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Four genes located on a SSC2 meat quality QTL region are associated with different meat quality traits in Landrace × Chinese-European crossbred population

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Summary

Several quantitative trait loci (QTL) for different meat quality traits have been localized on the q arm of porcine chromosome 2 at position 55–78 cM. Association analyses were performed in a commercial Landrace × Chinese-European (LCE) crossbred population (n = 446) slaughtered at approximately 127 kg and an average age of 198 days with records for performance (growth, fat and meat accretion) and meat quality [intramuscular fat (IMF), Minolta L^*, Minolta a^*, Minolta b^* and pH at 45 m]. Polymorphisms within positional candidate genes cloned from homologous regions on human chromosome 19, *ubiquitin-like 5* (*UBL5* – AM950288:g.566G>A), *resistin* (*RETN* – AM157180:g.1473A>G causing substitution p.Ala36Thr), *insulin receptor* (*INSR* – AM950289:g.589T>C) and *complement factor D (adipsin)* (*CFD* – AM950287:g. 306C>T) were located at positions 62.1, 64.0, 68.0 and 70.7 cM respectively on the current USDA USMARC map of porcine chromosome 2 and had the following allele frequencies in the LCE: *UBL5* 566G – 0.57; *RETN* 1473G – 0.84; *INSR* 589C – 0.70; and *CFD* 306C – 0.73. The effects of alleles within the candidate genes on the recorded traits were estimated using an animal model. Significant effects ($P < 0.05$) were found for pH_{45} in m. semimembranosus (m. sm.) (*UBL5*), IMF (*RETN*) and Minolta L^* (*RETN*, *CFD*). Differences between phenotypic means of homozygotes at *UBL5*, *RETN* and either *RETN* or *CFD* explained 0.34 SD for pH_{45} in m. sm., 0.47 SD for IMF and 0.68 SD for Minolta L^* respectively. Suggestive effects ($P < 0.10$) on IMF (*UBL5*, *CFD*), Minolta a^* (*INSR*, *CFD*) and Minolta b^* (*INSR*) were also observed. Our results support the localization of further QTL for meat quality traits in this region and suggest that there are several genes affecting different meat quality traits.

Keywords association study, *complement factor D (adipsin)*, *insulin receptor*, meat quality, pig, porcine chromosome 2, *resistin*, *ubiquitin-like 5*.

Several QTL for meat quality traits have been mapped to the central part of porcine chromosome 2 (PigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, November 2010). The 95% confidence intervals of QTL for different meat quality traits are located in chromosome region 55–77.9 cM, with peaks scattered between 63.6 and 74.8 cM on the USDA USMARC linkage map (Fig. 1). According to Meyers *et al.* (2005), this chromosome region encompasses homologous regions of human chromosomes (HSA) 19 (0.75–17.59 Mb), HSA1 (243.68–224.92 and 176.25–179.16 Mb) and HSA5 (176.25–80.30 Mb) and spans approximately 25.4 Mb on porcine chromosome 2 [Sscrofa9 (April 2009 assembly), Ensembl release 61, Feb 2011].

The aim of this work was to clone or partially PCR clone four porcine genes involved in glucose and fat metabolism, located in the human homologous segment on HSA 19, as candidate genes for QTL affecting meat quality, to search for gene-tagged SNPs and to perform linkage mapping and association analyses in a commercial Landrace × Chinese-European (LCE) synthetic population.

We detected the following mutations. In the *ubiquitin-like 5* (*UBL5*) gene: AM950288:g.566G>A (NCBI-ss2755 23695) in the *resistin* (*RETN*) gene: AM157180:g.1473A>G

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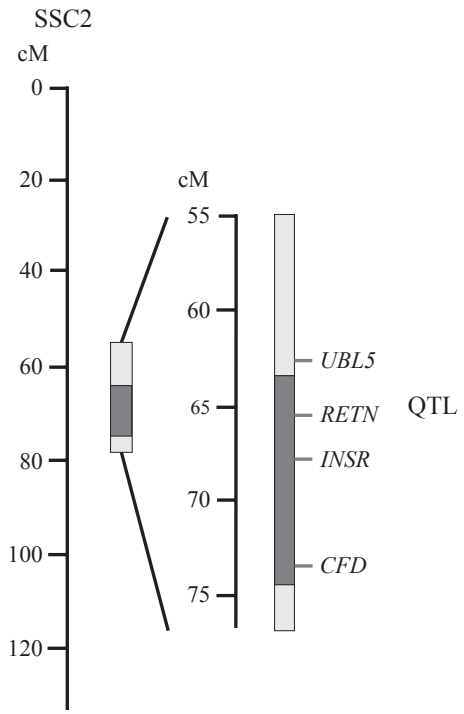

Figure 1 Map of the porcine chromosome 2 showing chromosome regions with 95% confidence interval of QTL for meat quality traits (light grey); the chromosome region with peaks of QTL for meat quality traits (dark grey); and positions of studied genes.

(NCBI-ss275523696), causing substitution p.Ala36Thr; in the *insulin receptor* (*INSR*) gene: AM950289:g.589T>C (NCBI-ss275523697) in intron 12 and in the *complement factor D* alias *adipsin* (*CFD*) gene: AM950287:g.306C>T (NCBI-ss275523698) in intron 3. For PCR amplification, sequencing and SNP detection see Appendix S1 and Table S1.

Allele frequencies of these SNPs in unrelated animals of eight breeds and wild boar are given in Table S2. In the LCE population, the SNPs had the following allele frequencies: *UBL5* 566G – 0.57, *RETN* 1473G – 0.84, *INSR* 589C – 0.70 and *CFD* 306C – 0.73.

Multipoint linkage analysis of SNPs within the *UBL5*, *INSR* and *CFD* genes, performed as described by Rohrer *et al.* (1994), placed the genes at the positions 62.1, 68.0 and 70.7 cM respectively on the current USDA USMARC linkage map of SSC2 (Fig. 1). The *RETN* gene was previously mapped to position 64.0 cM on the same linkage map (Čepica *et al.* 2002).

Statistical association analyses for the four SNPs were performed on 446 animals of the 12th–15th generations of a commercial Landrace × Chinese-European (LCE) synthetic population, with records for 15 traits described by Óvilo *et al.* (2006). Main statistics of the traits are given in Table S3. Animals were slaughtered at 127 ± 11.6 kg and an average age of 198 days.

The allele effects of the examined loci on the recorded production and meat quality traits were estimated with a standard animal model. The following univariate model was used for a separate analysis of each trait:

$$y = X\beta + Zu + e,$$

where y is a vector of trait records, β, u and e are vectors of fixed, additive genetic and residual effects, respectively, and X and Z are known incidence matrices. Fixed effects in X include slaughter batch (five levels) and the examined locus, with values 1, 0 and −1 for the genotypes. The slope of this covariate, b, estimates the additive allele substitution effect in the direction given in Table 1. Other covariates depend on the analysed trait: age at slaughter for growth traits and carcass weight for the rest of the traits.

The *UBL5* polymorphism was significantly associated with pH_{45} in m. semimembranosus (m. sm.) and suggestive for IMF. Animals with the *UBL5* g.566A allele had lower pH_{45} values in m. sm. in relation to animals carrying the *UBL5* g.566G allele ($P < 0.038$). Polymorphism within the *UBL5* gene explained 0.34 standard deviation (SD) for pH_{45} in m. sm. To date, suggestive QTL for pH_{45} have been reported at position 66 cM on SSC2 in Duroc × Landrace F_2 (Rohrer *et al.*2006) and White Duroc × Chinese Erhualian (Duan *et al.* 2009) resource populations. QTL for other pH values, such as pH_{24}, have been positioned previously on SSC2 at both ends and between positions 54–68 cM, but records for pH_{24} were not available for this population (pigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, November 2010). In search of novel factors involved in the regulation of energy metabolism, Collier *et al.* (2000) reported that the *UBL5* gene was over expressed in hypothalami of obese Israeli sand rats in comparison with lean littermates. Moreover, intracerebroventricular administration of *UBL%* resulted in a dose-dependent increase in food intake, body weight and neuropeptide Y gene expression in the hypothalamus. The human homologue, ubiquitin-5-like protein, has been shown to be prominently involved in the control of energy metabolism in humans (Bozaoglu *et al.* 2006; citations therein). In contrast, Sentinelli *et al.* (2008) reported that the *UBL5* gene is unlikely to play a major role in the genetic susceptibility to early onset obesity in children.

The *RETN* polymorphism was significantly associated with IMF ($P < 0.026$) and Minolta L^* ($P < 0.029$). Animals with the *RETN* g.1473A allele had lower values for IMF and Minolta L^* compared to animals with the *RETN* g.1473G allele, and phenotypic differences between homozygotes were equal to 0.47 and 0.68 SD for IMF and Minolta L^* in m. longissimus lumborum et thoracis (m.l.l.t.) respectively. A QTL for IMF was detected at position 67 cM, and QTL for L^*colour were reported at positions 62.4 and 72.4 cM (pigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, November 2010) respectively. Human plasma resistin levels are affected by polymorphisms in the

Table 1 Mean allele substitution effects of *UBL5*, *RETN*, *INSR* and *CFD* polymorphisms for the significantly affected traits in the Landrace × Chinese-European synthetic population (LCE).

		UBL5 566A>G		*RETN* g.1473A>G		*INSR* 589g.T>C		*CFD* g.306T>C	
Trait/locus	*n*	*b* (SD)	LR (*P* value)	*b* (SD)	LR (*P* value)	*b* (SD)	LR (*P* value)	*b* (SD)	LR (*P* value)
Meat quality									
pH_{45}[1]	329	**0.058 (0.028)**	**4.290 (0.038)***	0.028 (0.036)	0.565 (0.452)	−0.003 (0.036)	0.007 (0.935)	−0.058 (0.037)	2.370 (0.124)
Intramuscular fat, %[2]	335	−0.132 (0.075)	3.194 (0.074)†	**0.228 (0.104)**	**4.934 (0.026)***	0.023 (0.098)	0.061 (0.805)	0.175 (0.100)	3.140 (0.076)†
Minolta L^*[2]	176	−0.752 (0.521)	2.093 (0.148)	**1.529 (0.692)**	**4.791 (0.029)***	0.223 (0.660)	0.117 (0.733)	**1.527 (0.672)**	**5.071 (0.024)***
Minolta a^*[2]	176	−0.086 (0.170)	0.259 (0.610)	−0.162 (0.229)	0.521 (0.470)	−0.400 (0.206)	3.742 (0.053)†	−0.372 (0.221)	2.814 (0.093)†
Minolta b^*[2]	176	−0.155 (0.169)	0.865 (0.352)	0.081 (0.228)	0.127 (0.721)	−0.394 (0.205)	3.631 (0.057)†	0.153 (0.221)	0.490 (0.484)

Apart from the significantly associated traits, the following traits were tested: live weight 187 days, backfat 160 days, backfat 187 days, carcass backfat P2, carcass backfat GM, carcass backfat S, shoulder weight.
[1]m. semimembranosus, [2]m. longissimus lumborum et thoracis; significant association at level $^*P < 0.05$ (in bold); †$P < 0.10$.

promoter region of its gene and are slightly but significantly associated with reduced glucose uptake in adipocytes (Nogueiras *et al.* 2010; citations therein). Over expression of a resistin transgene in rats has been associated with increased muscle triglycerides (Pravenec *et al.* 2003). The porcine *RETN* gene was reported to be associated with backfat thickness in Polish Large White populations (Cieslak *et al.* 2009) and with total loin lipids in a Berkshire × Yorkshire F_2 population (Otieno *et al.* 2005). Our finding of an association between the *RETN* gene and IMF in the commercial population is important, as IMF is considered to play a key role in pork quality (Fernandez *et al.* 1999).

The *CFD* polymorphism was significantly associated with Minolta L* in m.l.l.t. ($P < 0.024$) and suggestively associated with IMF ($P < 0.076$) and Minolta a^* ($P < 0.093$). Homozygous animals for the *CFD* g.306T allele showed lower Minolta L^* and IMF and higher Minolta a^* values as compared to animals carrying the *CFD* g.306C allele. Phenotypic differences between homozygotes explained 0.68 SD for Minolta L^* in m.l.l.t. The *CDF* gene encodes a serine protease adipsin that participates in the formation of acylation-stimulating protein (ASP) from complement C3 protein and factor B. Acylation-stimulating protein, which is produced by adipocytes, is a main anabolic stimulator of triglyceride storage in adipose tissue (Cianflone *et al.* 2003).

The *INSR* gene was suggestively associated with Minolta a^* ($P < 0.053$) and Minolta b^* ($P < 0.057$). The *INSR* g.589T allele resulted in higher values for Minolta a^* and Minolta b^* values compared to the *INSR* g.589C allele. *INSR* is a key gene of the insulin signalling pathway. Muscle-specific *INSR* knockout mice examined in other studies exhibited elevated fat mass, serum triglycerides and free fatty acids (Brüning *et al.* 2000).

For fine mapping of quantitative trait loci (QTL), it is important to know the amount of linkage disequilibrium (LD) between the markers and the extent of LD in populations. To estimate the amount of LD between the studied loci, the two-locus LD measure r^2 (Hill & Robertson 1968), which is used for evaluation of LD for association studies, was used. The r^2 ranges from 0 (no LD) to 1 (complete LD), and it is supposed that $r^2 > 0.3$ is an appropriate threshold for a whole genome association study between marker and causative loci in pigs (Du *et al.* 2007). Estimated two-locus LD were as follows: *UBL5* – *RETN* ($r^2 = 0.048$), *RETN* – *INSR* ($r^2 = 0.072$) and *INSR* – *CFD* ($r^2 = 0.494$), implying that association of the *UBL5* gene with pH_{45} and association of the *RETN* gene with IMF and Minolta L^* revealed independent QTL, as only a negligible amount of LD exists between these markers. Contrary to this, associations of the *CFD* and the *INSR* genes could be related to just one QTL because of existing LD ($r^2 = 0.494$) between these genes.

The SNPs investigated here are potential markers for meat quality traits, which may be located within causative genes. Our results support localization of QTL for meat

quality traits to the chromosome region homologous with a HSA19 and suggest that there are at least three genes (*UBL5*, *RETN* and *CFD*) affecting different meat quality traits. A higher number of gene-tagged markers with a known gene order are needed for detailed multimarker linkage disequilibria mapping of these QTL.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Further details of the methodology.

Table S1 Description of source sequences used for cloning or PCR cloning, sequences referring to the SNPs, primers, PCR conditions and restriction enzymes used for genotyping of the examined loci.

Table S2 Allele frequencies at four gene-tagged SNPs in eight pig breeds and European wild boar.

Table S3 Main statistics of the recorded traits in Landrace × Chinese-European synthetic population.

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