

DEVELOPMENT AND UTILIZATION OF GENOMIC INFORMATION FOR EFFECTIVE PIG PRODUCTION – RECENT ACHIEVEMENTS AND FUTURE PROSPECTS

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ABSTRACT

In Japan, pork is highly demanded meat by the consumers relative to beef and chicken, while the degree of self-sufficiency is just above 50%. Additionally, the feed for pig production is greatly depended on imported crops from overseas countries. Therefore, it has been an important issue to improve productivity of pigs with emphasis on efficiency of reproduction and feeding cost in the industry. From that viewpoint, we sought to exploit genetic capacity of reproduction of dams and feed efficiency of pork producing pigs. Recently, completion of porcine whole genome sequence provided a broad picture of genetic information of the species and was followed by development of state-of-the-art technology to investigate genetic aspect of pigs with fully using the genomic information. Here we presented our recent studies for development of DNA markers which are associated with economically important physiological characteristics such as number of piglets born alive, feed intake, daily gain, and growth rate of piglets to improve overall productivity of pigs in Japan. For the implementation of the investigations, we used phenotypic data obtained from the pig population of commercial herds and also administered by local public authorities. In addition, we used genomic DNA from the populations and applied for recently available genotyping method namely SNP-array: more than 60,000 of single nucleotide polymorphisms (SNP) in all over the porcine genome can be simultaneously analyzed. By using the data of phenotype and genotype, we aimed to detect meaningful genetic polymorphisms through an estimation method called genome-wide association study (GWAS), which allowed us to predict the genomic region to be closely associated with the variance of economically important traits. Consequently, we identified genetic polymorphisms which were closely related to each production trait targeted in our studies. Additionally, using the polymorphisms, we developed DNA markers which are validated in the other pig populations to confirm the effect of the DNA markers. In the reproductive trait, we identified candidate gene in the genomic region on chromosome 7 (SSC7). The candidate gene is considered to be associated with placental development, which was validated with animal experiment using mouse model. Additionally, with regard to feed efficiency of pork producing pigs, we detected genomic region on chromosome 6 (SSC6) in association with average daily feed intake and average daily gain. Furthermore, we identified three chromosomal regions related to growth rate of pre-weaning piglets. Taken together, we successfully accomplished to develop DNA markers which are expected to be available for facilitating effective pork production in Japan, although validation of the effect of those DNA markers in every kind of pig population is the challenge in near future.

Keywords: feed efficiency, genome-wide association study, pig, reproduction

INTRODUCTION

Pork is acknowledged as the most popular meat in Japan. However, the degree of self-sufficiency of pork meat is just above 50%. Additionally, the feed for pig production is greatly depended on imported crops from overseas countries: the feeding cost is attributed to economic conditions of the countries producing the crops. Hence, circumstances of livestock production in Japan has involved as such a harsh situation which is consisted of in part unpredictable factors. Due to the aforementioned difficulties, it is basically important while hard to raise the levels of feeding efficiency and administrative skills at farms. To address this issue, we aimed to improve overall productivity of pigs through investigation of genetic factors which account for variance of production traits. Therefore, genetic improvement of breeding stock - grand-grand-parents (GGP) and grand-parents (GP) - is supposed to have greater spreading effect for general pig farmers than the other efforts.

With respect to the unique food culture in Japan, people prefers to have properly marbled pork meat and to cook thin-sliced and seasoned pork meat at home. Therefore, previous genetics studies focused on meat quality related traits (Nii et al., 2005; Taniguchi et al., 2010). At that time, the genetic studies were carried out by linkage analysis using relatively low-density DNA markers. That used to be an effective method to detect genomic region(s) which are related to targeted traits, in case the heritability of the targeted trait is high enough and major gene(s) are expected to associate with the variance of the trait. On the other hand, reproduction related traits are expected to be low heritability (Lamberson, 1990). Additionally, feed efficiency related traits are difficult to collect phenotypic data due to requiring for specific kind of equipment with a number of manpower: it costs a lot.

Once the whole pig genome sequence became available (Groenen et al., 2012), recently available method – genome-wide association study (GWAS) – is expected to be capable of detection of genomic region responsible for such traits with the low heritability (Tribout et al., 2008; Bergfelder-Drüing et al., 2015). Once the DNA marker(s) closely associated with those traits were identified, it would be possible to estimate the genetic ability of the dam or sire without measurement of phenotypic data. Recently we established research consortium comprised with a University, GGP producers and local public authorities producing pigs from regional to nationwide pig farmers in Japan to perform genetics studies for improvement of overall pig production level through research activities targeted on 1) reproductive traits, 2) feed efficiency and 3) growth rate of pre-weaning piglets.

In addition to our recent approaches for genetic improvement of pig productivity, we would like to propose near future prospect of recently advocated concept namely “genomic selection” which also utilizes SNP array data to evaluate genetic capacity of livestock with high accuracy.

DEVELOPMENT OF DNA MARKERS TO FACILITATE EFFECTIVE PIG PRODUCTION

Reproductive traits

It is primarily required to increase the number of piglets born alive and shorten the litter birth interval (number of delivery/year) at most. Pig productivity is simply attributed to the abilities of those traits. However, recent reproductive records of pigs in Japan are not as good as the indices from the other countries where have performed boar selection for many years using large scale population genetics approach (Table 1). In other words, the proper breeding scheme steadily has certain effect to improve reproductive traits even if the heritability is low.

Index	JPN	USA	CAN	DEN
No. of Delivery/year (A)	2.2	2.37	2.32	2.27
No. of piglet weaned (B)	20.2	24.94	23.59	29.62
No. of piglets weaned/litter (B/A)	9.2	10.5	10.2	13.0

Table 1 Comparison of reproductive performance between countries

JPN: Japan Pork Producers Association (2009)

Others: BPEX "2012 Pig Cost of Production in Selected Countries"

We, therefore, sought to detect genomic region(s) which are responsible for total number of piglets and number of piglets born alive of Large White breed of pigs from commercial herds through the GWAS approach (Figure 1). The result indicated that genetic polymorphisms related to the total number of piglets were identified on chromosomes 7 (SSC7) and 15 (SSC15). In addition to those polymorphisms, chromosome 14 (SSC14) also contained polymorphisms related to the trait so that we then investigated the effect of genotypes of each genomic region to the traits (Table 2). We defined the genetic polymorphisms as haplotypes from 1 - 4 on SSC7 and 15, and 4 and the others on SSC14. Our results indicated that haplotype 4 had an additive effect to increase the total number of piglets in each chromosome, while haplotype 1 was no positive effect on the trait. We then investigated the effect of combination of haplotype 4 on all three chromosomes that indicated 1.847 additive effect of total number of piglets on the basis of estimated breeding value – an index of genetic capacity of breeding stock. Furthermore, we identified a candidate gene around DNA marker position on SSC7 which is considered to be associated with placental development. We carried out a gene expression analysis of the candidate gene using mice model. The result suggested that reduction of the candidate gene supports implantation by blocking the inhibitory effect from T-cells. Consequently, the DNA markers are available for selection of favorable sires and/or elimination of unfavorable sires to increase the total number of piglets.

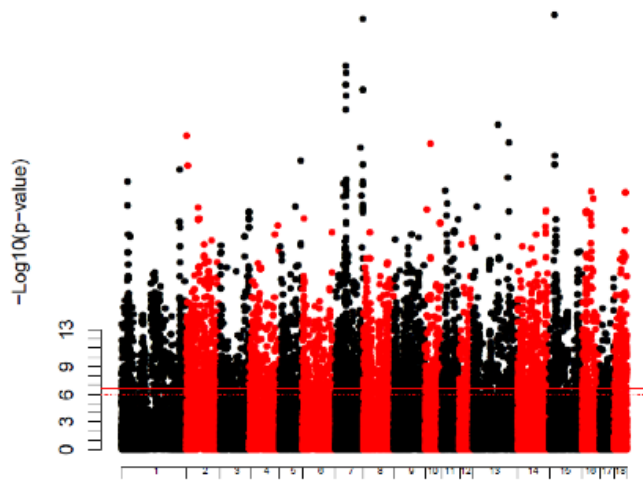


Figure 1 Genome-wide association study for total number of piglets of Large White pigs
Horizontal axis and vertical axis indicate chromosomes and probability of SNP to the targeted trait, respectively.

Chr.	Haplotype	EBV	N
SSC7	44	1.448	32
	34	1.724	15
	24	1.138	51
	13	0.656	33
	12	0.262	43
SSC15	11	0.124	72
	44	1.528	96
	24	1.045	19
	12	0.398	26
SSC14	11	0.035	84
	4*	1.448	79
	**	0.642	283

Table 2 Effect of SNP genotypes at each chromosome on total number of piglets

Multiple genotypes on each chromosome formed haplotypes 1-4.

*: Haplotypes on SSC14 were 4 and other than 4.

EBV: Estimated Breeding Value of total number of piglets

N: Number of pigs

Feed efficiency

It is expected the average feeding cost of pig farmers in Japan is almost 70%. Given that the price of imported feeding crops is increasing, it would be essential to improve feed efficiency of pigs. Feed conversion ratio (Amount of feeding/1kg body weight gain) of Duroc, Landrace and Large White breeds are now 3.1, 3.0 and 3.0, respectively. To address this issue, the Ministry of Agriculture, Forestry and Fisheries Japan formulated that those values are going to be improved to 2.9 to each breed by 2020. In order to accomplish to reach the goal, we aimed to detect genetic polymorphisms associated with feed efficiency related traits including average daily feed intake and average daily gain through GWAS approach. Remarkably we utilized Feed Intake Recording Equipment (FIRE®, Osbourne Industries Inc.) system which enables to automate the measurement of individual animal daily feed intake and other pig performance characteristics of growing animals (Figure 2). The results of GWAS indicated that there were five genetic polymorphisms significantly associated with average daily feed intake and average daily gain of Duroc population identified on SSC6 (Figure 3). Of four SNPs from markers B to E, out of five markers were in linkage disequilibrium. Moreover, the genotype AA of the four markers was favorable effect on not only average daily feed intake and average daily gain, but also on backfat thickness as well. The effect of those SNPs in the other Duroc population also showed quite similar so that those SNPs could be used as available DNA markers for improvement of feed efficiency, even if it is hard to obtain the feeding record of a give population.



Figure 2 Osbourne's Feed Intake Recording Equipment (FIRE®)
<https://osbornelivestockequipment.com/products/fire-pig-performance-testing-system/>

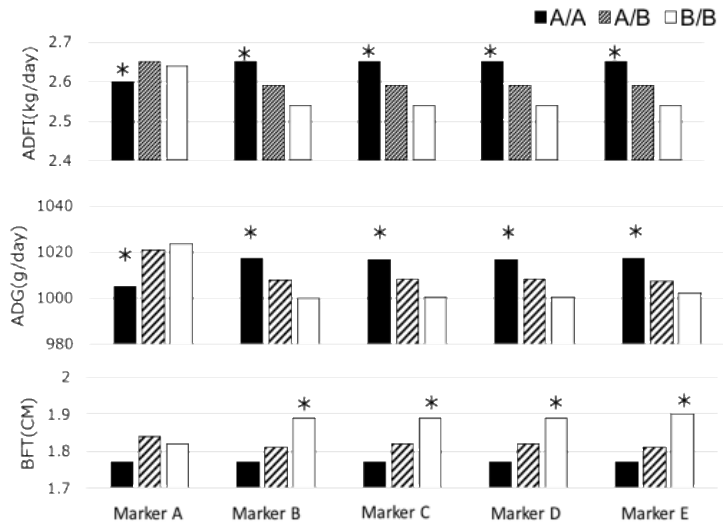


Figure 3 Effect of genotypes on feed efficiency traits
 ADFI, average daily feed intake; ADG, average daily gain; BFT, backfat thickness.
 Genotypes are shown in A/A, A/B and B/B.
 Asterisks show significant difference.

Growth rate of pre-weaning piglets

As mentioned above, it is essential to improve total number of piglets for overall increase of pig production. However, size of each piglet can be relatively small in the case of large number of piglets born compared to that of small number of piglets born. If so, piglets born from the large litter size will take longer time period to grow up, indicating they need to get more feedings to reach the regular body weight to ship: it simply means costly. Therefore, in addition to number of piglets born, also growth rate of pre-weaning piglets is important viewpoint to accomplish at the same time. We, then, sought to detect genetic polymorphisms related to the average body weight of piglets at birth, 1 week, 2 weeks and 3 weeks through the GWAS approach using Landrace population administered at a prefectural research station. The results showed that one SNP on SSC13 was associated with average body weight at birth, two SNPs on SSC10 and SSC14 were associated with average body weight at 2 weeks old piglets (Figure 4). Furthermore, we validated the effects of those SNPs using progeny line of Landrace population. Hence the SNPs can be available DNA markers for growth rate of pre-weaning piglets in Landrace pigs.

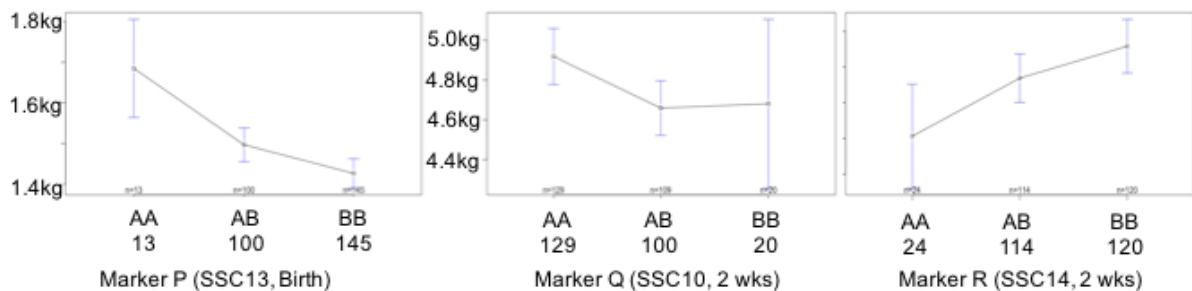


Figure 4 Comparison of average body weight of piglets between genotypes of DNA markers

Future prospects -genomic selection for commercial breeds-

As mentioned above, consumers in Japan tend to prefer meat with high fat content, such as thick backfat or high Intramuscular fat percentage (IMF), and therefore, most Japanese pig breeders have diligently improved commercial breeds (Landrace, Large White and Duroc breeds) originated from Europe or north America to have high performances for the fat related traits. Thus far, many quantitative trait loci (QTL) regions regarding with fat related traits have been detected by GWAS (Hu et al., 2016). However, those fat related traits are typically dominated by vast number of polygenic QTLs, where each QTL contributes very small degree of total phenotypic variation, and it would be difficult to use the detected QTL of the fatty traits to use improvement of the western breeds.

Recently, with the advancement of genotyping technology, we can simultaneous obtain huge amount of SNP markers. In addition to GWAS, the SNP markers have been used to evaluate genetic performance, or genomic prediction, for many traits, and the genomic prediction is a one of choice for improving the fat related traits in the imported commercial breeds. The expected benefit of the genomic prediction is that, if an individual has no phenotypic information, the genomic prediction gives high accuracy genetic evaluations for the potential performances of these traits. Therefore, we will be able to measure the genetic performance for fatty traits of the imported commercial breeds at the time of introduction of these breeds.

Commercial pigs are generally produced using a terminal crossbreeding system with three breeds. In this system, maternal line in Japan is F1 sows crossed between Large White and Landrace pure breeds, and paternal line is a boar of pure Duroc breed. Currently, the genetic performances of fatty traits have been predicted within each of these breeds using pedigree information. However, we sought to gain the genetic performances of commercial pigs produced by the three-way cross. It is often difficult to apply the pedigree-based prediction system to the evaluation for the genetic performance in commercial pigs by the three-way cross, because the pigs by the three-way cross are influenced by non-additive genetic factors, such as dominance and epistasis effects or heterosis. Those effects are not fully accounted for by pedigree information. One of advantages of the genomic prediction is considered to incorporate these effects into the genetic evaluation system of the three-way cross.

CONCLUSION

Taken together, our recent GWAS approaches to address issues on reproduction, feed efficiency and growth rate of piglets successfully developed DNA-markers which are potentially available for breeding scheme for commercial pig production, even though those traits were low heritability and/or difficult to record phenotypes. Additionally, we proposed future prospect of exploitation of SNP data for genomic selection which may allow us to fully gain the genetic capacity of breeder stocks for further efficient pig production in Japan.

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