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Intramuscular fat selection in rabbits modifies microbial genes for energy metabolic routes (#499)

Marina Martínez Alvaro, Agostina Zubiri, Agustín Blasco, Pilar Hernández

Univesitat Politècnica de València, Institute for Animal Science and Technology, Valencia, Spain

Introduction

Intramuscular fat content (IMF) improves meat quality by increasing the tenderness, juiciness and flavour. An experiment to study the genetic basis of IMF deposition was developed in rabbits, consisting of two lines from a common genetic origin and divergently selected for IMF for ten generations. The IMF difference between lines in the 9th generation was 0.44 g/100g. In this study we investigate the changes on microbe's genome in cecum, identifying the metabolic routes modified by the selection for IMF. Out hypothesis is the existence of cause consequence link between the host genetic profile and their metagenomics composition.

Methods

Thirty-three rabbits from the 10th generation of selection; 16 and 17 from each of the two selected lines for high (H) and low (L) IMF, respectively, were slaughtered at 9 wk of age after a 4 hours of fasting period. Cecum content samples were collected immediately after slaughter, frozen in liquid nitrogen and stored at -80ºC. Samples were sequenced with an Illumina NextSeq instrument. Generated paired-end reads were in average of 2 x 150 bp of length. Reads were aligned to KEGG genes database (http://www.kegg.jp), and 6230 microbial genes were identified. Metagenomic data were pre-processed using the centered log ratio (clr) transformation due to their compositional nature. Distinct microbial genes between the two lines were identified using Projection to Latent Structures Discriminant Analysis (DA-PLS, SIMCA, P+ 15.0.1, Umetrics (Umea, Sweden)) with a H/L categorical vector as dependent variable and the microbial genes as independent variables. The selection of variables was based on the variable importance for projection criterion (VIP) where we consider microbial genes with a VIP<1.0 contribute little to the prediction and are removed until the DA-PLS model starts losing predictive ability (Q^2). The final model was built with 5 latent components after a cross validation procedure, including 65 microbial genes. The metabolic pathways involving these genes were identified.

We focused our attention in a set of 10 genes involved in energy metabolic routes in the gut microbiome. A new DA-PLS model was analysed including only these 10 genes. Only one latent component was selected after cross validation. Then, we estimated the marginal posterior distributions of the differences between H and L lines for the relative abundance of these genes using the Rabbit software (Institute for Animal Science and Technology, Universitat Politècnica de València). From these marginal posterior distributions, we obtained their median and the probability of the difference being positive when the difference was higher than zero or negative when it was lower than zero (P).

Results

A correlated response to selection for IMF was shown in the relative abundance of 65 microbial genes in rabbit cecum, identified by DA-PLS. These 65 genes were able to explain a 90.0% of the H and L classification variability after a cross validation model test (Q^2). These 65 microbial genes coded for proteins involved in several metabolic pathways, being the most important: energy metabolism (10 genes), signal transduction (6), lipids (1), nucleotides (1) and aminoacids metabolism (3), membrane transport (3), replication and repair (2), translation (1) or metabolism of cofactors and vitamins (2). In this work, we focused in the microbial genes involved in the energy metabolic pathways. Analysing these genes in DA-PLS model, they explained a 51% of the total variability (Q^2).

Table 1 shows microbial genes involved in the energy metabolism showing different relative abundance between lines. Line H showed a higher abundance of three genes, one involved in methane metabolism (K14067 malate-CoA ligase subunit beta, P=1.00), the second involved in carbon metabolism (K00029 malate dehydrogenase, P =0.92) and the last in the metabolism of mannose and fructose (K01813 L-rhamnose isomerase, P = 1.00). In L line, seven genes were more abundant, two of them involved in amino sugar and nucleotide sugar metabolism (K15897 UDP-2,4-diacetamido-2,4,6-trideoxy-beta-L-altropyranose hydrolase and K13015 UDP-N-acetyl-D-glucosamine dehydrogenase with P = 0.95 and P = 0.98, respectively).

Conclusion

This preliminary analysis highlights the importance of the gut microbiome in the muscular lipid deposition in rabbits and shows that selection for IMF led to a correlated response in their metagenomics profile. In this study, we focus on the correlated response to selection in the microbial genes involved in the energy metabolism, and we show an enrichment of different metabolic routes in H or L lines. These results imply a link between the genes of the individual and the genes of its gut microbes.

Notes

Table 1. Microbial genes involved in the energy metabolism showing different relative abundance in the line selected for high (H) or low (L) IMF.

KEGG	Gene description	H-L ($x10^{-5}$)	P
K01813	L-rhamnose isomerase	3.73	1.00
K00029	malate dehydrogenase (oxaloacetate-decarboxylating)(NADP+)	3.21	0.92
K14067	malate-CoA ligase subunit beta	0.67	1.00
K01208	cyclomaltodextrinase	-3.98	1.00
K01573	oxaloacetate decarboxylase, gamma subunit	-2.25	0.98
K00362	nitrite reductase (NADH) large subunit	-2.05	0.99
K15897	UDP-2,4-diacetamido-2,4,6-trideoxy-beta-L-altropyranose hydrolase	-2.00	0.95
K03079	L-ribulose-5-phosphate 3-epimerase	-1.12	1.00
K13015	UDP-N-acetyl-D-glucosamine dehydrogenase	-0.75	0.98
K16951	anaerobic sulfite reductase subunit B	-0.52	0.99

H-L = median of the difference between lines measured in units of SD; P = probability of H-L being greater than 0 when positive or lower than 0 when negative.

Table 1.

Notes