EMA ($p= 0.002$).

Figure 4 shows a histogram of the EMA of gilts per *ADRB3* genotypes. The median EMA of the T6-homozygotes (39.8 cm^2) was larger than that of the T5-homozygotes (37.7 cm^2) and the heterozygotes (38.7 cm^2). Similarly, the mode of the EMA of T6-homozygotes (39.2 cm^2) was larger than that in T5-homozygotes (38.8 cm^2) and heterozygotes (35.3

cm^2). There was no significant difference in variance of eye muscle area between each genotype (Bartlett's test, $\chi^2 = 0.05$, $p = 0.976$). *ADRB3* genotypic frequencies were significantly different between upper 20% and lower 20% of EMA values in gilts ($\chi^2 = 12.78$, df = 2, $p = 0.0017$; two-tailed Fisher's exact test). The results indicate that *ADRB3* polymorphisms may affect EMA.

Table 7 shows data related to the breeding value of EMA (BVEMA). A highly significant association was detected between *ADRB3* genotypes and BVEMA ($p = 0.002$); the BVEMA of T6-homozygotes (0.83 cm^2) was larger than that in both the T5-homozygotes (−0.37 cm^2) and the heterozygotes (0.11 cm^2). The contribution of the *ADRB3* genotypes to the variance of the BVEMA was 25.4%. These results suggest that T6 is a female specific quantitative trait locus (QTL) allele that can increase EMA in the period prior to achievement of a 90 kg body weight in Duroc pigs.

2-4. Discussion

The T6 allelic frequency was 56.7% in our Duroc population. Tanaka *et al.* (2007) reported a 12.5% T6 frequency in another Duroc line, and Chikuni *et al.* (2008) reported that the frequency of T6 allele was only 2.1% in Duroc × Jinhua crossbred. Furthermore, T6 has been reported to be a minor allele in European breeds of pigs. The results indicate substantial differences in *ADRB3* allelic frequencies among breeds and lines.

In this study, porcine *ADRB3* thymine polymorphism was examined to determine whether performance traits in pigs were associated with T5 and/or T6 *ADRB3* genotypes. In our Duroc line, T6-homozygote gilts had a

significantly larger EMA (p = 0.003) than T5-homozygous and heterozygote gilts. A significant effect on EMA was detected in gilts. In boars and barrows, we did not detect any association between performance traits and T5/T6 *ADRB3* genotype. Rodríguez *et al.* (2001) reported that sex - related differences at expression of some different adrenogic receptor that include beta-1, -2, -3 adrenogic receptor were found in the rat's brown adipose tissue. In their study, the levels of beta 3-adrenogic receptor expression in male rats were higher than in females in the situation of overfeeding. Therefore, the difference of *ADRB3* genotypes might be associated with expression of porcine beta 3 adrenogic receptor in the muscle of female pigs. Moreover, sex specific associations between QTL alleles and performance traits have been previously reported in pigs. For example, de Oliveira Peixoto *et al.* (2006) demonstrated that Leptin genotypes were associated with average daily weight gain and feed conversion in male, but not female. Our present results suggest that the T5/T6 *ADRB3* polymorphism is the location of a female specific QTL allele that can increase EMA by the time of achievement of a 90 kg body weight. These results indicate that *ADRB3* can be useful as a genetic marker when improving to the loin eye muscle area in Duroc pigs.

The exact mechanisms of how *ADRB3* polymorphisms lead the differences in EMA are unknown. One possible mechanism would be differential regulation of receptor activities by the mutations. We expected that the T5/T6 *ADRB3* polymorphisms would affect energy metabolism and fatness in pigs. However, there was no association between *ADRB3* genotypes and fat deposition or rate of growth. Cieslak *et al.* (2009) also detected no correlation with fat deposition in Polish multi-breed panel (e.g.

Landrace, Duroc, Pietrain and so on). In humans, *ADRB3* polymorphisms have been linked to increased body mass indices, obesity, and more recently to dietary and nutrients preferences in adults (Emorine *et al.,* 1989; Clement *et al.,* 1995). On the other hand, the effects of *ADRB3* polymorphisms on obesity and weight gain are reported to very small in children (Cecil *et al.,* 2007). In this study, assessments were done between the time when the pigs were approximately 30 kg body weight to when they reached an approximate 90 kg body weight; an elapsed time of about 120 days. In piggeries, most pigs are butchered for meat by the age of six months, i.e. prior to reaching maturity. We were unable to determine the effects of porcine T5/T6 *ADRB3* polymorphisms on fat deposition and growth traits in matured pigs.

The effects of QTL alleles have been reported to shift among breeds and populations. For example, alleles of the porcine melanocortin-4 receptor (*MC4R*) gene were associated with growth and fatness traits in Landrace, Large White, and synthetic pig lines that had been generated by crossing Large White and Duroc pigs (Kim *et al.*, 2000; Hernández-Sánchez *et al.*, 2003; Houston *et al.*, 2004). In contrast, studies on a Large White × Wild Boar reference family did not reveal any significant effects of *MC4R* variants (Park *et al.*, 2002). One reason for the contrasting results may be genetic interaction between different QTLs. Recent literature indicates that genetic interactions involving ADRB3 and other loci influence human obesity (Corella *et al.*, 2001; Mentuccia *et al.*, 2002; Cecil *et al.*, 2007). In our study, the T6 allele is associated with increased pig EMA. This is the first study to report a relationship between porcine *ADRB3* variants and productive traits. As only one Duroc

population was assessed, studies on other breeds and populations are needed in order to clarify the effects of T5/T6 *ADRB3* polymorphism on the porcine productive traits.

Figure 2. The position of mutation for genotyping *ADRB3* gene in this study. The protein coding sequences are indicated by dark-stained boxes. . The numbering of base position was adhered to the reference NC_010457 (Sus scrofa 10.2; g.55482168..g.55484072, chromosome 15) sequence. The mutation in exon region were identified based on Genbank accession no. NM_001099927.1. Arrow shows the position of c1211 T(5_6).

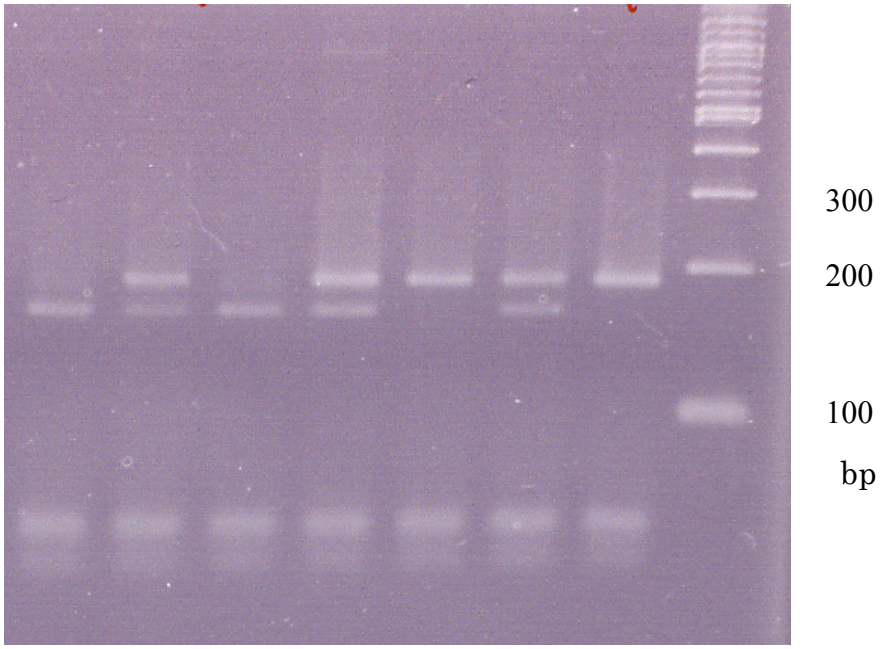

Figure 3. Genotyped patterns of polymorphism digested by restriction enzym *Xag*I in the exon2 of the porcine *ADRB3* gene on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. The M is a 100-bp DNA Ladder molecular size mark (Toyobo, Osaka, Japan).

Table 5 Genotypic and allelic frequencies of the T5 and T6 *ADRB3* gene polymorphism

	Genotypic frequency[1]			Allelic frequency[2]	
	T5/T5	T5/T6	T6/T6	T5	T6
Boar (323)	16.1 (52)	53.6 (173)	30.3 (98)	42.9 (277)	57.1 (369)
Gilt (359)	17 (61)	51 (183)	32 (115)	42.3 (305)	57.7 (413)
Barrow (53)	30.2 (16)	41.5 (22)	28.3 (15)	50.9 (54)	49.1 (52)
Total (735)	17.6 (129)	51.4 (378)	31 (228)	43.3 (636)	56.7 (834)

[1] Percentage of each genotype. Given in parentheses are number of pigs.

[2] Percentage of each allele. Given in parentheses are number of alleles.

Table 6 Association between porcine *ADRB3* polymorphism and economic traits in Duroc pigs

Traits[1]	Sex	Total[2]	Genotype[3]			*P*	Effect[4]		
			T5/T5 (N)	T5/T6 (N)	T6/T6 (N)		Additive	Dominant	Recessive
ADG (g/day)	Boar	1003 ± 7 (323)	998 ± 13 (52)	1004 ± 11 (173)	1010 ± 16 (98)	0.827	2.2 ± 7.9	3.0 ± 5.7	1.2 ± 7.2
	Gilt	933 ± 6 (359)	932 ± 11 (61)	939 ± 8 (183)	917 ± 15 (115)	0.432	-10.2±6.7	-2.2±5.0	-13.6±6.1*
	Barrow	1033 ± 92 (53)	1058 ± 26 (16)	1025 ± 22 (22)	1025 ± 25 (15)	0.549	-10.1±17.5	-16.4±14.8	-6.5±14.7
	Total	964c4 (735)	984±7 (129)	986±6 (378)	970±9 (228)	0.209	3.19±5.20	0.02±3.84	-5.17±4.70
BFT (cm)	Boar	1.44 ± 0.02 (323)	1.46 ± 0.03 (52)	1.46 ± 0.02 (173)	1.42 ± 0.04 (98)	0.635	-0.02±0.02	-0.00±0.02	-0.02±0.02
	Gilt	1.69 ± 0.02 (359)	1.65 ± 0.03 (61)	1.69 ± 0.03 (183)	1.67 ± 0.04 (115)	0.641	0.01 ± 0.03	0.01 ± 0.02	-0.00±0.02
	Barrow	1.77 ± 0.04 (53)	1.86 ± 0.10 (16)	1.71 ± 0.08 (22)	1.87 ± 0.09 (15)	0.321	0.01 ± 0.07	-0.04±0.06	0.05 ± 0.05
	Total	1.58±0.01 (735)	1.63±0.02 (129)	1.64±0.02 (378)	1.63±0.03 (228)	0.888	0.00±0.02	0.00±0.01	-0.00±0.02
EMA (cm^2)	Boar	37.2 ± 0.3 (323)	37.0 ± 0.4 (52)	37.4 ± 0.3 (173)	37.8 ± 0.6 (98)	0.52	0.41 ± 0.37	0.26 ± 0.27	0.28 ± 0.34
	Gilt	39.0 ± 0.2 (359)	38.1 ± 0.4[a] (61)	38.8 ± 0.3[a] (183)	40.6 ± 0.6[b] (115)	0.003	1.22 ± 0.36**	0.66 ± 0.27*	1.02 ± 0.33**
	Barrow	37.9 ± 0.6 (53)	38.2 ± 1.3 (16)	37.4 ± 1.1 (22)	40.3 ± 1.3 (15)	0.32	1.09 ± 0.91	0.20 ± 0.78	1.31 ± 0.74
	Total	38.2±0.2 (735)	37.4±0.4 (129)	38.0±0.3 (378)	39.2±0.4 (228)	0.002	0.83±0.25**	0.44±0.18*	0.68±0.22**
IMF (%)	Boar	4.35 ± 0.18 (176)	4.35 ± 0.18 (36)	4.54 ± 0.16 (82)	3.87 ± 0.23 (58)	0.057	-0.18±0.15	0.01 ± 0.11	0.31 ± 0.13
	Gilt	4.75 ± 0.16 (228)	4.75 ± 0.16 (38)	4.70 ± 0.13 (115)	4.87 ± 0.22 (75)	0.807	0.02 ± 0.13	-0.02±0.10	0.08 ± 0.02
	Total	4.63±0.07 (404)	4.53±0.12 (74)	4.60±0.10 (197)	4.23±0.15 (133)	0.519	-0.07±0.10	-0.01±0.07	0.10±0.09

[1] ADG: Average daily gain; BF: Backfat thickness; EMA: Eye muscle area; IMF: Intermuscular fat content.

[2] Mean values (±SE) of all pigs in each sex.

[3] Least square mean values (±SE). Different letters denoting significant difference between genotypes. Given in parentheses are number of pigs.

[4] Additive effect: T5/T5 = −1, T5/T6 = 0, T6/T6 = 1. Dominant model effect: T5/T5 = −1, T5/T6 and T6/T6 = 1. Recessive model effect: T5/T5 and T5/T6 = −1, T6/T6 = 1.

*: significant difference ($P < 0.05$), **: highly significant difference ($P < 0.01$).

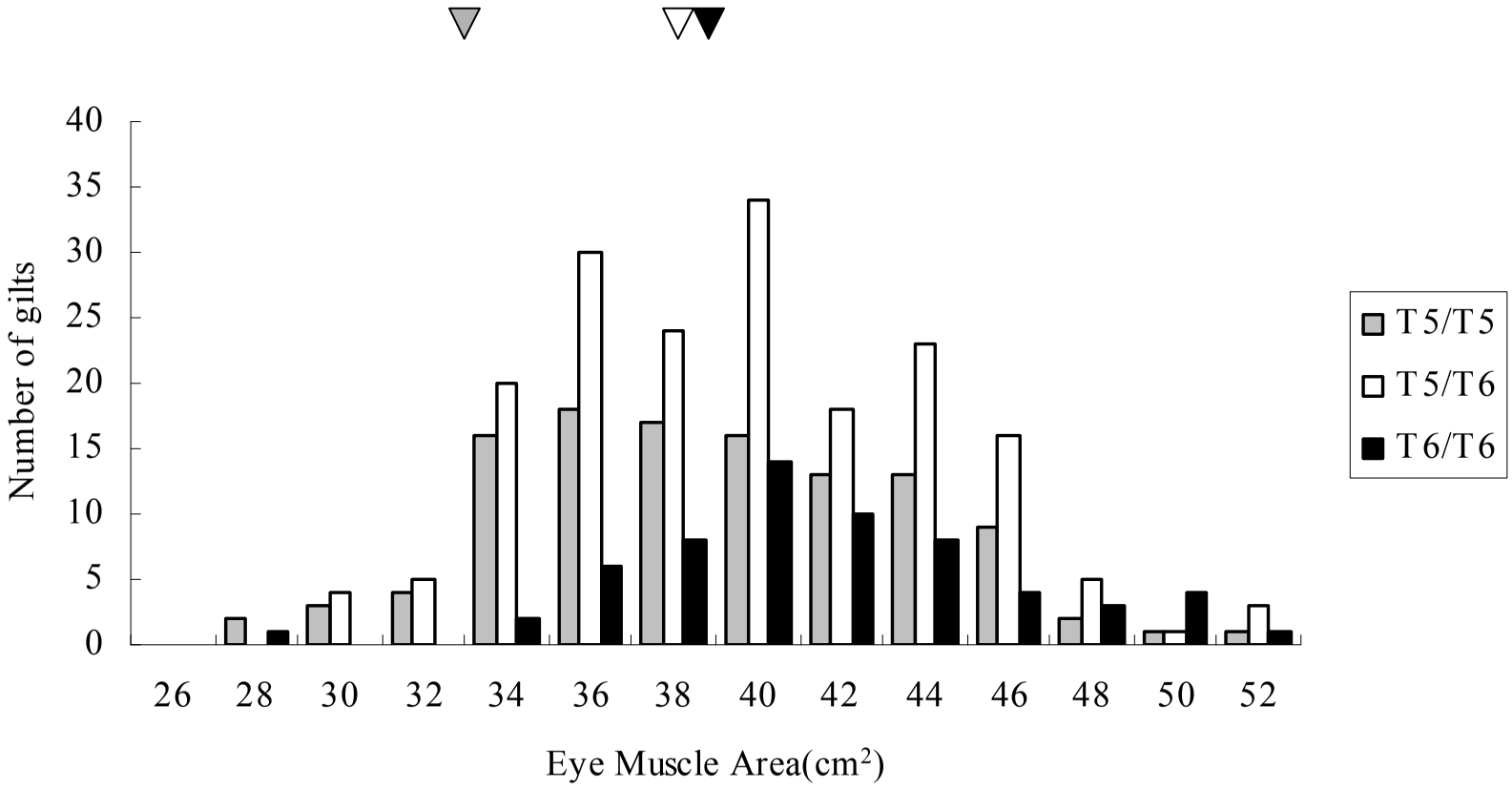

Figure 4. Distribution of loin eye muscle area in gilts per *ADRB3* genotype. Histogram represents loin eye muscle area measured in the middle of pigs' body length in 95 kg gilts at 2 cm^2 intervals. Mode of loin eye muscle area indicated by arrowheads. There was no significant difference in variance of eye muscle area between genotype (Bartlett's test, $\chi^2 = 0.05$, $p = 0.976$).

Table 7 Association between porcine *ADRB3* polymorphism and breeding value of eye muscle area (cm^2)

Trait	n	Total[1]	Genotype[2]			*P*
			T5/T5	T5/T6	T6/T6	
BVEMA	735	0.04 ± 0.06	−0.37± 0.11[a]	0.01 ± 0.01[b]	0.83 ± 0.15[c]	0.002

[1] Mean values(±SE) of all pigs.

[2] Least square mean values (±SE). Different letters denoting significant difference between genotypes.

CHAPTER 3: Association of porcine class 3 phosphoinositide-3-kinase (*PIK3C3*) gene with production traits in Duroc pigs

3-1.Introduction

The genetic improvements have been driven by measuring phenotypes of selection candidate traits and predicting genetic values based on phenotypes in the nucleus population. Although this method has led to increase the performance of several traits, the phenotype-based approaches suffer from several important limitation, for instance, meat quality traits cannot be measured only through the slaughter of animals. It is required for developing new molecular genetics by using individual's genotype to overcome the limitations and establish more efficient evaluating method.

Many studies have detected various genetic markers that have potential to associated with production traits, but the number of publicly available genetic marker in the industry are still very limited. It is required the evaluation for practical availability of these genetic markers in commercial pig population.

Class 3 phosphoinositide-3-kinase (*PIK3C3*) is a member of the phosphoinositide 3-kinase family, which is involved in both receptor-mediated signal transduction and intracellular trafficking (Shepherd *et al.*, 1998; Czech & Corvera., 1999; Prasad *et al.*, 2002; Stopkova *et al.*, 2004). The porcine *PIK3C3* gene (GeneBank accession no. NM_001012956) is composed of 109 kbp and 25 exons (Figure 5) and has been mapped at 117.948 Mb on SSC 6 in Sscrofa10.2 (http://www.ncbi.nlm.nih.gov/gene/503700). Several quantitative trait loci

(QTL) affecting the growth rate, amount of backfat (BF) thickness, and other production traits in this area have been identified by many researchers (Bidanel *et al.,* 2001; Óvilo *et al.,* 2000, 2002; Sato *et al.,* 2003; Yue *et al.,* 2003; Zhang *et al.,* 2007).

Kim *et al.* (2005b) sequenced full-length porcine *PIK3C3* cDNA and found five SNPs (C339G, C1401T, A2058G, A2256G, and C2604T; the number indicates the position of the SNP in the *PIK3C3* cDNA coding region) between Korean native and Large White pigs. All five SNPs are synonymous substitutions. Using the F2 generation between Korean native boars and Landrace sows, they also analyzed SNP C2604T, which is located on exon 24 and is associated with growth and fat deposit traits, and found that the C allele had a positive and significant effect on fat content. Furthermore, a study was performed based on a resource family between two breeds with a large phenotypic difference. However, the correlation between *PIK3C3* gene polymorphisms and economic traits has not been investigated in Duroc populations.

The effects of genotype, even if it has not responsible mutation, have been reported to shift among breeds and populations. For example, the porcine melanocortin-4 receptor (*MC4R*) gene were associated with growth and fatness traits in Landrace, Large White, and synthetic pig lines (Kim *et al.,* 2000; Hernández-Sánchez *et al.,* 2003; Houston *et al.,* 2004). In contrast, studies on a Large White × Wild Boar reference family did not reveal any significant effects of *MC4R* variants (Park *et al.,* 2002). In addition, although Gerbens *et al.* (1999) detected that an allele of *H-FABP* gene had increasing effect on intramuscular fat in the loin in one Duroc population, Uemoto *et al.* (2007) indicated that the allele had opposite

effect on intramuscular fat in other Duroc population. Therefore, the effects of the C2604T SNP in *PIK3C3* polymorphism are need to be determined before utilizing this gene as a genetic marker for our breeding program in Duroc.

In this study, we investigated the relationship between *PIK3C3* gene polymorphisms and economic traits in a Duroc population. We also tested the usefulness of *PIK3C3* genotyping for estimating the breeding values of porcine productive traits in statistical models.

3-2. Materials and Methods

3-2-1. Animals and data collection

The pig population used in this study was a pure Duroc strain that was part of an improvement program by nucleus breeding system at the Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan). The details of data collection methods, selection criteria, and selection methods in this strain were described in Chapter1.

In this part, we analyzed the four production traits (ADG, BFT, EMA, and IMF) that were collected from 739 Duroc pigs across three generations from second to fourth generation. All animals were provided unlimited access to food and water during the experimental period, and all experiments were performed in accordance with our institutional guidelines for animal management.

3-2-2. Genotyping

Genomic DNA was extracted from tail tissue clips of each pig using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Hilden, Germany). All

animals were genotyped for the T–C *PIK3C3* polymorphism using the PCR-restriction fragment length polymorphism (PCR-RFLP) method as described by Kim *et al* (2005a). Primer sets (forward: 5′-ATTTCGTC TAGACCTGTCCG-3′, reverse: 5′-TGAATCTGTTCTACCACCGC-3′) were used for PCR detection. The PCR reaction was performed in reaction mix (total volume 25 μL) containing 25 ng of genomic DNA, 12.5 μL of GoTaq® Green Master Mix (Promega Corp., Madison, WI, USA), and 0.25 μmol of each PCR primer. The PCR conditions were as follows: denaturation at 94°C for 5 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. For the PCR-RFLP assays, 10 μL of the PCR products was used for restriction digestion with 10 U of *Hpy*8I (Fermentas Inc., Glen Burnie, MD, USA) in 1x digestion buffer added to a total volume of 14 μL (Figure 6).

3-2-3. Statistical analysis

We used the Minitab GLM procedure (Version 14.12.2; Minitab Inc., State College, PA, USA) in order to analyze the effect of genotype. The following linear model was used to analyze the data:

$$Y_{ijk} = \mu + Sex_i + Generation_j + Genotype_k + \beta Weight_{ijk} + e_{ijk},$$

where Y_{ijk} is the phenotypic value of each trait, μ is the overall mean for each trait, Sex_i is the effect of gender, $Genotype_j$ is the effect of *PIK3C3* genotype, $Generation_k$ is the effect of generation, β is the regression coefficient of the covariate weight measurement for each trait, $Weight_{ijk}$ is the covariate of the measurement weight, and e_{ijk} is the random residual

effect. BFT and EMA correlated with the measurement weight; therefore, these traits were analyzed using the weight measurement as a covariate.

Additive and dominance effects of the *PIK3C3* alleles were estimated using Minitab using the REG procedure. The additive effect of the alleles was defined as −1, 0, and 1 for homozygous (T/T, C/C) and heterozygous (T/C) genotypes. The dominant model was defined as −1, 1, and 1 for T/T and T/C/C/C, whereas the recessive model was defined as 1, 1, and −1.

In order to investigate whether it was available for estimating breeding value considering the *PIK3C3* genotype in the mathematical model, we compared the Akaike's information criterion (AIC) of the each model. AIC 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 suitable model. AIC was obtained using GenStat (Version 8.1.0.152; VSN International Ltd., Hempstead, UK) using the restricted maximum likelihood (REML) method (Patterson & Thompson, 1971).

The mathematical model used for the analysis was as follows:

Model A (containing the genotype model)

$$Y_{ijk} = \mu + Sex_i + Generation_j + Genotype_k + \beta Weight_{ijk} + Sire_l\,(Dam_l) + e_{ijk}$$

Model B (Null model)

$$Y_{ijk} = \mu + Sex_i + Generation_k + \beta Weight_{ijk} + Sire_l\,(Dam_l) + e_{ijk},$$

where Y_{ijk} is the phenotypic value of each trait, μ is the overall mean for each trait, Sex_i is the effect of gender, $Genotype_j$ is the effect of the

PIK3C3 genotype, $Generation_k$ is the effect of generation, β is the regression coefficient of the covariate weight for each trait, $Weight_{ijk}$ is the covariate of the weight measurement, $Sire_l$ (Dam_l) is the random effect of the lth sire or dam, and e_{ijk} is the random effect of residual error. In a population bred using the closed nucleus breeding system, the effect of sire should be considered when examining the effect of genotype on traits. Therefore, we used the effect of sire as a random effect in the REML model. The dam model was used instead of the sire model for calculating IMF because the variance component estimated by REML using the sire model did not converge. The heritability of each trait was calculated simultaneously with the variance component estimated by model A.

3-3. Results

3-3-1. *PIK3C3* gene allele frequencies

The allelic and genotypic frequencies for the T and C *PIK3C3* gene polymorphisms are presented in Table 8. The T and C allele frequencies were 32.1% and 67.9%, and the T/T, C/T, and C/C allele frequencies were 7.4%, 49.3%, and 43.3%, respectively. Kim *et al.* (2005b) reported that the C allele frequency was 72.5% in 20 Duroc pigs, and there was no difference between their results and those obtained in this study ($\chi^2 = 0.374$, df = 1, $p = 0.541$). Although there was no significant difference in genotype distribution between genders ($\chi^2 = 0.967$, df = 4, $p = 0.915$), the C/C genotype frequency increased through the generations, and there was a significant difference between each generation ($\chi^2 = 26.841$, df = 4, $p < 0.001$). Particularly, the frequencies of C/C genotype (54.8%) and C allele (75.2%) at fourth generation significantly increased from second

generation (C/C genotype ; 43.3%, C allele ; 67.9%).

3-3-2. Association of genotypes with economic traits

Table 9 shows the phenotypic values of the measured traits for the *PIK3C3* genotypes. Pigs with the C/C genotype exhibited larger ADG, BFT, and IMF than those with the T/T and C/T genotypes in each gender (Table 9). Pigs with the C/C genotype exhibited lower EMA than those with the T/T and C/T genotypes in each gender (Table 9).

The *PIK3C3* genotype was significantly associated with ADG (P = 0.002), BFT ($p < 0.001$), EMA ($p < 0.001$), and IMF content ($p = 0.049$). The homozygous C/C pigs had a significantly higher mean DG (1018 ± 6 g/day) than the pigs with T/T (977 ± 12 g/day, $p = 0.0047$) and C/T genotypes (1000 ± 5 g/day, $p = 0.0263$). Pigs with the C/C genotype had a significantly higher mean BFT (1.72 ± 0.02 cm) than those with the T/T (1.57 ± 0.04 cm, $p = 0.0013$) and C/T genotypes (1.64 ± 0.02 cm, $p = 0.0014$). Pigs with the C/C genotype had a significantly lower mean EMA (36.8 ± 0.2 cm^2) than those with the T/T genotype (37.8 ± 0.5 cm^2, $p = 0.0162$). Pigs with the T/T genotype had a significantly lower mean IMF (3.88 ± 0.32%) than those with the C/C (4.71 ± 0.12%, $p = 0.0103$) and C/T genotypes (4.70 ± 0.13%, $p = 0.0086$). These results suggest that the C allele have increasing effects on ADG, BFT, and IMF in the period prior to 90 kg body weight in Duroc pigs.

3-3-3. Comparison of statistical model fitness

Table 10 showed the AIC values of each trait estimated by the two different models. The AIC values in the model A that estimated by

applying the *PIK3C3* genotype for production traits (ADG, BFT, EMA, and IMF) were smaller than those in model B that estimated by not including the *PIK3C3* genotype.

Table 11 shows the Wald test results for fixed effects in the REML variance components analysis using model A.

The *PIK3C3* gene genotype had a highly significant effect on BF (p = 0.004), EMA ($p < 0.001$), and IMF (p = 0.045). Although the effect of the *PIK3C3* genotypic did not reach statistical significance for DG, the *P* value was marginally significant (p = 0.051).

The estimated heritability of each trait by the sire model (four times the sire component variance ratio) ranged from 0.265 to 0.541.

3-4. Discussion

The Duroc population used in this study was a line established to improve economic traits such as average ADG, BFT, and IMF content using a closed nucleus breeding system for four generations. At the fourth generation, the ADG, BFT, and IMF breeding values increased 41 g/day, 0.19 cm, and 0.30%, respectively, compared with those at the second generation.

Although we did not consider the *PIK3C3* genotype for animal selection, the C allele frequency significantly increased through three generations (Table 8). This result suggests that the C allele is enriched by the phenotype-based approach to genetic improvement for fat deposition and growth traits.

It is usually used the BLUP method to predict individual's breeding values for improving quantitative traits such as ADG and BFT in the

livestock. Selecting an appropriately fixed and random effect is very important before adopting the BLUP method in order to evaluate genetic value more correctly (Fukawa *et al.,* 2001). Wada and Kashiwagi (1990) suggested that the AIC value is a useful indicator for choosing a model to calculate breeding values by BLUP method. In this study, AIC values of the model that in including the *PIK3C3* genotypes were smaller than those estimated without considering the *PIK3C3* genotype in each trait. Accordingly, our results indicate that the *PIK3C3* genotype can influence the model fitness and that it is worth considering the *PIK3C3* genotype to construct a more appropriate model for genetic evaluation.

Association analysis showed that the *PIK3C3* gene polymorphism had significant effects on multiple production traits in our Duroc population. Pigs with the C/C genotype had higher ADG, BFT, and IMF and less EMA than those with the T/T genotype. In this study, there were gender related differences for *PIK3C3* effect on all traits in case of analyzing by each sex. For example, a significant effect for *PIK3C3* genotypes on ADG was detected on gilts in this study, whereas the *PIK3C3* effect did not reach significance in boars and barrows. As described in Chapter 3, some genes that show gender variations in gene expression or genotype effect have been previously reported (de Oliveira Peixoto *et al.* 2006; Rodríguez *et al.* 2001). Therefore, the levels of effect caused by *PIK3C3* genotypes on traits might be difference in gender. However, the ranks of least square mean values for each *PIK3C3* genotype in each trait were conformed at all sex in the present study. Pigs with the C/C genotype had a higher mean values for ADG, BFT, IMF and lower mean value for EMA than those with the T/T and C/T genotypes in

all sex. These results suggest that *PIK3C3* might play an important role in both fat deposition and muscle development in pigs.

However, as the C2604T SNP in *PIK3C3* is a synonymous substitution, it must be considered whether this point mutation directly leads to such differences in multiple traits or is a result of linkage disequilibrium with other mutations.

QTL related to fatness and meat quality traits, such as intramuscular fat content (IMF) (de Koning *et al.*, 1999; de Koning *et al.*, 2000; Gerbens *et al.*, 1999, 2000; Grindflek *et al.*, 2001; Lee *et al.*, 2012; Ovilo *et al.*, 2000; Uleberg *et al.*, 2005), and backfat thickness (BFT) (Lee et al., 2012; Malek *et al.*, 2001; Ovilo *et al.* 2002; Szyda *et al.*, 2003; Soma *et al.*, 2011) have been detected on SSC6. In particular, Ovilo *et al.* (2002) detected a significant backfat related QTL at the area, which containing S0228 maker whose place was identified nearby *PIK3C3* gene (Kim et al. 2005a). In addition, QTL related productivity traits, such as average daily weight gain (ADG)(Sato *et al.*, 2003) and the size of eye muscle area (EMA)(Edwards *et al.*, 2008a, b) have also been identified on SSC6.

Thus, additional DNA sequence analysis is required to unravel all possible nucleotide polymorphisms on and around the porcine *PIK3C3* locus and to survey linkage disequilibrium blocks on SSC6 at this region.

The effects of *PIK3C3* genotype on production traits detected in an experimental cross of Korean native breeds and Landrace breeds (Kim et al. 2005) and those in Duroc breeds of this study showed same result, which suggest that the SNP C2604T on exon 24 in PIK3C3 might be linkage disequilibrium with functional mutation. Therefore, although C2604T is silent mutation and can not influence directly on the traits, it

has potential as a genetic marker for implicating in pig breeding program.

As only this study and Kim *et al.* (2005) indicated that the relationship between *PIK3C3* and pig production traits, this RFLP mutation may be linkage equilibrium with the functional mutation in population-wide until now. Therefore, the effect of allele might be depend on other population, it needs to preliminary research the correlation between marker and the traits before incorporating into breeding program.

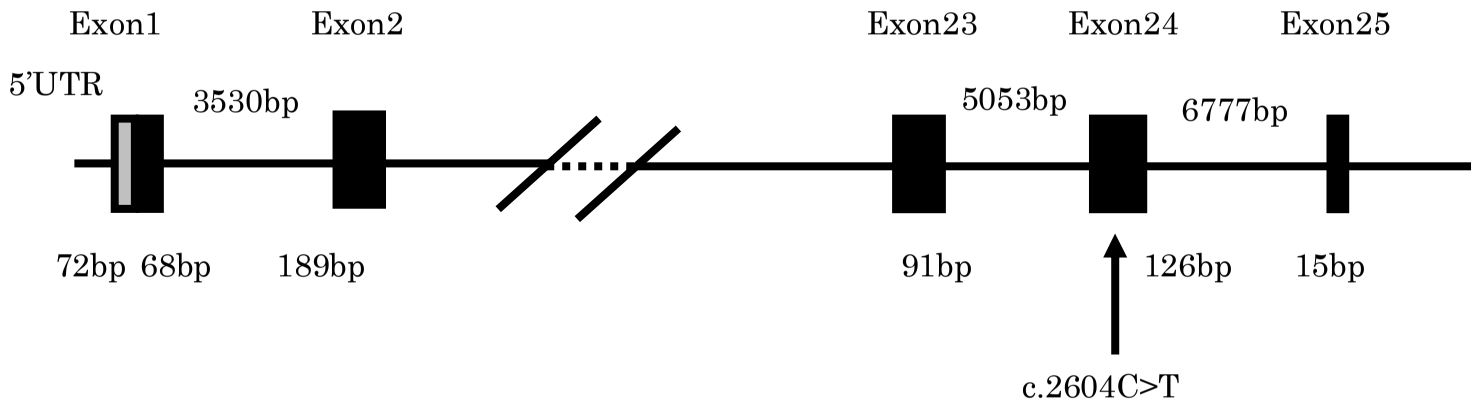

Figure 5. The position of mutation for genotyping *PIK3C3* gene in this study. The protein coding sequences and untranslated region are indicated by dark-stained boxes and grey-stained box, respectively. The numbers of base sequence were taken from the reference NC_010448 (Sus scrofa 10.2; g.117947967..g.118097705, chromosome 6) sequence. The mutation in exon region was identified based on Genbank accession no. NM_001012956.2. Arrow shows the position of c2604C>T mutation.

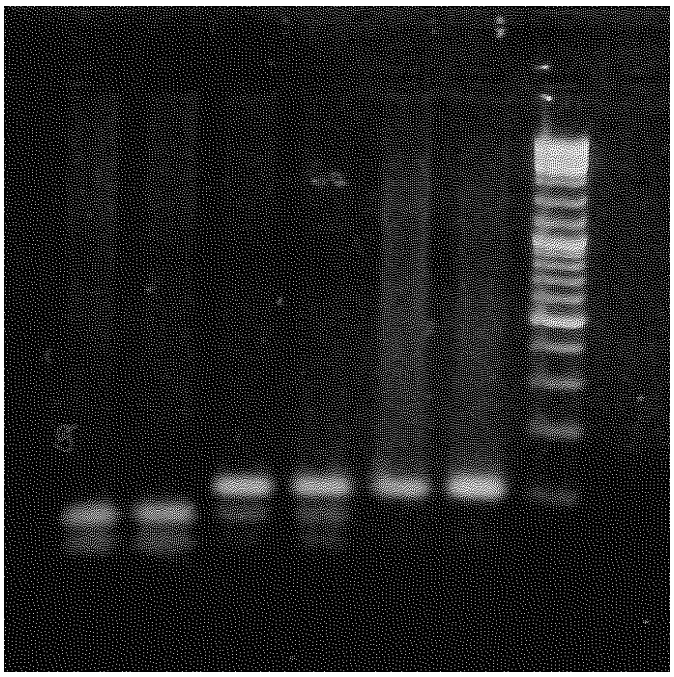

Figure 6. Genotyped patterns of polymorphism at c.2604C>T mutation of porcine *PIK3C3* gene digested by restriction enzym *Hpy*8I on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. Allele C produced 67- and 35-bp fragments, and allele T produced a 102-bp fragment. The heterozygote has both allele C and allele T fragments. The M is a 100-bp DNA Ladder molecular size mark (Fermentas Inc., Glen Burnie, MD, USA).

Table 8 Genotypic and allelic frequencies of the T and C *PIK3C3* gene polymorphism

Generation	Number of pigs				Genotypic frequency[1]			Allelic freque	
	Total	boar	gilt	barrow	T/T	T/C	C/C	T	
Total	739	303	346	90	7.4 (55)	49.3 (364)	43.3 (320)	32.1 (474)	6 (1(
G2	277	111	127	39	7.6 (21)	57.8 (160)	34.7 (96)	36.5 (202)	6 (3
G3	212	88	110	14	10.8 (23)	48.1 (102)	41 (87)	34.9 (148)	6 (2
G4	250	104	109	37	4.4 (11)	40.8 (102))	54.8 (137)	24.8 (124)	7 (3

[1] Percentage of each genotype. Given in parentheses are number of pigs.

[2] Percentage of each allele. Given in parentheses are number of alleles.

Table 9 Association between porcine *PIK3C3* polymorphism and economic traits in Duroc pigs

Traits[1]	Sex	Total[2]	Genotype[3]			*P*	Effect[4]		
			T/T	T/C	C/C		Additive	Dominant	Recessive
ADG (g/day)	Boar	1019 ±5 (303)	987 ± 17 (23)	1019 ± 7 (143)	1027 ± 8 (137)	0.134	13.2 ± 8.2	33.6 ± 19	10.9 ± 10.3
	Gilt	951 ± 5 (346)	924 ± 17[a] (26)	943 ± 7[a] (175)	970 ± 7[b] (145)	0.006	24.9 ± 7.8	30.4 ± 18.1	26.8 ± 9.7*
	Barrow	1044 ± 11 (90)	1036 ± 44 (6)	1039 ± 16 (46)	1060 ± 18 (38)	0.659	17.2 ± 18.5	14.7 ± 43.8	21.7 ± 22.6
	Total	990 ± 4 (739)	977 ± 12[a] (55)	1000 ± 5[a] (364)	1018 ± 6[b] (320)	0.002	19.5 ± 5.4*	29.8 ± 12.7*	21.7 ± 6.8*
BFT (cm)	Boar	1.52 ± 0.02 (303)	1.45 ± 0.06 (23)	1.52 ± 0.02 (143)	1.53 ± 0.02 (137)	0.434	0.03 ±0.03	0.08 ± 0.06	0.03 ± 0.03
	Gilt	1.71 ± 0.02 (346)	1.63 ± 0.06 (26)	1.69 ± 0.02 (175)	1.75 ± 0.03 (145)	0.12	0.06 ± 0.03	0.08 ± 0.07	0.06 ± 0.04
	Barrow	1.82 ± 0.04 (90)	1.63 ± 0.16 (6)	1.79 ± 0.06 (46)	1.93 ± 0.06 (38)	0.094	0.15 ± 0.07*	0.23 ± 0.16	0.16 ± 0.08*
	Total	1.64 ± 0.01 (739)	1.57 ± 0.04[a] (55)	1.64 ± 0.02[a] (364)	1.72 ± 0.02[b] (320)	<0.001	0.06± 0.02*	0.11 ± 0.05	0.06 ± 0.63*
EMA (cm^2)	Boar	37.3 ± 0.2 (303)	37.6 ± 0.7[ab] (23)	38.0 ± 0.3[a] (143)	36.5 ± 0.3[b] (137)	0.002	-0.97 ± 0.34*	-0.24 ± 0.79	-1.45± 0.42*
	Gilt	37.8 ± 0.2 (346)	38.1 ± 0.7 (26)	38.2 ± 0.3 (175)	37.3 ± 0.3 (145)	0.091	-0.52 ± 0.31	-0.28 ± 0.71	-0.76 ±0.38*
	Barrow	37.3 ± 0.4 (90)	38.2 ± 1.5 (6)	38.2 ± 0.6 (46)	36.5 ± 0.6 (38)	0.106	-1.24 ± 0.65	-0.59 ± 1.57	-1.68 ± 0.79*
	Total	37.5 ± 0.1 (739)	37.8 ± 0.5[ab] (55)	38.0 ± 0.2[a] (364)	36.8 ± 0.2[b] (320)	<0.001	-0.82 ± 0.21*	-0.28 ± 0.50	-1.18 ± 0.26*
IMF (%)	Boar	4.28 ± 0.12 (192)	3.75 ± 0.42 (15)	4.40 ± 0.18 (82)	4.46 ± 0.18 (95)	0.304	0.24 ± 0.19	0.69 ± 0.45	0.18 ± 0.24
	Gilt	4.87 ± 0.12 (251)	3.98 ± 0.48 (16)	5.00 ± 0.48 (130)	4.94 ± 0.19 (105)	0.133	0.22 ± 0.20	1.07 ± 0.49*	0.06 ± 0.25
	Total	4.61 ±0.09 (443)	3.88 ± 0.32[a] (31)	4.71 ± 0.12[b] (212)	4.70 ± 0.13[b] (200)	0.049	0.23 ± 0.14	0.88 ± 0.33	0.11 ± 0.17

[a-b] Means within a row with no common superscript differ significanly (P<0.05)

[1] ADG: Average daily gain; BFT: Backfat thickness; EMA: Eye muscle area; IMF: Intramuscular fat content.

[2] Mean values(±S.E.) of all pigs in each sex.

[3] Least square mean values(±S.E.). Different letters denoting significant difference between genotypes. Given in parentheses are number of pigs.

[4] Additive effect: T/T = −1, T/C = 0, C/C = 1. Dominant model effect: T/T = −1, T/C and C/C = 1. Recessive model effect: T/T and T/C = −1, C/C = 1.

*: significant difference (P < 0.05), ** : highly significant difference (P < 0.01).

Table 10 Comparison of Akaike's Information Criterion values obtained two alternative models for each trait

	Traits			
	ADG	BFT	EMA	IMF
Model A[1]	8769	2431	4034	1844
Model B[2]	8788	2437	4047	1848

[1] Model A :
Fixed effects : Generation, sex, and genotype of *PIK3C3*
Random effect : Sire (except for IMF), Dam (for IMF)
Covariate : End test weight (BFT and EMA)

[2] Model B :
Fixed effects : Generation and sex
Random effect : Sire (except for IMF), Dam(for IMF)
Covariate : End test weight (BFT and EMA)

Table 11 Wald tests for fixed effects of each traits estimated by model A contains *PIK3C3* genotype

	Traits			
Fixed term	ADG	BFT	EMA	IMF
Generation	0.013	0.002	0.075	<0.001
Sex	<0.001	<0.001	0.041	0.001
Measurement weight	-	<0.001	<0.001	-
Genotype	0.051	0.004	<0.001	0.045
Variance component				
Sire (Dam)	949	0.0134	1.18	-0.213
Residual	7168	0.0856	11.13	3.002
Heritability[1]	0.468	0.541	0.383	0.265

[1] Each heritability for ADG, BFT, and EMA was estimated by Sire model, that for IMF was estimated by Dam model

CHAPTER 4. Association of porcine vertnin (*VRTN*) gene with production traits in Duroc pigs

4-1. Introduction

The national improvement goal of sire line in Japan has been based on Japanese carcass grading regulations. For example, the national improvement goal of backfat thickness of carcass was configured at 2.0cm, because the backfat thickness at the prime grade was defined the range within 1.3 cm above and 2.4 cm bellow. At the same time, the carcass length that correlates with backfat thickness have been also improved to be assumed a fixed form. These improvement goals was set to be high quality and uniformly sized of domestic pork carcass.

The total number of thoracic and lumbar vertebrae varies among pigs. Wild boars have 19 vertebrae, whereas European commercial breeds have 21–23 vertebrae. The number of vertebrae is correlated with carcass length, back rib contents, loin meat contents and backfat thickness. In case of pig shipment in same weight, it is possible that changing carcass length by the number of vertebrae might lead change the size of loin eye muscle area.

Recently, the needs for carcass traits are not constant, for example, some farmer desired the pig that can produce rich lean meat rate and well productivity, and other farmer desired the pig that can produce high meat quality, unless have less productivity. For responding the request of customers, pig breeding companies have been supplied the boars and sows by selecting body style, however, it is not correctly reflected the genetic talent by only body style-based selection. For evaluation of genetic talents

such as fat deposition traits or carcass length, it is prefer to add the individual's the vertebral number data, however, measuring it at living individuals are very cumbersome and complicated.

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*). *VRTN* has two exons (Figure 7), and there are 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; Hicks *et al.*, 1998; Stewart & Schinckel., 1989). 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.

4-2. Materials and Methods

4-2-1. 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. The details of data collection methods, selection criteria, and selection methods of this strain were described in Chapter 1.

In this part, we analyzed the phenotypic production traits (ADG, BFT, EMA, IMF) that were collected from 1414 Duroc pigs through four generations, from the second to fifth generation. Furthermore, we measured four body composition traits at the same time when measuring previous four production traits.

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.

4-2-2. 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* (g.24801_24802insAA), *NV123* (g.20311_20312 ins291, which means insertion of a 291 nucleotide sequence between g.20311 and g.20312), and *NV149* (g.11051A>T) (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 12. 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, 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 (Figure 8).

4-2-3. Statistical analysis

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:

Model A

$$Y_{ijkl} = \mu + Sex_i + Genotype_j + Generation_k + \text{Group}_l + \beta Weight_{ijkl} + e_{ijkl}$$

where, Y_{ijkl} is the phenotypic value of each trait, μ is the overall mean for each trait, Sex_i is the effect of gender, $Genotype_j$ is the effect of the *VRTN* genotype, $Generation_k$ is the effect of generation, $Group_l$ is the effect of group, β is the regression coefficient of the covariate weight measurement for each trait, $Weight_{ijkl}$ is the covariate of the measurement weight, and e_{ijkl} is the random residual effect. BFT and 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 & Mitsztal., 2004) using the following Model:

Model B

$$Y_{ijkl} = \mu + Sex_i + Genotype_j + Generation_k + Group_l + \beta Weight_{ijkl} + u_{ijkl} + e_{ijkl}$$

where, $Genotype_j$ 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, $A\sigma_u^2$) (A 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, total 1,744 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:

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

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) .

We compared Akaike's information criterion (AIC) values of the mathematical model for the full Model A and the model where the *VRTN* genotypic effect was removed from Model A in order to evaluate the more suitable model. In this study, we calculated AIC value by using GenStat software (Version 8.1.0.152; VSN International Ltd., Hempstead, UK) with the restricted maximum likelihood (REML) method (Patterson & Thompson., 1971).

4-3. Results

4-3-1. *VRTN* allele frequencies

Table 13 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 (χ^2 = 6.163, df = 1, p = 0.013; χ^2 = 7.962, df = 2, p = 0.019, respectively).

5-3-2. Association of *VRTN* genotype and economic traits

Table 14 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 ± 0.16%) than those with the Q/Q genotype (4.79 ± 0.13%, p = 0.013). This effect was observed only in boars (Wt/Wt: 5.06 ± 0.19%, Q/Q: 4.38 ± 0.14%, p = 0.008), whereas the differences in gilts were not statistically significant (Wt/Wt: 5.22 ± 0.18%, Q/Q: 5.02 ± 0.12%, p = 0.543). There was no evidence of any effects of the

VRTN genotype on other traits such as ADG, BFT, 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.

4-3-3. Association of *VRTN* genotype and body composition traits

Table 15 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, 0.015, 0.001, 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.

4-3-4. Additive and dominant effects of *VRTN* on each trait

Table 16 shows the additive and dominant effects of *VRTN* on economic traits and body composition traits. The *VRTN* genotype did not significantly affect IMF in 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 16).

4-3-5. 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.

4-4. Discussion

This association analysis suggests that the porcine *VRTN* genotype had an effect on the phenotypic value of IMF and BL in Duroc. Wt/Wt pigs tend to have a higher IMF content and a shorter BL compared with Q/Q pigs. In this study, we detected a significant effect on IMF in only boar. Fat deposition in pig have gender bias, boars have generally less backfat thickness and intramuscular fat content in the loin than gilt, because some gene expression pattern during fat development is different in sex. Therefore, *VRTN* might relate with such as gene that has sex specific effect. The IMF breeding value was significantly greater in Wt/Wt pigs compared with Q/Q pigs, because even though it did not reach statistical significance, Wt/Wt gilts had greater IMF phenotypic value than Q/Q gilts. Furthermore, the Wt allele frequency increased, suggesting that it was probably synchronized with an increase in the average breeding value for the IMF content through four generations

(from the second to the fifth). This also suggests that *VRTN* may affect intramuscular fat deposition.

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). The present study suggests that *VRTN* could be a useful genetic marker for improving meat quality in Duroc population.

Stewart and Schinckel (1989) reported that the pig carcass length was positively correlated with the total lean content and negatively correlated with the BFT. The *VRTN* genotype affected the IMF, but it did not significant affect the BFT 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 the IMF without affecting the BFT 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.

There was a significant difference between the *VRTN* genotype and the phenotypic value of CF. The circumference of the foreleg might be influenced by various components such as the size of cannon bone or the muscle content around cannon bone. It needs to further research in order to clarify the relationship between the *VRTN* genotype and the circumference of the foreleg.

Although the proportion of additive genetic variance for IMF accounted for by *VRTN* genotypes were not high, the AIC value that

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 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 have potential to correlate 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.

The present results suggest that one *VRTN* allele might produce an increase of 0.54 cm in terms of BL in a 90kg 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 90kg 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 in this study was defined 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 suggests that vertebral number is not always consistent with the body length. Further investigations involving measurements of carcasses traits are needed to confirm the relationship between the *VRTN* genotype, carcass length, and vertebrae number. We performed 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.

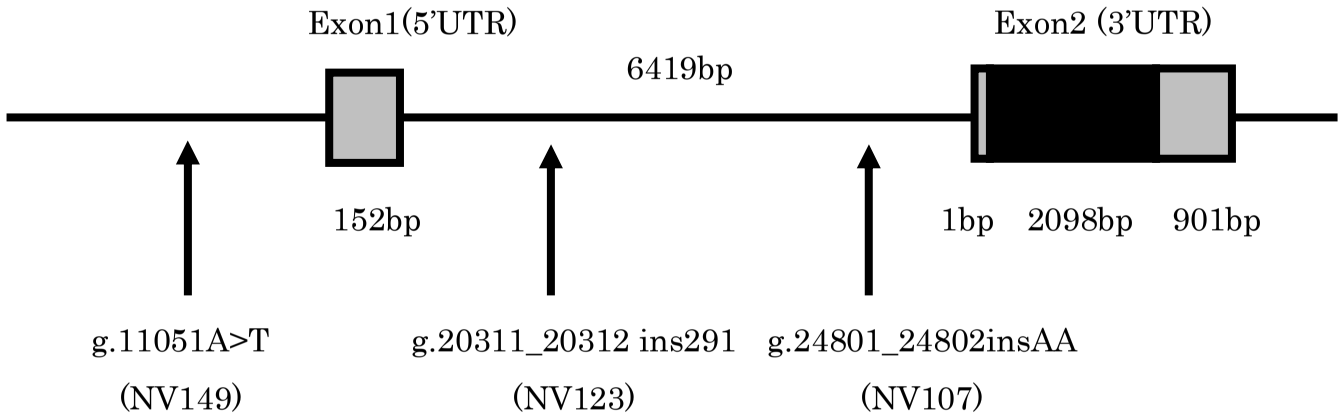

Figure 7. The position of mutation for genotyping *VRTN* gene in this study. The protein coding sequences and untranslated regions are indicated by dark-stained boxes and grey-stained box, respectively. The numbers of base sequence in each region were taken from the reference NC_010449 (Sus scrofa 10.2; g.10345706..g.103467076, chromosome 7) sequence. The mutation in intron region was identified based on Genbank accession no. AB554652.1. Arrows show the position of g.11051A>T, g20311_20312ins291, g.24801_24802insAA mutation, respectively.

Table 12 PCR primer sequence and size of allelic polymorphism

Primer name	Primer sequence (5'-3')	Annealing temperature (℃)	Final concentration rate (μM)	SNP indel	PCR product length (base pairs) Wt	 Q
NV107	Forward; CGA CAG GAA CTC TGC ATC AA Reverse; CAA ATA AAA TAG GTC TTT TTC C	57	0.30	Wt;AAAAA Q;AAA	295	
NV123	Forward; GAT CCT TGG TGA GCT CGA AT Reverse1; TCG TCA ACC CAC TGA GCA Reverse2; CCT TCC TCC TCC TGG AGT CT		0.15	Wt;del (291bp) Q;ins(291bp)	213	242
NV149	Forward; GGA CAC CAG GCC TGA GAT TA Reverse; AAG AGG TTT CAA GGG CTT GA		0.15	Wt; A Q; T		146

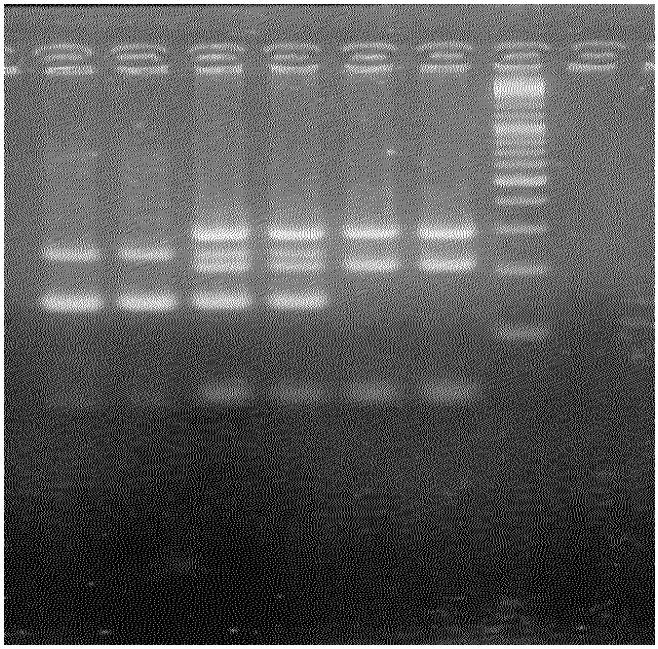

Figure 8. Genotyped patterns of polymorphism of porcine *VRTN* gene on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. Allele Q produced 246- and 142-bp fragments, and allele Wt produced 295-and 213-bp fragments. The heterozygote has both allele Q and allele Wt fragments. The M is a 100-bp DNA Ladder molecular size mark (Fermentas Inc., Glen Burnie, MD, USA).

Table13 Genotypic and allelic frequencies of the Wt and Q gene polymorphism

Generation	Number of pigs				Genotypic frequency[1]			Allelic frequency[2]	
	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)

[1] Percentage of each genotype. Given in parentheses are number of pigs.

[2] Percentage of each allele. Given in parentheses are number of alleles.

Table14 Association between *VRTN* genotype and economic traits in Duroc pigs

Traits[1]	Sex	Total[2]	Genotype[3]			*P*
			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
BFT (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 (cm^2)	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 significanly (P<0.05)

[1] ADG: Average daily gain; BFT: Backfat thickness; EMA: Eye muscle area; IMF: Intramuscular fat content; BVIMF: Breeding value of intramuscular fat content.

[2] Mean values(±S.E.) of all pigs in each sex.

[3] Least square mean values(±S.E.). Different letters denoting significant difference between genotypes. Given in parentheses are number of pigs.

Table 15 Association between *VRTN* genotypes and body composition traits in Duroc pigs

Traits[1]	Sex	Total[2]	Genotype[3]			*P*
			W/W	W/Q	Q/Q	
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 ± 0.3^{a} (96)	99.6 ± 0.2^{ab} (317)	99.9 ± 0.2^{b} (214)	0.041
	Barrow	99.6 ± 0.3 (196)	97.6 ± 0.7^{a} (19)	99.1±0.3^{ab} (99)	100.1 ± 0.4^{b} (74)	0.005
	Total	100.1 ± 0.1 (1414)	98.9 ± 0.2^{a} (210)	99.5 ± 0.1^{b} (710)	100.0 ± 0.2^{c} (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 ± 0.3^{b} (96)	106.1 ± 0.2^{a} (317)	106.3 ± 0.2^{ab} (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 ± 0.2^{b} (96)	61.7 ± 0.1^{b} (317)	61.2 ± 0.1^{a} (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 ± 0.1^{ab} (210)	18.2 ± 0.0^{b} (710)	18.1 ± 0.0^{a} (482)	0.044

[a-b] Means within a row with no common superscript differ significanly (P<0.05)

[1] BL: Body length; CC: Chest circomstance; BH: Body hight; CF: Cannon circumference of

[2] Mean values(±S.E.) of all pigs in each sex.

[3] Least square mean values(±S.E.). Different letters denoting significant difference between genotypes. Given in parentheses are number of pigs.

Table16. Additive and dominance effects of *VRTN* on economic traits and body composition traits[1]

Traits[2]	Sex	Model[3]	LRT[4]	*P*	a ± SE[4]	d ± SE[4]	Varicance (%)[4]
EMA	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

[1]Only those for which statistically significant ($P < 0.05$) gene effects were detected are listed for each trait.

[2] EMA: Eye muscle area; IMF: Intramuscular fat content; BL: Body length; CC: Chest circumference; CF: Cannon circumference of the foreleg

[3]a + d: Model includes both additive and dominance effects as *VRTN* effect; a: Model includes only additive effect as *VRTN* effect

[4]Additive and dominace effects were genotypic values of (WtWt-QQ)/2 and WtQ-(WtWt+QQ)/2, respectively. LRT: Liklihood ratio test. Variance (%) = the propotin of additive genetic variance accounted for by the *VRTN* genotypic effect.

CHPTER 5 Evaluation of effects of multiple candidate genes (*LEP*, *LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) on production traits in Duroc pigs

5-1. Introduction

Use of molecular genetic information can enhance livestock breeding programs by increasing selection accuracy and decreasing generation intervals (Dekkers. 2004). Many studies have reported an association between gene polymorphisms and desirable traits in pig production, which have become available on porcine QTL database (PigQTLdb http://www.animalgenome.org/cgi-bin/QTLdb/SS/index). For example, several dozen of candidate gene (*CTSD*, *FTO, GHRH, HMGA1, HMGA2, IGF2, LEP, LEPR, MC4R. PIK3C3* and so on) was reported as genetic markers might be related with backfat thickness. However, most studies evaluated only single gene effects. Production traits such as backfat thickness (BFT) and growth rate are typical quantitative traits under the control of multiple genes. Thus, it is important to elucidate combination or interaction effects between multiple candidate genes for establishing more effective breeding methods in pigs because there are relatively few reports regarding this. Moreover, in most studies, the association between gene polymorphisms and traits was investigated within limited experimental crosses, such as a Duroc × Pietrain population composed of only one generation. Genetic improvement of the pig strain is generally conducted through several generations, thereby the pig population has more complex genetic structure than that of experimental population. It is more available to verify genetic effect in actual breeding population in order to incorporating genetic effect into actual breeding program.

In the present study, we selected previously reported polymorphisms of *LEP*, *LEPR*, and *MC4R* gene.

The porcine Leptin (*LEP*) gene (GeneBank accession no. NM_213840) is composed of 38 kbp and 5 exons (Figure 9) and has been mapped at 21.201 Mb on SSC18 in the Ensembl genome browser 70: *Sus scrofa* comparative genomic database (Sscrofa10.2; http://www.ncbi.nlm.nih.gov/gene/396832). Leptin receptor (*LEPR*) gene (GeneBank accession no. NM_001024587) on SSC 6 (positional data has not yet been added to Sscrofa10.2)is composed of 80 kbp and 20 exons (Figure 10). Melanocortin 4 receptor (*MC4R*) gene (GeneBank accession no. NM_214173) is composed of 2.8 kbp and 2 exons (Figure 11) and has been mapped at 2at 178.553 Mb on SSC1 in Sscrofa10.2 (http://www.ncbi.nlm.nih.gov/gene/397359). These genes reportedly have significant effects on pig production traits (Kim *et al.* 2000; Kennes *et al.* 2001; Obilio *et al.* 2002; Chen *et al.* 2004; Munoz *et al.* 2009, 2011; Rodriguez *et al.* 2010; Uemoto *et al.* 2012). Furthermore, we added two loci; class 3 phosphoinositide 3-kinase (*PIK3C3*) and vertnin (*VRTN*), whose correlations for traits were detected in chapter 4 and 5.

Furthermore, we analyzed the effects of multigenic combination and interaction effects between the five loci linked to four production traits, which were average daily weight gain (ADG), backfat thickness (BFT), size of loin eye muscle area (EMA), and intramuscular fat content (IMF) in the loin, in five generations of a Duroc pig population improved using a closed nucleus breeding system.

5-2. Materials and Methods

5-2-1. Animals and data collection

The Duroc pig population used in this study was maintained at the Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan) from 2004 to 2010. A complete description of this population was previously provided in the Chapter 1.

In this study, we analyzed the phenotypic values of four production traits (ADG, BFT, EMA, IMF) that were collected from 1,414 Duroc pigs from the second to fifth generation of this strain. All animals were provided unlimited access to food and water during the experimental period, and all experiments were performed in accordance with our institutional guidelines for animal management.

5-2-2. Genotyping of *LEP*, *LEPR*, *MC4R*, *PIK3C3*, and *VRTN*

Genomic DNA was extracted from tail tissue clippings of each pig using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Hilden, Germany) or QuickGene DNA Tissue Kit (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Genotyping of the *LEP* (c.3469T>C), *MC4R* (c.1426A>G), and *PIK3C3* (c.2604C>T) polymorphisms were performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method as described by Stratil *et al.* (1997), Kim *et al.* (2000), and Kim *et al.* (2005), respectively (Table 17, Figure9 and 10). To genotype the *LEPR* polymorphism c.2002C>T in exon14, we developed a novel miss-match PCR–RFLP method using miss-match PCR primers (forward on exon 14 and reverse on intron 14) from the *S. scrofa* Ensembl genomic database (Sscrofa10.2; Ensembl gene ID, NW_003540913). These

miss-match primers were set to create a new *Ape*KI restriction site in the allele C. The PCR conditions were as follows: denaturation at 94°C for 9 min; 33 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. The 133-bp amplicon was digested using the restriction endonuclease *Ape*KI (New England BioLabs, Inc., Ipswich, MA, USA). The amplicon from the c.2002 C allele, but not that from the T allele, contained an *Ape*KI cleavage site (Table 17, Figure11). We also added *VRTN* genotype data as reported by Hirose *et al.* (2012).

5-2-3. Statistical analysis

To evaluate the genotypic trend and allelic frequency in this population, Pearson's chi-square test was performed between the second and fifth generation animals. As *LEPR* and *PIK3C3* were located on the same chromosome (SSC6), we measured the level of linkage disequilibrium of alleles at the two loci. The coefficient of linkage disequilibrium (D), standardized disequilibrium coefficients (D'), and squared allele-frequency correlations (r^2) was estimated using the Expectation-Maximization (EM) algorithm and the likelihood ratio test (LRT) (Montgomery *et al.*, 1996).

In the present study, we evaluated the associations between each genotype and trait in our mixed-inheritance animal model. The snp_ad option of Qxpak software (Perez-Enciso and Misztal. 2004) was used to assess individual genotypes. The following model was used to analyze the data:

Model A (full model)

$$Y_{ijkl} = Sex_i + Generation_j + Group_k + \sum_{l=1}^{n} Genotype_l + \beta Weight_{ijkl} + u_{ijkl} + e_{ijkl},$$

Model B (null model)

$$Y_{ijkl} = Sex_i + Generation_j + Group_k + \beta Weight_{ijkl} + u_{ijkl} + e_{ijkl},$$

where Y_{ijkl} is the phenotypic value of each trait, Sex_i is the fixed effect of ith gender(i = 1, 2, 3), $Generation_j$ is the fixed effect of jth generation(j = 1, 2, 3, 4), $Group_k$ is the fixed effect of the kth group(k = 1, 2), $Genotype_l$ is the effect of the lth genotype of each gene, and n is the number of genotype pairs (n=1, 2, 3, 4, 5). The effects of each gene were assumed to convey additive (a) and dominant (d) effects. β is the regression coefficient of the covariate for weight measurement of each trait and $Weight_{ijkl}$ is the covariate of the measurement weight of each trait. Because BFT and EMA were correlated with weight, an analytical model for two traits included weight measurement as a covariate. u_{ijkl} is the infinitesimal genetic effect of the $ijkl$ animals, which is distributed as $N(0, A\sigma_u^2)$, where A is the additive relationship matrix constructed using pedigree information. e_{ijkl} is the random residual effect. The number of pedigree animals was 1,744, including animals in the first generation.

To perform LRT, log-likelihood values were calculated using the full and null models (Model A and B, respectively) to eliminate each genotypic effect. A nominal p value was calculated assuming the chi-square distribution with the likelihood ratio and the degrees of freedom between

the number of parameters in the full and null models. To determine the effect of each gene on the genotype, the proportion of additive genetic variance of each gene was calculated as follows:

$$Variance\ percentage = [2pq\ (a + d(q - p))^2]/V_A,$$

where p and q are the allelic frequencies of each gene and V_A is the additive genetic variance of the trait obtained from the animal model analysis, without considering the genetic effects of each gene (Falconar, 1989)

The epi_snp option of Qxpak software (Perez-Enciso and Misztal., 2004) was used to evaluate the interactive effect of each gene. The following model was assumed:

Model C (epitasis model)

$$Y_{ijklm} = Sex_i + Generation_j + Group_k + Genotype1_l + Genotype2_m + Genotype1_l*Genotype2_m + \beta Weight_{ijklm} + u_{ijklm} + e_{ijklm},$$

where $Genotype1_l$ and $Genotype2_m$ are single genotypic effects and $Genotype1_l*Genotype2_m$ is the interactive effect of two genotypes. These interactive effect were assumed four epistatic effects (additive*additive, additive*dominant, dominant*additive, dominant*dominant) as described in Cockerham's decomposition method (Cockerham., 1954). For this analysis, LRTs were conducted to compare the epistasis and decreased models (Models C and A), which were used to eliminate the interactive effects of the models. Nominal p values were calculated according to the

chi-square distribution.

The false discovery rate (FDR) was considered as the threshold to determine a significant LRT to account for the multiple tests used in this study, and *q* values were calculated using R software (www.r-project.org/) and the BH method (Benjamini and Hochberg., 1995). The FDR procedure was separately for multiple genotype effect analysis (31 genotype combinations × 4 traits = 124 tests) and for epistatic analysis (10 genotype combinations × 4 traits = 40 tests).

We calculated Akaike's information criterion (AIC) values for each model to investigate whether the inclusion of some candidate gene effects into the mathematical model could increase the accuracy of the model to predict BVs of individual traits. Thus, we compared the difference in the AIC value (ΔAIC) of models A and B using the following equation (Akaike., 1974):

$$\begin{aligned}\Delta AIC_{AB} &= AIC_{mldelA} - AIC_{modelB} \\ &= -2\log\lambda_A + 2npr - (-2\log\lambda_B + 2npr') \\ &= -2(\log\lambda_A - \log\lambda_B) + 2npr - 2npr' \\ &= -2\log\lambda_A/\lambda_B + 2npr - 2npr' \\ &= -2LRT + 2(npr - npr'),\end{aligned}$$

where ΔAIC_{AB} is the difference in AIC values between Models A and B, LRT is the log-likelihood ratio between Models A and B, and npr and npr′ are the number of independently adjusted parameters within Models A and B, respectively, thereby npr-npr' shows the difference of genotype pairs number within Models A and B.

5-3. Results

5-3-1. Generational allelic and genotypic frequencies

The allelic and genotypic frequencies for the four loci (*LEP*, *LEPR*, *MC4R*, and *PIK3C3)* in each generation are presented in Table 18. Except for *LEP*, the distributions of genotypic and allelic frequencies for these loci in the fifth generation were significantly different from those in the second generation ($p < 0.05$). The allelic frequencies of c.1426A in *MC4R*, c.2002T in *LEPR*, and c.2604C in *PIK3C3* in the fifth generation were increased compared with those in the second generation.

5-3-2. Effects of single genes on pig production traits

In our study population, a non-random association of alleles at *LEPR* and *PIK3C3* was detected ($p < 0.001$). However, the values of D, D', and r^2 showed extremely low levels ($D = -0.038$, $D' = 0.202$, $r^2 = 0.032$); therefore, we treated each effect of *LEPR* and *PIK3C3* independently.

Results obtained from association analysis fitting each gene effect are included in Table 19. The *LEPR* and *MC4R* genotype effects were pleiotropic and significantly influenced ADG and BFT. Similarly, *PIK3C3* was associated with ADG and BFT. The effect of allele T in *LEPR*, allele G in *MC4R*, and allele C in *PIK3C3* increased ADG ($p = 0.102 \times 10^{-9}$, 0.859×10^{-5}, and 0.033, $q = 0.506 \times 10^{-9}$, 0.304×10^{-4}, and 0.076, respectively) and BFT ($p = 5.18 \times 10^{-33}$, 9.14×10^{-5}, and 0.015, $q = 0.107 \times 10^{-30}$, 0.252×10^{-3}, and 0.036, respectively) when they were substituted for each opposite allele. Only the independent dominant effect of *LEPR* on ADG resulted in a value greater than that of its additive effect.

An additive genetic variance of 16.99% in ADG was attributed to *LEPR*, *MC4R*, and *PIK3C3* (7.62, 7.84, and 1.53%, respectively). Similarly, the proportion of additive genetic variance on BFT by these three markers was 22.51%, in which the contribution of *LEPR* (19.76%) was significantly larger than that of *MC4R* (1.11%) and *PIK3C3* (1.64%).

As shown in Table 19, *LEP* polymorphisms showed no effect on any traits in this population. Although *LEPR* on IMF and *PIK3C3* on EMA induced marginally significant effects (p = 0.066 and 0.074, respectively), both p values did not reach significance after FDR correction.

5-3-3. Combination effects on pig production traits

The results obtained from the present association analysis of combination effects on pig production traits are presented in Table 20. We detected significant effects of all combinations of *LEPR*, *MC4R*, or *PIK3C3*, with the exception of *LEP/PIK3C3* on ADG and BFT. All p values indicated significance after FDR correction.

The largest influence on the ADG and BFT phenotype was induced by the genetic combination include *LEPR*, *MC4R, PIK3C3*, which was consistent with the alleles that were most likely to independently increase the phenotypic values. For IMF, only the genetic combination of *LEPR*/*VRTN* showed a statistically significant association, but this q-value did not reached significance after FDR correction. Lastly, we detected no significant association between any genotype pairs and EMA.

Epistatic effects between all gene pairs on the production traits are presented in Table 21. As shown, only the *LEPR* and *PIK3C3* pair on EMA reached significance (p = 0.026), however, this significance did not remain

after FDR correction.

5-3-4. Comparison of statistical model fitness

The *Δ*AIC values between the full model, which included single gene effects, and the null model, which did not, are presented in Table 19, and comparative results between the models that produced more than two gene effects and the null model are presented in Table 20. The *Δ*AIC values between the epistasis and null models are presented in Table 21.

A comparison within the single gene effect models showed that the *Δ*AIC values of the model that included the effect of *LEPR* were the least on both ADG and BFT and were extremely less compared with those of the model that contained the effects of *MC4R* or *PIK3C3*.

An intermodel comparison showed that the *Δ*AIC values estimated by applying the combination model of *LEPR*/*MC4R*/*PIK3C3* (*Δ*AIC = −62.0) for ADG had the smallest value, whereas the model that included *LEPR/MC4R/PIK3C3/VRTN* had the smallest value (*Δ*AIC = −160.3) for BFT.

The differences in the genetic effects on ADG induced by the combination of *LEPR*-TT/*MC4R*-GG/*PIK3C3*–CC and a model containing substitutions of each opposite allele was calculated for 131.4 g/day. For BFT, the combination of *LEPR*-TT/*MC4R*-GG/*PIK3C3*-CC/*VRTN*-Wt/Wt showed the largest value and the difference in genetic effects between the combinations of the alleles and the combination of the opposite alleles was 0.38 cm.

For EMA, although the *Δ*AIC value of the model that included the effect of *PIK3C3* was slightly negative (*Δ*AIC =−1.2), the values of the

models that contained the effects of more than two genes were positive. The ΔAIC value of the model that included the independent effects of *LEPR* and *PIK3C3* as well as the epistatic effects between those genes was slightly negative (ΔAIC = −1.1), however, this value was greater than that of the model that included only the *PIK3C3* genotype.

For IMF, only the ΔAIC value of the model that included *LEPR*, which showed a marginally correlation, was slightly lesser than that of the null model (ΔAIC = −1.4).

5-4. Discussion

The present study focused on combination effects of candidate genes known to be associated with economically important production traits, such as ADG and BFT, which were located in previously identified porcine quantitative trait loci regions, to predict the genetic value of animals more accurately.

The association analysis of single candidate genes demonstrated that *LEPR*, *MC4R*, and *PIK3C3* had pleiotropic effects on growth and fat deposition. c.2002T of *LEPR*, c.1426G of *MC4R*, and c.2604C of *PIK3C3* had positive additive effects on ADG and BFT in our Duroc pig population (Table 19), which were in accordance with previous reports regarding allelic effects on these pig production traits (Kim *et al.*, 2000, 2005; Houston *et al.*, 2004; Ovilio *et al.*, 2005, 2006; Bruun *et al.*, 2006; Munoz *et al.*, 2009; Hirose *et al.*, 2011; Uemoto *et al.* 2012). On the other hand, we could not detect any significant correlation between the *LEP* genotype and growth traits that had been reported in a Landrace strain (Kennes *et al.*, 2001). This discrepancy suggests that the effects of the *LEP*

c.3469T<C variants diverge depending on the genetic background.

BVs of ADG, BFT, and IMF of the fifth generation of the present Duroc pig population significantly increased compared with those of the first and second generations by improving our breeding program (Hirose *et al.* 2012). The changes in allelic frequencies for each of the *LEPR* and *PIK3C3* in this population appeared to have responded to the selective breeding (Table 18).

The present joint analysis determined that the genetic combinations of *LEPR*/*MC4R*/*PIK3C3* for ADG, *LEPR*/*MC4R*/*PIK3C3*/*VRTN* for BFT, and *LEPR*/*VRTN* for IMF had the smallest AIC values. For BFT, the combination effect of *VRTN* to the combination of *LEPR*/*MC4R*/*PIK3C3*, in which each gene significantly influenced the phenotypic traits described in this study, further enhanced the monitored pig production parameters. However, the effect of *VRTN* alone did not reach statistical significance but indicated a marginally influence (p = 0.066, data not shown) on BFT in our previous study (Hirose *et al.* 2012). Thus, a combination of the four genes analyzed herein might induce the smallest AIC value. For IMF, the AIC value of the *LEPR*/*VRTN* effect was smaller than that of the single *LEPR* effect. In our previous study, *VRTN* showed a significant effect on IMF in an additive effect model and GLM procedure (Hirose *et al.* 2012). Therefore, our results suggest that combination effect of between *LEPR* and *VRTN* might be useful for improving IMF in our Duroc population.

Although some models that included multiple genetic effects showed higher LRT values than those with fewer genetic effects, they were less adequate because of the higher AIC value in the present study. For

example, the LRT value of the *LEPR/PIK3C3* model for ADG was higher than that of the single *LEPR* model, but the AIC value of the former model was higher than that of the latter. Moreover, the model that included all genotype pairs showed the highest LRT values in this study, but it did not showed the smallest AIC value. With regard to estimation of the AIC value of the model, including more parameters leads to increased AIC values. Therefore, the increased number of markers used as parameters in the model includes some genes that affect the AIC value. These results suggest that it is useful to construct a more appropriate model to predict BVs by addition of other marker effects simultaneously. Nonetheless, it is important to choose the marker combinations carefully, even if each marker independently affects the traits because the model fitness may become unfavorable depending on the combination of markers.

We also detected marginally epistatic effects by *LEPR*/*PIK3C3* on EMA in this population. But this epistatic effect did not reach significance after FDR correction. The presence of epistatic effect between *LEPR* and *PIK3C3* for EMA were not clear in this study. On the other hand, although strong effects by *LEPR, MC4R, and PIK3C3* on ADG and BFT were detected in this study, they were independent. It is not clear about the mechanisms of interactive effects between these genes in this study. Therefore, further studies are needed in order to clarify the interactive effects within the gene polymorphisms on the porcine productive traits.

In this study, we compared the models to select favorable model with considering AIC value. However, some models had equivalent AIC value in the present study. In this study, difference of AIC value between the

most favorable model and the second most favorable one for ADG, BFT, and IMF were 2.1, 1.1, and 0.5, respectively. It is not clarified the correlation between difference of AIC value among models and difference of prediction accuracy for breeding values. Therefore, further studies are needed to confirm relationship between AIC value of the model and prediction accuracy.

Here we determined that addition of multiple appropriate markers might be useful to predict individual genetic traits more accurately.

In present study, it is suggested that utilizing the models which included three genes effects (*LEPR*, *MC4R*, and *PIK3C3*) for ADG, four genes effects (*LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) for BFT, and two genes effects (*LEPR* and *VRTN*) for IMF as fixed effects is the most favorable in order to implicate breeding our Duroc population. However, these marker effects are different from populations. Further studies using other breeds and populations are warranted to clarify the effects of multiple genes.

Furthermore, there are no reports about genetic gains in breeding pig population that are utilized gene marker effects as selection methods. It is need to study about practical values in the case of using genetic information to clarify the availability of breeding based on molecular information.

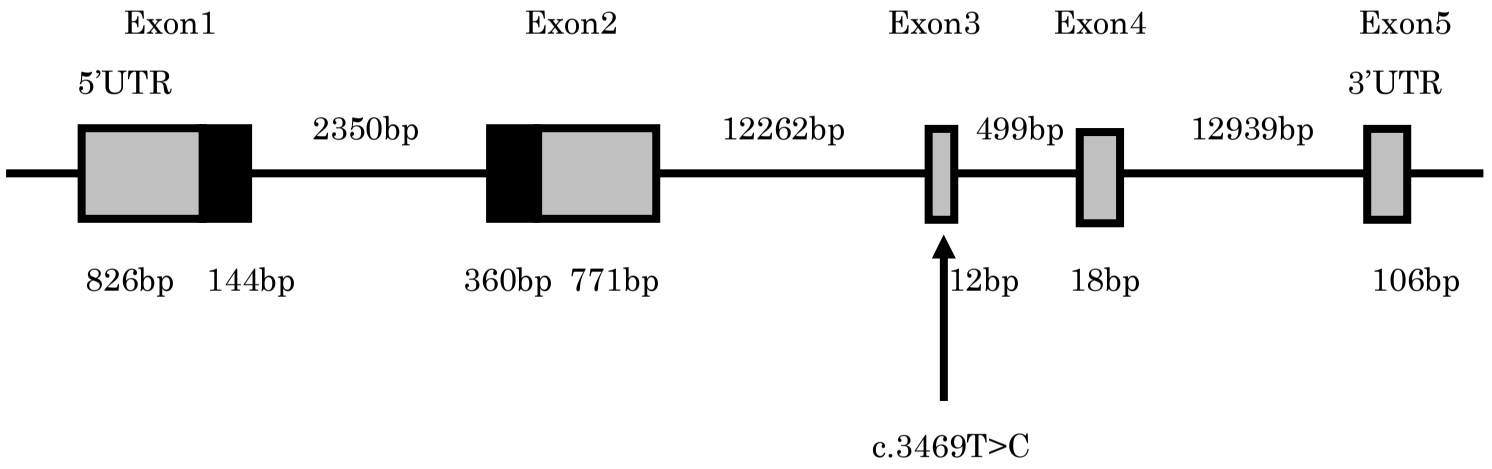

Figure 9. The position of mutation for genotyping *LEP* gene in this study. The protein coding sequences and untranslated region are indicated by dark-stained boxes and grey-stained box, respectively. The numbers of base sequence in each region were taken from the reference NC_010460 (Sus scrofa 10.2; g.21200988..g.21231274, chromosome 18) sequence. The mutation in exon region was identified based on Genbank accession no. NM_213840.1. Arrow shows the position of c.3469T>C mutation.

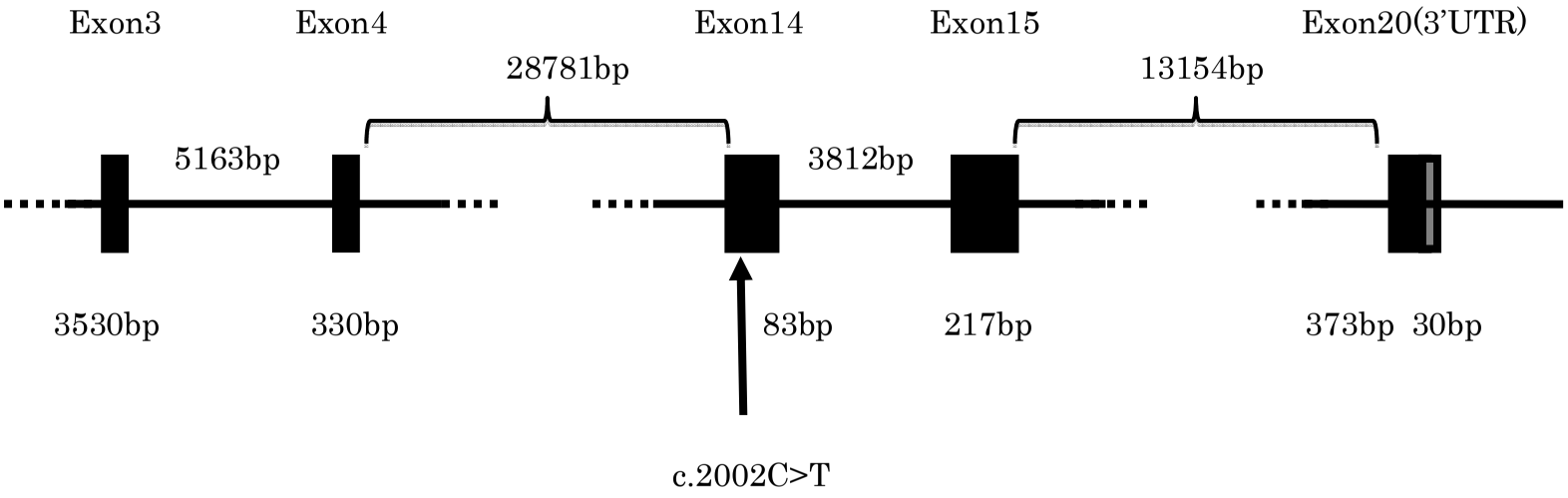

Figure 10. The position of mutation for genotyping *LEPR* gene in this study. The protein coding sequences and untranslated region are indicated by dark-stained boxes and grey-stained box, respectively. The numbers of base sequence in each region were taken from the reference NW_003541095 (Sus scrofa 10.2; unplaced scaffold reference) sequence. The mutation in exon region was identified based on Genbank accession no. NM_001024587. Arrow shows the position of c2002C>T mutation.

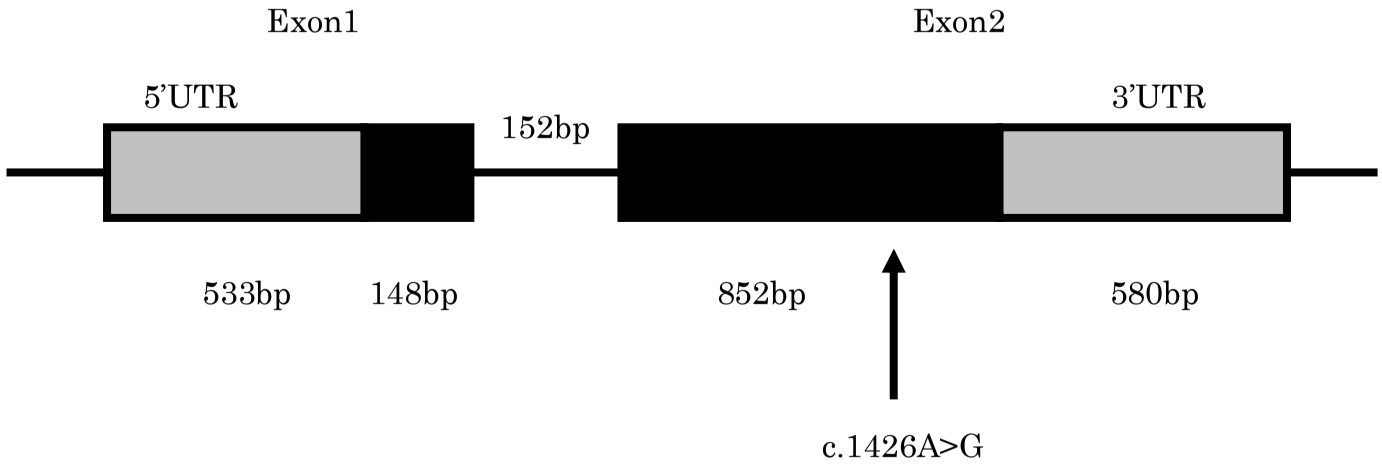

Figure 11. The position of mutation for genotyping *MC4R* gene in this study. The protein coding sequences and untranslated region are indicated by dark-stained boxes and grey-stained box, respectively. The numbers of base sequence in each region were taken from the reference NC_010443 (Sus scrofa 10.2; g.178553488..178555752, chromosome 1) sequence. The mutation in exon region was identified based on Genbank accession no. NM_214173.1. Arrow shows the position of c.1426A>G mutation.

Table 17 PCR primer sequence and size of allelic polymorphism

Gene	Primer sequences (5′-3′)	Annealing Temperature (℃)	Polymorphism location	Mutation type	Enzyme	Fragment size (bp)
LEP	TGCAGTCTGTCTCCTCCAAA CGATAATTGGATCACATTTCTG	55	exon3	silent	*Hinf*I	T: 152 C: 84,68
LEPR	CCACTAAAAAAGAGAGGAATATCACGCTG ATGGCAGAGTCCAGAAATGACT	54	exon14	missence	*Ape*KI	C: 107,26 T: 133
MC4R	TACCCTGACCATCTTGATTG ATAGCAACAGATGATCTCTTTG	56	exon2	missence	*Taq*I	A: 226 G: 156, 70
PIK3C3	ATTTCGTCTAGACCTGTCCG TGAATCTGTTCTACCACCGC	57	exon24	silent	*Hpy8*I	C: 67,35 T: 102

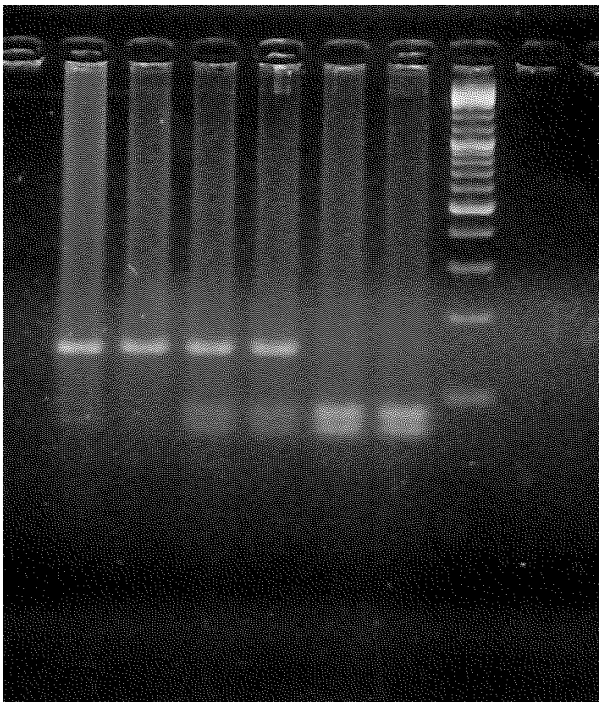

Figure 12. Genotyped patterns of polymorphism at c.3469C>T mutation of porcine *LEP* gene digested by restriction enzym *HinfI* on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. Allele T produced a 152-bp fragment, and allele C produced 84- and 68-bp fragments. The heterozygote has both allele T and allele C fragments. The M is a 100-bp DNA Ladder molecular size mark (Fermentas Inc., Glen Burnie, MD, USA).

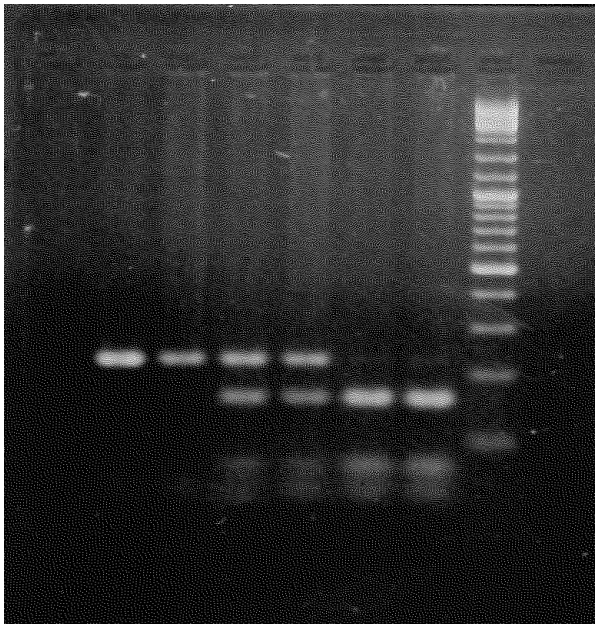

Figure 13. Genotyped patterns of polymorphism at c.1426A>G mutation of porcine *MC4R* gene digested by restriction enzym *Taq*I on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. Allele A produced a 226-bp fragment, and allele G produced 156- and 70-bp fragments. The heterozygote has both allele A and allele G fragments. The M is a 100-bp DNA Ladder molecular size mark (Fermentas Inc., Glen Burnie, MD, USA).

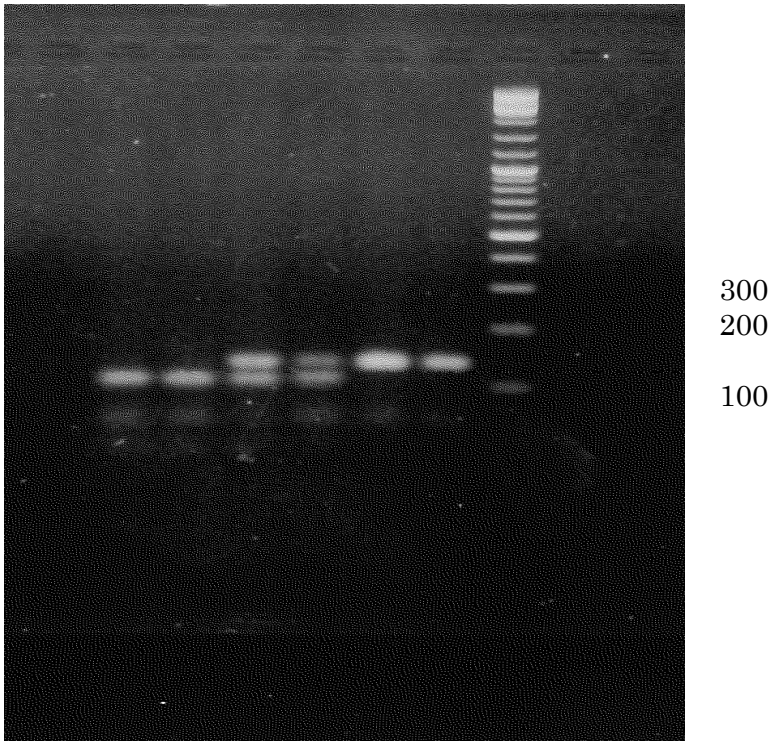

Figure 14. Genotyped patterns of polymorphism at c.2002C>T mutation of porcine *LEPR* gene digested by restriction enzym *Ape*KI on a 3.0% agarose gel. The genotypes indicate under lanes of the gel. Allele C produced 107- and 26-bp fragments, and allele T produced a 133-bp fragment. The heterozygote has both allele C and allele T fragment. The M is a 100-bp DNA Ladder molecular size mark (Fermentas Inc., Glen Burnie, MD, USA).

Table 18 Number of animals, genotypic, and allelic frequencies of markers in each generation

Marker	Generation		Total		G2		G3		G4		G5		G2–G5	
	Number of pigs	Total	1414		283		344		366		420			
		boar	588		113		131		154		190		c^2	p
		gilt	630		129		165		163		173			
		barrow	196		41		48		49		58			
LEP	Genotypic frequency[1]	T/T	0.561	(793)	0.569	(161)	0.578	(199)	0.557	(204)	0.545	(229)		
		T/C	0.414	(585)	0.424	(120)	0.407	(140)	0.423	(155)	0.405	(170)	9.848	0.007
		C/C	0.025	(35)	0.007	(2)	0.015	(5)	0.019	(7)	0.050	(21)		
	Allelic frequency[1]	T (q)	0.768	(2171)	0.781	(442)	0.782	(538)	0.769	(563)	0.748	(628)	2.062	0.151
		C (p)	0.232	(655)	0.219	(124)	0.218	(150)	0.231	(169)	0.252	(212)		
LEPR	Genotypic frequency[1]	C/C	0.108	(153)	0.198	(56)	0.134	(46)	0.077	(28)	0.055	(23)		
		C/T	0.455	(643)	0.530	(150)	0.520	(179)	0.445	(163)	0.359	(151)	23.369	<0.001
		T/T	0.437	(618)	0.272	(77)	0.346	(119)	0.478	(175)	0.587	(247)		
	Allelic frequency[1]	C (p)	0.336	(949)	0.463	(262)	0.394	(271)	0.299	(219)	0.234	(197)	20.878	<0.001
		T (q)	0.664	(1879)	0.537	(304)	0.606	(417)	0.701	(513)	0.766	(645)		
MC4R	Genotypic frequency[1]	A/A	0.766	(1083)	0.714	(202)	0.727	(250)	0.735	(269)	0.862	(362)		
		A/G	0.226	(319)	0.283	(80)	0.262	(90)	0.251	(92)	0.136	(57)	46.83	<0.001
		G/G	0.008	(11)	0.004	(1)	0.012	(4)	0.014	(5)	0.002	(1)		
	Allelic frequency[1]	A (p)	0.879	(2485)	0.855	(484)	0.858	(590)	0.861	(630)	0.930	(781)	35.476	<0.001
		G (q)	0.121	(341)	0.145	(82)	0.142	(98)	0.139	(102)	0.070	(59)		
PIK3C3	Genotypic frequency[1]	C/C	0.490	(693)	0.332	(94)	0.442	(152)	0.536	(196)	0.595	(250)		
		C/T	0.450	(636)	0.594	(168)	0.474	(163)	0.421	(154)	0.360	(151)	7.798	0.02
		T/T	0.060	(85)	0.074	(21)	0.084	(29)	0.044	(16)	0.045	(19)		
	Allelic frequency[1]	C (p)	0.715	(2022)	0.629	(356)	0.679	(467)	0.746	(546)	0.775	(651)	6.096	0.014
		T (q)	0.285	(806)	0.371	(210)	0.321	(221)	0.254	(186)	0.225	(189)		
VRTN	Genotypic frequency[1]	Q/Q	0.340	(481)	0.406	(115)	0.360	(124)	0.311	(114)	0.305	(128)		
		Q/Wt	0.511	(722)	0.466	(132)	0.474	(163)	0.549	(201)	0.538	(226)	78.969	<0.001
		Wt/Wt	0.149	(210)	0.127	(36)	0.166	(57)	0.139	(51)	0.157	(66)		
	Allelic frequency[1]	Q (p)	0.596	(1684)	0.640	(362)	0.597	(411)	0.586	(429)	0.574	(482)	80.735	<0.001
		Wt (q)	0.404	(1142)	0.360	(204)	0.403	(277)	0.414	(303)	0.426	(358)		

[1]Percentage of each genotype and each allele. The number of alleles is given in parentheses.

[2]Chi-square test for genotypic and allelic frequencies between G2 and G3

Table 19 Single gene effects for pig production test traits

Trait[1]	Marker	LRT[2]	*p* value	*q* value[3]	⊿AIC[2]	Additive effect		Dominance effect		Variance[4] (%)
						Mean	SE	Mean	SE	
ADG	*LEP*	0.63	0.728	0.778	3.37	3.18	7.97	-0.94	8.39	0.07
	LEPR	46.01	0.102×10^{-9}	0.506×10^{-9}	-42.01	-15.23	4.60	-20.78	5.22	7.62
	MC4R	23.33	0.859×10^{-5}	0.304×10^{-4}	-19.33	34.06	13.85	4.92	14.31	7.84
	PIK3C3	6.82	0.033	0.076	-2.82	14.35	5.68	5.7	6.27	1.53
	VRTN	0.78	0.678	0.798	3.22	-1.10	4.32	-3.86	4.97	0.00
BFT	*LEP*	0.55	0.759	0.793	3.45	0.01	0.02	0.02	0.03	0.31
	LEPR	148.68	0.518×10^{-32}	0.107×10^{-30}	-144.68	-0.11	0.01	-0.10	0.02	19.76
	MC4R	18.60	0.914×10^{-4}	0.252×10^{-3}	-14.60	0.01	0.04	-0.08	0.04	1.11
	PIK3C3	8.39	0.015	0.036	-4.39	0.04	0.02	-0.01	0.02	1.63
	VRTN	5.63	0.06	0.165	-1.63	0.02	0.01	-0.03	0.02	0.64
EMA	*LEP*	0.96	0.618	0.710	3.04	0.05	0.31	0.24	0.33	0.21
	LEPR	1.13	0.569	0.672	2.87	0.12	0.18	-0.21	0.21	0.02
	MC4R	0.80	0.670	0.755	3.20	-0.45	0.55	-0.33	0.57	1.94
	PIK3C3	5.20	0.074	0.151	-1.20	-0.24	0.22	0.25	0.25	0.92
	VRTN	2.10	0.350	0.583	1.90	0.24	0.17	-0.11	0.2	0.61
IMF	*LEP*	0.32	0.851	0.858	3.68	0.07	0.15	0.09	0.17	0.56
	LEPR	5.44	0.066	0.138	-1.44	-0.16	0.10	-0.10	0.11	1.87
	MC4R	1.52	0.467	0.568	2.48	0.34	0.28	0.35	0.29	8.78
	PIK3C3	2.39	0.303	0.433	1.61	0.16	0.11	0.04	0.13	0.95
	VRTN	4.29	0.117	0.234	-0.29	0.18	0.09	-0.06	0.10	2.00

[1]ADG, average daily weight gain during the test period. BFT, backfat thickness. EMA, loin eye muscle area. IMF, intramuscular fat content in the loin.

[2]LRT; likelihood ratio test. ⊿AIC; the difference of the AIC value between the full model that includes the gene effect and the null model

[3]The *q* value of a test measures the proportion of false positives incurred when that particular test is called significant.

[4]Variance(%): the proportion of additive genetic variance accounted for by each genotype

Table 20 Combination effects of the genotype for pig production test traits [1]

Trait[2]	Genotype pairs					LRT[3]	*p* value	*q* value[3]	⊿AIC[3]	Variance[4] (%)
	Gene1	Gene2	Gene3	Gene4	Gene5					
ADG	*LEP*	*LEPR*				47.17	0.141×10^{-8}	0.672×10^{-8}	-39.2	0.083
	LEP	*MC4R*				23.90	0.838×10^{-4}	0.258×10^{-3}	-15.9	0.051
	LEPR	*MC4R*				67.88	0.635×10^{-14}	0.437×10^{-12}	-59.9	0.138
	LEPR	*PIK3C3*				44.95	0.407×10^{-8}	0.187×10^{-7}	-37.0	0.061
	LEPR	*VRTN*				40.37	0.363×10^{-7}	0.150×10^{-6}	-32.4	0.054
	MC4R	*PIK3C3*				31.29	0.267×10^{-5}	0.974×10^{-5}	-23.3	0.070
	MC4R	*VRTN*				24.34	0.683×10^{-4}	0.202×10^{-3}	-16.3	0.051
	PIK3C3	*VRTN*				13.36	0.963×10^{-2}	0.024	-5.4	0.023
	LEP	*LEPR*	*MC4R*			68.91	0.682×10^{-12}	0.403×10^{-11}	-56.9	0.142
	LEP	*LEPR*	*PIK3C3*			45.98	0.299×10^{-7}	0.128×10^{-6}	-34.0	0.064
	LEP	*LEPR*	*VRTN*			41.25	0.259×10^{-6}	0.100×10^{-5}	-29.2	0.057
	LEP	*MC4R*	*PIK3C3*			31.88	0.172×10^{-4}	0.100×10^{-6}	-19.9	0.070
	LEP	*MC4R*	*VRTN*			24.78	0.376×10^{-3}	0.992×10^{-3}	-12.8	0.051
	LEP	*PIK3C3*	*VRTN*			13.89	0.0309	0.072	-1.9	0.025
	LEPR	*MC4R*	*PIK3C3*			74.01	0.613×10^{-13}	0.437×10^{-12}	-62.0	0.152
	LEPR	*MC4R*	*VRTN*			68.59	0.795×10^{-12}	0.448×10^{-11}	-56.6	0.136
	LEPR	*PIK3C3*	*VRTN*			45.27	0.413×10^{-7}	0.165×10^{-6}	-33.3	0.059
	MC4R	*PIK3C3*	*VRTN*			32.67	0.121×10^{-4}	0.398×10^{-4}	-20.7	0.071
	LEP	*LEPR*	*MC4R*	*PIK3C3*		75.06	0.478×10^{-12}	0.297×10^{-11}	-59.1	0.156
	LEP	*LEPR*	*MC4R*	*VRTN*		69.45	0.632×10^{-11}	0.327×10^{-10}	-53.5	0.141
	LEP	*LEPR*	*PIK3C3*	*VRTN*		53.16	0.100×10^{-7}	0.447×10^{-7}	-37.2	0.089
	LEP	*MC4R*	*PIK3C3*	*VRTN*		33.12	0.585×10^{-4}	0.177×10^{-3}	-17.1	0.072
	LEPR	*MC4R*	*PIK3C3*	*VRTN*		75.16	0.459×10^{-12}	0.297×10^{-11}	-59.2	0.151
	LEP	*LEPR*	*MC4R*	*PIK3C3*	*VRTN*	76.02	0.300×10^{-11}	0.162×10^{-10}	-56.0	0.153
BFT	*LEP*	*LEPR*				149.59	0.249×10^{-30}	0.238×10^{-29}	-141.6	0.206
	LEP	*MC4R*				19.12	0.744×10^{-3}	0.192×10^{-2}	-11.1	0.022
	LEPR	*MC4*				163.78	0.226×10^{-33}	0.157×10^{-33}	-155.8	0.224
	LEPR	*PIK3C3*				152.62	0.560×10^{-31}	0.631×10^{-31}	-144.6	0.178
	LEPR	*VRTN*				155.22	0.155×10^{-31}	0.155×10^{-31}	-147.2	0.213
	MC4R	*PIK3C3*				28.05	0.122×10^{-4}	0.398×10^{-4}	-20.1	0.038
	MC4R	*VRTN*				26.57	0.243×10^{-4}	0.753×10^{-4}	-18.6	0.034
	PIK3C3	*VRTN*				15.95	0.309×10^{-2}	0.782×10^{-2}	-8.0	0.029
	LEP	*LEPR*	*MC4R*			164.57	0.637×10^{-32}	0.113×10^{-30}	-152.6	0.219
	LEP	*LEPR*	*PIK3C3*			153.42	0.146×10^{-29}	0.113×10^{-28}	-141.4	0.198
	LEP	*LEPR*	*VRTN*			156.43	0.337×10^{-30}	0.298×10^{-29}	-144.4	0.213
	LEP	*MC4R*	*PIK3C3*			28.55	0.741×10^{-4}	0.214×10^{-3}	-16.5	0.037
	LEP	*MC4R*	*VRTN*			27.34	0.125×10^{-3}	0.337×10^{-3}	-15.3	0.035
	LEP	*PIK3C3*	*VRTN*			16.70	0.010	0.025	-4.7	0.028
	LEPR	*MC4R*	*PIK3C3*			168.33	0.102×10^{-32}	0.316×10^{-30}	-156.3	0.213
	LEPR	*MC4R*	*VRTN*			171.18	0.253×10^{-33}	0.157×10^{-31}	-159.2	0.225
	LEPR	*PIK3C3*	*VRTN*			159.65	0.702×10^{-31}	0.725×10^{-30}	-147.7	0.203
	MC4R	*PIK3C3*	*VRTN*			36.69	0.202×10^{-5}	0.759×10^{-5}	-24.7	0.052
	LEP	*LEPR*	*MC4R*	*PIK3C3*		169.02	0.207×10^{-31}	0.257×10^{-30}	-153.0	0.217
	LEP	*LEPR*	*MC4R*	*VRTN*		172.26	0.433×10^{-32}	0.107×10^{-30}	-156.3	0.233
	LEP	*LEPR*	*PIK3C3*	*VRTN*		160.74	0.112×10^{-29}	0.926×10^{-29}	-144.7	0.211
	LEP	*MC4R*	*PIK3C3*	*VRTN*		37.43	0.958×10^{-5}	0.330×10^{-4}	-21.4	0.053
	LEPR	*MC4R*	*PIK3C3*	*VRTN*		176.30	0.615×10^{-33}	0.254×10^{-31}	-160.3	0.226
	LEP	*LEPR*	*MC4R*	*PIK3C3*	*VRTN*	177.27	0.862×10^{-32}	0.134×10^{-30}	-157.3	0.232
IMF	*LEPR*	*VRTN*				9.872	0.043	0.096	-1.9	0.038

[1]Only those pairs for which statiscally significant gene effects were detected are listed for each trait.

[2]ADG, average daily weight gain during test period. BFT, backfat thickness. IMF, intramuscular fat content in the loin.

[3]LRT:likelihood ratio test. The q value of a test measures the proportion of false positives incurred when that particular test is called significant. ⊿AIC: Diferrence between AIC of the model that includes genetic effects and that of the exclusive model

[4]Variance(%): the proportion of additive genetic variance accounted for by each genotype

Table 21 Epistatic effects of genotype and the models that include epistaitc effects for pig production test traits[1]

Trait[2]	Genotype pairs		LRT[3]	*p* value	*q* value[4]
	Genotype1	Genotype2			
EMA	*LEPR*	*PIK3C3*	4.978	0.026	0.97

[1]Only those pairs for which statiscally significant gene effects were detected are
[2]EMA, Eye muscle area.

[3]LRT = likelihood ratio test

[4]The *q* value of a test measures the proportion of false positives incurred when that particular test is called significant

General Discussion

There have been numerous research to detect the genetic markers relate with production traits, and it is provided that some examples of genetic markers that have been available and/or used in pig breeding. But examples of implement breeding program by using genetic markers in commercial pig lines were limited, and the methods for incorporating genetic markers into routine genetic evaluation scheme in pig breeding organizations are not clear.

In this work, we implicated the investigation to detect genetic markers on production traits and the evaluation the multiple effects of genetic markers in commercial Duroc population developed by closed nucleus breeding system. From first to third part, we researched the association between gene polymorphism and pig production traits, and detected that the polymorphism in *ADRB3* might affect to eye muscle area (Chapter 2), the polymorphism in *PIK3C3* might effect to growth rate, fat deposition, and eye muscle area (Chapter 3), and polymorphism in *VRTN* might affect intramuscular fat content and body length (Chapter 4). Thereby, these results suggest that such markers are useful in our Duroc population.

The application strategies for incorporating marker data into improvement program are depending on the maker data (as described below), the traits (e.g. Low heritability traits, sex limited traits, or slaughter traits), population (e.g. within-breed like a synthetic line, pure breed line), and the breeding method (e.g. closed nucleus system, open nucleus system). Therefore, for the purpose of the use of genetic markers for selection, it needs to clarify the marker types and selection strategies.

Dekkers *et al.* (2004, 2010) distinguished that the genetic marker type for three types, and selection strategies for four types.

About the genetic marker, Dekkers *et al.* (2004) defined below three types.

1) Direct markers: loci that code for the functional mutation.
2) LD markers: loci that are in population-wide linkage disequilibrium with the functional mutation.
3) LE markers: loci that are in population- wide linkage equilibrium with the functional mutation in outbred populations.

Direct markers can be the most useful marker in the three, but it is difficult to identify, because its causality with quantitative traits is difficult to prove and, as a result, a number of available examples are still very limited. Whereas direct markers, LD markers have less degree, which can be useful markers because there are consistent association between genotype and phenotype. On the other hand, the LE markers can be readily detected on a genome-wide basis by using experimental crosses, and there are many examples of successful reports for detection of QTL regions. However, it needs to reanalyze whether these previously reported LE markers is available in other population before it is applied to actual selection. Although we detected correlation between gene mutation of *ADRB3*, *PIK3C3*, *VRTN* and pig production traits in this study, these associations have been assessed in a few breeds and strain. Therefore, this mutation should be considered as LE markers at present stage.

After that, it needs to decide when and how the maker data should

be incorporate into individual selection. Dekkers *et al.* (2010) proposed that four strategies at the selection stage.

1) Strategy 1(Marker-alone selection); Selection on marker data or marker-based BV alone.
2) Strategy 2 (Tandem selection); Selection of candidates on marker genotype or marker-based BV, followed by selection on phenotype-based BV.
3) Strategy 3 (Index selection); Simultaneous selection on a combination of marker data and phenotype-based BV.
4) Strategy 4 (Preselection); Preselection on maker data at a young age, followed by selection on phenotype-based BV

Most genetic improvement in pig is based on selection for economical traits that are quantitative traits in pure breed in Japan. These traits are dominated by numerous polygenes that have respective little effect. Although several QTLs that may have relatively large effect were reported, it is much limited the proportion of genetic variance accounted for by those QTLs. Therefore, Index selection (Strategy3) is expected to be the most advantageous for incorporating the marker information into genetic evaluation in practical breeding program to combine selection on the marker data with selection on the phenotype-based BV. It is because that can capture the all QTL and polygenic effect, which include those are not captured by the marker data (Dekkers *et al.*, 2010). Fernando and Grossman (1989) established the method that optimally combines marker and phenotypic information, which is called MABLUP (Marker assisted

BLUP) and that could predict BV by directly incorporating genetic markers in routine mixed-animal model BLUP.

In the approach of Fernando and Grossman (1989), LE maker data was treated as random effect for each QTL in order to incorporate into MABLUP model. On the other hand, Van Arendonk *et al* (1999) reported that LD or direct marker data could be incorporated in existing genetic evaluation procedures as fixed effects. However, the object of the molecular genetics for improvement of livestock is not to identify the responsible gene that can directly influence traits, but to select the animals that have superior genetic talent from own population. Therefore, it is sufficient to utilize the LE marker as the DNA marker, which segregates in specific population for increasing selection efficiency, particularly, in the inbreeding such as Japanese closed nucleus breeding systems that have no genetic introduction from other populations except for base population.

LE marker might not be completely consistent the traits at the early generation in closed nucleus population, because animals in base population are generally introduced from various strains and the genetic background of individuals might be much different. However, Uemoto *et al.* (2010) indicated that LE marker information might be available as selection criteria by simultaneously using with polygenic breeding values in the limited number of animals at early generation in the closed nucleus breeding population, if the marker effect is sufficiently high.

Furthermore, the study in the chapter 5 suggested that simultaneous addition of multiple appropriate markers might be useful to predict individual genetic traits more accurately. Thus, our study suggests that

the two step strategy lead the efficient implicating the strain development with using genetic marker, which is consisted by step 1; choosing the appropriate markers at early generation, and step 2; predicting individual genetic talent by phenotype based BLUP which include some marker effects as fixed effect.

Circumstances surrounding the pork industry in Japan are extremely severed, which is under the increase in production costs due to higher feed, declining market price in increasing cheap imports pork. In order to overcome this situation, there is a strong demand for the development of technology that can improve efficiently pig strain that can be produced safe delicious pork consumers expect at low cost.

In the breeding of pigs, the genetic evaluation under the mathematical procedure after collecting traits data for several generations has been conducted as the main method, regardless of large or small population. However, recent techniques that can incorporate the genetic information into practical breeding program and perform individual genetic evaluation correctly have been developed. This study presented important implications for efficiency improvement by breeding methods using marker effects, even with not identifying the responsible gene. Therefore, it is important that future improvements carried out breeding using the information these markers.

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Summary

General introduction

In recent years, the business performance in Japanese pig industry have deteriorated by increasing production costs due to higher feed prices and imports of low price pork from abroad, and pig herd size in Japan which recorded 12 million heads in 1989 is a steadily decreasing every year. It is required the efficiency of pig productivity by improving of feeding and management technology, and breeding pigs that have superior genetic talent in order to stabilize the domestic pig production management.

For conducting improvements of breeding pigs, pure breed such as Landrace, Large White, or Duroc have been mainly used in Japan. The methods in breeding scheme are distinguished between open nucleus breeding system and closed nucleus breeding system. Open nucleus breeding system is the method conducted by the improvement of desired traits of the population with introducing genetic resources continuously from other populations, which is mainly performed in private breeders in Japan. On the other hands, closed nucleus breeding systems is uniquely developed in Japan, which is mainly performed in public breeders such as local governmental institute or National Agricultural Co-operative (ZEN-NOH). The closed nucleus breeding scheme is performed by repeating the selection superior pigs through about five generations in the isolated population, in which new genetic resources are not introduced from other populations.

In any case, the traits for improvement are mainly quantitative traits such as production traits, fertility traits, or meat quality, which are

dominated by numerous polygenes that have complicated relationship. In modern breeding works, most genetic progress for quantitative traits in livestock has been performed by predicting the genetic value which is called an estimated breeding value that are calculated by means of statistical model such as BLUP (Best linear unbiased prediction) method.

Recently, many approach that implicate genetic evaluation of individuals by not only phenotype-based method, but by gaining insight into the "black box" of quantitative traits with using molecular genetic information have been investigated in order to increase selection accuracy and decreasing generation intervals. Although many candidate genetic markers have been detected, the genetic makers that are available in the industry were very limited. The aims of this study were to evaluate genetic effect of genes that might affect productive traits in the Duroc population, and to establish the breeding scheme by adding genotype information. Duroc pigs used entirely in this study were from a line selected through five generations at Central Research Institute for Feed and Livestock ZEN-NOH (Hokkaido, Japan) from 2004 to 2010.

CHAPTER 1. Breeding experiments on strain development in Duroc pigs

The Strain development with Duroc was performed through five generations to develop a line which has excellent production trait and meat quality to be used as terminal sire. Average daily weight gain (ADG), backfat thickness (BFT), and intramuscular fat content in the loin (IMF) were configured as improvement traits in this population. The pigs were selected mainly based on animal model BLUP of breeding value of each traits. Average phenotypic values of the ADG and BFT of boars at the fifth

generation significant increased by 44g/day, 0.21cm compared with first generation, respectively. But phenotypic value of IMF decreased 0.31%. The each breeding value of ADG, BFT, and IMF at the fifth generation significantly increased by 82 g/day, 0.32cm, and 0.83% compared with those of the first generation, respectively. An average inbreeding coefficient and average relationship coefficient of selected pigs at fifth generation were 3.02%, 10.09%, respectively. And all selected pig at fifth generation had relationship each other.

CHAPTER 2. Association of porcine beta 3-adrenergic receptor gene (*ADRB3*) gene with production traits in Duroc pigs

An insertion/deletion variant of a thymine base (T5 and T6) in exon 2 of porcine beta 3-adrenergic receptor (*ADRB3*) gene has been described. In the current study, we made an association study between the *ADRB3* polymorphisms and production traits in 735 Duroc pigs. The allele frequencies for the T5 and T6 alleles in our study population were 0.567 and 0.433, respectively. Any associations were not detected between *ADRB3* genotype and average daily weight gain during test period, or backfat thickness and intramuscular fat content in either sex. However the size of the loin eye muscle area (EMA) was significantly associated with *ADRB3* genotypes in gilts. T6-homozygous gilts had a 2.5cm^2 higher mean of EMA than T5-homozygous gilts. This association was not detected in males. In addition, a multiple traits animal model best linear unbiased predictor (BLUP) analysis revealed that the T6-homozygous genotype had positive effects on breeding value of EMA. Accordingly, we suggest that *ADRB3* polymorphism has the potential to be an important genetic

marker for prediction of EMA in Duroc pigs.

CHAPTER 3. Association of porcine class 3 phosphoinositide-3-kinase (*PIK3C3*) gene with production traits in Duroc pigs

A C↔T SNP on exon 24 of the porcine class 3 phosphoinositide - 3 - kinase (*PIK3C3*) gene is considered a possible genetic marker for selecting backfat (BF) thickness and carcass fat, although only one study has published results on its effects by performing experiments on a single resource family. In this chapter, we analyzed the association of this *PIK3C3* polymorphism with production traits in our Duroc line in order to reveal the utility of this gene as a genetic marker. The C allele frequency at fourth generation was 75.2%, and significantly increased from second generation (63.5%). *PIK3C3* polymorphism showed significant effects on ADG, BFT, IMF, and EMA, and the C alleles have increase effect on ADG, BFT, and IMF, and decrease effect on EMA. The predicted differences in traits between the homozygous pigs of the C and T alleles were 40 g/day for DG, 1.2 mm for BF, 0.44% for IMF, and 1.6 cm^2 for EMA. Furthermore, the statistical models for estimating the breeding values of each trait had lower Akaike's information criterion (AIC) values when adding *PIK3C3* genotype information. We therefore confirmed that the c.2604C<T polymorphism in *PIK3C3* has the potential to be a genetic marker for production traits in our Duroc line.

CHAPTER 4. Association of porcine Vertnin(*VRTN*) gene with production traits in Duroc pigs

Vertebral number is related to body size in pigs, and many reports

have suggested presence of an association between body length and meat production traits. Previous study revealed that variation in the vertebral number of Western breed (Duroc, Landrace, and Large white) is strongly associated with haplotype of Vertnin (*VRTN*) gene that is located on *Sus scrofa* chromosome 7. However, the relationship between Q and Wt haplotypes of *VRTN* gene and the production traits such as growth rate, fat deposition, or meat quality have not been investigated. In this chapter, we analyzed the association between the *VRTN* genotype and the production and body composition traits in Duroc pigs. The *VRTN* genotype was closely related to body length in a similar to previous studies, and 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). 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, the Wt allele had a positive effect on the IMF breeding value. No associations were observed between the *VRTN* genotype and other production traits. These results suggest *VRTN* has the potential to act as a genetic marker of IMF in Duroc population.

CHAPTER 5. Evaluation of effects of multiple candidate genes (*LEP*, *LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) on production traits in Duroc pigs

Numerous studies have been detected the genetic marker for the improvement of traits. Although several dozen of candidate genes were detected as genetic marker might be related with backfat thickness, most

study reported single marker effect only. Production traits such as average daily weight gain (ADG) or backfat thickness (BFT) are typical quantitative traits under the control of multiple genes. In this chapter, we evaluated single and combination effects of genetic variations of five candidate loci (*LEP*, *LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) on four production traits (ADG, BFT, EMA, IMF) in 1414 Duroc pigs. Polymorphisms in *LEPR*, *MC4R*, and *PIK3C3* had significant single gene effects on ADG and BFT. The additive genetic variance in ADG and BFT (16.99% and 22.51%, respectively) was explained by genetic effects of these three loci. No correlations were observed between the *LEP* genotype and production traits in this study. There were no epistatic effects between all selected combinations of loci pairs and analyzed traits, except for a pair of *LEPR* and *PIK3C3.* A marginally epistatic effect on EMA was detected between this loci pair, however, this effect did not reach statistical significance after FDR correction. These results suggested that *LEPR*, *MC4R*, *PIK3C3*, and *VRTN* may influence ADG and fat deposition independently of each other loci. Furthermore, we compared the fitness of the statistical models for predicting the breeding values that included multiple gene effects by Akaike's information criterion (AIC) values. The models which included three genes effects (*LEPR, MC4R*, and *PIK3C3*) for ADG, four genes effects (*LEPR*, *MC4R*, *PIK3C3*, and *VRTN*) for BFT, and two genes effects (*LEPR* and *VRTN*) for IMF as fixed effects showed the most favorable in this study, respectively. This study suggested that addition of multiple appropriate markers into mathematical model as fixed effect might be useful to predict individual genetic traits more accurately.

General discussion

Most genetic improvements in Japanese pig industry are based on selection for economical traits in pure breed population such as Landrace, Large White, or Duroc. These traits are quantitative traits dominated by numerous polygenes that have respective little effect. Although several QTLs that may have relatively large effect were reported, it is much limited the proportion of genetic variance accounted for by those QTLs. Therefore, index selection method which is called MABLUP (Marker-Assisted BLUP) is expected to be the most advantageous for incorporating the marker information into genetic evaluation in practical breeding program to combine selection on the marker data with selection on the phenotype-based BV. It is because that can capture the all QTL and polygenic effect, which include those are not captured by the marker data. Furthermore, it is sufficient to utilize the LE marker (loci shows linkage equilibrium with the functional mutation in particular population) as the DNA marker, which segregates in specific population for increasing selection efficiency in the inbreeding such as Japanese closed nucleus breeding systems that have no genetic introduction from other populations except for base population. Present study in the Chapter 5 suggested that simultaneous addition of multiple appropriate markers might be useful to predict individual genetic traits more accurately. Thus, the present study suggested that the two step strategy lead the efficient implicating the strain development with using genetic marker, which is consisted by step 1; choosing the appropriate markers at early generation, and step 2; predicting individual genetic talent by phenotype based BLUP which include some marker effects as fixed effect. This study presented useful

genetic maker information and the statistical methods for incorporating genetic marker effects into improvement pigs by closed nucleus breeding scheme.