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A CLASSICAL QTL SCAN SUPPORTS THE COMPLEXITY OF THE GENETIC BASIS OF COAT COLOUR IN PIG

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ABSTRACT

Although an important progress on the pig coat colour genetics has been experienced during recent years, the genetic basis of this trait has not been completely clarified. The aim of the present study was to identify chromosomal regions with effects on coat colour extension and to examine the effects of relevant colour genes in F2 pigs of a Black Iberian × Landrace experimental population. The QTL scan has allowed us to detect two genome-wide significant QTLs on SSC6 and SSC8, six chromosome-wide significant QTLs on SSC1, SSC7, SSC9, SSC12, SSC13 and SSC17 and one suggestive QTL on SSC15. In addition, *MC1R*, *OCA2* and *KIT* genes have been investigated. The results point out that the *KIT* gene is the main responsible of the coat colour extension and *MC1R* also plays a major role. However, other loci seem to have significant effects on the determination of this trait.

Key words: coat colour pattern / QTL / *KIT* / *MC1R* / pig

1 INTRODUCTION

In the processes of pig domestication and selection the coat colour patterns were crucial, providing phenotypic patterns characteristic of breeds and varieties (Legault, 1998). Currently, the coat colour is an important trait in pig production since there are still preferences for specific colour patterns from the consumer and breeders. There isa large number of coat colours in pig, from white, such as it appears in Landrace or Large White, different shades of red and blond, as in Red Mangalitza or Red Iberian pigs, to black, as in Large Black or Black Iberian pigs. Additionally, several colour patterns can be also found, including uniform colour over the whole coat and different types of spotted and belted patterns. An important progress on the pig coat colour genetics has been experienced during recent years. In fact, several causal mutations of colour and colour pattern variants have been identified in pigs. The *Melanocortin receptor 1* (*MC1R*), also known as *Extension* locus, and *v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog* (*KIT*), also known as *I* locus, are considered the key genes of the coat colour variations. In pig, at least six different *MC1R* alleles have been described, which correspond to four *Extension* alleles: wilt type (E^+), dominant black (E^d), spotted (E^p) and red (*e*) (Kijas *et al*., 1998; Giuffra *et al*., 2000). Furthermore, six different alleles, at least, have also been identified for the *KIT* gene (Kijas *et al*., 2001; Rubin *et al*., 2012), which correspond to four *I* alleles: recessive allele (*i*), black spots (I^p), belt (I^{Be}) and dominant white (*I*). However, several other genes participate in the colour determination (Cieslak *et al*., 2011). Moreover, interactions among coat colour genes have been revealed as important part in the pigmentation process of pig coat (Marklund *et al.,* 1998, Hirooka *et al*., 2002). The aim of the present study was to identify the genes and causal mutations responsible of the coat colour extension in a Black Iberian × Landrace experimental population. For this purpose, a QTL scan was conducted in order to identify the chromosomal regions with effects on this trait

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and subsequent analyses of underlying colour genes have been performed.

2 MATERIAL AND METHODS

2.1 ANIMALS AND COAT COLOUR

Phenotypic data for coat colour extension were registered on the F2 pigs of a Black Hairless Iberian (Guadyerbas) × Landrace experimental cross (IBMAP). The phenotypes for 127 F2 pigs were scored by splitting the animal body in four parts (Fig. 1a) and applying a four-digit coding system to each of four parts (0-1-2-3). The 0 digit indicates no pigmentation (completely white), the 1 and 2 digits were used for marks or spots and the 3 digit was used for entirely pigmented (completely black or red). The sum of the four scored parts provided the final phenotypic register, ranging from 0 to 12. The phenotype distribution across the F2 pigs (n = 127) is shown in Fig. 1b.

2.2 MARKERS GENOTYPING AND STATISTICAL ANALYSES

The F2 animals, their ancestors from F1 and the parental populations, Landrace and Black Hairless Iberian (Guadyerbas) purebreds, were genotyped for 114 microsatellites and 17 SNPs distributed along autosomes. The marker information and linkage maps were used in previous studies on the same animal material (Estellé *et al.*, 2008). The QTL scan was performed in intervals of 1 cM, testing by LR tests a full model fitting additive and dominant QTL effects besides of a gender systematic effect and a polygenic random effect versus a reduced model without QTL effects. All the statistical analyses were performed using the Qxpak v.5.2 software (Perez-Enciso and Misztal 2011). Genome and chromosome significance thresholds at 5% and 10% were calculated using the procedure described by Nezer *et al.* (2002). The 95% confidence intervals (CI) for the QTLs were calculated according to Mangin *et al.* (1994).

2.3 *MC1R*, *OCA2* AND *KIT* POLYMORPHISM GENOTYPING AND ASSOCIATION ANALYSIS

Polymorphisms on the *MC1R* (nt67insCC), *OCA2* (nt2039G>C) and *KIT* (gene duplication-DUP1 and intron 17 splice mutation) genes were genotyped in the 127 F2 and ancestors according to previously reported protocols (Fernández, 2003; Fernández *et al.*, 2006; Fontanesi *et al.*, 2010). Association analyses were carried out using an animal model.

3 RESULTS AND DISCUSSION

The experimental population analyzed in the present study, a F2 intercross between Guadyerbas and Landrace lines, constitutes a great animal material for the study of the coat colour genetic basis. The Guadyerbas strain corresponds to an ancient Iberian pig variety completely black and hairless, which has been maintained as a closed herd since 1945 at 'El Dehesón del Encinar' (Oropesa, Toledo, Spain). By contrast, the Landrace lean line proceeds from a widely used European breed, characterized by its white coat colour. The cross of both pig breeds has allowed us to obtain many colour patterns.

The QTL scan has allowed us to detect two genome-wide significant QTL regions on SSC6 and SSC8, six chromosome-wide significant QTL regions on SSC1,

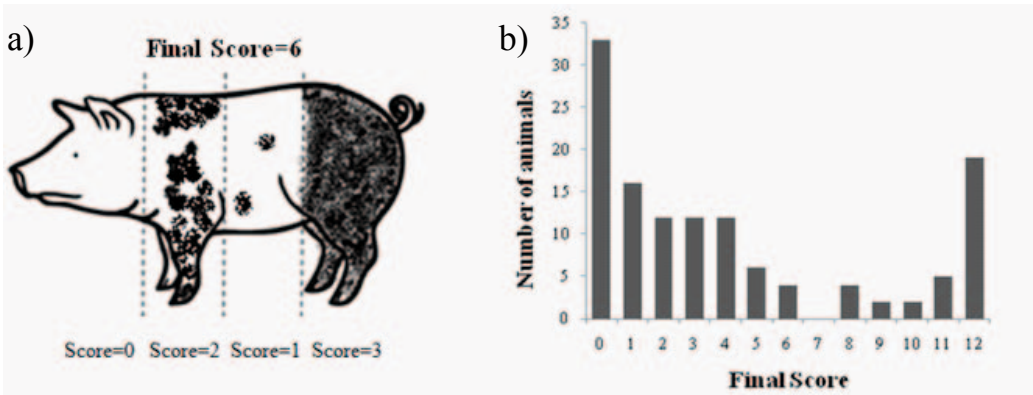

Figure 1: *Phenotypic scoring for coat colour patterns in 127 F2 pigs, a) Illustration of colour pattern scoring method, b) Number of animals displaying each final phenotypic score*

Table 1: *Significant results of the QTL scan for coat colour patterns conducted with phenotypic and genotypic data from 83 F2 pigs and their ancestors*

SSC	Position (CI) (cM)	LR	P-value	***a*** (SE)	***d*** (SE)
Genome wide significant QTL (5%)					
6	1 (1–2)	13.69	0.22×10^{-3}	2.20 (0.65)	-
8	60 (58–63)	82.57	$<1 \times 10^{-16}$	5.32 (0.46)	−3.37 (0.66)
Chromosome wide significant QTL (5%)					
1	56 (51–58)	7.57	0.59×10^{-2}	-	−2.85(1.29)
7	75 (72–84)	10.75	0.10×10^{-2}	-	3.19 (1.12)
9	96 (79–124)	9.33	0.14×10^{-2}	1.89 (0.74)	-
12	37 (29–76)	10.25	0.14×10^{-2}	−1.88 (0.68)	-
13	97 (85–112)	12.29	0.45×10^{-3}	2.79 (0.89)	-
17	1 (1–10)	8.78	0.30×10^{-2}	-	−2.20 (0.89)
Chromosome wide suggestive QTL (10%)					
15	39 (27–52)	6.93	0.85×10^{-2}	−1.29 (0.63)	-

SSC7, SSC9, SSC12, SSC13 and SSC17 and one suggestive QTL region on SSC15 (Table 1).

The most significant result was found on SSC8, with huge additive and dominant effects (Table 1). This region, 58–63 cM, corresponds approximately to a physical distance of 10–72 Mb, according to the microsatellites flanking the QTL region. As expected, this region includes *KIT* gene (43.55 Mb), considered main responsible of melanocytes survival and distribution. The second most significant QTL was identified on SSC6 and also revealed an important additive effect. This QTL maps at the beginning of the chromosome and overlaps with the *MC1R* gene position at 0.25 Mb. These results support the key role of both genes on determining coat colour.

Interestingly, in spite of the limited number of animals, other QTL regions identified on SSC7, SSC13 and SSC17 revealed also important effects, proving the complex genetic architecture of this trait. Several of the regions here identified overlap with known colour genes. The *tyrosinase-related protein 1 (TYRP1*) gene codes for a melanosomal enzyme that plays a known role in mammalian pigmentation (Javerzat and Jackson, 1998), and it maps at 233.66 Mb on SSC1, close to the QTL detected on this chromosome (62–210 Mb). The *microphthalmia associated transcription factor (MITF*) gene codes for a transcription factor responsible for pigment cell-specific transcription of the melanogenesis enzyme genes. Moreover, it has been studied as candidate to be responsible of porcine melanoma (Bourneuf *et al*., 2011). This gene maps at 56.59 Mb on SSC13, also close to the QTL region on that chromosome (58–194 Mb). Finally, the O*culocutaneous albinism II* (*OCA2*) gene, which codes for integral membrane protein involved in tyrosine transport, a precursor of melanin, has been previously associated to pigment dilution in pigs (Fernández *et al*., 2006). This gene maps at 63.92 Mb on SSC15, within the confidence interval of the suggestive QTL identified on this chromosome.

In addition, three coat colour genes have been investigated: *MC1R, OCA2* and *KIT*. Two *MC1R* alleles appear segregating in the IBMAP population: Guadyerbas pigs carry the *MC1R**3 allele and Landrace carry the *MC1R*6* allele (allelic discrimination was performed based on the indel: nt67insCC, MAF = 0.41). Association analysis revealed significant association of *MC1R* on coat colour extension (additive effect = 1.73 ± 0.55; P-value = 0.0018), showing that *MC1R* seems to be the responsible of the QTL effect previously detected. Even more, these results support the hypothesis that *MC1R* has a role not only in coat colour but also in the colour pattern determination. One of the polymorphisms identified on *OCA2* gene (Fernández *et al*., 2006) was genotyped in the IBMAP population (nt2039G>C, MAF = 0.17). The association analysis did not show any significant association, stating that the analyzed mutation is not responsible of the suggestive QTL effect. Finally, we have analyzed two of the most relevant polymorphisms of the *KIT* gene (gene duplication-DUP1 and intron 17 splice mutation). Both polymorphisms appear segregating in the IBMAP population: Guadyerbas pigs carry the wild *KIT* allele (one copy of the gene and no mutation in intron 17), while Landrace pigs carry three different haplotypes: one copy of the gene and no mutation in intron 17, gene duplication and no mutation in intron 17, and gene duplication and mutation in intron 17. A linkage map was built with *KIT* gene duplication polymorphism in order to

confirm its localization. The result validated *KIT* position between *Swr110* and *S0017*, flanking markers of the QTL. However the determination of the haplotypes was not clear for all the individuals and association analysis could not be conducted. In fact, it has recently been published that the allelic forms of this gene are even more complex than stated up to now, three new duplications of gene segments (DUP2, 3 and 4) provide new alleles responsible of the white, belted and spotted phenotypes (Rubin *et al*., 2012). Further studies are being conducted in order to identify specific *KIT* alleles segregating in this material.

The results of this study point out that the *KIT* gene is the main responsible of the coat colour pattern but *MC1R* also plays a major role in colour extension. Moreover, other loci seem to have significant effects on the determination of this trait, showing the complexity of the genetic basis of this trait, simple in its appreciation on the animals.

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5 REFERENCES

Bourneuf E., Du Z.Q., Estellé J., Gilbert H., Créchet F., Piton G., Milan D., Geffrotin C., Lathrop M., Demenais F., Rogel-Gaillard C., Vincent-Naulleau S. 2011. Genetic and functional evaluation of MITF as a candidate gene for cutaneous melanoma predisposition in pigs. Mammalian Genome, 22: 602–612

Cieslak M., Reissmann M., Hofreiter M., Ludwig A. 2011. Colours of domestication. Biological reviews of the Cambridge Philosophical Society, 86: 885–899

Estellé J, Gil F, Vázquez JM, Latorre R, Ramírez G, Barragán MC, Folch JM, Noguera JL, Toro MA, Pérez-Enciso M. 2008. A quantitative trait locus genome scan for porcine muscle fiber traits reveals overdominance and epistasis. Journal of Animal Sciences, 86: 3290–3299

Fernández A.I. 2003. Estudio de la base genetica del color de la capa y aplicaciones practicas en porcino. PhD Thesis, Universidad Complutense de Madrid, Madrid.

Fernández A., Silió L., Rodríguez C., Ovilo C. 2006. Characterization of OCA2 cDNA in different porcine breeds and analysis of its potential effect on skin pigmentation in a red Iberian strain. Animal Genetics, 37: 166–170

Fontanesi L., D'Alessandro E., Scotti E., Liotta L., Crovetti A.,Chiofalo V., Russo V. 2010. enetic heterogeneity and selection signature at the KIT gene in pigs showing different coat colours and patterns. Animal Genetics, 41: 478–492

Giuffra E., Evans G., Törnsten A., Wales R., Day A., Looft H., Plastow G., Andersson L. 1999. The Belt mutation in pigs is an allele at the Dominant white (I/KIT) locus. Mammalian Genome, 10: 1132–1136

Hirooka H., de Koning D.J., van Arendonk J.A., Harlizius B., de Groot P.N., Bovenhuis H. 2002. Genome scan reveals new coat color loci in exotic pig cross. Journal of Heredity, 93:1-8Javerzat S., Jackson I.J. 1998. White-based brown (Tyrp1B-w) is a dominant mutation causing reduced hair pigmentation owing to a chromosomal inversion.Mammalian Genome, 9: 469–471

Kijas J.M., Wales R., Törnsten A., Chardon P., Moller M., Andersson L. 1998. Melanocortin receptor 1 (MC1R) mutations and coat color in pigs. Genetics, 150: 1177–1185

Kijas J.M.H., Moller M., Plastow G., Andersson L. 2001. A frameshift mutation in MC1R and a high frequency of somatic reversions cause black spotting in pigs.Genetics, 158:779–785

Legault C.1998. Genetics of colour variation. In: The genetics of the pig. Rothschild M.F., Ruvinsky A. (ed.). New York: CAB International: 51–70

Mangin B., Goffinet B., Rebaï A. 1994. Constructing confidence intervals for QTL location. Genetics, 138: 1301–1308

Marklund S., Kijas J., Rodriguez-Martinez H., Rönnstrand L., Funa K., Moller M., Lange D., Edfors-Lilja I., Andersson L.1998. Molecular basis for the dominant white phenotype in the domestic pig.Genome Research, 8: 826–833

Nezer C., Moreau L., Wagenaar D., Georges M. 2002. Results of a whole genome scan targeting QTL for growth and carcass traits in a Piétrain × Large White intercross. Genetics Selection and Evolution, 34: 371–387

Pérez-Enciso M., Misztal I. 2011. Qxpak.5: old mixed model solutions for new genomics problems. BMC Bioinformatics, 25, 12: 202

Rubin C.J., Megens H.J., Martinez Barrio A., Maqbool K., Sayyab S., Schwochow D., Wang C., Carlborg Ö., Jern P., Jørgensen C.B., Archibald A.L., Fredholm M., Groenen M.A., Andersson L.2012. Strong signatures of selection in the domestic pig genome. In: Proceedings of the National Academy of Sciences of the USA, 109, 48: 19529–31956