GENOME WIDE ASSOCIATION STUDY OF GROWTH AND FEED EFFICIENCY TRAITS IN RABBITS

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ABSTRACT

We performed a genome wide association study for growth and feed efficiency using the GEMMA software on 679 rabbits genotyped with the Affimetrix Axiom Rabbit 200K Genotyping Array . No significant SNP was found for growth traits or feed intake. Two and 89 chromosome-wide significant SNPs were detected for feed conversion ratio and residual feed intake, respectively. The 89 significant SNPs for residual feed intake were all located on chromosome 18, where the putative functional candidate gene *GOT1* could be identified.

Key words: feed efficiency, SNP, GWAS, genetics, rabbit

INTRODUCTION

Improvement of feed efficiency is essential to increase the competitiveness of the rabbit industry but also to reduce animal excretion, and consequently decrease the environmental impact of the production. It can be achieved by selection on lower residual feed intake (RFI) (Drouilhet *et al.*, 2013, 2015). Approaches including linkage analyses, genome-wide association studies (GWAS) and candidate gene association for RFI have been performed to unravel the genetic background behind the complex trait in many species. Using the newly available Axiom Rabbit 200K Genotyping Array A GWAS was performed for the first time on feed efficiency and growth traits in rabbits to identify genetic variants and candidate genes.

MATERIALS AND METHODS

Animals and phenotypes

The experimental rabbit population was issued from the paternal INRA 1001 line (Larzul and De Rochambeau, 2005) and bred in the experimental INRA farm Pôle d'Expérimentation Cunicole Toulousain (Castanet-Tolosan, France), in accordance with the national regulations for human care and use of animals in agriculture. Two related genetic lines were used in this design: the G10 line, selected for 10 generations for decreased RFI (Drouilhet *et al.*, 2013, 2015), and the G0 control line produced from frozen embryos of the ancestor population of the selected line. The 296 G10 and 292 G0 rabbits were produced in the same 3 batches, with a 42 days interval between batches. In each batch, half of the kits was fostered to G0 does and the second half was fostered to G10 does. Does adopted alternatively kits from one line and from the other line in successive batches. At weaning (32 days), kits were placed in individual cages. More details about the experiment can be found in Garreau *et al.* (2019). Animals were weighed at weaning (BW32) and at 63 days of age (BW63). Total individual feed intake (FI) was recorded. Average daily gain (ADG) was obtained by dividing the body weight gain during the test by the number of days of the growing period (31 days). Feed conversion ratio (FCR) was calculated as total individual feed intake divided by the body weight gain. The RFI was computed as the residual of the multiple linear regression of total individual feed intake on average metabolic body weight (average body weight between weaning and end of the test to the power 0.75) to account for maintenance requirements, and ADG to account for production requirements (REG procedure; SAS software), as in Drouilhet *et al.* (2015).

Genotyping and genotype quality control

Ear biopsies were sampled at 63 days of age. The DNA was extracted from ear biopsies of 711 animals (588 kits and their 123 parents). Six hundred and ninety six animals were genotyped using the Affimetrix Axiom Rabbit 200K Genotyping Array (Santa Clara, CA, USA) containing 199 692 SNPs, at the Centro Nacional de Genotipado (CeGen) platform (Santiago de Compostela, Spain). The order and position of the SNPs on the genome were based on the Rabbit OryCun2.0 assembly released by the Broad Institute of MIT and Harvard (Carneiro *et al*., 2014). The Plink software (Purcell et al, 2007) was used for the quality control. After removing SNPs exhibiting a MAF below 5% and individuals with a call rate below 95%, 679 animals and 133,333 SNPs remained. Additionally 5486 SNPs were discarded based on a departure from Hardy-Weinberg equilibrium assessed by a P-value below 5% after Bonferroni correction. Finally, 679 animals and 127,847 SNPs were retained for association analyses (i.e. an average of 1 SNP every 20 Kb).

Statistical analyses

The phenotypes of the 679 kits were analyzed to test systematic effects using the GLM procedure (SAS Inst., Inc., Cary, NC). The fixed effects tested for each trait were: sex (2 levels), batch (3 levels), dam parity (4 levels), litter size at birth (4 levels), litter size at weaning (4 levels). The fixed effects were considered significant if *P* value ≤ 0.05, and were included in the final model (Table 1).

Table 1: Significance[1] of the fixed effects in linear models for growth and feed efficiency traits

	BW32	BW63	ADG	FI	FCR	RFI
Sex	ns	**	**	*	ns	ns
Batch	***	***	**	***	***	***
Parity of dam	ns	ns	ns	ns	**	*
Litter size at birth	***	***	**	***	***	***
Litter size at weaning	***	***	ns	***	***	ns

BW32: BW at 32 days, BW63: body weight at 63 days, ADG: average daily gain, FI: total feed intake, FCR: feed conversion ratio, RFI: residual feed intake. [1]Significance levels from linear models including all effects *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

Genome wide association studies

The GWAS were performed using GEMMA version 0.94.1 (Zhou and Stevens, 2012). For each trait, SNP effects were tested with the following animal mixed model:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{W}a + \boldsymbol{u} + \boldsymbol{\epsilon} \qquad \text{with} \quad \boldsymbol{u} \sim \text{MVN}_{\boldsymbol{n}}(0, \mathbf{K}\sigma_u^2) \quad \text{and} \quad \boldsymbol{\epsilon} \sim \text{MVN}_{\boldsymbol{n}}(0, \mathbf{I_n}\sigma_\epsilon^2)$$

where $\boldsymbol{y}$ is the phenotype observation vector, $\boldsymbol{X}$ is the incidence matrix of fixed effects and $\boldsymbol{b}$ stands for the estimation of these covariates, a is the marker effect and $\boldsymbol{W}$ stands for the vector of genotypes, $\boldsymbol{u}$ is the random polygenic effect and $\boldsymbol{\epsilon}$ is the random residual effect. Residual effects are supposed normally distributed and independent, while additive genetic effects are structured after $\mathbf{K}$, the centered relatedness matrix computed from the genotypes. Each SNP position was tested successively. GEMMA outputs under the null hypothesis ($\beta = 0$) were also used to estimate genomic based heritability coefficients for each trait.

Significance was assessed for each tested SNP. To account for multiple tests, a Bonferroni correction was applied per chromosome as α/K_l, where α = 0.05 and K_l is the number of SNPs on chromosome *l*.

RESULTS AND DISCUSSION

Heritability estimates

Heritability estimates ranged between 0.15 (±0.05) and 0.31 (±0.07) for growth traits and between 0.45(±0.07) and 0.47 (±0.06) for feed efficiency traits (table 2). These estimates are higher than those reported by Drouilhet *et al.* (2013) from data recorded during 6 generations of selection in the same rabbit experimental population.

Table 2: Heritability estimates and standard errors for growth and feed efficiency traits.

Trait	BW32	BW63	ADG	FI	FCR	RFI
Heritability estimate	0.14	0.25	0.30	0.38	0.45	0.47
Standard error	0.05	0.06	0.07	0.06	0.07	0.06

BW32: BW at 32 days, BW63: body weight at 63 days, ADG: average daily gain, FI: total feed intake, FCR: feed conversion ratio, RFI: residual feed intake.

GWAS results

No significant SNP was found for growth traits or for FI. Two and 89 chromosome-wide significant SNPs were detected for FCR (figure 1) and RFI (figure 2), respectively. The 2 SNP for FCR were somewhat isolated, and no functional candidate gene could be identified. The 89 significant SNPs for RFI were located on chromosome 18. Despite a limited annotation of the rabbit genome, the putative functional candidate gene *GOT1* was identified in this region. Glutamic-oxaloacetic transaminase is a pyridoxal phosphate-dependent enzyme that exists in cytoplasmic and inner membrane mitochondrial forms, *GOT1* and *GOT2*, respectively. *GOT* plays a role in amino acid metabolism and in urea and tricarboxylic acid cycles (Mavrides & Christen 1978). A significant positive correlation between RFI and fecal N was described by Aggrey *et al.* (2014) in broilers: low RFI birds need to generate sufficient nucleotides to maintain growth despite reduced FI, that then results in reduced fecal N. The same authors reported different gene expression levels of *GOT1* and *GOT2* between two broilers lines divergently selected for RFI. Both *GOT1* and *GOT2* were downregulated in four tissues (duodenum, muscle, liver and kidney) of the low RFI line. Mukiibi *et al.* (2018) also found differential expression of *GOT*1 between six extreme high and six extreme low RFI steers from three beef breed populations.

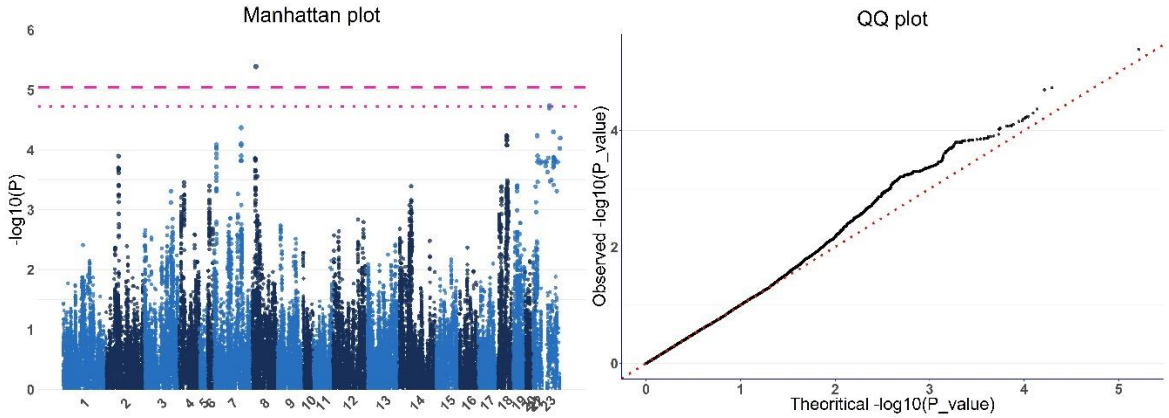

Figure 1: Manhattan plot and Quantile-Quantile (QQ) plot of FCR**.** Dotted and dashed lines correspond to the 5% chromosome wide threshold for chomosome 23 and chromosome 8, respectively.

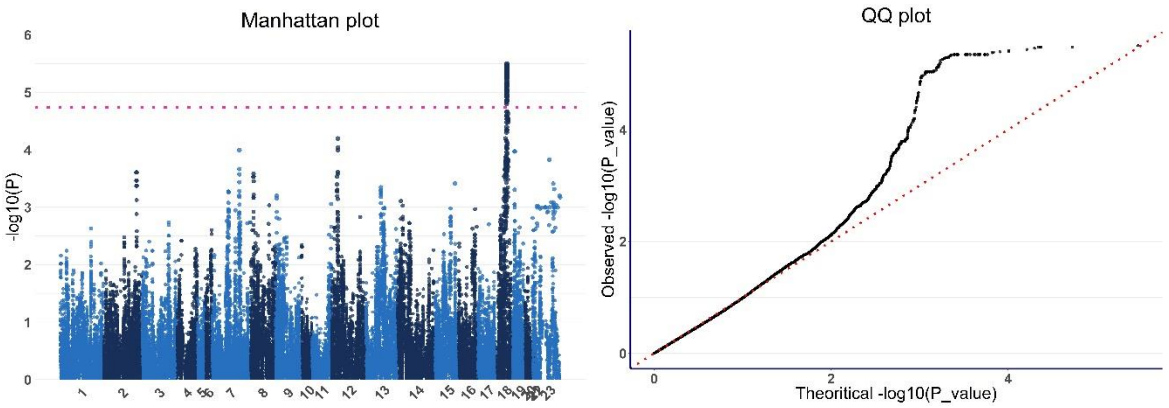

Figure 2: Manhattan plot and Quantile-Quantile (QQ) plot of RFI**.** Dotted line correspond to the 5% chromosome wide threshold for chomosome 18.

To our knowledge these are the first published GWAS results on feed efficiency traits in rabbits using the Axiom Rabbit 200K Genotyping Array. In this species, using a restriction-site associated DNA (RAD) sequencing, Gilbert *et al.* (2018) identified significant SNPs on chromosome 2 in association with the Craniosynostosis disease occurrence and on chromosomes 14 and 19 in association with the disease onset.

CONCLUSIONS

For the first time, a genome association study reveals a QTL for feed efficiency on chromosome 18 in rabbit. The putative candidate gene *GOT1* was identified in this region.

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