GENOME-WIDE ASSOCIATION STUDY FOR FATTY ACID COMPOSITION IN RABBITS

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ABSTRACT

Fatty acid composition is a key trait influencing the nutritional and organoleptic quality of meat. A divergent selection experiment for intramuscular fat content in rabbits was performed during nine generations, showing a correlated response on the fatty acids profile. A genome-wide association study was carried out on the selected lines in order to identify genomic regions associated with the fatty acid composition. The studied traits were saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the ratios PUFA/SFA, MUFA/SFA, and N6/N3. Bayesian statistics under the Bayes B model were used to analyze the genomic data of 475 rabbits from the two lines. Main genomic regions harboring genes related to lipid metabolism were identified in rabbit chromosomes (OCU) OCU1, OCU3, OCU8, OCU9, OCU17, and OCU18. A relevant region at 46.0-49.2 Mb on OCU18 overlapped for SFA, MUFA, and the ratio MUFA/SFA. This region explained 7.91%, 1.90%, and 0.55% of the genomic variances of MUFA/SFA, MUFA, and SFA, respectively. In addition, an important genomic region at 34-37.9 Mb on OCU1 overlapped for SFA, PUFA, PUFA/SFA, and N6/N3. This region explained up to 11.32 %, 1.89%, 2.78%, and 1.26% of the genomic variance of SFA, PUFA, PUFA/SFA, and N6/N3, respectively. Main genes retrieved by functional analyses were: *SCD, PLIN2, ERLIN1*, and *LIPC*. These genes bear a crucial role in lipid metabolism. The main genomic regions in which we found genes related to lipid metabolism were not previously detected in our experiment for IMF. *MTMR2* is the only gene that was associated with both the fatty acid composition and the IMF content. Further analyses would be necessary in order to corroborate the associations identified by this study.

Key words: divergent selection, fatty acids, genomic analysis, intramuscular fat, rabbits.

INTRODUCTION

Intramuscular fat (IMF) and the fatty acid composition are closely related to meat nutritional and organoleptic quality. A divergent selection experiment for IMF content in rabbits was carried out at the Universitat Politècnica de València during nine generations. This selection led to a correlated response on the fatty acid composition. A companion paper has reported that the correlated response to selection was higher in the high-IMF line for SFA and MUFA fatty acids percentages than in the low-IMF line. The correlated response was negative for PUFA percentage, with higher values for the low-IMF line than for the High-IMF line (Laghouaouta et al., 2020). Genomic studies reported genetic markers and genes having major effects on the fatty acid composition in pigs (Ros-Freixedes et al., 2016) and in beef cattle (reviewed by Gotoh et al., 2018). Besides, we have previously identified genomic regions associated with IMF content in our divergent selection experiment (Sosa-Madrid et al., 2019).

In this study, we carried out genome-wide association studies (GWAS) using the previously mentioned divergently selected rabbit lines, in order to identify genomic regions associated with the fatty acid composition, and also to generate a list of putative candidate genes.

MATERIALS AND METHODS

Animals and experimental design

Rabbits came from the ninth generation of a divergent selection experiment for IMF content (Sosa-Madrid et al., 2019). The study was carried out on 475 individuals (high-IMF line: 239 and low-IMF line: 236). Each individual was slaughtered and dissected to obtain samples from the left *longissimus thoracis et lumborum* muscle (LM). We used the chemical method described by O'Fallon et al. (2007) to obtain fatty acid methyl esters (FAME) from the lyophilised LM. After that, we determined the fatty acid composition by gas chromatography (FOCUS, Thermo, Milan, Italy). The studied traits were SFA, MUFA, PUFA, and the ratios PUFA/SFA, MUFA/SFA and N6/N3.

Rabbits were genotyped using the Affymetrix Axiom OrcunSNP Array. The SNP array contains 199,692 molecular markers. Quality control and genotype calling were performed with Axiom Analysis Suite v. 4.0.3 of Thermo Fisher Scientific. The SNP data were filtered using the following quality control criteria: call rate ≥ 0.95, minor allele frequencies ≥ 0.05, and only SNPs with known chromosome position. Further, animal samples with a missing genotype frequency greater than 0.05 were excluded from the data set. After quality control, 475 animals and 90,235 SNPs were used for the association analysis.

Statistical analysis and functional enrichment analysis

The GWAS were performed using the Bayes B model:

$$\boldsymbol{y} = \boldsymbol{Xb} + \sum_{j=1}^{k} \boldsymbol{z}_j \alpha_j \delta_j + \boldsymbol{e}$$

where $\boldsymbol{y}$ is the phenotypes vector; $\boldsymbol{X}$ is the incidence matrix for fixed effects; $\boldsymbol{b}$ is the vector with the fixed effects of month (five levels), sex (two levels) and parity order (three levels); $\boldsymbol{z}_j$ is the vector of genotypes for a SNP at locus j (j = 1,…, k, where k is the number of SNPs after quality control); α_j is the random substitution effect for SNP j; δ_j is a random 0/1 variable that represents the presence ($\delta_j = 1$ with probability 1-π) and the absence ($\delta_j = 0$ with probability π) of the SNP j and $\boldsymbol{e}$ is the vector of the random residuals. The proportion of SNPs with zero effects in each iteration was determined using predefined values for the parameter π (0.9988) (Ros-Freixedes et al., 2016, Sosa-Madrid et al., 2019). Residuals were considered to be independent and normally distributed. The marginal posterior distributions of the model parameters were estimated using Monte Carlo Markov chains. A total of 500,000 iterations were performed, with a burn-in of 100,000, and a lag of 40. The genomic variance was computed as the posterior distribution of the percentage of the total genetic variance explained by the SNP markers within 1Mb non-overlapping windows. In this study, 1982 non-overlapping genomic windows of 1-Mb were a priori allocated to the 21 autosomes. The association between a trait and a genomic region was assessed by the window contribution to the genomic variance. Genomic windows that explained at least 1.0% of the genomic variance of a particular trait were considered to be associated. In addition, the genomic windows exceeding 0.5% of the genomic variance and having SNPs with a Bayes factor greater than 10 were also considered as associated. The associated regions were extended to ± 500 Kb from the first and latest associated SNP in order to consider nearby associated SNPs. Genes were retrieved from these extended regions. The GenSel software (Garrick & Fernando, 2013) was used for the analysis.

The gene annotations were obtained from the Ensembl Genes 98 database using the Biomart Software (https://www.ensembl.org/biomart/martview/f73c148e17e81673ab0eab4444a2586c). The metabolic pathways of these genes were retrieved from DAVID (Database for Annotation, Visualization and Integrated Discovery) v.6.8 (Jiao et al., 2012). We focused on the functions and pathways related to lipid metabolism.

RESULTS AND DISCUSSION

The associated regions with the fatty acid composition of LM muscle were spread across different chromosomes (OCU) (OCU1, OCU3, OCU5, OCU6, OCU8, OCU9, OCU10, OCU13, OCU14, OCU15, OCU16, OCU17, and OCU18; Table 1).

A large genomic region at 34-37.9 Mb on OCU1 accounted for the highest genomic variance of SFA (11.32%), with a Bayes factor up to 115. The same region was associated with PUFA, PUFA/SFA, and N6/N3, explaining 1.89%, 2.78%, and 1.26% of the genomic variance, respectively. This region included two important genes, *ACER2* and *PLIN2*. The *ACER2* gene is involved in lipid metabolism, while *PLIN2* is implicated in the accessibility of lipases to the stored fats in response to the energy demands of the cells. In addition, Gol et al. (2016) reported that *PLIN2* gene could be a useful marker for lean growth in pigs. The 148.5-150.2 Mb genomic region on OCU3 was associated with SFA, MUFA, PUFA, and the ratio PUFA/SFA. This region corresponds to the location of *ENSOCUG00000000157* gene related to lipid biosynthesis. This novel gene is also known as *ST3GAL1* in humans, pigs, and mice. Another important region on OCU8 at 20.5-22.3 Mb was found to be associated with both MUFA and PUFA, with six genes. Among them, *PIK3C2G, PLCZ1*, and *PLEKHA5* are related to lipid metabolism and binding. On the same chromosome, the 14.7-16.2 Mb region was associated with SFA. Although our previous results showed that it was also associated with IMF content (Sosa-Madrid et al., 2019), the putative candidate genes did not show functional annotations related to lipid metabolism. The genomic region at 63.5-66.0 Mb on OCU9 was associated with SFA, PUFA, and PUFA/SFA (Table 1). This region corresponds to the location of *NPC1* gene involved in lipid transport. On OCU17, the 12.5-14.4 Mb region was associated with MUFA and PUFA. Three genes *ALDH1A2, LIPC*, and *MYOE1*, related to lipid metabolism, mapped to this region. *LIPC* catalyzes the hydrolysis of phospholipids, and converts fat transporting molecules such as intermediate-density lipoprotein (IDL) to low-density lipoprotein (LDL).
The genomic region at 46-49.2 Mb on OCU18 was associated to SFA, MUFA, and MUFA/SFA. Among the forty candidate genes, *ERLIN1*, *ENSOCUG00000001375*, and *ENSOCUG00000014801* are highly involved in lipid metabolism. *ERLIN1* promotes lipid binding, while the novel genes are known in pigs and humans as the *Stearoyl-CoA Desaturase (SCD)*. This latter is responsible for the biosynthesis of MUFA from SFA in adipocytes. A strong association between *SCD* and the fatty acid composition was reported in pigs (Ros-Freixedes et al., 2016) and cattle (Gotoh et al., 2018). The MUFA were associated with two more regions, at 120.5-121.9 Mb on OCU1, and at 8.7-10.2 Mb on OCU6. The first region corresponds to the *MTMR2* gene involved in lipid metabolic process, and was also associated with the IMF content in the same rabbit population (Sosa-Madrid et al., 2019).

Taken together, the present study underlines different genes associated with the fatty acids due to the polygenic nature of the group of traits. Other genes were identified in the associated genomic regions presented in Table 1; however, their functional annotations did not show a direct relationship with the fatty acids metabolism.

CONCLUSIONS

The present study highlighted several genomic regions associated with fatty acid composition. The main associations were found on OCU1 at 34-37.9 Mb, on OCU3 at 148.5-150.2 Mb, and on OCU18 at 46.0-49.2 Mb. The associated regions harbor several genes related to lipid metabolism such as *SCD, PLIN2, ERLIN1*, and *LIPC*. Further analyses on the associated regions would be needed to assess the causal variants.

Table 1: Candidate regions for fatty acid composition of *longissimus thoracis et lumborum* in rabbits

OCU[1]	Region (Mb)[2]	Number of genes[3]	TRAIT (% var[4])
1	28.7-30.4	3	PUFA(0.56), PUFA/SFA (0.53)
	30.7-32.1	7	PUFA (0.6), PUFA/SFA (0.59)
	34-37.9	13	SFA (11.32), PUFA (1.89), PUFA/SFA (2.78), N6/N3 (1.26)
	120.5-121.9	12	MUFA (0.61)
3	118.0-119.2	11	MUFA/SFA (0.58)
	148.5-150.2	12	SFA (0.98), MUFA (1.38), PUFA (2.25), PUFA/SFA (2.45)
	154.0-155.4	4	SFA (0.91)
5	6.9-8.3	1	MUFA (0.99), PUFA (0.91), PUFA/SFA (0.84)
6	0.01-1.2	-	PUFA (0.55), PUFA/SFA (1.09)
	8.7-10.2	1	MUFA (0.56)
8	14.7-16.2	7	SFA (0.52)
	20.5-22.3	6	MUFA (1.01), PUFA (0.78)
	51.01-52.26	7	N6/N3 (0.67)
	53.0-54.3	14	MUFA/SFA (0.53)
9	63.5-66.0	16	SFA (0.96), PUFA (1.9), PUFA/SFA (2.44)
	68.8-69.8	4	SFA (0.56)
10	4.9-6.1	4	MUFA /SFA (0.63)
	41.9-44.1	3	SFA (0.59), MUFA (0.53), PUFA (0.58), PUFA/SFA (0.52)
13	125.9-127.0	23	MUFA (0.99)
14	77.8-79	12	MUFA/SFA (0.52)
15	7.56-9.37	13	N6/N3 (0.83)
	9.7-11.1	9	SFA (0.63), PUFA (0.87), PUFA/SFA (1.6)
16	61.7-62.9	8	MUFA/SFA (0.59)
	71.05-72.99	6	N6/N3 (1.04)
17	12.5-14.4	13	MUFA (0.65), PUFA (0.59)
18	11.0-12.3	4	MUFA/SFA (0.86)
	21.0-22.4	2	MUFA/SFA (0.93)
	46-49.2	40	SFA (0.55), MUFA (1.9), MUFA/SFA (7.91)

[1]Rabbit chromosome, [2]Megabases, [3]Protein coding genes, [4]percentage of the genomic variance explained by the associated region,
SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

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