Selective Genotyping Analysis of 677 SNPs to Identify Markers Associated with Back Fat Thickness in Italian Large White Pigs

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Introduction

Fatness in pigs is an important trait that affects carcass value and consumers’ acceptance of pork. On the other hand, an appropriate fat coverage of the legs is needed for dry-cured ham production (Bosi and Russo (2004)). In addition, considering fat deposition traits, the pig could represent an interesting animal model for human obesity (Lunney (2007)). Back fat thickness (BFT) is a measure that can be easily recorded and that is usually included as a target trait in selection programs in purebred pig populations and commercial pig lines. Estimated breeding values (EBVs) for this trait are routinely calculated in pig nuclei under selection.

Several human obesity-related genes have been also investigated in pigs with the objective to identify DNA markers associated with fat deposition traits. For example, variability in the fat mass and obesity associated gene (FTO), that is a major determinant in predisposing common obesity in human (Dina et al. (2007); Frayling et al. (2007)), affects intermuscular fat content in Italian Duroc pigs and fatness traits in other pig populations (Fan et al. (2009a); Fontanesi et al. (2009); (2010)).

Selective genotyping for QTL mapping in farm animals has been proposed to reduce the cost of genotyping without losing much power (Darvasi and Soller (1992)). In pigs, this approach can be applied within populations and lines taking advantage from the large number of animals that are evaluated in selected nuclei and choosing for genotyping the animals with extreme high and low EBV for a recorded trait (Fontanesi et al. (2009); Heuven et al. (2009)). This approach is similar to the case and control experiment designs applied in human genetics studies (Cardon and Bell (2001)) but the differences are that i) in animals it is possible to use EBVs for the constitutions of the two groups and for this reason they can be much more informative in terms of genetic differences and that ii) the extreme tails maximize the distance in terms of trait value.

Here we identified, combining different approaches (sequencing, literature mining, and in silico porcine expressed sequence tag database mining), a few hundreds of single nucleotide polymorphisms (SNPs) in candidate genes for fat deposition traits and genotyped these
markers in Italian Large White pigs using a selective genotyping approach based on EBV for BFT.

**Material and methods**

**Animals.** This study was conducted on Italian Large White pigs individually performance tested at the Test Station of the National Pig Breeder Association (ANAS). These animals are structured on triplets of siblings from the same litter (two females and one castrated male). Data are used for the genetic evaluation of a boar from the same litter (sib-testing). Two extreme and divergent groups of females (evaluated in the period 1996-2007) were chosen according to their EBV for BFT (280 with most negative and 280 with most positive EBV) among a population of ~12,000 pigs. Average EBVs for the most negative and positive selected animals were $-9.4 \pm 1.6$ and $+8 \pm 5.95$, respectively. All animals did not have common parents. For the chosen 560 pigs, genomic DNA was extracted from blood.

**SNP identification and genotyping.** SNPs were identified by resequencing or de-novo sequencing of parts of 60 obesity candidate genes. Gene fragments were amplified from a panel of 10-12 pigs belonging to different breeds and from four pools of DNA constituted each by 5 equimolar genomic DNA samples obtained from Italian Large White pigs with extreme divergent EBVs for BFT. PCR primers were designed on porcine sequences identified through BLAST analysis using homologous human cDNA sequences. Identification of SNPs was also conducted systematically analysing literature for pig markers associated with fat deposition traits. Mining porcine expressed sequence tags for in silico SNP identification was obtained using a specifically designed pipeline that can recruit genes involved or predicted to have a role in fat metabolism and fat deposition traits (Fronza et al. (2009)). Selection of candidate genes was based on the human interactome. This module was designed to expand a core of genes associated with obesity, derived from a literature and database survey, in order to define an enlarged candidate gene dataset putatively involved in this disease. Mapping of the identified SNPs was obtained by BLAST analysis on the Sscrofa9 genome assembly at the Ensembl database and/or by analysing the INRA-Minnesota 7000 rads radiation hybrid panel (Yerle et al. (1998)). SNP genotyping was carried out for the chosen pigs using GoldenGate (Illumina) platform.

**Statistical analyses.** Breeding values for BFT (expressed in mm), were estimated for the performance tested pigs using a BLUP-multiple trait animal model including fixed factors of age at the beginning of test, weight at slaughter, age at slaughter, day of slaughtering and inbreeding coefficient. SNPs with minor allele frequency (MAF) >0.05 (calculated in the whole genotyped population) were evaluated for allele frequency differences between the two extreme groups of pigs using a chi-square test implemented in R. Correction for multiple testing was obtained controlling the Proportion of False Positives (PFP; Fernando et al. (2004)). PFP thresholds were calculated as described in Bagnato et al. (2008). A PFP threshold of 0.20 was used to declare significance for single marker tests.

**Results and discussion**

Sequencing of fragments amplified from 60 obesity related genes identified 84 SNPs, 67 of which were chosen for genotyping. Other 131 SNPs obtained from published literature on
markers associated with fat deposition traits in pigs were included in the genotyping SNP panel. In silico expressed sequence tags database mining reported 7976 SNPs. Of these SNPs, 565 were analysed. On the whole adding the three different sources from which we identified SNPs, 763 SNPs were included in the GoldenGate panel that was used to genotype the 560 Italian Large White pigs. For 86 SNPs the designed GoldenGate tests were not successful leaving 677 SNPs: 169 SNPs were monomorphic in the analysed pigs; 187 SNPs had a MAF<0.05; and 321 had MAF >0.05. Allele frequency differences between the two extreme groups of pigs chosen according to their BFT EBV were evaluated for 321 SNPs with MAF >0.05. Of these SNPs, 65 showed P nominal value <0.10. PFP thresholds of 0.10 and 0.20 corresponded to P nominal values of 0.003 and 0.025, and included 8 and 30 SNPs, respectively. For brevity, only genes whose polymorphisms were significantly associated with BFT at PFP<0.10 are reported in Table 1.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>P &lt;sup&gt;α&lt;/sup&gt;</th>
<th>SNP type</th>
<th>SNP location</th>
<th>Chromosome</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBC1D1</td>
<td>0.000137</td>
<td>G&gt;A</td>
<td>Intron 2</td>
<td>SSC8&lt;sup&gt;β&lt;/sup&gt;</td>
<td>Resequencing</td>
</tr>
<tr>
<td>CALR</td>
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<td>A&gt;G</td>
<td>Exon 1</td>
<td>SSC2</td>
<td>In silico</td>
</tr>
<tr>
<td>PPARG</td>
<td>0.000690</td>
<td>A&gt;G</td>
<td>5′-UTR</td>
<td>SSC13</td>
<td>Literature&lt;sup&gt;γ&lt;/sup&gt;</td>
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<td>T&gt;C</td>
<td>Exon 23</td>
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<td>In silico</td>
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<td>Exon 7</td>
<td>SSC1</td>
<td>In silico</td>
</tr>
<tr>
<td>RRAGD</td>
<td>0.002064</td>
<td>T&gt;C</td>
<td>Intron 3</td>
<td>SSC1</td>
<td>Literature&lt;sup&gt;δ&lt;/sup&gt;</td>
</tr>
<tr>
<td>MC4R</td>
<td>0.002220</td>
<td>A&gt;G</td>
<td>Exon 1</td>
<td>SSC1</td>
<td>Literature&lt;sup&gt;ε&lt;/sup&gt;</td>
</tr>
<tr>
<td>VCAM1</td>
<td>0.002281</td>
<td>T&gt;C</td>
<td>Exon 5</td>
<td>SSC4</td>
<td>In silico</td>
</tr>
</tbody>
</table>

<sup>α</sup>P nominal value.
<sup>β</sup>Mapped using the radiation hybrid panel.
<sup>γ</sup>Fan et al. (2009b).
<sup>δ</sup>Grapes and Rothschild (2006).
<sup>ε</sup>Kim et al. (2000).

Most of these genes map in chromosome regions in which QTL for BFT and other related traits have been localized. Interestingly, the MC4R missense mutation (p.Asp298Asn) already shown by different studies to affect growth rate, feed efficiency and fatness (Kim et al. (2001)) is significantly associated with BFT in the Italian Large White breed, confirming its role in affecting carcass and performance traits. The role of the other significant genes on BFT remains to be further evaluated as they could have a direct or indirect effect on the physiological mechanisms that regulate energy metabolism, adipocyte formation and related aspects.

**Conclusion**

This large scale genotyping of SNPs in candidate genes for fat deposition traits provided DNA markers that could be applied in selection programs in the Italian Large White pig breed. Moreover, the recruitment strategy of candidate genes based on the human interactome followed by an association study of *in silico* detected SNPs represents an
innovative approach that may open new possibilities for the identification of genes affecting obesity-related traits in pig enforcing the role of this species as a model for human obesity.

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References