Genome wide perspective of genetic variation in pig metabolism and production traits

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ABSTRACT: In this study we present a first genomics-metabolomics approach in pigs. About 900 performance tested Italian Large White pigs were genotyped with the Illumina PorcineSNP60 BeadChip. Blood was collected at slaughtering for haematologial analyses. Plasma was used for the determination of 186 metabolites using mass spectrometry. Performance and carcass traits were measured on the same animals. Heritability of metabolotypes and haematological parameters ranged from 0.04 to 0.93. A Graphical Gaussian Model (GGM) was generated including partial correlation coefficients. The structure of the obtained network represented clearly the general biological relationships among metabolites. GGM analyses suggested that pig metabolism is very close to the human metabolism. Genome wide association studies, carried out for a total of 153 traits, identified genetic variation in genes directly involved in the metabolism of several metabolites that could open interesting applications in pig nutrigenomics.

Keywords: metabolomics; systems biology; genome wide association study

Metabolomics is the study of a large range of metabolites generated from the characterization of biological samples (Suhre and Gieger (2012)). Metabolites represent phenotypes (metabotypes) that are the direct products of the activities of enzymes included in all metabolic pathways. Therefore, they represent internal phenotypes that can be used to dissect more complex phenotypes like performance traits in animals (Houle et al. (2010)).

In this study we present a first genomics-metabolomics approach in highly phenotyped pigs. This dataset provided different levels of information for GWAS with about 230 different traits (performance and carcass traits, haematological parameters and metabolotypes). Metabotype data were also used to model a systems biology approach that identified close metabolic similarities between humans and pigs.

Materials and Methods

Animals. About 900 performance tested Italian Large White pigs were included in this study. These pigs were from the national selection program of the Italian Large White breed that is based on triplets of siblings from the same litter (two females and one castrated male) individually performance tested at the Central Test Station of the National Pig Breeder Association (ANAS) for the genetic evaluation of a boar from the same litter (sib-testing). Pigs were slaughtered in a commercial abattoir in different groups of 30-60 pigs.

Data. Several performance traits (average daily gain and feed:gain ratio), carcass traits (ham weight, backfat thickness, weight of several cuts) were determined on all animals in vivo or after slaughtering.

In addition, blood was collected at slaughtering for biochemical and haematological analyses. Plasma was used for the determination of 186 metabolites using a combined Direct Flow Injection (DFI-) and liquid chromatography (LC-) coupled with tandem mass spectrometer (LC-MS/MS) Triple Quadrupole. Metabolite data were filtered using inter- and intra-plates coefficient of variation <0.20. The normalization of metabolite and haematological data

Introduction

Holistic approaches based on the integration of different omics technologies are clarifying the biological processes underlying many phenotypic and pathological traits in humans and in many other species.

In livestock, high throughput genotyping platforms have changed the possibility to dissect genetic variability of performance traits applying genome wide association studies (GWAS). In pigs, GWASs were mainly conducted for a few performance traits (e.g. Fontanesi et al. (2012)). However, despite this approach has produced a quite large number of single nucleotide polymorphisms (SNPs) associated to the target traits, only in few cases significant SNPs could be directly linked to a biological process explaining variability of the investigated parameter. Therefore it seems that a quite large distance exists between the genotype space (genome information of the animals) and the final production traits that dilutes the effects of the markers reducing the proportion of variability explained in GWAS, usually conducted with a relatively low number of animals.
was performed with a BoxCox transformation using a lambda which presented the best log likelihood.

Pigs were genotyped with the Illumina PorcineSNP60 BeadChip (Ramos et al. (2009)).

**Statistical analyses.**
The Pearson phenotypic correlation coefficients among metabotypes and performance and carcass traits were calculated with the CORR procedure of SAS (SAS Inst. Inc., Cary, NC). Variance components, genetic parameters and their standard errors were estimated by using VCE software (Neumaier and Groeneveld (1998); Groeneveld et al. (2010)). Bivariate mixed linear animal models, for each parameter with backfat thickness, considered the significant effects of date of slaughtering (26 levels), sex (2 levels), and carcass weight (covariates). Animal (22785 pigs) and residual random effects were assumed to follow normal distributions with zero mean.

Gaussian Graphical Model (GGM) was obtained with metabolomic data. GGM is based on pairwise Pearson correlation coefficients corrected for the correlations with the other metabolites. The partial correlation coefficients were computed with R package “corpcor”. Then the list of all the correlations coefficient was extracted from the matrix and used as input in Cytoscape (Version. 3.0.1).

GWAS was carried out with GEMMA (Zhou and Stephens (2012)) after filtering for minor allele frequency <0.05 and Hardy Weinberg equilibrium <0.001. Different covariates were included in the model according to the traits (sex, weight, slaughtering date (range: 1-25), kit plate (range:1-14) and the centered genomic matrix, to exclude the stratification of the population due to the relatedness.

**Results and Discussion**

**Correlations and heritability.** After quality control and filtering 113 metabolites were selected for further analyses. Figure 1 shows the Pearson phenotypic correlation coefficients among metabotypes and production and carcass traits. Metabolites within classes were usually more correlated to each other than with metabolites of other classes. Heritability of metabolites and haematological parameters within the same classes varried substantially (Table 1). Heritability of backfat thickness was similar to previous estimates for this trait (0.60).

**Table 1. Summary of heritability (h², minimum and maximum) of metabolites, haematological parameters and backfat thickness**

<table>
<thead>
<tr>
<th>Classes</th>
<th>h² min</th>
<th>h² max</th>
<th># of metabolites/parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylcarnitines</td>
<td>0.07</td>
<td>0.34</td>
<td>10</td>
</tr>
<tr>
<td>Amino acids</td>
<td>0.07</td>
<td>0.45</td>
<td>20</td>
</tr>
<tr>
<td>Biogenic amines</td>
<td>0.21</td>
<td>0.50</td>
<td>9</td>
</tr>
<tr>
<td>Hexoses</td>
<td>0.14</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>PC ae</td>
<td>0.05</td>
<td>0.72</td>
<td>27</td>
</tr>
<tr>
<td>PC aa</td>
<td>0.16</td>
<td>0.73</td>
<td>27</td>
</tr>
<tr>
<td>Lyso PC a</td>
<td>0.05</td>
<td>0.48</td>
<td>9</td>
</tr>
<tr>
<td>Sphingomyelines</td>
<td>0.14</td>
<td>0.47</td>
<td>10</td>
</tr>
<tr>
<td>Haematological parameters</td>
<td>0.04</td>
<td>0.93</td>
<td>33</td>
</tr>
<tr>
<td>Backfat thickness</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Gaussian Graphical Model.** A GGM was generated including all partial correlation coefficients (PCC) that were above 0.21. This threshold corresponded to a Bonferroni corrected threshold of 0.05. The structure of the obtained network represented clearly the general biological relationships among metabolites (within classes) and among different classes of metabolites and included several substructures (Figure 2). The highest PCCs were obtained between metabolites that are very close in terms of positions in the metabolic pathway. In particular, SM C18:0 and SM C18:1 that showed a PCC of 0.77 are separated to each other by just one enzymatic reaction. These results,
together with many other PCC between different pairs of metabolites (data not shown) confirmed in pigs the metabolic relationships and pathways already described in humans (Krumsiek et al. (2011)). GGM analyses suggested that pig metabolism is very close to the human metabolism.

Figure 2. Gaussian Graphical Model obtained using pig metabolomic data.

**Genome Wide Association Studies.** GWAS was carried out for a total of 153 traits (113 metabolotypes, 33 haematological parameters and 7 production and carcass traits. Significant SNPs (P<0.10, Bonferroni corrected) were obtained for 20 metabolotypes, 6 haematological parameters and 4 production/carcass traits. In particular, for most metabolotypes, significant SNPs were close or within genes directly involved in the catabolic or anabolic pathways of the targeted metabolites. A few of these markers were associated (P nominal value <0.01) with production and carcass traits.

**Conclusion**

This study reports for the first time the analysis of heritability of a large number of metabolotypes in pigs and describes a systems biology analysis based only on metabolomics data (GGM). GGM identified biological relationships between metabolites already described in humans supporting indirectly the quality of the data we obtained using a targeted metabolomic approach. GWAS identified genetic variation in genes directly involved in the metabolism of several metabolites that could open interesting applications in pig nutrigenomics. GWAS using metabolomics data can help to dissect the biological complexity of performance traits in livestock.

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**Literature Cited**


