Meat Science 95 (2013) 368–375

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Association mapping of quantitative trait loci for carcass and meat quality traits at the central part of chromosome 2 in Italian Large White pigs

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ARTICLE INFO

Article history:
Received 5 February 2013
Received in revised form 24 April 2013
Accepted 1 May 2013

Keywords:
Pig
Association
Chromosome 2
Carcass quality
Meat quality

ABSTRACT

Association mapping of the central part of porcine chromosome 2 harboring QTLs for carcass and meat quality traits was performed with 17 gene-tagged SNPs located between 44.0 and 77.5 Mb on a physical map (Sscrofa10.2) in Italian Large White pigs. For the analyzed animals records of estimated breeding values for average daily gain, back fat thickness, lean cuts, ham weight, feed conversion ratio, pH_1, pH_u, CIE L^*, CIE a^*, CIE b^* and drip loss were available. A significant QTL for fat deposition (adjusted $P = 0.0081$) and pH_1 (adjusted $P = 0.0972$) to *MYOD1* at position 44.4 Mb and a QTL for growth and meatiness (adjusted $P = 0.0238$–0.0601) to *UBL5* at position 68.9 Mb were mapped. These results from association mapping are much more accurate than those from linkage mapping and facilitate further search for position candidate genes and causative mutations needed for application of markers through marker assisted selection.

1. Introduction

Carcass and meat quality traits are amenable to marker assisted selection (MAS) as the majority of such traits can be measured post mortem. Earlier studies of quantitative trait loci (QTLs) for carcass and meat quality based on linkage mapping in experimental F_2 crosses with high level of linkage disequilibrium (LD) and lack of recombination usually have been able to map QTLs in intervals of 20–40 centimorgans (cM) (Georges, 2007). Fine mapping of QTLs that leads to identifying the mutations underlying phenotypic variation is necessary for use in selection programs.

Association or LD mapping that uses correlations between QTL alleles and marker alleles in the whole population is likely to be a more effective tool than linkage studies for examining complex traits because it can have greater statistical power to detect several genes of small effect. LD may occur if a marker allele and QTL allele were on the same chromosome in an ancestor of the current population and, due to chance effects and finite population size, that the chromosome segment is now common in the population. Since recombination will separate the marker and QTL alleles unless they are tightly linked, LD is expected and more often observed between genes that are tightly linked (Goddard, 2003). Consequently, LD mapping can only detect a QTL in the vicinity of a marker. If a QTL is close, however, it can map its position much more accurately than can linkage mapping because it uses all recombination events that have occurred since the common ancestor.

Generally, the amount of LD between a marker and a QTL useful for association mapping is assumed to be $r^2 \geq 0.3$ (Du, Clutter, & Lohuis, 2007; Jungerius et al., 2005). Association mapping enables precise QTL mapping in commercially exploited populations when the mapping is based on a sufficient number of markers in a specific region.

In addition to the imprinted *IGF2*–intron3–G3072A substitution with a major effect on body composition that maps to the proximal tip of pig chromosome 2 — SSC2 (Van Laere et al., 2003) other QTLs for fat deposition, growth (de Koning et al., 1999; Geldermann, Čepica, Stratil, Bartenschlager, & Preuss, 2010; Lee et al., 2003; Liu et al., 2008; Qiu et al., 2010; Rattink et al., 2000; Stearns et al., 2005; Thomsen, Lee, Rothschild, Malek, & Dekkers, 2004; Tortereau et al., 2010) and meat quality traits such as pH_u (Heuven et al., 2009; Lee et al., 2003; Liu et al., 2007; Qu et al., 2002; Rohrer, Thallman, Shackelford, Wheeler, & Koohmaraie, 2006; Su et al., 2004), pH_1 (Čepica et al., 2012), water holding capacity and meat color (Malek et al., 2001), drip loss (H.D. Li et al., 2010) and

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http://dx.doi.org/10.1016/j.meatsci.2013.05.002

intramuscular fat (Čepica et al., 2012; Stearns et al., 2005) have been mapped at the central part of porcine chromosome 2. The QTLs for carcass and meat quality traits and 95% confidence intervals are located in the chromosome region 55.0–77.9 cM (PigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index).

The aim of this work was to perform association mapping of carcass and meat quality traits in the Italian Large White (ILW) population using multiple gene-tagged single nucleotide polymorphisms (SNPs) spread over the chromosome 2 region harboring QTLs for carcass and meat quality traits.

2. Material and methods

2.1. Animals

Two groups of sib-tested ILW pigs were used. Three siblings of the same litter (2 females and 1 castrated male) were performance-tested at the Central Test Station of the National Association of Pig Breeders (Italy) and slaughtered for genetic evaluation of a boar from the same litter. Animals were slaughtered at average live weight of 155 kg at about 8 months of age.

Group 1 used for the selective genotyping approach comprised 200 pigs (138 females and 62 castrated males) and consisted of at least two-generation-unrelated performance-tested animals with highest and lowest estimated breeding values (EBVs) for average daily gain (ADG; high tail for ADG EBVs [N = 100] and low tail for ADG EBVs [N = 100]). These animals were selected from 3591 sib-tested pigs slaughtered between 1996 and 2007 (Table S1).

Group 2 comprised a random sample of 633 animals (422 females and 211 castrated males) encompassing two subgroups, of which subgroup 2A consisted of 277 animals (184 females and 93 castrated males) slaughtered in 2003 (6 different slaughter batches) and subgroup 2B consisted of 356 animals (238 females and 118 castrated males) slaughtered in 2008 (11 different slaughter batches). Subgroups 2A and 2B were analyzed as independent groups, as they were separated by 5 years of selection. The characteristics of subgroups 2A and 2B are presented in Table 1.

Animals of both populations had EBVs for average daily gain, measured in grams, from 30 to 155 kg of live weight with quasi ad libitum feeding (ADG); feed conversion ratio calculated as feed intake/weight gain from 30 to 155 kg (FCR); back fat thickness measured in mm and recorded post mortem at the *m. gluteus medius* (BFT); ham weight measured in kg (HW); and weight of lean cuts in kg including weight of neck, loin and HW (LC). EBVs for the traits reported above were calculated and provided by the National Association of pig breeders as described by Russo et al. (2000, 2008) using a BLUP multiple-trait animal model (Henderson & Quaas, 1976). Briefly, models were different for each trait and included fixed effects of batch in test, sex, age at beginning of test, age of sow, weight at slaughter, age at slaughter, and inbreeding coefficient as well as the random effects of litter, individual permanent environment, and animal. Pigs' genetic merit for the considered traits was calculated taking into account the additive relationship matrix. EBVs were expressed as differences from the genetic mean value for the considered trait in 1993. In addition, meat quality traits such as pH_1 (measured about 1 h post mortem), pH_u (measured 24 h post mortem), CIE L*a*b* color (1976 CIE L*a*b* Color Space, CIELAB; http://www.cie.co.at/index.php/index.php?i_ca_id=485), drip loss (DRIP; Grau & Hamm, 1957; Hofmann, Hamm, & Blüchel, 1982), and glycolytic potential (GP; Monin et al., 1987; Nanni Costa et al., 2009) were measured in *m. semimembranosus*.

Table 1
Mean breeding values for ADG, BFT, LC, HW and FCR; phenotypic values for pH_1, pH_u, CIE L*, CIE a*, CIE b*, DRIP, GP and CW; age at slaughter; and their standard deviations (SD) of subgroups 2A and 2B of Italian Large White pigs used for association studies.

Trait	Subgroup 2A			Subgroup 2B		
	N	Mean	SD	N	Mean	SD
ADG (EBV)	276	35.17	29.06	350	41.50	19.83
BFT (EBV)	276	−2.24	3.80	350	−2.31	2.85
LC (EBV)	276	2.03	1.91	350	3.36	1.62
HW (EBV)	276	0.55	0.59	350	0.64	0.43
FCR (EBV)	276	−0.14	0.16	350	−0.16	0.10
pH_1	275	5.94	0.24	347	6.21	0.27
pH_u	274	5.67	0.21	313	5.77	0.23
CIE L*	–	–	–	349	40.04	4.98
CIE a*	–	–	–	349	7.75	2.56
CIE b*	–	–	–	349	3.79	0.98
DRIP	–	–	–	350	69.54	19.33
GP	275	103.44	23.06	–	–	–
CW	276	120.05	9.57	350	114.58	8.41
AGE	275	239.56	7.92	350	238.21	7.76

EBV — estimated breeding value, ADG — average daily gain, BFT — back fat thickness, LC — weight of lean cuts, HW — hams weight, and FCR — feed conversion ratio.
Traits: pH_1 — meat pH measured at about 1 h post mortem in *m. semimembranosus*, pH_u — meat pH measured at 24 h post mortem in *m. semimembranosus*, color parameters according to 1976 CIE L*a*b* Color Space, DRIP — drip loss, GP — glycolytic potential, CW — carcass weight, and AGE — age at slaughter.

2.2. SNPs and their genotyping

To optimize the number of genotyped animals, selective genotyping as a preliminary approach followed by sequential sampling was used. Selective genotyping involves phenotyping a large population of individuals, but the actual genotyping involves only those individuals whose phenotypes deviate substantially from the mean (Van Gestel et al., 2000). The genotyped SNPs were either retrieved from the NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_batchSearch.cgi?org=9823&type=SNP) and literature or obtained by comparative sequencing of the gene-tagged polymerase chain reaction (PCR) products prepared on DNA from 12 animals (4 Czech Large White, 4 Czech Landrace, and 4 Duroc). All 17 SNPs were gene-tagged. Of these, 14 were located in introns, 2 in exons, and 1 in the 3′ UTR. The SNPs used for genotyping, including reference sequences, PCR primers, PCR conditions, restriction enzymes used for PCR-restriction fragment length polymorphism, and literature references, are listed in Table 2. Pairwise measures of LD (r^2) were calculated using the Haploview software package accessible at www.broad.mit.edu/mpg/haploview/ (Barrett, Fry, Maller, & Daly, 2005).

Group 1 was genotyped first for all considered SNPs in order to verify their segregation and preliminarily assess association with records for EBVs in ILW population. Apart from that the segregating SNPs were subjected to sequential sampling, meaning that a subset of animals of Group 2 was genotyped and associations were preliminarily tested. Further genotyping in Group 2 was performed for SNPs with MAF > 0.05 and positive results of selective genotyping in Group 1 and sequential sampling in subgroups 2A and 2B.

2.3. Statistical analyses

Associations between genotypes and EBVs (obtained by the National Association of pig breeders) were assessed using the general linear model (GLM) procedure of SAS, release 9.2 (SAS Institute Inc., Cary, NC). The model included the fixed effects of genotype and sex (Group 1) or genotype, sex and day of slaughter (Group 2, subgroups 2A and 2B). The number of slaughter days was not available for Group 1.

The model for Group 1 is as shown below:

$$EBV = \mu + SNP_i + Sex_j + e_{ij},$$

where EBV stands for estimated breeding value for ADG, BFT, LC, HW and FCR, respectively; μ = overall mean; SNP = fixed effect of each