Genetic linkage mapping of quantitative trait loci for behavioral and neuroendocrine stress response traits in pigs[1]

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ABSTRACT: A QTL analysis of behavioral and neuroendocrine responses to a "novel environment" stress was conducted in a three-generation experimental cross between Meishan and Large White pig breeds. A total of 186 F2 males and 182 F2 females were studied for their behavioral and neuroendocrine reactivity to a novel environment test at 6 wk of age. Locomotion, vocalization, and defecation rate, as well as exploration time, were measured for 10 min. Blood samples were taken immediately before and after the test to measure plasma levels of ACTH, cortisol, and glucose. Animals were typed for a total of 137 markers covering the entire porcine genome. Analyses were performed using two interval mapping methods: a line-cross regression method, where founder lines were assumed to be fixed for different QTL alleles, and a half-/full-sib maximum likelihood method where allele substitution effects were estimated within each half-/full-sib family. Both methods revealed a highly significant gene effect for poststress cortisol level ($P < 0.001$) and a significant effect for basal cortisol level ($P < 0.05$) at the end of the q arm of chromosome 7, explaining, respectively, 20% and 7% of the phenotypic variance. Meishan alleles are associated with higher cortisol levels and are partially dominant (for poststress levels) over Large White alleles. Other significant gene effects on biological measures were detected on chromosomes 1 and 17 (ACTH response to stress), 3, 5, and 8 (glucose levels). The SSC 17 QTL explains 12% of the phenotypic variance of poststress ACTH levels, with a suggestive evidence of imprinting effects. Meishan alleles are associated with lower poststress ACTH levels. Gene effects of low amplitude only were found for behavioral reactivity traits. Considering the effects of stress neuroendocrine systems on energy fluxes and protein deposition, and the importance of stress reactivity for meat quality and animal welfare, these results open new perspectives for pig selection.

Key Words: Behavior, Hypothalamus, Large White, Meishan, molecular genetics, stress

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Introduction

The management of stress in modern husbandry is an important goal for the economics of animal production, product quality, and animal welfare. Most efforts have been directed toward the adjustment of the environment to animal needs, but the use of genetic selection is an alternative approach to increase adaptive abilities of livestock and animal well-being (Muir and Craig, 1998; Newman, 1994). Indeed both behavioral and neuroendocrine adaptive responses are widely variable and under genetic influence (Dantzer and Mormède, 1983; McGlone et al., 1998; Mormède et al., 2002). They are therefore amenable to genetic selection. One example of the successful use of molecular genetics is the identification of the halothane-susceptibility gene in pigs (Fuji et al., 1991).

The development of genetic maps in livestock species has allowed the detection of genomic regions contributing to the genetic variation of quantitative traits, such as growth, body composition, meat quality, or reproduction (Bidanel et al., 2001; Malek et al., 2001a,b). In spite of the accumulating evidence for

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Table 1. Distribution of F2 pigs in full-sib families. Number of male (M) and female (F) offspring per sire (sires are numbered from 1 to 6 and lines in the table correspond to the respective full-sib families)

	Sire											
	1		2		3		4		5		6	
Sex:	M	F	M	F	M	F	M	F	M	F	M	F
	0	3	4	2	14	9	19	10	13	7	10	17
	8	6	1	4	4	6	4	4	5	4	12	14
	15	7	10	10	7	9	14	11	0	8	4	4
	3	3	6	7	3	3	13	16	10	6		
									7	12		
Total (per sex/sire)	26	19	21	23	28	27	50	41	35	37	26	35
Total (per sire)	45		44		55		91		72		61	

genetic variation in pig behavior and for a genetic control of stress neuroendocrine responses (McGlone et al., 1998; Mormède et al., 2002), these traits have not so far been reported in pig QTL experiments.

As compared to western breeds, Chinese Meishan pigs have been shown to have very different stress responses, with low behavioral activity and reactivity, and an hyperactive adrenal gland (Mormède et al., 1984; Bergeron et al., 1996; Désautés et al., 1997, 1999; Hay and Mormède, 1998; Weiler et al., 1998). Therefore, behavioral and neuroendocrine responses to an environmental challenge were measured in an experiment developed at INRA to map QTL that explain differences between the Meishan and Large White breeds for a large number of traits (Bidanel et al., 2001). In this paper, we identify several genomic regions related to variability of stress reactivity.

Experimental Procedures

Animals and Data Recording

A three-generation resource population was developed at the INRA experimental research farm of Le Magneraud (Surgères, France) by first mating six unrelated Large White boars to six lowly related Meishan sows (one boar/sow). The 12 founder animals were tested and found to be free of the mutation at the ryanodyne receptor locus, which is responsible for halothane susceptibility. One boar and four gilts were kept for breeding in each of the six litters produced (except in one litter where only three females were available). Three or four F1 females were assigned to each of the F1 boars and were mated to produce large families of F2 piglets. Matings were assigned so as to minimize relationships. Six F1 females were culled early and were removed from the experiment. The 17 remaining sows were allowed to produce up to 13 litters. Two of the six males were culled before the end of the experiment. Their females were reassigned to the four remaining males in order to produce new full-sib families. Only 368 piglets were studied for the traits presented here and Table 1 gives their family structure. A detailed description of the breeding protocol and further characterization of the whole population can be found in Bidanel et al. (2001).

The F1 sows were managed under a batch farrowing system, with a 3-wk interval between contiguous batches. Piglets were weighed at birth and at 3 wk of age, weaned at 3 wk of age and then allocated in groups of 25 ± 1 animals in 3.65-m $\times$ 1.70-m pens with free access to food and water. Lights were on from 0600 to 2000.

Piglets were submitted to a "novel environment" challenge at 6 wk of age as described by Mormède et al. (1994). They were introduced for a 10-min session into a van (2.4 m $\times$ 1.2 m) with the top and sides covered that was kept immobile. The floor was divided by painted lines into 8 sections (0.6 m $\times$ 0.6 m). Wood shavings were spread on the floor and refreshed between two successive animals. Behavioral activities were recorded by a single observer: locomotion (number of sections entered), vocalizations (squeals and grunts), defecations, and exploration (time spent rooting and sniffing the floor or the sides of the test arena). The 368 piglets tested in this experiment were distributed in 15 experimental groups, corresponding to different breeding batches. Each group was tested between 0800 and 1200 on two successive mornings (15 to 17 animals per day).

Blood samples (5 mL) were collected on EDTA in evacuated tubes (Venoject, Terumo, CML, Nemours, France) immediately before and after the test for basal and poststress plasma ACTH, cortisol, and glucose determinations, as described in Désautés et al. (1997). Plasma total cortisol was measured by radioimmunoassay. Tritiated cortisol was used as the tracer and dextran-coated charcoal as the adsorbant for the unbound fraction. Plasma ACTH concentrations were determined by immunoradiometric assay with a commercial kit (Allegro HS-ACTH, Nichols Institute Diagnostics, San Juan Capistrano, CA), validated for pig plasma (Mormède et al., 1994). Plasma glucose concentration was measured by spectrophotometry using an enzymatic method (PAP 1200, Biomérieux, Craponne, France).

Traits Analyzed

A total of 13 traits were defined from the abovementioned measurements:

1. basal ACTH (**ACTH1**), cortisol (**CORT1**), and glucose (**GLU1**) levels measured in the blood sample collected immediately before the stress test
2. poststress ACTH (**ACTH2**), cortisol (**CORT2**), and glucose (**GLU2**) levels measured in the blood sample collected at the end of the 10-min stress test
3. variation of ACTH (Δ**ACTH**), cortisol (Δ**CORT**), and glucose (Δ**GLU**) levels computed as the difference between poststress and basal levels
4. locomotion (**LOC**), vocalizations (**VOC**), number of fecal boli (**DEF**), and exploration time (**EXPL**) measured during the 10-min stress session

Data were first checked for the normality of distributions. Cortisol and ACTH values had log-normal distributions and data were transformed to logarithmic scores before analysis. A Box-Cox power transformation, which has the form $y(t) = (x^t - 1)/t$, where t ($t \neq 0$) is a parameter to estimate and x the variable values, was used for VOC, LOC, EXPL, and DEF with 0.346, 0.238, 0.595, and 0.839 as t-values. Optimal t-values were found by maximum likelihood as proposed by McLean et al. (1976).

Genotyping and Map Construction

A whole-genome scan was performed using a panel of 123 microsatellite markers and the major histocompatibility complex (SLA). The panel was complemented by 13 additional microsatellite markers used in families with homozygous markers in QTL chromosomal regions. The markers covered all autosomes and the X chromosomes, with 3 to 12 markers on each. Genotypes were obtained for all F0, F1, and F2 pigs as described by Bidanel et al. (2001). Multipoint linkage analyses were carried out for males, females and both sexes with version 2.4 of the CriMap software (Green et al., 1990). Recombination units were then transformed to map distances using the Haldane mapping function. The average distance between adjacent markers was 22.0 cM on the sex-averaged map (see Bidanel et al., 2001, for more details).

Phenotypic data were first adjusted for systematic environmental effects. Adjustment factors were obtained using a mixed linear model assuming a polygenic inheritance, as described in Bidanel et al. (2001). The model included contemporary group, sex, age at measurement as fixed effects, birth litter, the additive genetic value of each animal and a residual error term as random effects and the order of piglets in the novel environment test as a covariate. The data $\tilde{\mathbf{y}}$ used for QTL mapping were obtained as $\tilde{\mathbf{y}} = \mathbf{y} - \mathbf{X}\hat{\mathbf{b}} - \mathbf{W}\hat{\mathbf{p}}$. Estimates of fixed effects ($\hat{\mathbf{b}}$) and of common birth litter effects ($\hat{\mathbf{p}}$) were obtained as back-solutions from restricted maximum likelihood analyses (Patterson and Thompson, 1971). The computations were performed using the VCE software (Neumaier and Groeneveld, 1998).

Two types of interval mapping analyses were performed: 1) a line-cross analysis assuming that founder populations are fixed for different QTL alleles (referred to as **LC** model hereafter), 2) a model assuming that the F2 population is a mixture of full and half-sib families and making no assumption about the number of QTL alleles and allele frequencies within the founder populations (referred to as **HFS** model hereafter).

The LC analysis was performed using the software developed by Haley et al (1994). The model used assumed a diallelic QTL with alternative alleles fixed in founder breeds, i.e., QQ in Meishan (with effect a) and qq in Large White (with effect -a) animals. The adjusted performance $\tilde{y}_i$ of an F2 offspring i could be written as

$$\tilde{y}_i = \mu + c_{ai}\, a + c_{di}\, d + e_i \qquad [1]$$

where μ is the population mean, c_{ai} and c_{di} are the coefficients of additive (a) and dominance (d) components, respectively, for animal i at a given position and e_i is the residual error. Coefficients c_{ai} and c_{di} were computed as $c_{ai} = \text{Prob}(QQ_i) - \text{Prob}(qq_i)$ and $c_{di} = \text{Prob}(Qq_i)$, where $\text{Prob}(XX_i)$ is the probability of animal i to have the genotype XX. The genotypic probabilities were computed as described in Haley et al. (1994) considering only the most probable phases. At each location (each centimorgan), an *F*-ratio was computed comparing Model [1] with one QTL to an equivalent model without any linked QTL. Estimates for a and d were calculated at the location with the highest *F*-ratio.

Additional line-cross analyses were performed to test the presence of sex × QTL and family × QTL interactions, of imprinting effects and of linked QTL. The sex × QTL interaction was investigated by estimating additive and dominance effects for each sex separately. This model was first compared with a model with no QTL, giving an *F*-test (F_{4df}) with 4 d.f. in the numerator. When F_{4df} reached significance, a second test was performed comparing the model with interaction with the best single QTL model (F with 2 d.f. in the numerator).

Models with either sire × QTL or dam × QTL interactions were also run to test differences in QTL effects between families, which would suggest the existence of different QTL alleles in founder populations. Models with sire × QTL and dam × QTL interactions were first compared with a no-QTL model, giving *F*-tests with 12 and 48 d.f., respectively. They were then compared to the best single QTL model, giving *F*-tests with 10 and 46 d.f., respectively. The interactions were also considered significant only when both statistics reached significance.

The presence of imprinting effects was investigated as suggested by Knott et al. (1998) by adding a third effect to the model in order to test differences between two classes of heterozygotes, defined according to the paternal or maternal origin of grandparental (MS or LW) alleles. This model was first contrasted with a no-QTL model (*F*-test with 3 d.f. in the numerator). When significant, it was compared with the best QTL model to test the significance of imprinting effects.

The presence of two QTL in the same linkage group was tested by adding additive and dominance effects for a second QTL in the model and carrying out a two-dimensional search, fitting the coefficients for all possible combinations of two positions on the chromosome. Two *F*-statistics were computed. The first F-value with 4 d.f. in the numerator was obtained by contrasting the two-QTL with a no-QTL model. When F_{4df} was significant, a second *F*-value (F_{2df} with 2 d.f. in the numerator) was computed by contrasting the two-QTL model with the best single QTL model.

Only a single QTL model was considered in the HFS approach. The F2 population was supposed to be structured in 24 full-sib families nested within 6 independent sire families. Hence, dams mated to different sires were considered as different dams. The test statistic was computed as the ratio of likelihoods (L-ratio) under the hypothesis of one (H1) vs no (H0) QTL linked to the set of markers considered. Under H1 hypothesis, a QTL with a gene substitution effect for each sire and each dam was fitted to the data. Sire genotypes were considered as correctly determined due to the large family size, so that only the most probable sire phase was considered. Conversely, all probable enough (above 0.10) dam phases were considered, so that the likelihood could not be entirely linearized. Further details on likelihood computation procedures can be found in Le Roy et al. (1998) and Bidanel et al. (2001). Average substitution effects, which in the present case are equivalent to additive values (a), were estimated within each sire and dam family, as explained by Bidanel et al. (2001), at the location with the highest likelihood ratio.

The distribution of *F*- and *L*-ratios being unknown, chromosomewide significance thresholds were determined empirically by data permutation as described by Churchill and Doerge (1994) for the line-cross analyses and by simulating the data assuming a polygenic infinitesimal model and a normal distribution of performance traits for the HFS analysis (Le Roy et al., 1998). A total of 10,000 permutations or simulations were performed for each chromosome × trait combination. This number was increased to 50,000 when accurate thresholds for highly significant linkage were required. It has to be noted that the thresholds obtained are based on realized marker density and not on an infinitely dense marker map as proposed by Lander and Kruglyak (1995).

Thresholds for *suggestive*, genomewide *significant*, and *highly significant* linkage were defined. Suggestive linkage was defined as the probability of obtaining, by chance, one significant result per genome analysis (Lander and Kruglyak, 1995). Considering that 19 independent chromosomes were analyzed and assuming the number of significant chromosomes follow a binomial distribution, the required threshold for suggestive linkage on a chromosome level P_c is such that $19\ P_c = 1$; that is, $P_c \sim 0.05$ (Knott et al., 1998). To derive genomewide from chromosomewide significance levels, a Bonferroni correction was applied. The chromosomal test significance level P_c corresponding to a genomewide test probability P_g was obtained as a solution to $P_g = 1 - (1 - P_c)^{19}$, which gives $P_c = 0.0027$ and 5.3×10^{-5}, respectively, for genomewide significant ($P_g = 0.05$) and highly significant ($P_g = 0.001$) linkage (Knott et al., 1998). An equivalent number of independent traits was computed using canonical transformation (Weller et al., 1996) based on phenotypic correlation estimates in order to estimate the expected number of false-positive results. The canonical transformation showed that the first nine factors accounted for 95.8% of the total variation, so that 8.5, 0.46, and 9×10^{-3} false-positives can be expected based on the above mentioned suggestive and genomewide significant and highly significant levels, respectively.

Models with sex × QTL and family × QTL interactions, with imprinting and with two QTL, were tested using approximate significance thresholds obtained as described by Knott et al. (1998). The threshold *F*-ratio obtained from the null hypothesis was converted into a probability of the *F*-ratio under a standard *F*-distribution with 2 d.f. in the numerator. It was then possible to obtain the *F*-ratio that would give the same probability under a distribution with 1, 3, or 4 d.f. from standard *F*-tables. Genomewide suggestive and significant ($P < 0.05$) thresholds were, respectively, 8.0, 4.7, 4.1, 2.8, 2.0, 2.0 and 13.5, 6.9, 6.1, 3.6, 3.3, 2.1, 2.1 for *F*-ratios with 1, 3, 4, 10, 12, 46, and 48 d.f. Corresponding thresholds for likelihood ratios under HFS model ranged from 57 to 59 and from 66 to 68, respectively, according to the trait × chromosome combination considered.

Results

The overall means and phenotypic standard deviations of the 13 traits studied are given in Table 2. Associations between trait scores and markers with at least a suggestive level of significance obtained using either LC and HFS models are given in Table 3.

Both LC and HFS analyses showed genomewide (CORT1: $P_g < 0.05$) or highly significant genomewide (CORT2: $P_g < 0.001$) evidence for a QTL affecting cortisol levels on SSC7. The most likely location of the QTL is in the S0101–SW764 interval at the end of the q arm of SSC 7 and explains, respectively, 7.7% and 20.7% of the phenotypic variance of CORT1 and CORT2 (Figures 1 and 2). Meishan alleles are associated with higher cortisol levels and are partially dominant (CORT2) over Large White alleles.

Five other genomewide significant chromosomal regions were found using the HFS model, with two of them, located on SSC 8 and SSC 17 and affecting GLU2 and

Table 2. Overall means and phenotypic standard deviations of the 13 traits studied

Trait	Abbreviation	No. of pigs	Mean	Standard deviation
Basal ACTH level, pg/mL	ACTH1	368	73	89
Post stress ACTH level, pg/mL	ACTH2	365	206	131
Variation of ACTH level, pg/mL	ΔACTH	348	135	112
Basal cortisol level, ng/mL	CORT1	340	85	43
Poststress cortisol level, ng/mL	CORT2	342	162	49
Variation of cortisol level, ng/mL	ΔCORT	337	78	31
Basal glycemia, g/L	GLU1	366	1.18	0.25
Poststress glycemia, g/L	GLU2	363	1.29	0.26
Variation of glycemia, ng/mL	ΔGLU	363	0.11	0.19
Locomotion, no. of sections entered	LOC	367	24.7	17.5
Vocalizations	VOC	368	74.2	63.2
Exploration	EXPL	367	3.3	1.7
Defecation, no. of fecal boli	DEF	367	2.4	1.9

ACTH2, respectively, being highly significant. The SSC 17 QTL was also shown at a suggestive level of significance using the LC model. It is located in the S0359–SW2431 interval and explains 12% of the phenotypic variance of ACTH2 under the HFS model. Meishan alleles are associated with lower poststress ACTH levels. The most likely position of the SSC 8 QTL is very close to the S0376 marker. The additive genetic value (a) averaged over families is close to zero, due to a similar number of positive and negative within-family estimates of a. The QTL explains 7% of the phenotypic variance of GLU2 when computed from within-family estimates of a. A similar situation occurs on SSC 1, where another genomewide significant QTL explaining 6% of the phenotypic variance of GLU2 is located close to the S0113 marker. Yet, it should be noted that nonsignificant sire × QTL and dam × QTL interactions were obtained using the LC methodology in both cases. Two other genomewide significant QTL affecting glycemia were found at the end of the q arm of SSC 3 and SSC 5, respectively. Meishan alleles are on average associated with high GLU1 values on SSC 3 and with low GLU2 and ΔGLU values on SSC 5.

Three additional genomewide significant ($P < 0.05$) QTL were shown using the LC model. A QTL affecting ACTH2 and, at a suggestive significance level, ΔACTH, is located in the S0155–S0374 interval on SSC 1. Meishan alleles are dominant over Large White alleles and are associated with a lower poststress ACTH level mainly due to a reduced ACTH increase during environ-

Table 3. Results of QTL analyses

		Line-cross model				Half/full-sib model			
Trait[a]	SSC	Position, cM[b]	*F*-ratio[c]	Additive effect ± S.E.	Dominance effect ± S.E.	Position, cM[b]	*L*-ratio[c]	Additive effect[d]	% variance[e]
ACTH2	1	125	9.5*	–29.5 ± 8.4	–27.5 ± 11.2	135	55.2 ns	—[f]	3.2
ΔACTH	1	116	7.0†	–18.7 ± 7.0	–22.0 ± 9.2	143	52.0 ns	—	4.9
GLU2	1	99	2.5 ns	—	—	102	73.1 *	0.00	6.0
LOC	1	94	9.1*	–5.5 ± 1.5	0.6 ± 2.3	139	46.0 ns	—	8.6
GLU1	3	110	5.2 ns	—	—	121	70.5*	0.09	13.6
GLU2	5	17	2.1 ns	—	—	110	64.9†	–0.08	10.0
ΔGLU	5	123	4.6 ns	—	—	118	77.4**	–0.07	7.2
CORT1	7	149	11.3*	12.6 ± 2.7	6.2 ± 4.2	137	69.9*	10.1	7.7
CORT2	7	149	35.4***	22.9 ± 2.8	8.9 ± 4.2	153	101.5***	18.6	20.7
ΔCORT	7	156	8.3†	9.6 ± 2.4	–0.5 ± 3.8	100	44.1 ns	—	6.6
EXPL	8	112	10.6*	0.43 ± 0.12	0.50 ± 0.20	4	45.7 ns	—	8.8
GLU2	8	142	2.4 ns	—	—	71	85.5***	0.00	7.0
CORT1	11	5	7.9†	4.7 ± 2.4	19.8 ± 5.0	43	35.9 ns	—	7.1
ACTH2	17	54	6.3†	–30.4 ± 8.9	11.1 ± 14.4	58	79.6***	–51.8	4.6
CORT1	18	3	7.0†	–8.3 ± 2.2	0.9 ± 3.2	0	44.6 ns	—	4.1
ΔCORT	18	0	6.3†	9.4 ± 2.7	–3.3 ± 3.7	9	46.1 ns	—	5.0

[a]See Table 2 for the definition of the traits.
[b]Most probable location of the QTL.
[c]*; **; *** = 5%, 1%, and 0.1% genomewide significance levels, respectively. † = suggestive linkage; ns = not significant.
[d]Meishan - Large White alleles.
[e]Genetic variance at the QTL based on estimated additive and dominance effects and allele frequencies of 2 or 0.5, as a percentage of the phenotypic variance in the F2.
[f]Estimates are considered as meaningless and are consequently not indicated.

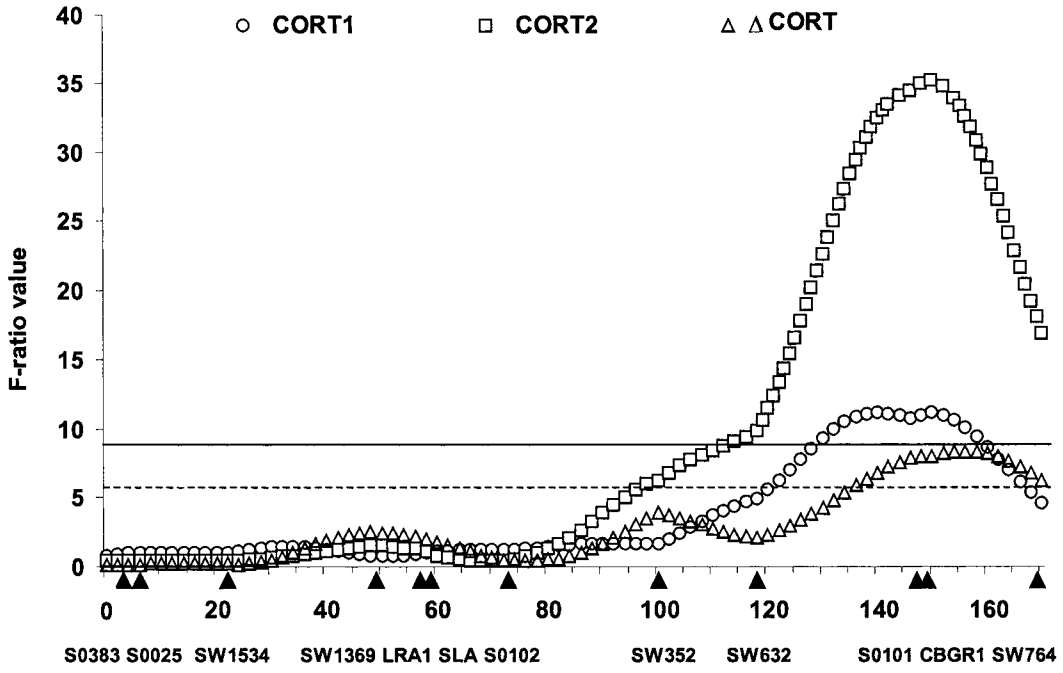

Figure 1. *F*-ratio curves for evidence of QTL on chromosome 7 under line-cross model. CORT1, CORT2, ΔCORT = basal, poststress, and variation of cortisol levels, respectively. The horizontal dashed and solid lines are the approximate 5% chromosomewide and genomewide significance levels, respectively. Arrows indicate microsatellite positions.

mental challenge. Another genomewide significant association was found for LOC on SSC 1. The most likely position of the QTL is in the S0396–S0113 interval, 23 cM away from the ACTH QTL. The QTL explains 6% of the phenotypic variance and has purely additive effects. Meishan alleles are associated with a lower locomotor activity than Large White alleles. The third QTL is located close to the SW1551 marker on SSC 8 and ex-

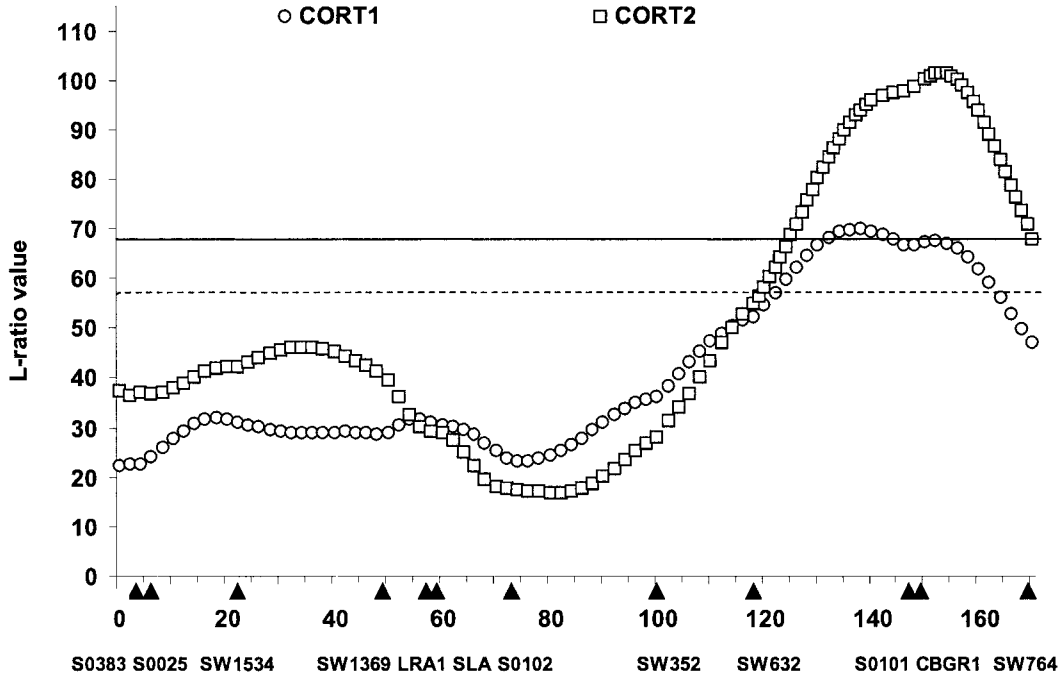

Figure 2. *L*-ratio curves for evidence of QTL on chromosome 7 under half/full sib model. CORT1, CORT2 = basal and poststress cortisol levels, respectively. The horizontal dashed and solid lines are the approximate 5% chromosomewide and genomewide significance levels, respectively. Arrows indicate microsatellite positions.

Table 4. Results from fitting a model with imprinting

Trait[a]	SSC	Location	F_{3df}[b]	F_{1df}[c]	i[d]	(S.E.)[d]	a[d]	(S.E.)	d[d]	(S.E.)
ACTH2, pg/mL	17	42	7.48*	10.66†	30.5	(9.3)	–32.7	(9.2)	6.0	(14.9)
ΔACTH, pg/mL	17	42	6.24†	12.10†	25.5	(7.3)	–19.3	(7.3)	4.2	(11.1)

[a]See Table 2 for the definition of the traits.
[b]Model [4] versus Model without QTL; † suggestive level *$P < 0.05$.
[c]Model [4] versus Model [1] with best single QTL; † $P < 0.05$ at chromosomewide level.
[d]i, a, d = estimates of imprinting, additive, and dominance effects, respectively; S.E. = standard error.

plains 4% of the phenotypic variance of EXPL. Meishan alleles are associated with more exploration time than Large White alleles.

Finally, suggestive associations were found for CORT1 at the end of the p arm of SSC 11 and for CORT1 and ΔCORT on SSC 18. Meishan alleles are associated with high CORT1 values on both chromosomes and with a lower ΔCORT value on SSC 18.

Additional genome scans using more complex models did not reveal any sex × QTL, sire × QTL or dam × QTL interactions. However, suggestive evidence of imprinting effects was obtained for the ACTH2 and ΔACTH QTL identified on SSC17 (Table 4). Estimated imprinting effects indicate that heterozygous individuals that received the LW allele from the male parent have higher ACTH2 and ΔACTH values than those who received it from the female parent. Additive and dominance values remained similar to those obtained using the model without imprinting.

Results from the two-QTL genome scan are shown in Table 5. Suggestive evidence for a second QTL (QTL2) affecting CORT2 and, to a lesser extent, ΔCORT was obtained on SSC7. The QTL located at 100 cM was overdominant on both traits and had nonsignificant additive effects. The most likely position of QTL1 and its additive and dominance effects remained very similar to those obtained under the single QTL model (Table 3).

Discussion

Methodology

With the exception of highly significant QTL that were detected in both cases, different QTL were detected using the line-cross (LC) and half/full-sib (HFS) methods. As discussed by Bidanel et al. (2001), this situation can often be explained by the fact that each method makes different assumptions about family structure and QTL genotypes in founder populations. The basic LC model assumes that different QTL alleles are fixed in founder populations. It is very powerful when this assumption holds to the true state of nature, but it will not be able to detect QTL with similar allele frequencies in founder populations. The HFS model, which does not make any assumption about allele distribution in founder populations, is more adapted to such situations. It has in the present case allowed detecting QTL such as those affecting glucose concentrations that would not have been detected using the LC model only. Conversely, the HFS approach involves a much larger number of parameters, which may in some instances reduce its power.

The above-mentioned limitation of the LC approach can be partly removed by adding sire × QTL or dam × QTL interactions in the model. However, some results from this latter model remained inconsistent with those obtained with the HFS model. One possible explanation is that the LC model with interactions does not account for the hierarchical structure of the population and does not simultaneously analyze the effects of sire and dam alleles, which may consequently be biased if sire and dams have different allele frequencies.

The use of more complex genetic models has provided suggestive evidence for a parent-of-origin specific expression of a QTL on SSC17. It should be noted that this effect cannot be unambiguously considered as an imprinting effect. Other hypotheses, such as mitochondrial gene effects or the existence of two or several alleles segregating in parental populations, combined with extreme QTL allele frequency differences between males and females might also explain the observed ef-

Table 5. Results from fitting two QTL

				Position[d] and estimates[e]								
Trait[a]	SSC	F_{4df}[b]	F_{2df}[c]	L_{QTL2}	L_{QTL1}	a_{QTL2}	(S.E.)	a_{QTL1}	(S.E.)	d_{QTL2}	(S.E.)	d_{QTL1}	(S.E.)
CORT1, ng/mL	7	22.2***	7.5+	100	149	–5.4	(3.0)	24.9	(2.9)	7.7	(4.2)	15.1	(4.4)
ΔCORT, ng/mL	7	6.8+	5.1 ns	100	147	–4.0	(2.4)	9.9	(2.3)	9.4	(3.3)	1.4	(3.0)

[a]See table 2 for the definition of the traits.
[b]Model [3] versus Model without QTL; + $P < 0.05$ at a chromosomewide level; *** $P < 0.001$ at a genomewide level.
[c]Model [3] versus Model [1] with best single QTL; + $P < 0.05$ at a chromosomewide level; ns = not significant.
[d]Most likely position of QTL1 and QTL2, respectively.
[e]Estimates of additive (a) and dominance effects of QTL1 and QTL2; S.E. = standard error.

fects. Similar "imprinting" effects of QTL had been reported in pigs by Knott et al. (1998), Jeon et al. (1999), Nezer et al. (1999), and de Koning et al. (2000; 2001) for growth and fatness traits, but in different chromosomal regions. It has to be noted that, contrary to other papers (e.g., de Koning et al., 2000; 2001), imprinting was tested against Mendelian inheritance, which may explain that few regions with evidence of imprinting were detected.

Neuroendocrine Traits

To our knowledge, this is the first reported attempt to identify the molecular bases of different stress neuroendocrine activity and reactivity by QTL analysis, not only in pigs but also in other species. It is not surprising that the strongest linkage was found with plasma cortisol levels, since the high circulating levels of cortisol in the Meishan breed as compared to Western genotypes is a robust finding in different experimental settings (Mormède et al., 1984; Bergeron et al., 1996; Désautés et al., 1997, 1999; Weiler et al., 1998). The partial dominance of the Meishan alleles is in accordance with previous quantitative analysis (Désautés et al., 1997). The directional dominance was also shown previously by the difference measured between reciprocal F1 cross between Meishan and Large White (Désautés et al., 1997) or Yorkshire (Bergeron et al., 1996) progenitors. This breed difference is most probably the result of an increased secretory activity of the adrenal gland, since urinary excretion of cortisol and its metabolite cortisone (and catecholamines) is much higher in Meishan as compared to Large White pigs (Hay and Mormède, 1998). Indeed, the adrenal reactivity to ACTH is increased in the Meishan pigs (Désautés et al., 1999). These neuroendocrine differences may have important functional consequences since glucocorticoids influence metabolic pathways (Dallman et al., 1993), the immune system (Dantzer and Mormède, 1995), and numerous brain functions. As an example, cortisol increases the catabolism of proteins in peripheral tissues, the synthesis of glucose in liver and fat deposition. Therefore, it may have a negative impact on growth and protein deposition, as observed in the Meishan pigs (Bidanel et al., 1993). The suggestive QTL on chromosome 18 has the same most likely position as a QTL related to growth traits (Bidanel et al., 2001). It may be hypothesized that they correspond to a single QTL, which would reflect the relationship between cortisol and metabolisms. However, it is worth noting that there is no other overlap between QTL for biological stress responses and QTL for growth and fatness (Bidanel et al., 2001), despite the existence of phenotypic correlations between poststress cortisol levels and adiposity (our unpublished observations).

At the present time, the molecular mechanisms responsible for the spontaneously occuring differences in hypothalamic-pituitary adrenal axis activity are largely unknown, except for some polymorphisms described in mineralocorticoid and glucocorticoid receptors (Mormède et al., 2001). The detection of chromosomal regions involved in this genetic variation will allow further exploration of underlying molecular mechanisms. On the other hand, selection against high cortisol levels should be advantageous for lean meat production and could be improved by the use of molecular markers since circulating plasma cortisol is a fluctuating parameter very sensitive to numerous environmental factors (Mormède, 1995). The comparison of genetic maps published in different species shows that the end of the long arm of porcine chromosome 7 is orthologous to the telomeric part of the long arm of human chromosome 14 (Goureau et al., 2000). In this region has been mapped the gene encoding corticosteroid-binding globulin, a glycoprotein specifically carrying cortisol in plasma (Billingsley et al., 1993). It has been shown in humans that differences in plasma corticosteroid-binding globulin levels are a major determinant of circulating cortisol concentrations (Bright and Darmaun, 1995). Therefore, *Cbg* is an interesting positional and functional candidate to explain the influence of the QTL on plasma cortisol levels.

The variables related to ACTH integrate more complex influences. Basal circulating levels do not differ between the parental breeds, and the response to stress (ACTH2 and ΔACTH) is larger in Large White pigs, due to both neuroendocrine and psychological factors. On one hand, the feedback action of corticosteroids on the hypothalamic-pituitary adrenal axis activity is probably reduced in Large White pigs, due to lower cortisol levels, and despite adaptive changes of glucocorticoid receptor density in the pituitary gland (Perreau et al., 1999). On the other hand, poststress ACTH levels are correlated with behavioral measures of the emotional responses, such as vocalization, locomotion, and defecation rates in the novel environment (Désautés et al., 1997). The location of a QTL on SSC1 for both poststress ACTH and locomotion rate in the test environment may reflect this relationship.

Finally, the increase of plasma glucose levels under stress was used as an index of sympathetic activation, acting in synergy with cortisol and pancreatic glucagon (Shamoon et al., 1981). The large variability among parental F1 (not shown), and within pure breeds (Désautés et al., 1997) suggests that numerous genetic factors are involved and not necessarily segregated between breeds.

Behavioral Traits

The search for molecular mechanisms of genetic influences on emotional behaviors is very active in rodents, and several QTL have been detected (see Mormède et al., 2001, for review), but no such data are available yet in farm animals. However, selection for different behaviors is a very potent means of improving adaptation of animals to the constraints of their environment, and this process has probably played a major

role in domestication (Price, 1999). In the present study, only two QTL have been found for behavioral traits, and with a limited gene effect. The simplest explanation is that behavioral traits are under polygenic influences without any gene with large effects, as well as very sensitive to environmental influences, as suggested by selection studies (Mormède et al., 2001). The search for underlying molecular mechanisms therefore appears to be a difficult task.

Implications

Behavioral and neuroendocrine adaptations to environmental demands are important components of animal production in terms of economic output, product quality and animal welfare. In the present study of a segregating population between the Meishan and Large White pig breeds, we could detect chromosomal regions (QTL) with strong effect on biological responses to a novel environment stress (poststress plasma cortisol, ACTH, and glucose levels). These QTL could be used to select animals with varying degrees of stress responses in order to analyze the correlated responses in terms of animal production. They can also represent the starting point in the search for genes responsible for these genetic influences and the underlying molecular mechanisms. Due to the very wide implications of adrenal steroids in physiology and physiopathology, the present results open new perspectives for animal production and human health.

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