NOTA BREVE

CHARACTERISATION OF SOME FATNESS CANDIDATE GENES IN BASQUE BLACK PIED AND LARGE WHITE PIGS

CARACTERIZACIÓN DE ALGUNOS GENES CANDIDATOS DE LA ADIPOSIDAD EN CERDOS PÍO NEGRO DEL PAÍS VASCO Y LARGE WHITE

Alfonso, L. and A. Arana

U.P.N.A. Departamento de Producción Agraria. 31006 Pamplona. Spain. E-mail: leo.alfonso@unavarra.es

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INTRODUCTION

Fatness is a highly heritable quantitative trait strongly influenced by the environment, so only mutations with very large effects are likely to be detected (Keightley, 1995). Breeds with a high adipose development offer an opportunity for this detection. An example is the Basque Black Pied, one of those pig breeds that have lost its productive role during the last century (Iriarte and Alfonso, 2000). The Basque breed exhibited an early and higher adipose development and a higher activity of enzymes responsible for lipid synthesis than selected pig populations (Alfonso *et al.*, 2005).

Laval *et al.* (2000), in a study of pig genetic diversity, indicated that the Basque breed appeared to be the most *unique* in the set of eleven pig breeds originating from six European countries they analysed. So, the Basque Black Pied can be considered as an interesting pig population to analyse candidate genes of fat tissue development. Several candidate genes, mapped functional genes related to the expression of a trait, have been suggested to explain pig fatness. Four of them are characterised in this work: the Heart Fatty Acid-Binding Protein (H-FABP), the Adipocyte Fatty Acid-Binding Protein (A-FABP), the Porcine Leptin Receptor (LEPR) and the Porcine Leptin (LEP). Associations between them and intramuscular fat content and backfat thickness have been found in different studies (Estany *et al.*, 2002; Gerbens *et al.*, 1998; Gerbens *et al.*, 1999; Kennes *et al.*, 2001).

MATERIALS AND METHODS

Samples from forty-two animals were available from a previous experiment carried out to characterise the adipose development of Basque pigs (n=20) by comparison with a commercial selected line of Large

White origin (n=22) (Alfonso *et al.*, 2005). All these animals were genotyped for A-FABP microsatellite and the following RFLP polymorphisms: *Hinf*I, *Hae*III and *Msp*I for the H-FABP gene, *Hinf*I, *Hpa*II and *Rsa*I for the LEPR gene and *HindIII* for the LEP gene.

*Hinf*I and *Hae*III H-FABP polymorphism were typed using the primers described by Gerbens *et al*. (1997). Primers for *Msp*I were Fw: 5'-ATT GCT TCG GTG TGT TTG AG-3' and Rev: 5'-GGC CAT CCC ATA GAA CT-3'. The PCR mixture contained 2 mmol MgCl2, 0.2 mmol of each dNTP, 6 pmol of each primer, 100 ng genomic DNA, 0.7 U of Taq DNA polymerase in a 25 µl final volume. *Hinf*I LEPR polymorphism was typed using the primers described by Vincent *et al*. (1997), and *Hpa*II and *Rsa*I polymorphism with those described by Stratil *et al*. (1998). The PCR mixture contained 1.5 mmol MgCl2 (2 for *Hinf*I), 0.2 mmol of each dNTP, 15 pmol of each primer, 100 ng genomic DNA, 0.7 U of Taq DNA polymerase in a 25 µl final volume. *Hind*III LEP polymorphism was typed using the primers described by Kennes *et al*. (2001). The PCR mixture contained 1 mmol MgCl2, 0.2 mmol of each dNTP, 5 pmol of each primer, 75 ng genomic DNA, 0.2 U of Taq DNA polymerase in a 25 µl final volume.

PCR products were digested with 3 U of the respective enzyme (37º C/3 h.) and after PCR amplification fragment lengths were determined upon electrophoresis on agarose gels (H-FABP: 2 percent *Hinf*I and *Hae*III, 3 percent *Msp*I; LEPR: 1 percent *Hpa*II, 3 percent *Hinf*I and *Rsa*I; LEP: 3 percent *Hind*III) and the bands visualised with ethidium bromide. Polymerase chain reaction conditions for amplification of the A-FABP microsatellite were as described by Gerbens *et al*. (1998). Resulting fragments were detected on an ABI PRISM 310 Genetic Analyzer (ABI, Perkin Elmer, USA) and analysed using Genescan software (Applied Biosystems, Perkin Elmer, USA).

Allele and genotype frequencies were determined by gene counting. A Chi-square test was carried out to compare genotype frequencies among breeds.

RESULTS AND DISCUSSION

Allele frequencies estimated for the polymorphism analysed are shown in **table I**. Biallelic polymorphisms were found with *Hae*III and *Msp*I for H-FABP gene, with *Hpa*II and *Hinf*I for LEPR gene and with *Hind*III for LEP gene. For *Hinf*I H-FABP and *Rsa*I LEPR polymorphisms the same allele was found fixed in both breeds.

Differences in genotype frequencies among breeds were significant for *Msp*I H-FABP, *Hinf*I LEPR and *Hind*III LEP polymorphisms **(table I)**. The *C Msp*I H-FABP allele frequency was lower in Basque than in Large White line. The *a Hinf*I LEPR allele was fixed in Basque but segregating, although at a high frequency, in Large White. The *A Hind*III LEP allele was segregating in Basque and absent in Large White.

Results for the A-FABP microsatellite polymorphism analysed also showed significant differences among populations. Alleles *A3* and *A5* were found in Large White but absent in

Basque, and frequencies in A_2 and A_4 alleles were markedly different, especially for A_4 allele (0.88 in Basque and 0.07 in Large White pigs).

To our knowledge, this study reports the first results regarding the characterisation of the Basque pig breed for H-FABP, A-FABP, LEPR and LEP genes. The results indicate that Basque breed shows differences with the Large White line analysed for all these genes. Differences can be also noticed in comparison with the characteristics reported on other populations. First, the *C Msp*I H-FABP allele frequency was lower in Basque breed than values estimated in Pietrain (1), Landrace (0.96), Torbiscal Iberian (0.8) and Black Hairless Iberian (1) populations (C. Ovilo, personal communication). Second, whereas the *a Hinf*I LEPR allele has been found fixed in Basque pigs it was found to be segregating in Landrance, Duroc, Hampshire and Meishan populations (Vincent *et al*., 1997) like in the Large White analysed in the present study. Third, Kennes *et al*. (2001) found that *A Hind*III LEP allele was segregating in Duroc and Landrace like observed in Basque breed, but at a lower frequency. Finally, it should be noticed that Ovilo *et al*. (2001) also found the A_2 and A_4 A-FABP alleles in a population of Iberian

Table I. *Allele frequencies at the H-FABP, LEPR, LEP and A-FABP gene polymorphisms and Chi-square test significance of genotype frequencies comparison among breeds.* (Frecuencias alélicas para los polimorfismos de los genes H-FABP, LEPR, LEP y A-FABP y significación de la prueba Chi-cuadrado de la comparación de las frecuencias genotípicas entre razas).

Gene	Polymorphism	Allele[a]	Breed Basque Allele frequency	Large White Allele frequency	Significance of differences in genotype frequencies[b]
H-FABP	*Hinf*I	*H*	1	1	ns
	*Hae*III	*D*	0.58	0.46	ns
	*Msp*I	*C*	0.28	0.98	***
LEPR	*Hpa*II	*b*	0.65	0.68	ns
	*Rsa*I	*d*	1	1	ns
	*Hinf*I	*a*	1	0.70	***
LEP	*Hind*III	*A*	0.73	0	***
A-FABP	*Microsatellite*	A_1	0	0	***
		A_2	0.12	0.61	
		A_3	0	0.07	
		A_4	0.88	0.07	
		A_5	0	0.25	

[a]*H* allele: 350+180+110+50+37 bp; *D* allele: 684+117+16 bp; *C* allele: 322+90 bp; *b* allele: 1450+550 bp; *d* allele: 750+349+334+300+250 bp; *a* allele: 2100+700+395+240+140+110 bp; *A* allele: 658 bp; A_1 allele 248 bp; A_2 allele 250 bp; A_3 allele 252 bp; A_4 allele 254 bp; A_5 allele 268 bp.
[b]χ^2 statistic significance. ns: not significant; ***p<0.001.

pig, but with intermediate frequencies between the values found in the Basque and Large White breeds in the present study.

The differences among breeds that have been reported could allow the design of future experiments interested in dealing with fatness genetic background, especially involving H-FABP, A-FABP and LEP genes.

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