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Pig KALRN, MYH1, MLC2V, SNX13, AK1, and PPIA loci RH mapping and chromosome position refining

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ABSTRACT. The suppressive subtractive hybridization technique was previously used by the authors to identify candidate genes for meat quality in pig. A set of ESTs homologous (>95%) to genes involved in muscle metabolism is reported in the present paper. Four ESTs homologous to MYH1, KALRN, MLC2V, and SNX13 genes plus two genes (AK1, PPIA) used as housekeeping for muscle tissue were assigned to porcine chromosomes using the INRA-Minnesota 7000 rads radiation hybrid panel (IMpRH). Our data confirm and refine the cytogenetic position of the KALRN, AK1, PPIA genes, improve the existing physical map of MYH1 and assign two new genes (MLC2V and SNX13) to swine chromosomes.

Key words: RH mapping; Pig; Meat quality

INTRODUCTION

During the last decades, research has focused on genetic improvement of pork quality, and studies on the RN (Rendement Napole; Le Roy et al., 1990) and halothane (Fujii et al., 1991) genes have already demonstrated the importance of allelic variation in economically important traits such as meat quality. Despite its economic importance and the number of ongoing studies, information regarding genes involved in pork quality is still poor. To identify candidate genes for pork quality, suppressive subtractive hybridization libraries (Diatchenko et al., 1996) starting from skeletal muscle tissue samples and a commercial population were created (Gorni C, Stella A, Restelli GL, Iacuaniello S, et al., unpublished data). Four ESTs homologous to genes involved in muscle metabolism, skeletal muscle myosin heavy polypeptide 1 (MYH1), kalirin (KALRN), myosin regulatory light chain (MLC2V), and sorting nexin 13 (SNX13), were identified and mapped to porcine chromosomes using the INRA-Minnesota 7000 rads radiation hybrid panel (IMpRH; Yerle et al., 1998). Two additional housekeeping genes for muscle tissue, adenylate kinase 1 (AK1) and cyclophilinA (PPIA), were also mapped.

MATERIAL AND METHODS

Primer pairs were designed using PrimerExpress 3.0 (Applied Biosystems) and were based on the EST sequences produced in the present study. AK1 and PPIA specific primers were designed based on mRNA pig sequences downloaded from GenBank (AY610127 and AY008846). Each primer pair was tested against hamster and pig control DNAs. Amplification conditions were optimized to produce single amplicons.

Following optimization, polymerase chain reactions were performed at 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Amplification products were visualized after electrophoresis on ethidium bromide-stained 2% agarose gels. Positive clones in the IMpRH panel were scored and polymerase chain reactions were repeated twice. Data were submitted to the IMpRH database (http://www.toulouse.inra.fr/lgc/pig/RH/IMpRH.htm) to assess map positions.

RESULTS AND DISCUSSION

As summarized in Table 1, all our sequences were successfully mapped at LOD >4 to pig genome, and all assignments are in accordance with *in-silico* human-pig comparative mapping data available at the PigQTL database (http://www.animalgenome.org/QTLdb/pig.html; Hu et al., 2005).

In particular, for three (KALRN, AK1, PPIA) of the six genes, the reported map position refines the existing cytogenetic chromosomal assignments. KALRN position was reported by GenBank as unpublished cytogenetic mapping data (13q41; Tang H, Ren J, Huang X, Yan X, et al., 2007, unpublished data) and our data assign this locus to SSC13 linked to S0075 marker at 34 cR. AK1 and PPIA genes had been previously mapped using a somatic cell hybrid panel to position 1q28-q213 and 18q24, respectively (Davoli et al., 2002).

The present study refines the position of AK1, by linking it to SW1301 (20 cR), and confirms the cytogenetic position of PPIA on SSC18 linked to S0177 at 50 cR distance.

Moreover, our data allow us to merge the two linkage groups (map1 and map2) identi-

fied by Shimogiri and co-workers (2006) as in our MYH1 study, which had been previously mapped to the linkage group "map2", is linked to the SWR1021 marker, which had been previously mapped to the linkage group "map1". Finally, our data, confirm the orientation of Shimogiri's map and the relative marker position.

Table 1. Physical map positions of the six porcine gene panels.

Gene	RH mapping assignment							
	Human accession No.	Human location	Porcine accession No.	Chromosome position[1]	Closest marker	Distance (cR)	LOD score	Retention (%)
KALRN	NM_001024660	3q21	EV825903	13q41	S0075	34	9.50	35
MYH1	NM_005963	17p13.1	AB025262	12q15	SWR1021	52	4.47	11
MLC2V	NM_000432	12q24.1	AJ487671	14q15	CATP3	19	15.15	22
SNX13	NM_015132	7p21.1	DY404375	9q14	SW1006	65	4.70	41
AK1	NM_000476	9q34.1	AY610127	1q213	SW1301	20	14.13	38
PPIA	NM_021130	7p13	AY008846	18q24	S0177	50	6.13	60

[1]Position inferred from the linked markers in the porcine cytogenetic map.

Two new genes were added to the swine physical map: MLC2V, which maps to SSC14 linked to CATP3 at 19 cR distance, and SNX13 located on SSR9 at 65 cR from SW1006.

In the same chromosomal regions where the six genes have been mapped, QTLs for ham weight (Milan et al., 2002; Yue et al., 2003), musculus biceps femoris and dissected ham ratio (Karlskov-Mortensen et al., 2006), conductivity 45 min post-mortem (Yue et al., 2003), average backfat (Rohrer and Keele, 1998) and shoulder weight (Milan et al., 2002) have been previously identified, suggesting these genes as positional candidate genes for swine meat quality. All genes investigated in the present paper are functional candidate genes for muscle metabolism: KALRN and MLC2V play an important role in muscular hypoxia and calcium ion binding pathways (Wardle et al., 2007; Smolensky et al., 2007); MYH1 is involved in oxidative muscle metabolism (Lefaucheur et al., 2004); MLC2V plays a role in calcium ion binding pathways (Smolensky et al., 2007), and SNX13 is one of the SNX family members involved in intracellular and vescicular trafficking (Carlton and Cullen, 2005).

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REFERENCES

Carlton JG and Cullen PJ (2005). Sorting nexins. *Curr. Biol.* 15: R819-R820.

Davoli R, Fontanesi L, Zambonelli P, Bigi D, et al. (2002). Isolation of porcine expressed sequence tags for the construction of a first genomic transcript map of the skeletal muscle in pig. *Anim. Genet.* 33: 3-18.

Diatchenko L, Lau YF, Campbell AP, Chenchik A, et al. (1996). Suppression subtractive hybridization: a method for generating differentially regulated or tissue-specific cDNA probes and libraries. *Proc. Natl. Acad. Sci. U. S. A.* 93: 6025-6030.

Fujii J, Otsu K, Zorzato F, de Leon S, et al. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253: 448-451.

Hu ZL, Dracheva S, Jang W, Maglott D, et al. (2005). A QTL resource and comparison tool for pigs: PigQTLDB. *Mamm. Genome* 16: 792-800.

Karlskov-Mortensen P, Bruun CS, Braunschweig MH, Sawera M, et al. (2006). Genome-wide identification of quantitative trait loci in a cross between Hampshire and Landrace I: carcass traits. *Anim. Genet*. 37: 156-162.

Le Roy P, Naveau J, Elsen JM and Sellier P (1990). Evidence for a new major gene influencing meat quality in pigs. *Genet. Res.* 55: 33-40.

Lefaucheur L, Milan D, Ecolan P and Le Callennec C (2004). Myosin heavy chain composition of different skeletal muscles in Large White and Meishan pigs. *J. Anim. Sci.* 82: 1931-1941.

Milan D, Bidanel JP, Iannuccelli N, Riquet J, et al. (2002). Detection of quantitative trait loci for carcass composition traits in pigs. *Genet. Sel. Evol.* 34: 705-728.

Rohrer GA and Keele JW (1998). Identification of quantitative trait loci affecting carcass composition in swine: I. Fat deposition traits. *J. Anim. Sci.* 76: 2247-2254.

Shimogiri T, Kiuchi S, Hiraiwa H, Hayashi T, et al. (2006). Assignment of 204 genes localized on HSA17 to a porcine RH (IMpRH) map to generate a dense comparative map between pig and human/mouse. *Cytogenet. Genome Res.* 112: 114-120.

Smolensky AV, Gilbert SH, Harger-Allen M and Ford LE (2007). Inhibition of myosin light-chain phosphorylation inverts the birefringence response of porcine airway smooth muscle. *J. Physiol.* 578: 563-568.

Wardle RL, Gu M, Ishida Y and Paul RJ (2007). Rho kinase is an effector underlying Ca^{2+}-desensitizing hypoxic relaxation in porcine coronary artery. *Am. J. Physiol. Heart Circ. Physiol.* 293: H23-H29.

Yerle M, Pinton P, Robic A, Alfonso A, et al. (1998). Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. *Cytogenet. Cell Genet.* 82: 182-188.

Yue G, Russo V, Davoli R, Sternstein I, et al. (2003). Linkage and QTL mapping for *Sus scrofa* chromosome 13. *J. Anim. Breed. Genet.* 120: 103-110.

