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## Genetic determinism of cortisol levels in pig

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### Summary

The plasma cortisol levels measured one hour after injection of ACTH reflects the hypothalamic-pituitary-adrenocortical (HPA) axis activity. The test was performed on piglets at 6 weeks of age. It was investigated as a potential selection criterion to improve pig robustness. From a base population of Large White pigs, two divergent lines were derived for 3 generations of selection, one selected for a high cortisol level (H line), one for a low cortisol level (L line). All breeders, as well as unselected animals, were also genotyped on a 70kSNP chip. At the 3<sup>rd</sup> generation of selection, the divergence between the two lines was about 5 genetic standard deviations. The high heritability of the trait was confirmed ( $h^2 = 0.64 \pm 0.03$ ). The genetic correlations with individual body weights and growth rates measured during lactation, post weaning and finishing periods were very low except for the genetic correlation between cortisol level and post-weaning growth rate ( $r_g = -0.22 \pm 0.10$ ). The genome-wide association study revealed a quantitative trait locus influencing post-ACTH cortisol level, located on chromosome 2. It was suggested that divergent selection modified the frequency of *NR3C1* alleles, favouring the hypersensitive glucocorticoid receptor in the low line.

*Keywords: pig, robustness, cortisol, genetics, GWAS*

### Introduction

Pig production is more and more challenged by antibiotics use, environment control with climate change, feed competition and animal welfare. To face these challenges, animal breeding and genetics can provide a sustainable option by improving animal robustness. However, the major issue is to provide new selection criteria related to robustness. The hypothalamic-pituitary-adrenocortical (HPA) axis plays a major role in metabolic regulations and adaptive responses and could provide a potential leverage for more robust animals (Mormede & Terenina, 2012). To undermine the genetic determinism of HPA activity and its influence on functional traits and robustness, a divergent selection experiment was carried out in a Large White pig population on the basis of plasma cortisol levels measured one hour after injection of ACTH.

### Material and methods

The experiment was carried out according to the French regulation; the protocols were evaluated and approved by an ethics committee (decision CE2013-1, 21012013).

### Animals and selection experiment

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The base population was constituted by the progeny of a foundation breeding stock of purebred French Large White pigs consisting of 30 unrelated AI boars and 30 sows. This base population (F0) has been described by Larzul *et al.* (2015). Two divergent lines were selected on the plasma cortisol level after ACTH injection. From the base population, 14 males were selected, 7 with the highest cortisol values (H line) and 7 with the lowest cortisol values (L line). They were mated at random with 62 unselected Large White sows. On the following generations, 8 males and 40 females were selected on extreme cortisol values, within line (table 1).

Table 1. Number of animals measured and mean plasma cortisol level after ACTH injection per line and generation.

Generatio n	N		Mean (ng/ml)	
	Low line	High line	Low line	High line
F0	298		99	
G1	201	152	105	121
G2	403	357	87	129
G3	330	313	73	158

Replacement boars and gilts were chosen among first-parity litter progeny. From the unselected 62 Large White sows, 30 were inseminated a second time with 30 AI Large White boars. A total of 120 animals G0 were phenotyped as representative of the base population for the selection experiment.

## Measurements

### Selection criterion

At 6 weeks of age, 2 weeks after weaning, piglets were injected in the neck muscles with synthetic ACTH(1–24) in generation G0 and G1 (222 µg/animal) and porcine ACTH(1–39) in generation G2 and G3 (333 µg/animal). Synacthen was replaced by ACTH(1-39) for drug regulation issues. The peak response was not different but the kinetics was a bit shorter with ACTH(1-39) with lower levels at 4h post-injection. A blood sample was collected 1 h after ACTH injection. The blood samples were centrifuged and plasma frozen at –20°C until assay. The dose of ACTH was chosen to be maximally stimulating the adrenal cortex. Plasma cortisol was measured by direct automated immunoassay (AIA-1800, Tosoh Bioscience, San Francisco, CA). The selection criterion was the plasma cortisol level measured 1 h after ACTH injection.

### Growth and carcass traits

Piglets were all weighed at birth, 21 days, and weaning at 4 weeks of age. Piglets did not receive feed supplement up to 21 days of age. After weaning, piglets were transferred in post-weaning units for 5 weeks and weighed at the end of the post-weaning period. Piglets not selected as breeders of the next generation were raised and slaughtered at an average weight of 100 kg. For G2 and G3 generation, animals were weighed on the day before departure to slaughterhouse. A measure of carcass lean percentage was provided by the slaughterhouse

(Image meater procedure, Blum *et al.*, 2014). The average daily gain was estimated for the lactating period (ADGI), the post-weaning period (ADGpw) and the fattening period (ADGf).

### Genotyping

The 62 unselected Large White sows, the 120 G0 piglets, the 14 F0 selected boars, the sows and boars selected as reproducers in the G1 and G2 populations as well as the extremes from the G3 population were genotyped with an Illumina Beadchip array. Up to G1, animals were genotyped with the 60kSNP array; pigs from G2 and G3 populations were genotyped with a 70kSNP GenSeek array.

## Statistical analyses

### Genetic parameters and breeding values

Preliminary least squares analyses were performed using the GLM procedure of SAS to estimate the effect of sex, batch nested within generation and line x generation combination on each trait. For weights measured at birth, 21 days of age and weaning, the litter size was added as a fixed effect (reduced to 4 levels). For the genetic analysis, the random effects of common litter and animal were also included in the model, without the line x generation effect. Genetic parameters were estimated with a two-trait animal model. All the ancestors of the recorded animals up to five generations from the F0 animals were taken into account to build the additive relationship matrix. The estimation of genetic parameters was performed with VCE6. Additive genetic breeding values were estimated with BlupF90. The response to selection was estimated by averaging predicted breeding values within line and generation.

### Association Study

In a first step, for each type of array, SNP information was removed for SNP with a minor allele frequency less than 5% and a call rate less than 90%. In a second step, missing SNP information was imputed with the FImpute software (Sargolzaei *et al.*, 2014). The markers were located on the genome assembly Sscrofa11.1. Association analyses were performed with the R package GenABEL (Aulchenko *et al.*, 2007).

## Results and discussion

### Genetic parameters and response to selection

The heritability value of cortisol level after ACTH injection was 0.64 ( $\pm 0.03$ ), which is very similar to the previous value (0.68) estimated in the base population (Larzul *et al.*, 2015) and confirmed the high heritability of this trait. As expected from the heritability value, the response to selection was large. The difference between the two divergent lines after 3 selection steps was around 5 genetic standard deviations (Figure 1).

*Figure 1. Genetic evolution of post-ACTH cortisol level in the high (H) and low (L) lines, in genetic standard deviation.*

The heritability values for other traits are reported in table 2 as well as genetic correlations with the selection criterion. The genetic correlations are low except for the genetic correlations between cortisol and average daily gain during the post-weaning period. The negative correlation shows that piglets with the lowest cortisol levels tended to have a higher average daily gain after weaning, which might be related to a better ability to go through the stress of weaning (Mormede et al., 2018).

Table 2. Genetic parameters: heritability ( $h^2$ ), genetic correlations with cortisol level ( $r_g$ ), common litter effect ( $c^2$ ) and their standard error ( $se$ )

Traits <sup>1</sup>	N	$h^2$	$r_g$	se $h^2$	se $r_g$	$c^2$	se $c^2$
BW	3405	0.18	-0.04	0.05	0.09	0.24	0.02
W21	2538	0.16	0.01	0.06	0.10	0.39	0.03
WW	2491	0.11	0.00	0.05	0.11	0.44	0.03
WPW	2486	0.06	-0.15	0.04	0.15	0.11	0.02
ADG <sub>l</sub>	2491	0.07	0.02	0.05	0.13	0.46	0.03
ADG <sub>pw</sub>	2464	0.18	-0.22	0.06	0.10	0.14	0.02
ADG <sub>f</sub>	984	0.21	0.01	0.08	0.11	0.12	0.03
Lean%	600	0.46	0.09	0.13	0.11	0.04	0.03

<sup>1</sup>BW: birth weight; W21: weight at 21 days of age; WW: weaning weight; WPW: post-weaning weight; ADG<sub>l,pw,f</sub>: average daily gain during lactation (l), post weaning (pw) and fattening (f), respectively; lean%: lean percentage.

### Genome Wide Association Study

A highly significant quantitative trait locus was located on chromosome 2 (Figure 2). The SNP most significantly associated with cortisol level was exactly the same as the one reported by Murani *et al.* (2012). It means that the selection process resulted in the segregation of 2 alleles of the *NR3C1* gene, with glucocorticoid receptor hypersensitivity in the animals from the low cortisol line. No SNP was significantly associated with any other trait.

Figure 2. Manhattan plot for post-ACTH cortisol level.

### Conclusion

The selection experiment confirmed that it was possible to select for divergent HPA axis activity. The two lines constituted a valuable resource to further study the influence of HPA axis activity on traits related to robustness. The consequences of selecting for GR sensitivity will also be fully evaluated.

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