Molecular Genetics of Neuroendocrine Stress Responses and Robustness in Pigs

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Genetics and STRESS

- STRESS is the set of non specific responses to environmental challenges, with **behavioural** and **biological** components
- Stress has profound consequences on sustainability of animal breeding
  - Level of production (growth rate, feed efficiency)
  - Product quality (carcass composition, meat quality)
  - Resistance to diseases (immune system and inflammatory processes)
  - Well-being
- Stress is the result of an **interaction** between the animal and its environment
  - Individual variability (**genetic** and/or acquired)
  - ROBUSTNESS

ROBUSTNESS

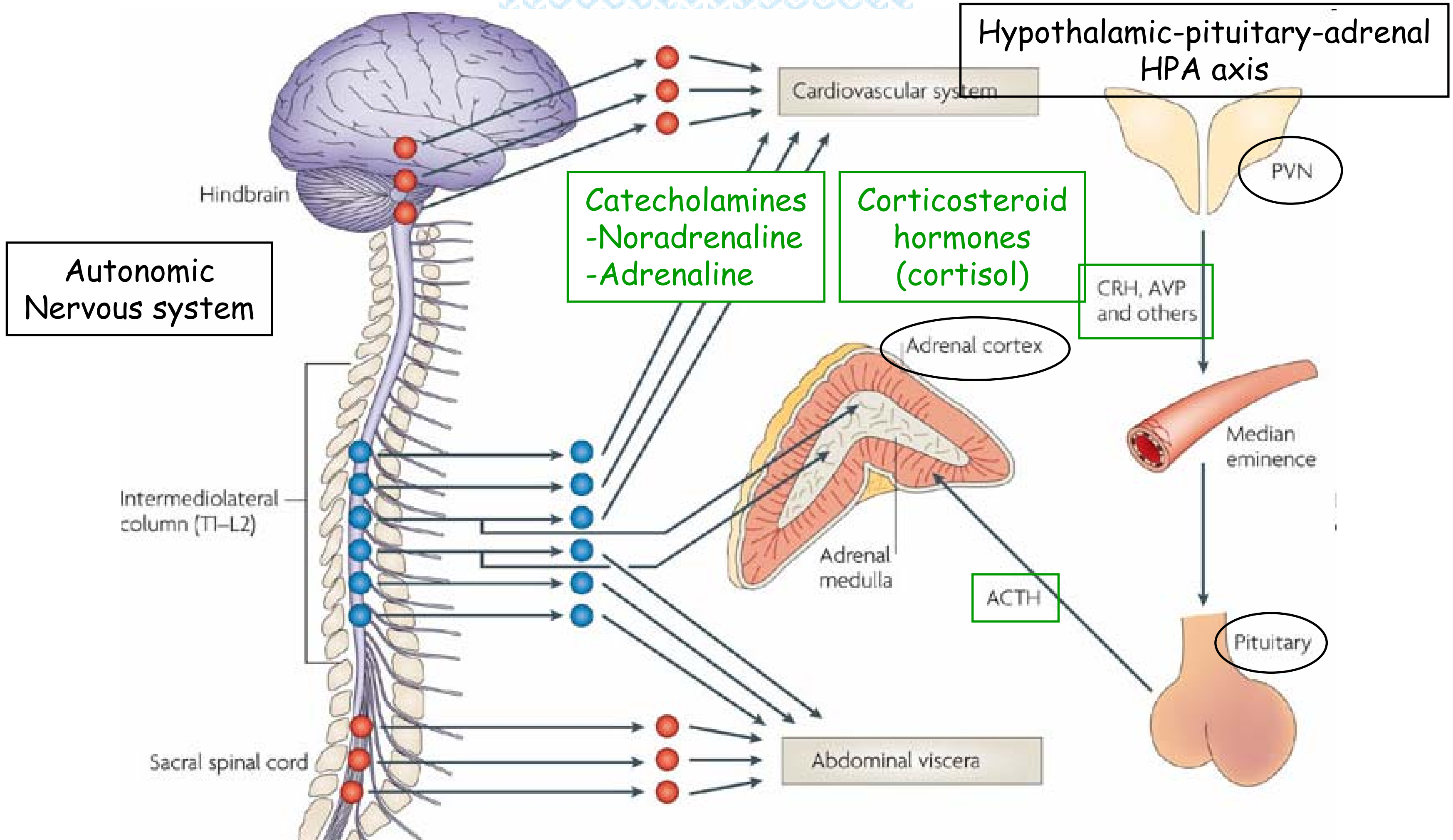
- 'The ability to combine a high production potential with resilience to stressors, allowing for unproblematic expression of a high production potential in a wide variety of environmental conditions' (Knap 2005)
- Includes several components
  - Sensitivity of the production potential to diverse commercial environments and external stressors (t°)
  - Quality of supportive tissues (e.g. leg soundness)
  - Newborn survival
  - Efficiency of the immune system (resistance to diseases)

Robustness as a breeding goal

'Sustainable breeding goals combine robustness traits with production traits to such an extent that selection balances genetic change in production potential with genetic change in environmental sensitivity' (Knap 2009)

Selection strategies

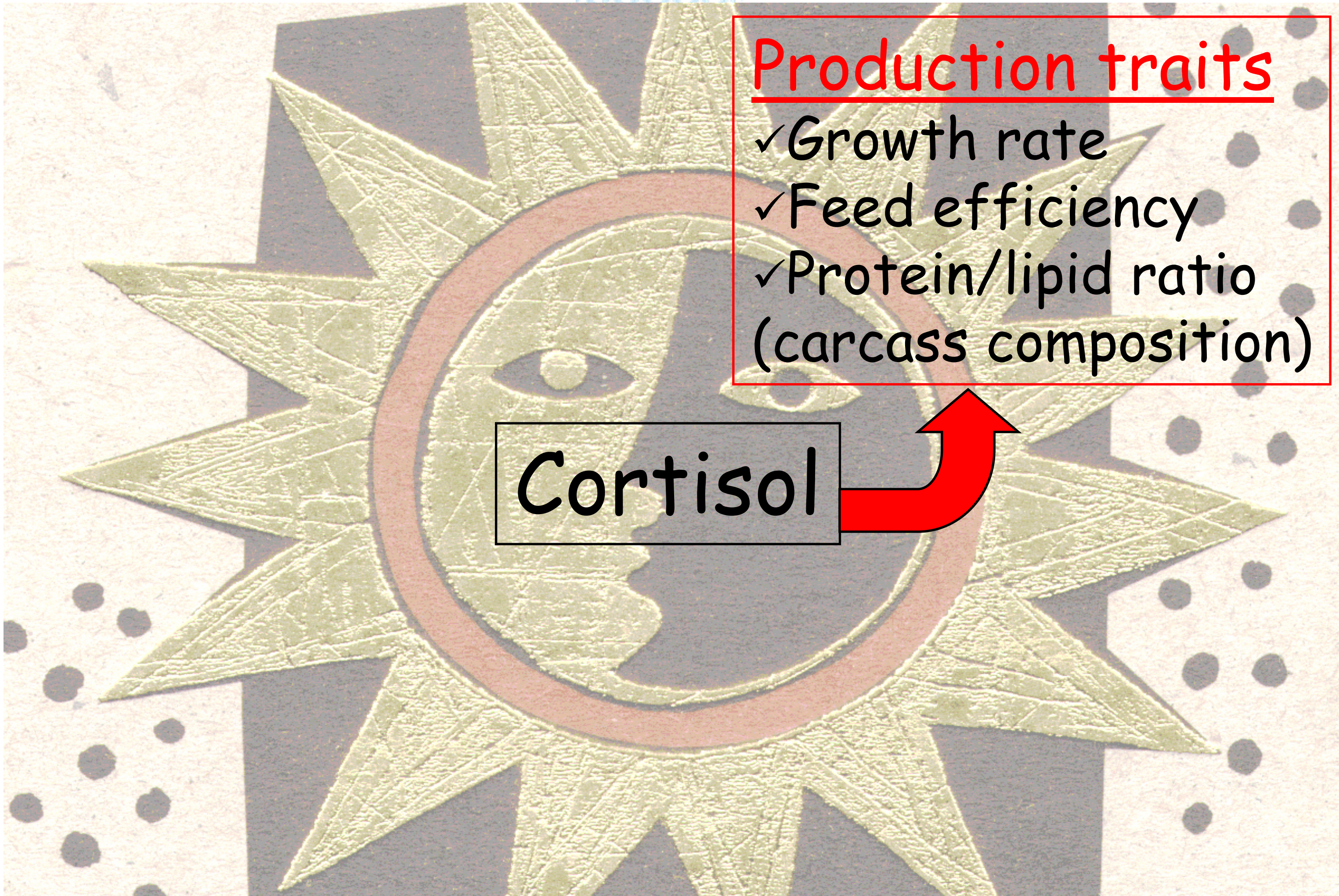
1. Inclusion of directly measurable **robustness traits** in the breeding objective and in the selection index (BLUP) – leg soundness, newborn survival rate, …
2. **Reaction norm** analysis – sensitivity to the environment (Lynch and Walsh 1998) – low heritability
3. Genetics of **neuroendocrine stress responses**



Neuroendocrine stress systems

Nature Reviews | Neuroscience

Effects of cortisol on production and robustness traits



Production traits

- ✓Growth rate
- ✓Feed efficiency
- ✓Protein/lipid ratio (carcass composition)

Cortisol

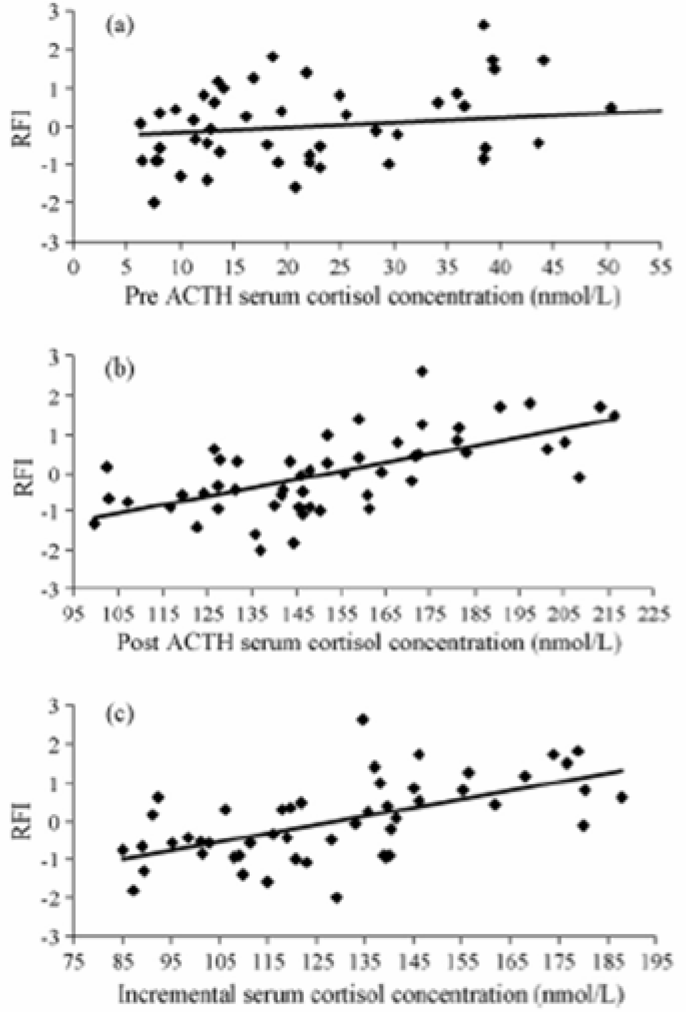
Body weight, growth rate and feed efficiency in pigs as a function of cortisol response to ACTH

Response to ACTH	Weight (kg) at			Total gain (kg) 9-21 wks	Feed conversion (kg feed/kg gain)
	3 wks	9 wks	21 wks		
FEMALES					
Low	4.44 ± 0.32^{a}	27.13 ± 1.16^{a}	78.02 ± 3.19^{a}	50.88 ± 2.59^{a}	2.84 ± 0.25^{a}
Median	3.98 ± 0.40^{b}	25.62 ± 1.54^{b}	74.61 ± 3.77^{b}	48.85 ± 2.39^{b}	3.18 ± 0.40^{b}
High	3.60 ± 0.42^{c}	23.56 ± 1.74^{c}	69.39 ± 2.98^{c}	45.73 ± 1.63^{c}	3.20 ± 0.27^{b}
MALES					
Low	4.51 ± 0.20^{a}	27.94 ± 0.63^{a}	82.73 ± 1.39^{a}	55.04 ± 1.84^{a}	2.74 ± 0.35^{a}
Median	4.07 ± 0.47^{b}	25.97 ± 1.51^{b}	77.73 ± 2.56^{b}	51.77 ± 1.91^{b}	2.90 ± 0.29^{b}
High	3.70 ± 0.24^{c}	24.45 ± 0.66^{c}	74.88 ± 1.02^{c}	50.59 ± 1.23^{b}	3.03 ± 0.30^{b}

Two- to three-week-old piglets (1,200) were injected with ACTH (6.25 I.U. i.m.) and plasma cortisol concentrations were measured one hour later. Animals were selected by the intensity of the response (15% low, median and high) and bred in groups of 22-23 (4 groups per sex and response level). Means with different letters in the same column and for the same sex are significantly different ($P < 0.01$).

Hennessy & Jackson 1987

Feed efficiency in sheep as a function of cortisol response to ACTH



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DOMESTIC ANIMAL ENDOCRINOLOGY

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Rams with poor feed efficiency are highly responsive to an exogenous adrenocorticotropin hormone (ACTH) challenge

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Fig. 2. Linear regression models $y = a + bx$ that predict residual feed intake (RFI) (y) based on serum cortisol concentrations in $n = 50$ sheep. (a) Serum cortisol levels for pre-ACTH with the coefficient values (±S.E.) for $a = -0.28$ (0.28) and $b = 0.012$ (0.010) and the adjusted R^2 (%) = 0.7 ($P > 0.05$). (b) Serum cortisol levels for post-ACTH, with the coefficient values (±S.E.) for $a = -3.38$ (0.59) and $b = 0.02$ (0.003) and the adjusted R^2 (%) = 39.5 ($P < 0.001$). (c) Incremental change in serum cortisol levels, with the coefficient values (±S.E.) for $a = -2.85$ (0.57) and $b = 0.02$ (0.004) and the adjusted R^2 (%) = 34.2 ($P < 0.001$).

Correlation between stress hormone levels in urine post-mortem, carcass composition and meat quality

	AD	NA	pH-AF	ADI	MBT	IMF
Cortisol	.42 ****	.15 *	.04	.24 ****	.23 ****	.02
Adrenaline (AD)		.55 ****	.27 ****	.29 ****	.25 ****	.07
Noradrenaline (NA)			.24 ****	.07	.07	-.07

pH-AF = pH_{24} *adductor femoris*
ADI = loin weight / back fat weight
MBT = mean backfat thickness
IMF = intramuscular fat %

F2 Duroc x Large White (n = 312)

Effects of cortisol on production and robustness traits

Production traits
- ✓Growth rate
- ✓Feed efficiency
- ✓Protein/lipid ratio (carcass composition)

Cortisol

Robustness traits
- ✓Resistance to stress
- ✓Resistance to diseases
- ✓Newborn survival

DECREASED HEAT TOLERANCE IS ASSOCIATED WITH HYPOTHALAMO–PITUITARY–ADRENOCORTICAL AXIS IMPAIRMENT

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Key words: rat, heat exhaustion, body core temperature, corticosterone, ACTH, inflammatory processes.

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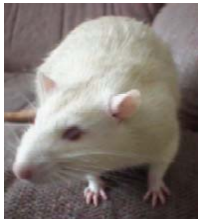
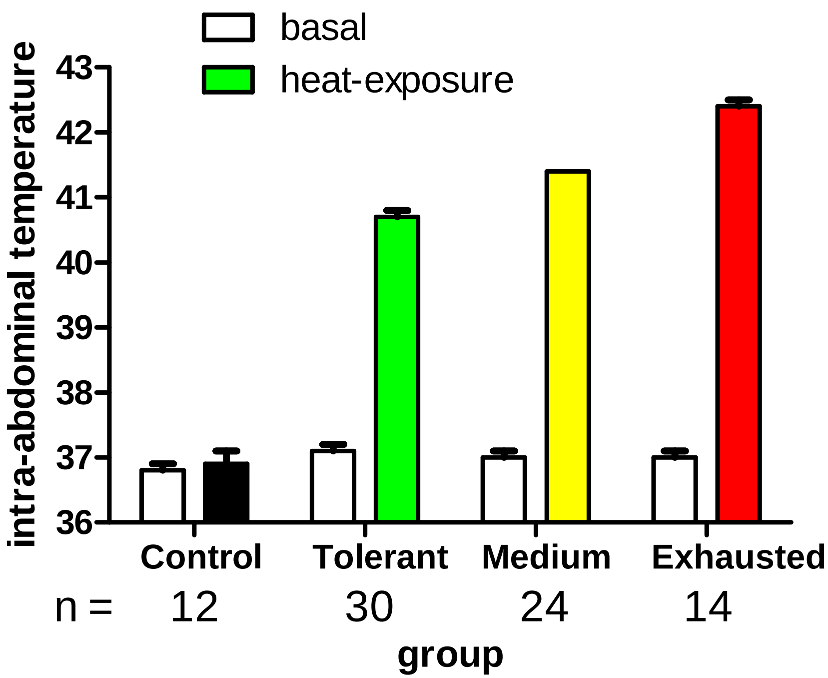
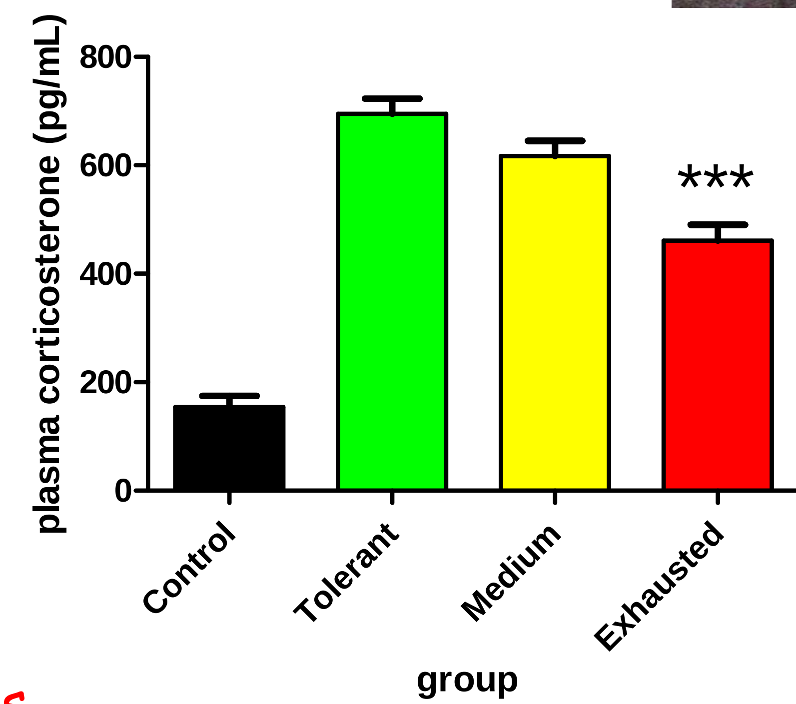


Ta=40 °C; r.h. 50±10%; 90 min





No data in farm animals

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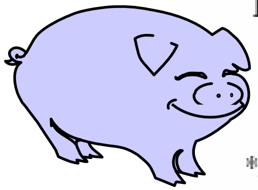
Genetics, HPA axis and resistance to diseases

- Chicken
  - Gross, 1972, 1976; Gross et Colmano, 1969, 1971; Gross et Siegel, 1973, 1975.
  - Divergent selection for plasma corticosterone response to social stress
  - High responders are more resistant to bacterial (sacculitis) and parasitic (*E. necatrix*) diseases, but more sensitive to viral disease and tumors (Marek's disease), and mount a lower antibody response to antigen challenge
- Turkey
  - Brown et Nestor, 1973
  - Divergent selection for plasma corticosterone response to cold stress
  - High line more sensitive to *Mycoplasma meleagridis*

→ Limited information available



Fetal development in the pig in relation to genetic merit for piglet survival[1]

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Table 3. Regression coefficients and *P*-values for the model: $\ln(Y) = \ln(a') + b_1 EBVps + b_2 \ln(BW)$[a]

		EBVps		ln(BW)	
Y	ln(a′)	Regression coefficient[b]	*P*-value	Regression coefficient[b]	95% C.I.[c]
Heart wt, g	–4.47	–0.0025	0.15	0.96	0.92 – 1.00
Liver wt, g	–3.32	0.0069	0.02	0.97	0.92 – 1.02
Lung wt, g	–3.30	–0.0031	0.30	0.99	0.92 – 1.06
Stomach wt, g	–4.14	0.0042	0.09	0.84	0.79 – 0.89
Spleen wt, g	–6.81	–0.0063	0.10	0.99	0.92 – 1.07
Kidney wt, g	–3.93	–0.0008	0.73	0.85	0.80 – 0.90
Adrenal wt, g	–5.80	0.0196	0.0001	0.55	0.47 – 0.63
Small intestinal wt, g	–4.02	0.0053	0.01	1.04	1.00 – 1.09
Small intestinal length, cm	2.88	0.0015	0.44	0.41	0.37 – 0.45

[a]Y = organ characteristic; ln(a′) = intercept; EBVps = estimated breeding value for piglet survival; b_1, b_2 = regression coefficients.
[b]Regression coefficients indicate the increase or decrease in the natural logarithm of the respective characteristic with every percentage increase in EBVps or unit ln(BW).
[c]The 95% confidence interval was calculated as the regression coefficient ± 1.96·SE of the regression coefficient.

Key Words: Animal Breeding, Breeding Value, Fetal Development, Perinatal Mortality, Pigs, Placenta

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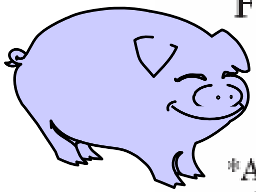

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Table 4. Relationships of blood characteristics with EBVps of the litter[a]

		Litter average			Within-litter variation[d]		
Characteristic	n[b]	Mean ± SE	Regression coefficient[c]	*P*-value	Mean ± SE	Regression coefficient[c]	*P*-value
Hematocrit, %	38	30.4 ± 0.5	0.08	0.41	2.5 ± 0.2	0.06	0.12
Plasma venous glucose, mg/100 mL	46	62.6 ± 1.4	0.14	0.62	8.5 ± 0.5	−0.03	0.75
Plasma arterial glucose, mg/100 mL	46	55.0 ± 1.4	0.11	0.70	9.7 ± 0.6	−0.03	0.77
Plasma arterial fructose, mmol/L	46	3.19 ± 0.10	−0.01	0.51	0.51 ± 0.02	0.002	0.65
Plasma arterial albumin, g/L	46	6.83 ± 0.14	0.04	0.16	1.03 ± 0.06	0.001	0.92
Serum arterial estradiol-17β, ng/mL	46	1.42 ± 0.09	−0.01	0.51	0.56 ± 0.04	−0.003	0.71
Serum arterial cortisol, ng/mL	46	51.5 ± 2.6	1.7	0.0001	15.3 ± 1.3	0.2	0.53

[a]EBVps = estimated breeding value for piglet survival.
[b]Number of litters.
[c]Regression coefficients indicate the increase or decrease in the respective characteristic with every percentage increase in EBVps.
[d]Calculated as within-litter SD.

Key Words: Animal Breeding, Breeding Value, Fetal Development, Perinatal Mortality, Pigs, Placenta

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Effects of cortisol on production and robustness traits

Selection for
- ✓Fast growth rate
- ✓High feed efficiency
- ✓Lean carcasses

Production traits
- ✓Growth rate
- ✓Feed efficiency
- ✓Protein/lipid ratio (carcass composition)

Cortisol

Robustness traits
- ✓Resistance to stress
- ✓Resistance to diseases
- ✓Newborn survival

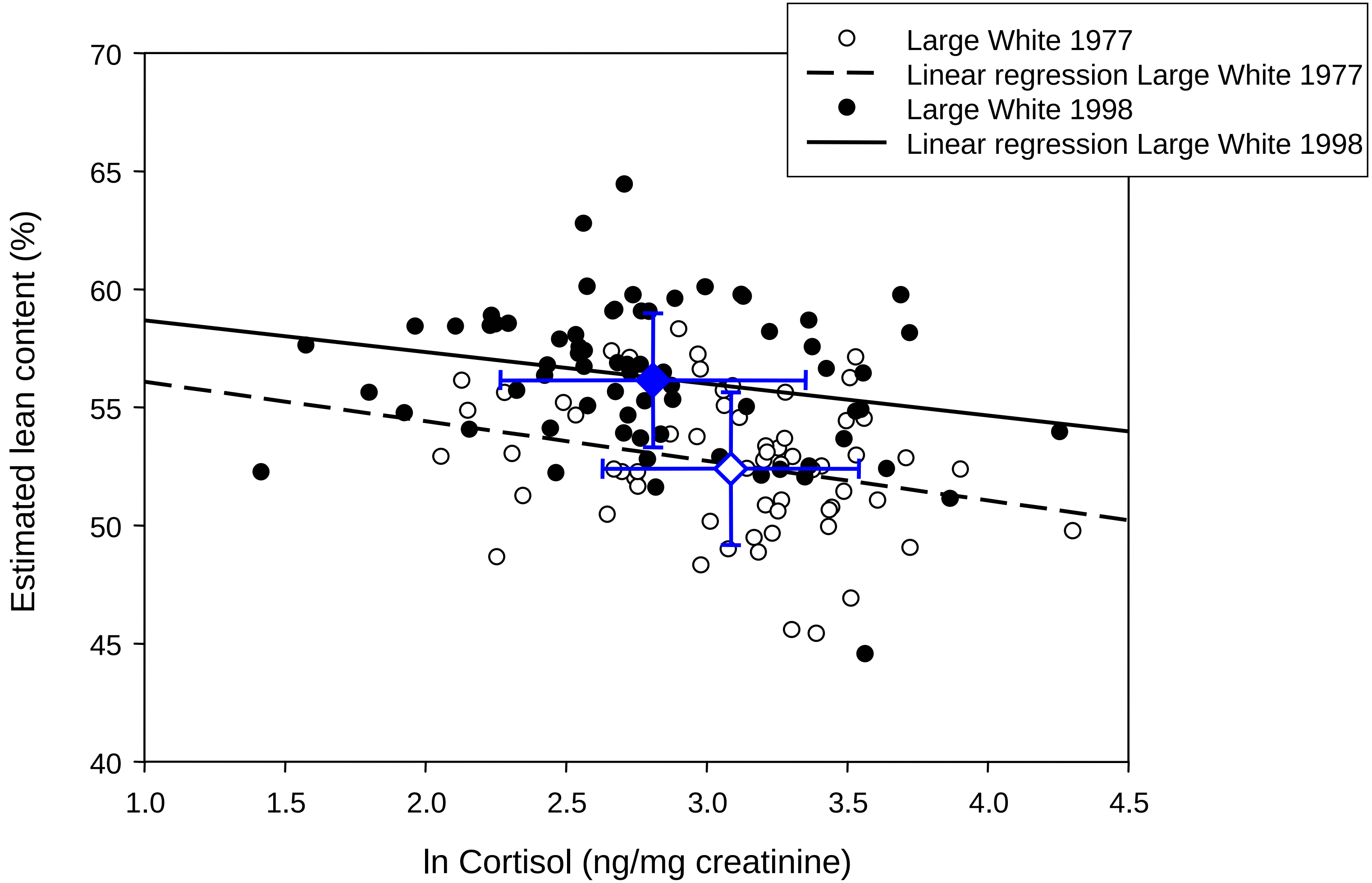
Least-squares means (LSM) and estimated genetic trends (ΔG) for endocrine, carcass composition and meat quality traits of G77 and G98 pigs (Large White breed)

Traits★	n G77	n G98	G77 LSM (s.e.)	G98 LSM (s.e.)	ΔG (s.e.)	Pr > |t| H0 : $\Delta G = 0$
Estimated lean content (%)	116	120	52.20 (0.30)	56.00 (0.29)	7.59 (0.82)	****
Mean backfat thickness (mm)	116	120	24.51 (0.28)	22.13 (0.28)	-4.76 (0.80)	***
pH24	116	120	5.84 (0.03)	5.76 (0.03)	-0.17 (0.07)	*
Cortisol urine (ng/mg creatinine), ln	63	70	2.97 (0.07)	2.69 (0.07)	-0.56 (0.17)	**
Adrenaline urine (ng/mg creatinine), ln	57	58	1.96 (0.10)	1.65 (0.10)	-0.63 (0.22)	**
Noradrenaline urine (ng/mg creatinine), ln	58	63	2.60 (0.08)	2.39 (0.08)	-0.42 (0.18)	*

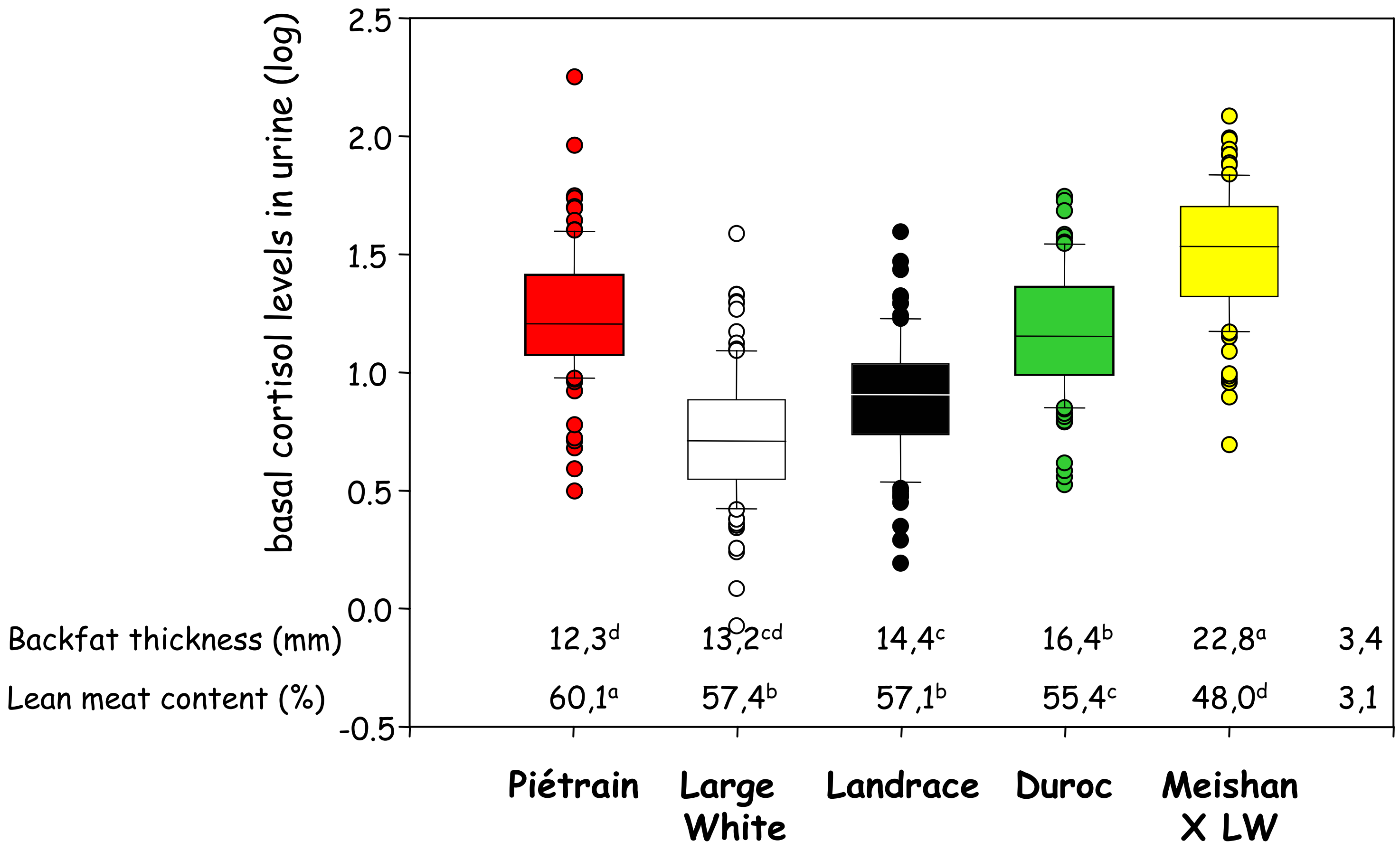
n = number of observations in each group
⁺$\Delta G = 2 \times$ (G98 LSM – G77 LSM)
★ECLC = Estimated Carcass Lean Content; Mean backfat thickness = average of the backfat thickness measured at the neck, back and rump levels; pH24 = ultimate pH in the *Semi membranosus* muscle.
* P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001

Foury et al. Animal (2009)
See poster # 26/13
by A. Foury et al.

Genetic trends 1977-1998, Large White



Between- and within-breed variation of cortisol production (n=100 female pigs / breed)



Breeding objectives to increase robustness by selection of a stronger HPA axis

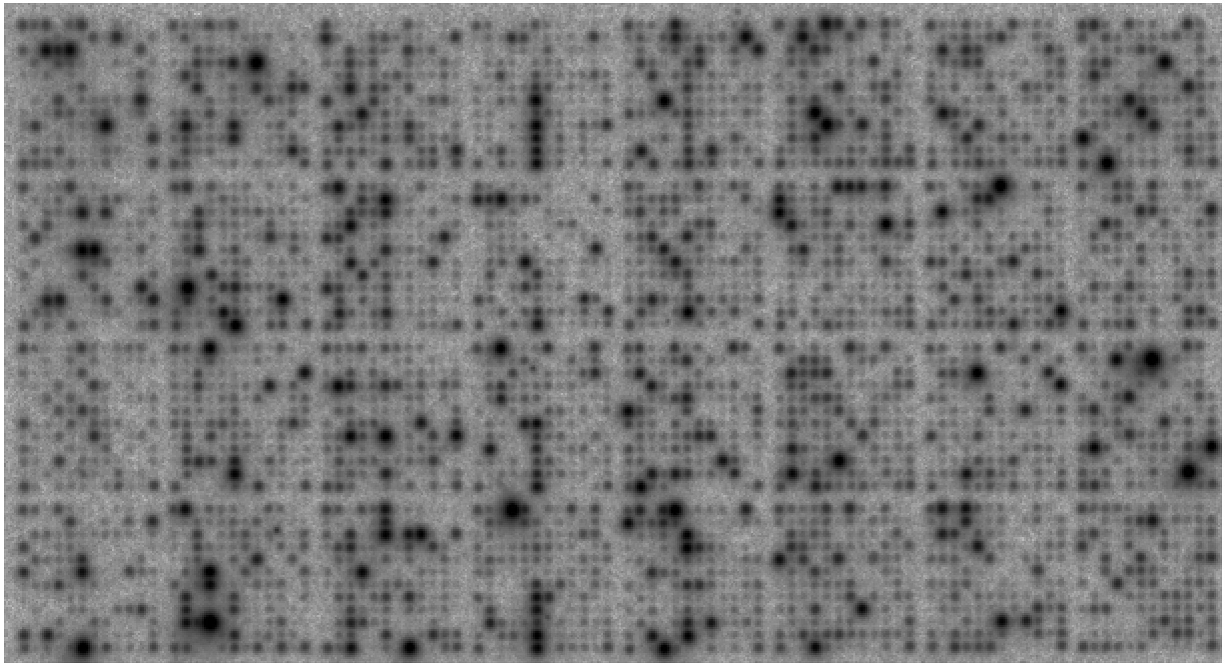
- Selection for fast growth rate, high feed efficiency and lean carcasses may have reduced HPA axis activity with negative consequences on robustness.
- Selection for a more active / reactive HPA axis should improve robustness with limited effects on production traits.
- Strong genetic influences on HPA axis activity. Divergent genetic selection in chicken (ACTH, social stress), turkey (cold), trout (confinement), mouse (restraint).
- Several sources of genetic variation
  - Cortisol production
  - Bioavailability
  - Receptor function

Response of the adrenal gland to ACTH

- Individual trait
  - Humans: 'Adrenal phenotype' (Bertagna *et al.* 1994 ; Coste *et al.* 1994)
  - Pig (Hennessy *et al.* 1988)
- Heritable
  - $h^2 = 0.26$; half-sib analysis of 357 litters from 24 boars (D.P. Hennessy, personal communication)
  - Divergent genetic selection
    - In chickens (Edens et Siegel, 1975)
    - In trout, confinement stress (Pottinger et Carrick, 1999)

Gene expression in the adrenal glands of pigs

- Large White / Meishan - Control / ACTH
- Collection of adrenal glands (1h), ARN extraction
- P^{33} labelling and hybridization on nylon membranes (9216 clones)



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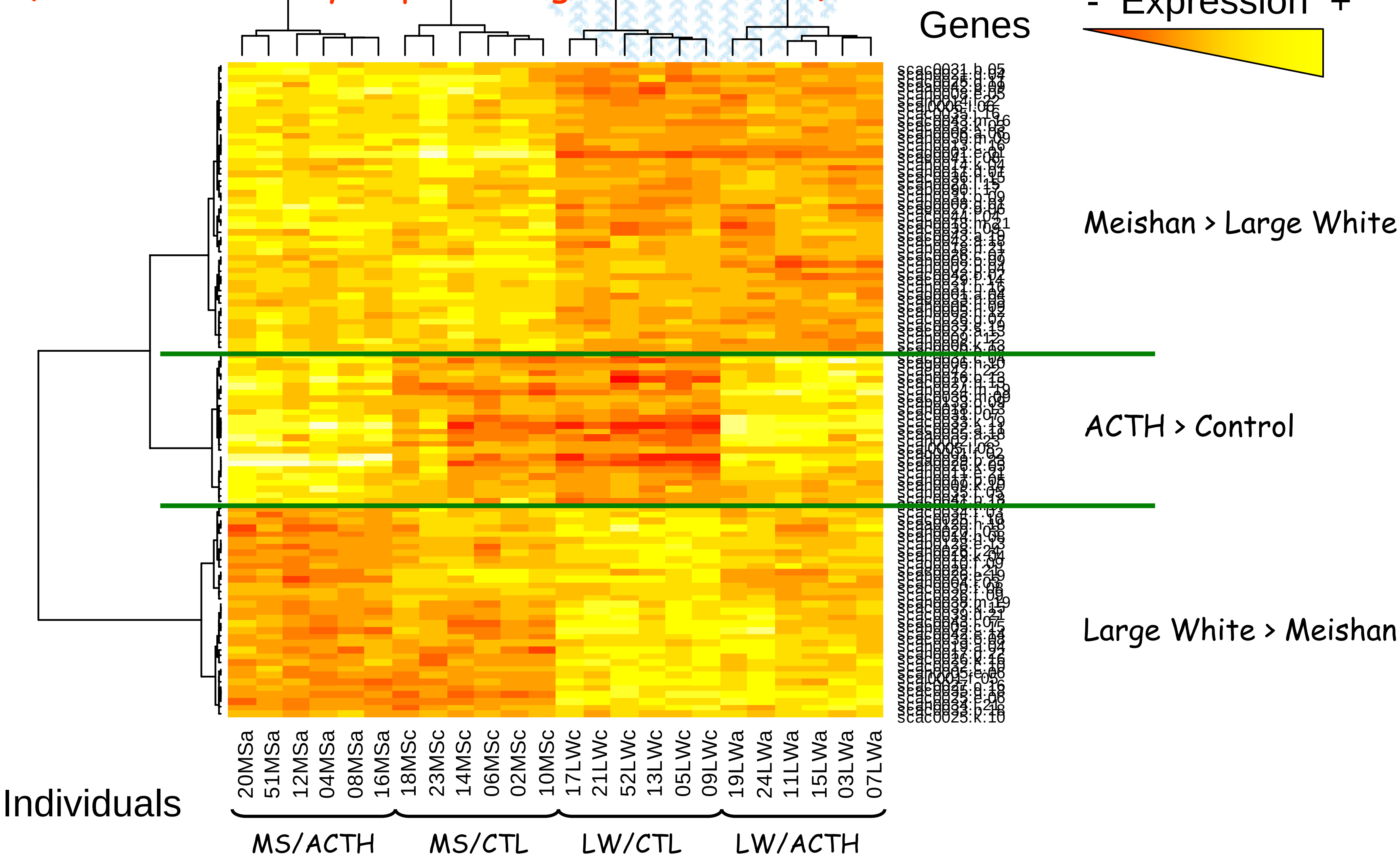
CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

Ascending hierarchical classification

(211 differentially expressed genes, FDR<5%)



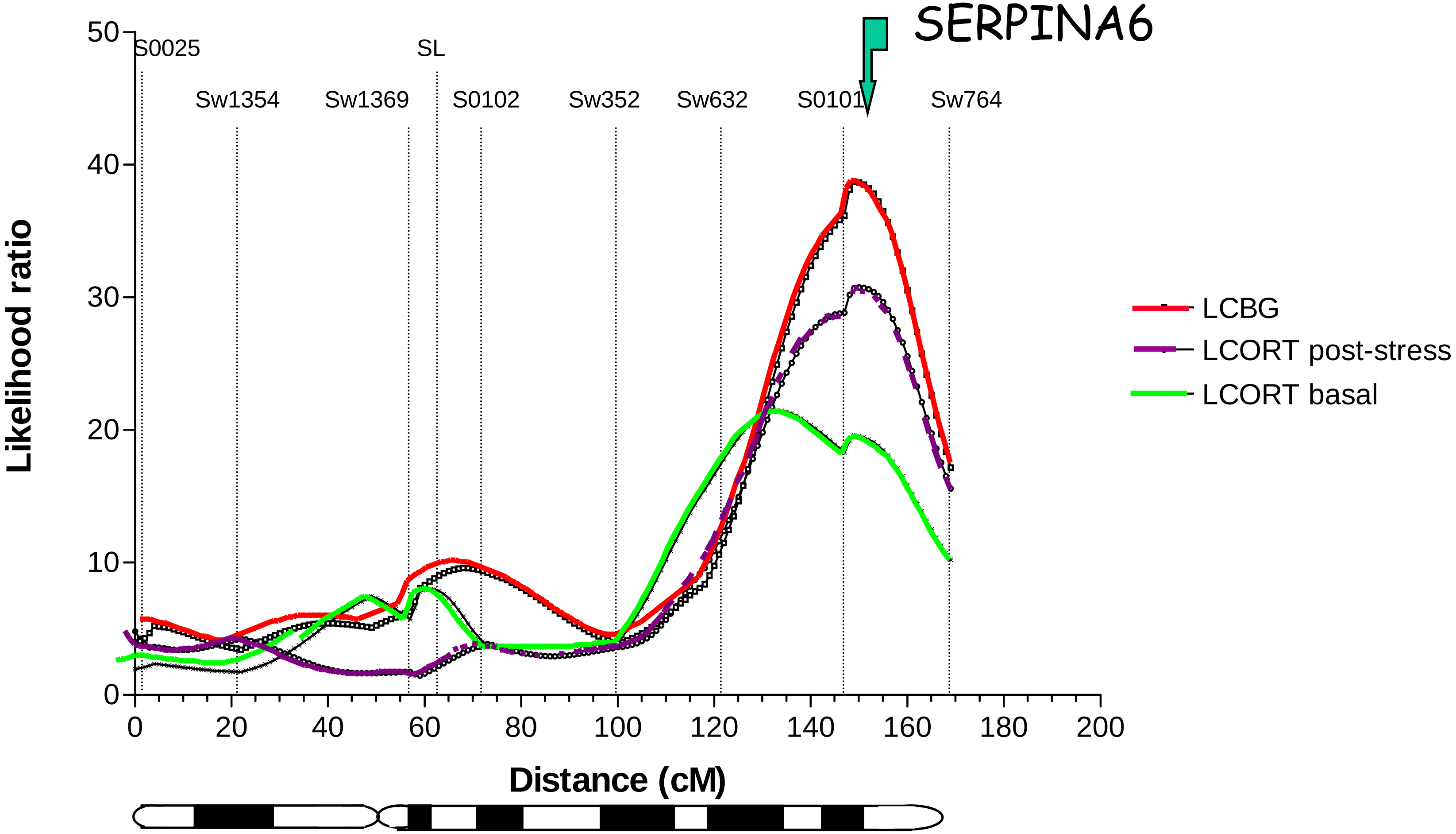
Hazard et al. BMC Genomics 9:101 (2008)

See poster # 26/11
by M. Rucinski et al.

Cortisol bioavailability

- Corticosteroid-binding globulin (CBG or transcortin)
  - Glycoprotein carrying cortisol. Plays a dual role of sequestration of cortisol in plasma and delivery to the target tissues.
  - Demonstrated to influence cortisol levels by linkage study in a Large White x Meishan F2 population (Désautés et al. J Anim Sci 80:2276, 2002)
  - Molecular polymorphisms influencing protein properties with consequences on adiposity and meat quality (Ousova et al. Mol Endocrinol 18:1687, 2004; Geverink et al. J Anim Sci 84:204, 2006)

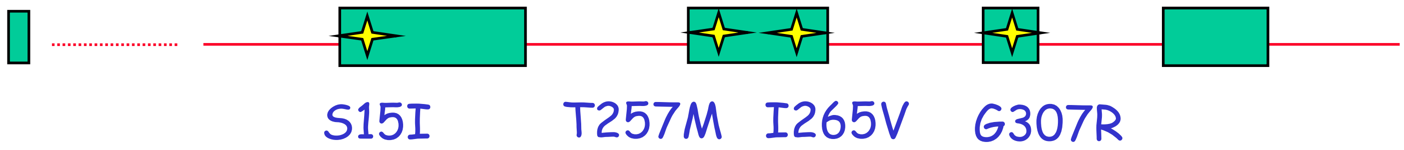
QTL cortisol / CBG on Ssr 7



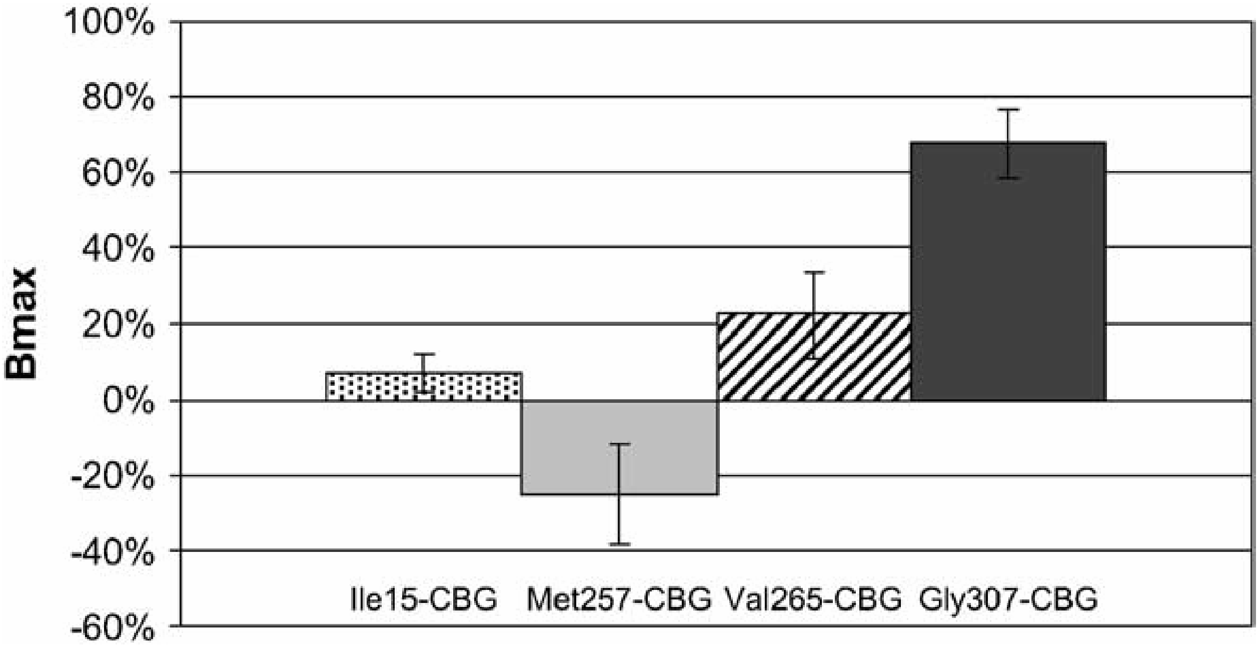
Désautés et al. J Anim Sci 80:2276 (2002)
Ousova et al. Mol Endo 18:1687 (2004)

Polymorphisms of SERPINA6 (Large White / Meishan)

Cbg gene



Transfection in human embryonic kidney (HEK-293T) cells



Cortisol bioavailability

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- Hormone metabolism
  - Cortisol – cortisone shuttle (hydroxysteroid dehydrogenases 1&2)
  - Local tissue bioavailability of cortisol may influence adiposity
  - Linkage/association between HSD11B1 polymorphism and carcass length and adiposity (Otieno et al. Anim Genet 36:36, 2004)

Variation in receptor function and transduction pathways

- Cortisol receptors are transduction factors
  - Glucocorticoid receptors, GR, gene NR3C1
  - Mineralocorticoid receptors, MR, gene NR3C2
- Genetic influences and gene polymorphisms well documented in humans and experimental animals
- Little information in pigs
  - Functional differences LW/MS
    (Perreau et al. Life Sci 17:1501, 1999)
  - Association study: presentation by E. Murani et al.

Sources of genetic variability in the HPA axis

CORTICOSTEROID HORMONES PRODUCTION
- **Response of the adrenal cortex to ACTH**
- 'Central' mechanisms

See presentation by Murani E. et al.

BIOAVAILABILITY
- Corticosteroid-binding globulin (CBG)
- 11β-hydroxysteroid dehydrogenase

ACTION ON TARGET TISSUES
Receptors or transduction pathways

FUNCTIONAL CONSEQUENCES
Production / Meat quality / Robustness

Conclusion and perspectives

- Glucocorticoid hormones have a negative effect on growth rate and feed efficiency, they increase lipid accumulation (fat) at the expense of protein accretion (muscle)
- Genetic selection for fast growth and lean carcasses has reduced the production of glucocorticoid hormones
- Low activity / efficiency of the HPA axis may have negative effects on various traits related to robustness such as adaptation to stress, fetus maturation and newborn survival, immune system and inflammatory processes
- A breeding objective is to increase robustness without negative effects on production traits
- Detailed knowledge on gene polymorphisms will allow fine-tune molecular selection for an optimal level of activity

Collaborators

- Neurogenetics and Stress, PsyNuGen, Bordeaux
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  - Marie-Pierre Moisan
  - Céline Désautés (Ph.D.)
  - Virginie Perreau (Ph.D.)
  - Véronique Guyonnet-Dupérat
  - Olga Ousova (Ph.D.)
  - Nicoline Geverink (post-doc)
  - Dominique Hazard (post-doc)
- INRA, SGQA, Jouy-en-Josas
  - Jean-Pierre Bidanel
  - Marie-Pierre Sanchez
  - Hervé Tribout
- INRA, LGC, Toulouse
  - Denis Milan
  - Nathalie Iannucelli
  - Laurence Liaubet
  - Magali San Cristobal
- INRA, GEPA, Le Magneraud
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  - Yvon Billon
- PIC/Sygen
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  - Gary Evans

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