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Copy number variation of amylase alpha 2B gene is associated with feed efficiency traits in Large White pigs

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Abstract: Copy number variation (CNV) of the *AMY* gene in humans has been enthusiastically studied for its association with starch digestibility and obesity. The alpha-amylase (AMY) is a major starch digestive enzyme in mammals. This study aimed to determine the association between CNV of the porcine pancreatic amylase (*AMY2B*) gene and feed efficiency. Improvement of feed efficiency in growing pigs is of great economic interest. We assayed the copy number of *AMY2B* by using real-time quantitative PCR (qPCR) in a Large White pig population. We identified three genotypes for *AMY2B* CNVs, namely *I*/*I* (homozygotes of haplotype *I*; a chromosome with one copy of *AMY2B*), *I*/*II* (heterozygotes of haplotype *I* and *II*; a chromosome with two copies) and *II*/*II* (homozygotes of haplotype *II*). We tested the genotypes of the parental generation consisting of six males, 21 females and 265 offspring piglets to validate the *AMY2B* CNV genotyping. With very few mistyping exceptions, copy numbers of *AMY2B* were transmitted to piglets in segregation ratios following Mendelian inheritance. Finally, we performed an association analysis between the CNV of the *AMY2B* gene and feed efficiency traits in 207 uncastrated male pigs. The generalised linear model analysis showed the significant effects of *AMY2B* CNV genotype on average daily feed intake, total feed intake and feed conversion ratio during growth from 30 kg to 100 kg body weight. However, it was not associated with average daily gain, backfat thickness and loin eye muscle area. Individuals with the genotype *I*/*I* had about 76.6 ± 27.1 g lower average daily feed intake, 5.35 ± 1.90 kg lower total feed intake and 0.089 8 ± 0.026 5 lower feed conversion ratio than individuals with *I*/*II* and *II*/*II* genotypes. Thus, *AMY2B* CNV has the potential to be an effective genetic marker that could reduce feed costs for pig farming.

Keywords: feed intake; gene duplication; genetic analysis, quantitative trait locus; pig breeding

Feed efficiency is an important economic trait in the pig industry. Feed costs account for 60–70% of the total cost in modern intensive pork production (Patience et al. 2015). Pigs are fed a carbohydrate-rich diet such as maize and soybean meal, a major energy source in pigs, accounting for 60–70% of total feed energy (Bach-Knudsen et al. 2013). Therefore, improving the carbohydrate efficiency (digestion and absorption) will reduce feed costs.

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The alpha-amylase (AMY) is a major starch digestive enzyme in mammals. In humans, there are two paralogous genes for AMY, salivary gland-specific amylase (*AMY1*) and pancreatic amylase (*AMY2*) (Meisler and Ting 1993). Molecular evolutionary studies have shown that the human *AMY1* gene derives from a recent duplication of the ancestral *AMY2* gene in a great ape ancestor (Samuelson et al. 1990; Pajic et al. 2019). Other than primates, mice also have a salivary gland-specific amylase gene (*Amy1*), but many other mammals do not have the *AMY1* gene (Mocharla et al. 1990; Meisler and Ting 1993; Pajic et al. 2019).

Copy number variations (CNVs) of the *AMY* gene family were found within and among many mammalian species (Inchley et al. 2016; Reiter et al. 2016; Pajic et al. 2019). The association between *AMY* gene CNV and starch digestive capability has been studied in humans and dogs. In either species, a high copy number of the *AMY* gene causes increased expression of amylase in the saliva, which is likely to enable more efficient digestion of starch-rich foods (Perry et al. 2007; Axelsson et al. 2013; Arendt et al. 2014; Falchi et al. 2014; Reiter et al. 2016). From these studies, it is considered that the *AMY* gene copy number increased as an adaptation to the gain in starch intake due to agricultural civilisation and domestication (Luca et al. 2010; Axelsson et al. 2013; Inchley et al. 2016).

The *AMY2* and *AMY*-like loci are co-localised on pig chromosome 4 in the pig genome assembly (Sscrofa11.1). In Sscrofa11.1, there are seven loci, *AMY2B*, LOC100521789 (*AMY2A*), LOC110258046, LOC100153854, LOC110260364, LOC100522672 and LOC100153446, including those present in unplaced genomic scaffold contigs. It has been reported that CNVs of the *AMY* gene are present in pigs and wild boars (Paudel et al. 2013; Pajic et al. 2019; Yoshidomi et al. 2021). However, unlike domesticated dogs, no clear difference was detected in the mean copy number of the *AMY* gene between pigs and wild boars (Paudel et al. 2013; Pajic et al. 2019; Yoshidomi et al. 2021). Based on these results, Pajic et al. (2019) pointed out that pig domestication did not require any increased *AMY* gene copies for feed adaptation due to the omnivorous nature of wild boars. However, major issues remain about CNVs in the porcine *AMY* gene. At least three of the seven loci in the Sscrofa11.1 are probably pseudogenes because some of the exons are missing, and the predicted coding sequence cannot be translated as an amylase protein. In previous studies, the copy number of pig *AMY* genes was established for functional and pseudogenised loci (Paudel et al. 2013; Pajic et al. 2019; Yoshidomi et al. 2021). Therefore, no association analysis between the CNVs of the *AMY* gene and the trait of pigs has been reported. This study investigated the CNVs of the *AMY2B* (amylase alpha 2B) gene by using real-time quantitative PCR (qPCR) with locus-specific primers in a Large White pig population. Then, we describe that the detected CNVs of *AMY2B* were transmitted to progenies according to Mendelian inheritance. Finally, we performed an association analysis between the CNVs of the *AMY2B* gene and feed efficiency traits in Large White pigs. Here we report that the *AMY2B* copy number was associated with average daily feed intake (ADFI), total feed intake (TFI) and feed conversion ratio (FCR) in pigs.

MATERIAL AND METHODS

Animals

Purebred Large White pigs raised on the East Japan Great Grandparent Farm of ZEN-NOH Livestock Co., Ltd. (GGP Farm; Iwate, Japan) and the Central Research Institute for Feed and Livestock ZEN-NOH (Research Farm; Hokkaido, Japan) were used in this study. Individuals on the GGP Farm were used for trait correlation analysis, and individuals on the Research Farm were used for the other experiments. All procedures for collecting porcine tissue samples were approved by the institutional animal care and use committee of ZEN-NOH (Research Code Number: 1501-766) and by the animal research committee of Azabu University (Permission Number: 201124-4).

Copy number measurement of *AMY2B* gene

Genomic DNA was extracted from tail tissue clippings or whole blood samples of each pig. QuickGene DNA Tissue Kit S (Fujifilm Corporation, Tokyo, Japan) was used for the tail tissue and QuickGene DNA Whole Blood Kit S (Fujifilm Corporation, Tokyo, Japan) was used for whole blood. The PCR primers were designed

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on the locus-specific regions of *AMY2B* (forward: 5'-TAGAGTGTTGCTAACAAGCA-3', reverse: 5'-CCACATATATACGCACCTGAAAG-3'; positions from 115178754 to 115178735 and from 115178645 to 115178667 on GenBank NC_010446.5: Sscrofa11.1, respectively), avoiding sequence overlap with *AMY*-like and *AMY2A* loci. The PCR primer pair for the leptin (*LEP*) gene (forward: 5'-AACAGAGGGTCACCGGTTTG-3' and reverse: 5'-GGTTCTCCAGGTCATTCGATATT-3'; positions from 20109753 to 20109734 and from 20109604 to 20109626 on NC_010460.4: Sscrofa11.1, respectively) was used as an internal standard for a single-copy gene (Yoshitomi et al. 2021). The quantitative real-time PCRs (qPCRs) were performed in a Thermal Cycler Dice® Real-Time System II (Takara Bio, Inc., Shiga, Japan) using THUNDERBIRD qPCR Mix (Toyobo Co., Ltd., Osaka, Japan), following the manufacturer's instructions. Thermal cycling conditions consisted of initial denaturation at 95 °C for 60 s, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s (data collection). The delta cycle threshold (delta CT) value was calculated by subtracting the CT value of *LEP* from that of *AMY2B*. The copy numbers of the *AMY2B* gene per haploid genome were measured as a relative ratio of amplification of the *AMY2B* to the *LEP* gene ($2^{-\text{delta CT}}$) within each sample. All samples were run in duplicate, and average values were used to calculate the *AMY2B* gene copy numbers.

Exploring the inheritance pattern of *AMY2B* copy number

We analysed the copy number of *AMY2B* in a sample set of parental generations consisting of six males and 21 females and 265 piglet generations bred on the Research Farm to confirm that the CNVs of *AMY2B* were transmitted to offspring and determine the mode of inheritance. The observed segregation ratio for *AMY2B* genotypes was compared with the expected Mendelian ratios using the chi-square test.

Measurement of pig traits

The growth and feed intake of 207 uncastrated male pigs were measured on the GGP Farm from 2016 to 2018. The pigs increased in weight from approximately 30 kg to 100 kg body weight (BW) during the test period. Pigs were housed in groups of 4–8 in pens with feed and water provided *ad libitum*. Feed Intake Recording Equipment (FIRE; Osborne Industries Inc., Osborne, KS, USA) electronic feeders were used to measure the daily individual feed intake and weight gain. ADFI and TFI were obtained from records of all sampled individuals collected by the FIRE system. However, the electronic feeder system occasionally malfunctioned and could not record the amount of feed intake on those days. Missing data on such daily feed intake were estimated using locally weighted regression equation (LOESS) on all records available for an animal, as described by Ito et al. (2018). The TFI value of each individual was calculated by supplementing the daily food intake records with estimates of the day when the measurement was missing. Average daily gain (ADG) was calculated based on the gained weight from 30 kg to 100 kg BW. FCR was calculated as ADFI/ADG. When the pigs reached approximately 100 kg BW, backfat thickness (BFT) and loin eye muscle area (EMA) were measured at a half-body length position using a real-time ultrasound machine (Exago; Echo Control Medical, Angouleme, France) and the BioSoft Toolbox II for Swine (Biotronics, Inc., Ames, IA, USA).

Association analysis

On the GGP Farm, 207 uncastrated male pigs were studied for associations between the *AMY2B* copy number and production characteristics. Statistical analyses were performed using the generalised linear model (GLM) procedure of the R software v3.6.1 (https://www.R-project.org/). The linear models for ADG, TFI, ADFI and FCR were as follows:

$$Y_{ijkl} = \mu + G_i + \beta D_j + S_k + e_{ijkl} \quad (1)$$

Conversely, the linear models for BFT and EMA were as follows:

$$Y_{imn} = \mu + G_i + \beta W_m + e_{imn} \quad (2)$$

where:

μ – the overall mean for each trait;

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Y_{ijkl} and Y_{imn} – the phenotypic values of each trait;
G_i – the effect of *AMY2B* genotype;
βD_j – the effect of the number of days of the test period [β is the coefficient of the days (D_j) for each trait];
S_k – the seasonal effect (month of initiating the testing of each individual);
βW_m – the effect of BW at the time of trait measurement [β is the coefficient of the BW (W_m) for each trait];
e_{ijkl} and e_{imn} – random error terms.

For the probability distribution in GLM for each trait, those with the smallest Akaike information criterion values were selected. The calculation of the least-squares means (LMS) of the trait values for each genotype and its pairwise comparison were performed using the emmeans R package v1.5.5-1 (https://github.com/rvlenth/emmeans). A *P*-value lower than 0.05 was considered to be statistically significant.

RESULTS

Copy number measurement of *AMY2B* gene

The histogram of the value of the $2^{-\text{delta CT}}$ as an *AMY2B* gene CNV genotype in 265 Large White pigs is shown in Figure 1. The *AMY2B* copy number had two distinct peaks. A small number of individuals showed even larger values than the second peak. Due to the inconsistent PCR efficiencies of the target *AMY2B* and the internal standard gene, the $2^{-\text{delta CT}}$ value cannot be directly treated as the copy number of *AMY2B* per haploid genome. However, three polymorphisms in the CNV of *AMY2B* were observed. Consequently, we hypothetically classified the genotypes as *I*/*I* (homozygotes of haplotype *I*, a chromosome with one copy of *AMY2B*) for less than 1.8, *I*/*II* (heterozygotes of haplotype *I* and *II*, a chromosome with two copies) for the range of 1.8 or more and less than 2.9 and *II*/*II* (homozygotes of haplotype *II*) for 2.9 or more (Figure 1). Table 1 shows the seg-

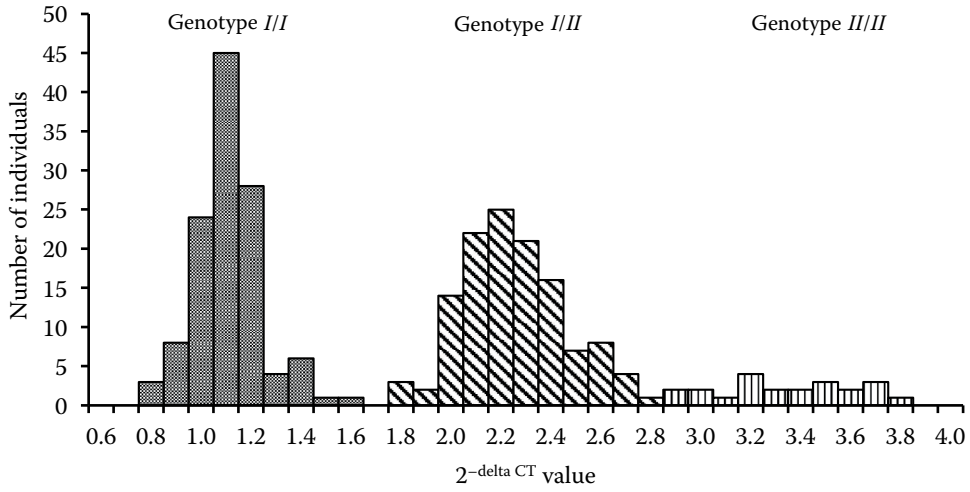

Figure 1. A histogram of the relative ratio of amplification of the *AMY2B* to the *LEP* gene ($2^{-\text{delta CT}}$) in 265 Large White pigs

The value smaller than 1.8 was classified as genotype *I*/*I* (two *AMY2B* copies per diploid), values higher than 1.8 but smaller than 2.9 were classified as genotype *I*/*II* (three copies per diploid) and values higher than 2.9 were classified as genotype *II*/*II* (four copies per diploid)

Table 1. Transmission and segregation ratio of *AMY2B* copy number variations from parents to offspring

Genotype of parents		Number of mating pairs	Number of offspring	Observed genotype of offspring			Expected segregation ratio
Sire	dam			*I*/*I*	*I*/*II*	*II*/*II*	*I*/*I*:*I*/*II*:*II*/*II*
I/*I*	*I*/*I*	2	23	23	0	0	1:0:0
I/*I*	*I*/*II*	4	49	22	26	1*	1:1:0
I/*II*	*I*/*I*	8	104	58	46	0	1:1:0
I/*II*	*I*/*II*	7	89	20	50	19	1:2:1

*One individual determined to have a genotype inconsistent with the hypothesized mode of inheritance

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regation analysis of the *AMY2B* CNVs among offspring from the genotyped parents. All offspring born to parents with *I*/*I* homozygotes occurred in the *I*/*I* genotype. From the parents of the *I*/*II* heterozygotes, three genotypes, *I*/*I*, *I*/*II* and *II*/*II*, were born according to Mendelian expected segregation ratio (1 : 2 : 1). Mating between genotypes *I*/*I* and *I*/*II* also followed Mendelian expected segregation ratios, but one animal contradicted the hypothesis and had the genotype *II*/*II* (Table 1). The $2^{-\text{delta CT}}$ value for this animal was slightly above the genotype *II*/*II* threshold. Although less frequently, genotype *I*/*II* may be misidentified as *II*/*II* in our measurement method.

Association analysis between pig traits and *AMY2B* CNVs

The trait values and genotypic frequencies of *AMY2B* CNVs in 207 uncastrated male pigs are presented in Table 2. The frequencies of *AMY2B* genotypes were 61.8% for *I*/*I*, 32.4% for *I*/*II* and 5.8% for *II*/*II* in this population. The GLM analysis showed the significant effects of *AMY2B* CNV genotype on TFI, ADFI and FCR (Table 2). Genotype *I*/*II* consumed about 5.56 ± 2.01 kg more feed during the period BW was 30 kg to when it was 100 kg compared to genotype *I*/*I* ($P < 0.05$). Although not statistically significant, genotype *II*/*II* also consumed about 4.24 ± 3.99 kg more feed than genotype *I*/*I*. For FCR, genotype *I*/*II* ingested about 0.095 kg more feed than genotype *I*/*I* for an increase in BW of 1.0 kg during the test period. Furthermore, when genotypes *I*/*II* and *II*/*II* were pooled, they ate 76.6 ± 27.1 g more feed per day than genotype *I*/*I*. However, the *AMY2B* CNV genotype did not affect ADG. Additionally, there was no significant difference in the number of days required to reach 100 kg of BW between the genotypes. Neither was there any significant effect on BFT and EMA. These results suggested that an increase in the copy number of the *AMY2B* gene worsened feed efficiency traits by increasing feed intake but did not affect weight gain or fat mass.

DISCUSSION

Copy number measurement of *AMY2B* gene

Insufficient accuracy of the qPCR has been pointed out in previous studies on the association analysis of human *AMY1* copy numbers (Carpenter et al. 2015; Usher et al. 2015). In particular, it has been reported that the precision of qPCR is not sufficient to accurately measure the high gene copy number (Hindson et al. 2011). We focused on the most reliable *AMY2B* locus rather than on the total *AMY* genes to minimise this problem. In this study, we measured the copy numbers of *AMY2B* in pigs and identified three polymorphisms. These polymorphisms could be explained by the presence of a chromosome with one copy of *AMY2B* (haplotype *I*) and a chromosome with two copies (haplotype *II*). We confirmed that the *AMY2B* CNV genotype was transmitted to progenies according to Mendelian inheritance. Thus, the *AMY2B* CNV genotype proposed in this study meets the conditions necessary for genetic markers that can be used for an association analysis. Of course, there are some limitations to this study. First, since the reference sequence of the pig *AMY* clustering region still contains ambiguous parts, it is unclear what structural changes have increased the *AMY2B* gene copy number. Also, it is not clear whether the additional copy of *AMY2B* has a functional structure. Thus, detailed whole-genome sequencing is required to understand structural changes concerning the pig *AMY* gene CNVs.

Association analysis between pig traits and *AMY2B* CNVs

This study identified that a high copy number of the *AMY2B* gene significantly worsened feed efficiency in pigs. Although the mechanism is not clear, the higher copy number of the *AMY2B* gene increased ADFI and TDF without affecting ADG. Individuals with the genotype *I*/*I* had about 5.35 ± 1.90 kg lower TFI than individuals with other genotypes. Thus, *AMY2B* CNVs identified in this study can potentially be an effective genetic marker to reduce feed costs. According to Pig QTLdb (Hu et al. 2019), a QTL for ADFI weight (QTL #22202) has been mapped on the SSC4 across the region spanning from 126.2 Mbp to 126.9 Mbp based on the previous assembly Sscrofa10.2 (Onteru et al. 2013). The *AMY2B* locus [positions from 126305825 to 126321854 in NC_010446.4 (GenBank) of the Sscrofa10.2] is exactly in the interval of this QTL. Thus, the CNV of *AMY2B* or *AMY* genes may be candidates for the previously reported QTL

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Table 2. Associations between the genotype of *AMY2B* copy number variations and feed efficiency, growth and fattening traits in male Large White pigs

Traits	Overall mean ± SD	Genotype *I/I*, *I/II*, *II/II*	*n*	Probabilistic distribution	LSM ± SE	Genotype effect ± SE	*P*-value	Genotype *I/I* vs *I/II* + *II/II*	Probabilistic distribution	LSM ± SE	Genotype effect ± SE	*P*-value
ADG (g/day)	975 ± 117.8	*I/I*	128	gaussian	977 ± 5.12	–	–	*I/I*	gaussian	977 ± 5.20	–	–
		I/II	67		972 ± 7.14	–5.371 ± 8.801	0.542 4	*I/II* + *II/II*		973 ± 6.58	–4.137 ± 8.341	0.620 5
		II/II	12		980 ± 16.73	2.741 ± 17.49	0.875 6	–		–	–	–
TFI (kg)	173.2 ± 21.5	*I/I*	128	gamma	171[a] ± 1.18	–	–	*I/I*	gamma	171[a] ± 1.17	–	–
		I/II	67		177[b] ± 1.65	5.556 ± 2.009	0.006 2	*I/II* + *II/II*		177[b] ± 1.52	5.351 ± 1.900	0.005 4
		II/II	12		175 ± 3.82	4.240 ± 3.986	0.288 7	–		–	–	–
ADFI (kg/day)	2.48 ± 0.237	*I/I*	128	gaussian	2.45[a] ± 0.017 0	–	–	*I/I*	gaussian	2.45[a] ± 0.016 9	–	–
		I/II	67		2.53[b] ± 0.023 2	0.079 2 ± 0.028 7	0.006 3	*I/II* + *II/II*		2.53[b] ± 0.021 4	0.076 6 ± 0.027 1	0.005 3
		II/II	12		2.51 ± 0.054 5	0.062 4 ± 0.056 9	0.274 3	–		–	–	–
FCR (kg/kg)	2.56 ± 0.262	*I/I*	128	gamma	2.53[a] ± 0.016 3	–	–	*I/I*	gamma	2.53[a] ± 0.016 3	–	–
		I/II	67		2.62[b] ± 0.023 1	0.094 8 ± 0.028 1	0.000 9	*I/II* + *II/II*		2.62[b] ± 0.021 2	0.089 8 ± 0.026 5	0.000 9
		II/II	12		2.59 ± 0.053 3	0.062 0 ± 0.055 6	0.266 1	–		–	–	–
BFT (cm)	1.58 ± 0.332	*I/I*	128	gamma	1.57 ± 0.027 5	–	–	*I/I*	gamma	1.57 ± 0.027 5	–	–
		I/II	67		1.61 ± 0.038 8	0.038 5 ± 0.047 4	0.418 0	*I/II* + *II/II*		1.59 ± 0.035 5	0.022 0 ± 0.044 8	0.623 0
		II/II	12		1.50 ±0.085 7	–0.072 3 ± 0.090 0	0.423 0	–		–	–	–
EMA (cm^2)	31.9 ± 2.89	*I/I*	128	gamma	31.7 ± 0.226	–	–	I/I	gamma	31.7 ± 0.227	–	–
		I/II	67		32.2 ± 0.317	0.462 4 ± 0.389 4	0.236 0	I/II + II/II		32.1 ± 0.291	0.347 0 ± 0.368 9	0.348 0
		II/II	12		31.4 ± 0.734	–0.300 9± 0.767 8	0.696 0	–		–	–	–

ADG = average daily gain; ADFI = average daily feed intake; BFT = backfat thickness; EMA = loin eye muscle area; FCR = feed conversion ratio; TFI = total feed intake

[a,b]The different letters indicate statistically significant difference within each comparison at $P < 0.05$ (Tukey-adjusted)

Differences in following traits between genotypes are shown as least squares means (LSM) ± standard errors (SE)

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for ADFI. Additionally, a QTL for residual energy intake has been reported around the *AMY2B* gene near the microsatellite *SW856* at 140.3 Mbp of SSC4 based on the Sscrofa10.2 (Shirali et al. 2013). In this study, we performed an association analysis in only one population of Large White pigs. Therefore, it is necessary to verify whether the results obtained in this study can be reproduced in other pig populations and breeds.

Evolutionary implications of the pig *AMY2B* gene CNV

Many studies indicated that the increased copy number of the *AMY1* gene in humans resulted from natural selection for starch-rich diets (Perry et al. 2007; Carpenter et al.2015; Inchley et al. 2016). Similar events have been reported for increased copy numbers of the *AMY2B* gene in dogs (Axelsson et al. 2013). However, no clear increase in the *AMY* gene has been detected in domesticated pigs (Paudel et al. 2013; Pajic et al. 2019; Yoshidomi et al. 2021). It is believed that this is because wild boars are omnivorous; therefore, they do not require an increase in the number of copies of the amylase gene to adapt to starch-rich feed (Pajic et al. 2019). From this study, it is suggested that individuals with high *AMY2B* copies have worse feed efficiency. Feed efficiency is one of the important economic traits in pig farming, and selection for this trait has been ongoing for many years. If increased copy numbers of the amylase gene worsen feed efficiency, haplotypes with high copy numbers of *AMY2B* may be excluded from the pig population through a negative selection for the unfavorable phenotype. A high copy number of the *AMY* gene has been shown to increase amylase secretion in humans and dogs (Arendt et al. 2014; Atkinson et al. 2018). However, in this study, we only analysed the correlation between the CNV genotype of *AMY2B* and traits for feed efficiency. In order to clarify the results of this study, we must first analyse the effect of the CNV genotype of porcine *AMY2B* on the gene expression and secretion of pancreatic amylase. In humans, the association between *AMY1* CNV and body mass index and metabolic health has been analysed (Falchi et al. 2014; Bonnefond et al. 2017; Atkinson et al. 2018; Elder et al. 2018). Human *AMY1* CNV affects the intestinal flora (Poole et al. 2019). However, the phenotypic effects of amylase CNV have not yet been fully elucidated. To date, there is no study regarding the association of human *AMY2B* CNV with the daily dietary intake of humans. In this study, pigs with high *AMY2B* gene copy numbers were shown to have an increased amount of feed intake (that is, the amount of feed energy ingested), but it did not affect the weight gain. This is the first report showing the relationship between the CNV of the amylase gene and food intake in mammals. Many research institutes on pig farming conduct studies on feed digestion and energy metabolism under controlled environmental factors (Noblet and van Milgen 2004; Patience et al. 2015). If such digestion and metabolism tests can be combined with *AMY2B* genotype analysis, the mechanism of changes in feed intake could be scientifically elucidated. Studying the mechanism by which the CNV of the *AMY2B* alters feed intake during pig growth will lead to the elucidation of the functional role of the CNV of amylase genes not only in pigs but also in other mammals including humans.

CONCLUSION

CNVs of the *AMY2B* gene were found in the Large White pig population. The three genotypes of *AMY2B* CNV could be explained by hypothesising the existence of two haplotypes, type *I* with one copy per chromosome and type *II* with two copies. The copy number of *AMY2B* was transmitted to offspring according to the segregation ratio expected from Mendelian inheritance. Significant effects of the *AMY2B* CNV were detected on ADFI, TFI and FCR during growth from 30 kg to 100 kg BW. However, *AMY2B* CNV was not associated with ADG, backfat thickness and loin EMA. Individuals with the genotype *I/I* (homozygotes of haplotype *I*) had about 76.6 ± 27.1 g lower ADFI, 5.351 ± 1.900 kg lower TFI and 0.089 8 ± 0.026 5 lower FCR than individuals with other genotypes. Thus, *AMY2B* CNV could potentially be an effective genetic marker to reduce feed costs in pig farming.

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Conflict of interest

The authors declare no conflict of interest.

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