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in

De Pedro E.J. (ed.), Cabezas A.B. (ed.).
7th International Symposium on the Mediterranean Pig

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 101

2012
pages 53-58

New genetic tests to select Iberian pigs

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Abstract. One of the main tasks in animal breeding is the identification of genes controlling economically important traits. Several polymorphisms (SNPs) in genes associated with growth, fatness, meat quality, disease resistance and prolificacy have been described in pigs and some of these SNPs have been incorporated in breeding programmes of pig industry. However, most of these SNPs are not functional and therefore they may not show any phenotypic effect in a particular population. Besides, most of them are fixed or at low frequency in *Iberian* pigs. We propose a new approach to identify new SNPs in complex candidate genes for designing useful genetic tests to select *Iberian* pigs. As an example, we have selected, from the PorcineSNP60 BeadChip genotype information, one panel of 96 SNPs flanking *FAT1* SSC4 QTL, Leptin Receptor (*LEPR*) and Acetyl Coenzime-A Carboxilase (*ACACA*) genes regions. A total of 334 *Iberian* pigs from AECERIBER performance tests were genotyped with this panel. Significant effects of five SNPs on growth, ham and loin weight were detected. The ability of this approach to identify genetic markers useful as a tool for selecting *Iberian* pigs is discussed.

Keywords. Iberian pig – Genetic tests – Selection.

Nouveaux tests génétiques pour la sélection des porcs Ibériques

Résumé. *Une des principales activités en amélioration génétique animale est l'identification de gènes responsables de caractères d'intérêt économique. Plusieurs polymorphismes (SNPs) localisés dans des gènes associés avec la croissance, l'engraissement, la qualité de la viande et la prolificité ont été décrits chez le porc. Certains de ces SNPs, conjointement avec les informations de performances, sont utilisés par la filière porcine. Toutefois, la plupart de ces SNPs ne sont pas fonctionnels, et selon la population étudiée, n'ont pas systématiquement un effet phénotypique. De plus, la plupart d'entre eux sont fixés ou ont une fréquence faible chez les porcs Ibériques. Nous proposons une nouvelle approche pour identifier de nouveaux SNPs dans des gènes candidats complexes, et pour réaliser des tests génétiques utiles à la sélection des porcs Ibériques. Nous avons sélectionné, à partir des résultats de génotypage de la puce PorcineSNP60, un panel de 96 SNPs flanquant le QTL FAT1 et les régions des gènes du Récepteur à la Leptine (LEPR) et de l'Acétyl-CoA Carboxylase (ACACA). 334 porcs Ibériques issus du programme de tests de performances AECERIBER ont été génotypés grâce à ce panel. Des effets significatifs de cinq SNPs ont été détectés pour les caractères de croissance, et de poids de jambon et de filet. La capacité de cette approche à identifier des marqueurs génétiques utiles à la sélection des porcs Ibériques est discutée.*

Mots-clés. *Porc Ibérique – Tests génétiques – Sélection.*

I – Introduction

In the last decades, one of the main tasks in animal breeding has been to identify genes controlling production traits. Single nucleotide polymorphisms (SNPs) have become the genetic markers of choice because they are the most frequent type of sequence variation of DNA being distributed over all the genome. In pigs, several polymorphisms in genes associated with diverse economic and morphological traits have been described and, some of them, together with traditional performance information, have been incorporated in breeding programmes of the pig industry. However, most of these SNPs were detected from quantitative trait loci (QTL) experiments in crosses between divergent breeds of pigs (Hu *et al*., 2007) and their effects need to be validated in commercial populations since they may not be the causal mutations of the phenotypic changes. Besides, the most relevant associations between SNPs and traits reported in porcine breeds have not been confirmed in *Iberian* pigs. In some cases this is due to

the fact that the studies are based on SNP present at low frequency or monomorphic in this breed (Pérez-Montarelo *et al*., 2010).

Two experimental crosses involving *Landrace* or *Meishan* as the maternal breed and Guadyerbas *Iberian* strain as the paternal line have been analyzed during the last years in order to identify chromosomal regions affecting economically important traits, particularly those related to growth, carcass composition, meat quality and reproduction (Varona *et al*., 2002; Noguera *et al*., 2009). These studies allowed detecting QTL located on chromosomes 4, 6 and 12 with important effects on live weight, back fat thickness and meat and fat quality traits (Pérez-Enciso *et al*., 2000; Ovilo *et al*., 2000; Muñoz *et al*., 2007).

New molecular technologies, such as the emerging high-throughput genotyping have allowed the development of high-density SNP genotyping platforms which are useful to identify mapped allelic variants in livestock animals. We propose to take advantage of these technologies for identifying genetic markers useful as a complementary selection tool for *Iberian* pigs. The first objective of this work was to build up a low-density panel of polymorphic SNPs mapped in the three quoted chromosomal regions, using the PorcineSNP60 BeadChip (Ramos *et al*., 2009. The second objective was to identify SNP effects on production traits in a representative sample of the *Iberian* pig breed, using the low-density panel developed.

II – Materials and methods

1. Animals and phenotypic records

Since 1993, the Spanish Association of *Iberian* Pig Breeders (AECERIBER) has carried out annual tests of carcass performance based on family groups of animals sampled at weaning from different herds which are transferred to a common testing farm. The pigs were under restricted feeding with concentrates until they reached a live weight of approximately 100 kg. Afterwards, they were fattened in 'Montanera' until an average weight of 160 kg. The live weight at the day before the slaughter and the weight of the warm carcass, trimmed hams, forelegs and loins, were individually registered. Samples of *longissimus dorsi* muscle at level of the fourth rib were collected to determinate, using near infrared spectroscopy (NIRS), the content of intramuscular fat, moisture and protein (Fernández *et al*., 2003).

Available records came from 334 barrows born in eight breeding nucleus from South-western Spain that maintain pigs representing the main morphological types of the breed. Data were collected from 2002 to 2005 and were grouped in four different batches according to the slaughter date. The elementary statistics of the analysed traits are reported in Table 1. Sire and dam identification was available for all animals included in the study.

2. SNP genotyping

Genomic DNA was extracted from blood or meat samples using a standard protocol. In a first step, samples of 26 *Iberian* pigs from 16 breeding nuclei registered in the Herd Book were genotyped for 62,163 SNPs using the Porcine SNP60 BeadChip (Illumina, San Diego, CA, USA). Genotyping reactions were performed on an "Infinium DNA Analysis Assay" at the Veterinary Service of Molecular Genetics (Universitàt Autonoma de Barcelona, Spain). We selected 85 SNP probes, from the above genotype information, with minor allele frequencies greater than 0.20 and overlapping the targeted regions: *FAT1* on chromosome 4 (SSC4), Leptin Receptor (*LEPR*) on SSC6 and Acetyl Coenzime-A Carboxilase (*ACACA*) on SSC12. These regions were selected because *FAT1* region corresponds to the main QTL region on SSC4 where several genes of the *FABP* family, related to lipid metabolism in different tissues are located (Szczerbal *et al*., 2007), *LEPR* gene has been studied as a candidate to underlay the main QTL on SSC6 for growth and body composition traits (Ovilo *et al*., 2005; Morhman *et al*., 2006) and *ACACA* is a key gene in fatty acid metabolism and it maps in the central region of the

QTL on SSC12 for fatty acid content of porcine back fat (Gallardo *et al*., 2007; Muñoz *et al*., 2007).

Table 1. Main statistics for the analysed traits

Trait	N	Mean	SD	Minimum	Maximum
Growth in extensive fattening period					
Starting weight, kg	171	98.38	10.58	73.00	121.00
Final weight, kg	325	162.88	13.39	131.00	225.00
Carcass weight, kg	325	129.33	11.08	106.50	178.50
Premium cuts yield					
Hams, kg	327	21.39	2.01	16.90	30.80
Forelegs, kg	329	14.52	1.37	11.50	19.70
Loins, kg	333	2.95	0.40	2.00	4.48
Meat quality					
Intramuscular fat, %	325	9.74	2.93	2.54	22.10
Moisture, %	324	69.39	2.28	56.46	74.44
Protein content, %	325	19.44	1.16	17.08	22.70

We increased the above set of 85 SNPs with eleven additional SNPs probes, which correspond to *LEPR* SNPs (five probes) and *ACACA* SNPs (six probes), previously identified by sequencing. The 96 SNPs covered a region of 146,146,887bp: 13,332,046bp on *FAT1* region of SSC4 (between INRA0014028 and ALGA0025271 probes), 13,020,811bp on SSC6 *LEPR* region (between ASGA0029534 and ALGA0037285) and 2,606,730bp on SSC12 *ACACA* region (between ALGA0066258 and ALGA0108055). The 96 finally selected SNPs loci were simultaneously interrogated with the GoldenGate Genotyping Assay.

3. Statistical analysis

Individual SNP effects on phenotypes were estimated fitting animal models, which besides the random polygenic effects included the following fixed effects: batch, additive and dominant SNP effects and the carcass weight as a covariate for premium cuts yield and meat composition traits. The analyses were performed using the Qxpak v.4 software (Pérez-Enciso and Misztal, 2004). The statistical significance of each SNP effects was tested comparing the full and reduced models by means of $\chi 2$ approaches to the distribution of log-likelihood ratios (LR). This quantity is distributed as a $\chi 2$ with degrees of freedom (d.f.) equal to the difference in the number of parameters between the alternative and the null models. We used one additional d.f. to calculate P-values stricter than the nominal ones. This extra d.f. accounts for the position of multiple SNPs tested.

III – Results and discussion

Seven out of the 96 selected probes failed to produce amplification in all samples: one out of the 30 located on SSC4, four out of the 35 corresponding to SSC6 and two out of the ones mapped on SSC12. Moreover, four of the five *LEPR* probes and one of the six *ACACA* probes were monomorphic. Sixty of the 89 probes showed SNPs allelic frequencies between 0.30 and 0.70. The average genetic distances between the genotyped SNPs was approximately 0.34 cM (340 kb, Build9) and the average value of linkage disequilibrium (LD) between adjacent SNPs was r 2 = 0.10.

SNP effects on several growth and carcass traits have been found in the three chromosomal regions analysed (Table 2). The most remarkable results corresponded to ALGA0123491 SNP probe mapping on SSC12. This SNP was associated with the live weights at the start and end of the extensive fattening period and also with the carcass weight. Additive effects explained about 2% of the mean of these traits. Dominant effects of similar magnitude were detected for final live weight and carcass weight. A stronger additive effect on starting weight, equivalent to 4.6% of the trait mean, was also detected on SSC6 (LIN3412 SNP probe). Interesting genetic effects on premium cuts yield were observed on the three chromosomal regions. An additive effect on ham weight of ASGA0019655 SNP probe (SSC4) was detected with a magnitude equivalent to a 1% of the mean. Two SNP probes (ALGA0037139 and H3GA0034325, on SSC6 and SSC12 respectively) showed significant additive effects on the loin weight, respectively equivalent to 4.4 and 2% of the mean. Moreover, a large dominant effect of the first probe on this trait was also estimated and was equivalent to a 7.4% of the mean. No significant SNP effects were identified on the composition of *longissimus dorsi* muscle.

Table 2. Significant additive (*a*) and dominant (*d*) effects on growth and carcass traits detected in the analysed chromosomal regions of SSC4, SSC6 and SSC12

Trait	SNP probe	SSC	a (SE)	d (SE)	LR*	d.f.†	P-value
Growth in fattening period							
Starting weight, kg	LIN3412	6	4.51 (1.23)	-	13.02	2	0.001
	ALGA0123491	12	3.11 (1.07)	-	8.23	2	0.016
Final weight, kg	ALGA0123491	12	2.14 (0.93)	2.91 (1.29)	9.98	3	0.019
Carcass weight, kg	ALGA0123491	12	2.10 (0.78)	2.01 (1.09)	10.04	3	0.018
Premium cuts yield‡							
Hams, kg	ASGA0019655	4	0.24 (0.09)	-	7.04	2	0.030
Loins, kg	ALGA0037139	6	0.13 (0.06)	-0.22 (0.07)	9.59	3	0.022
	H3GA0034325	12	0.06 (0.02)	-	7.06	2	0.029

*LR = Likelihood Ratio; † d.f. degrees of freedom; ‡ Adjusted for a common carcass weight.

The first question posed by the results concern the causal mutations underlying the observed genetics effects. Four probes (ALGA0123491, ASGA0019655, ALGA0037139 and H3GA0034325) correspond to SNPs located in intergenic regions, excluding ALGA0037139 that is an intronic mutation of *EFCAB7* gene. Only, the LIN3412 SNP probe corresponds to the sequence of a functional candidate gene. This SNP is an A/G substitution in the 3412 position of intron 11 of the *LEPR* gene sequence. Therefore, the potential functional effects of all these SNPs are not evident and their association with the phenotypic traits should be explained by linkage disequilibrium with the actual causal mutations.

It is interesting to compare the extent of LD in the *Iberian* population analysed with that observed in other pig breeds. Du *et al*. (2007) assessed the extent of LD in six lines of commercial pigs and found an average value of r^2 of 0.20 for SNPs separated by one cM. Despite the narrower distance between markers (0.34 cM), our results indicate a lower LD (r^2 = 0.10) in the *Iberian* population, which is a mixture of eight different *Iberian* breeding nuclei. A low LD is unfavourable to detect significant associations with quantitative traits, and more successful results may be expected within specific *Iberian* pig lines of lower effective size and greater LD.

A second relevant question is the usefulness of low-density SNP panels as tools for improving economically important traits of *Iberian* pigs. Breeding schemes for this breed are mainly focused on improvement of growth and carcass traits but quality traits should be also included in the breeding goal to take into account meat suitability for a long dry-curing process

(Fernandez *et al*., 2003). Meat quality and carcass performance traits are lately recorded on slaughtered pigs genetically related to the candidates to be selected as breeding animals. This reduces the intensity of selection and leads to a long generation interval. Methods for *in vivo* prediction of carcass and meat composition have been proposed for commercial pig breeds (Maignel *et al*., 2010) but, their application to heavy pigs as the *Iberian* may be difficult due to the differences in age, weight and body composition between tested candidates and commercial pigs. An alternative is the use of molecular markers associated to these traits. Our study explores an approach in this way combining results from QTL experiments and emerging high-throughput genotyping methods. The usefulness of this procedure will depend on its ability to provide early predictions of the genetic values for meat quality and carcass traits which would shorten the generation interval.

New high-throughput genotyping techniques are making a large amount of SNPs available that can be used to study patterns of genetic variation across the genome, to identify genome regions associated to traits of interest and to perform genetic selection with high accuracy. In particular, there is a growing interest in the application of genome wide selection (Meuwissen *et al*., 2001) that uses associations of large number of SNPs across the whole genome with phenotypes, without prior QTL detection. Genomic selection is the actual paradigm of animal breeding for traits difficult to improve by traditional methods. It could revolutionise *Iberian* pig breeding by producing early estimates of genetic merit of high accuracy for many more animals. However, some objections to this proposal may be outlined: (i) the instability of associations based on LD between neutral markers and traits limits its practical use to a reduced number of generations; (ii) many SNPs genotyped by the commercially available high-density porcine arrays are monomorphic in *Iberian* pigs, because this breed has not been taken into account in their design; and (iii) the development of high-density SNP arrays has excluded the information from QTL experiments and association studies performed in pigs along the last two decades; and (iv) finally, the high-density genotyping arrays have still a high cost for *Iberian* pig breeders.

Cheaper alternatives to genomic selection or genome wide association studies based on low-density SNPs panels have been suggested. Fontanesi *et al*. (2010) identified hundreds of SNPs in candidate genes for fat deposition traits from three different sources: sequencing, literature mining and expression database mining. To detect porcine genes affecting obesity related traits, they genotyped 677 selected SNPs in *Large White* pigs using a selective genotyping approach based on the estimated breeding value for back fat thickness (BFT). Eight genes have been associated with BFT, but possible SNPs effects on other traits related to fat metabolism have not been reported in their paper.

Our approach is also an alternative based on low-density SNPs panels but presents some singular features. The SNP identification was based on the results of previous QTL detection experiments which involved the *Iberian* breed as a parental breed, and the actual polymorphism of selected SNPs in *Iberian* pigs was verified using a moderate number of high-density genotyping arrays. The selective genotyping performed in the quoted study was exclusively based on one trait while we genotyped animals with many recorded traits for detecting marker-trait associations. Although this study is only a preliminary work, several interesting results have been obtained. Future research should focus in other chromosomal regions harbouring QTL for relevant traits and additional pigs with new records (e.g., fat thickness, back fat and muscle fatty acid composition or meat colour).

Acknowledgements

The authors wish to acknowledge the financial support received from the MICINN (Project AGL2007-65563-C02). Technical assistance of M.A. López, F. García, J. Salces, Y. Nuñez and A. Mercadé (UAB) and the facilities provided by E. de Pedro (UCO), J. García-Casco (INIA - Centro Nacional de Investigación en Cerdo Ibérico) and AECERIBER to obtain phenotypic records and samples are also recognized.

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