Quantitative Trait Loci Influencing Abdominal Fat Deposition and Functional Variability of the HPA Axis in the Rat

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Key words
- QTL
- metabolism
- glucocorticoid
- rat

Abstract

With the aim to reveal common genomic regions influencing phenotypes related to HPA axis function and metabolism, we did a quantitative trait loci (QTL) study in a F2 population obtained from the cross-breeding between 2 contrasted rat strains, LOU/C and Fischer 344. QTL determining phenotypes related first to corticotropic function were searched: plasma corticosterone (Cort) in control and stress conditions, after a dexamethasone suppression treatment (glucocorticoid receptor related-effect), and mineralocorticoid receptor-mediated urinary response to aldosterone. Then, phenotypes related to metabolism were studied on the same animals: body composition, basal and post-insulin plasma glucose, plasma free fatty acids, leptin, and insulin. Finally, we analyzed the overlapping regions between these QTL and looked for candidate genes within these regions. The gene NR3C1 encoding the glucocorticoid receptor was confirmed to be central in the link between hypothalamic-pituitary-adrenal (HPA) axis function and fat deposition, and its metabolic consequences. Among the other candidate genes detected, most contain a glucocorticoid responsive element, strengthening our hypothesis of common genetic determinism between HPA axis and metabolism.

Introduction

The hypothalamic-pituitary-adrenocortical (HPA) axis is involved in numerous biological processes including metabolism. Genetic variability in HPA axis function influences feeding behavior, metabolism, and energy expenditure [1,2]. Glucocorticoids act through 2 nuclear receptors: the mineralocorticoid (MR) and the glucocorticoid receptor (GR). Glucocorticoids regulate the release of glucose from hepatic and muscle stores in order to supply the body "fight or flight" response to face environmental challenges. They also induce insulin resistance directly by perturbing insulin signal transduction via GR and indirectly by promoting visceral fat deposition (via MR and GR) and loss of lean mass (via GR) [3,4]. Cushing's syndrome in humans and animals illustrates the link between high glucocorticoid levels and accumulation of central fat at the expense of lean mass and subcutaneous fat [5].

To explore the metabolic consequences of the genetic variations in HPA axis, we compared previously 2 contrasting inbred rat strains, Fischer 344 (F344) and LOU/C. The F344 strain is characterized by high HPA activity and reactivity to stress, abdominal fat deposition increasing with age and high-calorie diets, and insulin resistance. The LOU/C strain is lean and resistant to diet-induced obesity; its HPA axis is hypoactive and hyporeactive to environmental and metabolic challenges [4,6]. The aim of the present study was to find the genomic regions involved in the genetic influences on these traits, to determine whether the same genomic regions could be involved in HPA axis function and metabolic parameters in the rat and to propose candidate genes that could be involved in the relationship between these phenotypes. To this end, an F2 LOU/C×F344 population was genotyped with microsatellite markers, and a QTL analysis was conducted by multiple interval mapping (MIM) on a large panel of phenotypes. The phenotypes related to HPA axis function were plasma corticosterone (Cort) in control and stress conditions, after a dexamethasone suppression treatment (GR effect), and MR-mediated urinary response to aldosterone. Phenotypes related to metabolism were body composition, plasma glucose in control and after insulin treatment, plasma FFA, leptin, and insulin.

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Bibliography
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Materials and Methods

Animals

All animal experiments were conducted according to the INRA Quality Reference System, and to relevant French (Directive 87/148, Ministère de l'Agriculture et de la Pêche) and international (Directive 86/609, November 24th 1986, European Community) legislation. They adhered to protocols approved by Région Aquitaine Veterinary Services (Direction Départementale de la Protection des Populations, approval ID: A33-063-920). Our local ethics committee specifically approved this study. Every effort was made to minimize suffering and the number of animals used. LOU/C and F344 rats were first purchased from Charles River (L'Arbresle, France), and then produced in our laboratory. F1 hybrids were obtained by cross-breeding LOU/C with F344 rats, and then F1 were bred inter se to obtain the F2 population of which 93 males and 94 females were studied. All rats were housed in a temperature-controlled room (23 ± 1 °C) with a light/dark cycle of 12/12-h (lights on at 07:00 h). Food and tap water were provided ad libitum.

Rats were randomly submitted to different protocols aiming to characterize their HPA axis function and their metabolic parameters. Blood was collected on EDTA in all rats in the morning (09:00–11:00 AM) from a nick of the tail. Blood was centrifuged at 4000*g* for 10 min at 4 °C in order to extract the plasma and stored at −80 °C until use.

Tests relative to HPA axis function

Plasma Cort was measured in plasma samples obtained: in the morning between 09:00 h and 11:00 h (basal levels); 2 h after a (DEX) subcutaneous injection of dexamethasone [5 µg/100 g body weight (BW), "GR feedback effects"]; and at the peak (60 min) of the response to a 20-min restraint stress (in a plastic bag, Harvard Apparatus, Courtaboeuf, France [6]).

In order to test the functional properties of MR, the effect of an acute high dose of aldosterone on the urinary Na^+/K^+ ratio was measured in F2 rats, with a dose shown by most authors to induce a large decrease of the Na^+ and K^+ ratio 6–8 h after injection [7–9]. Aldosterone (100 µg/100 g BW) was injected subcutaneously. A water load (3 ml) was administered intraperitoneally, and urine was collected for 8 h after injection as previously described [10, 11]. Each test was randomly applied to rats during 6 weeks from adulthood (12 weeks of age) and separated by a minimum of 2 days. The reference body weight was taken at the end of the experiment (18 weeks-old).

Metabolic measurements

F2 rats were fasted overnight before the insulin test. Blood was taken from a nick of the tail in order to measure basal plasma glucose concentration (T0). Two minutes later, rats were injected i.p. with insulin (Actrapid Humarin®, Novo Nordisk, Mainz, Germany, 4 IU/kg). Then, blood samples were collected at T0 + 30 min as described in the literature (test of insulin resistance [12, 13]). Since basal plasma glucose showed high individual variation, insulin resistance was estimated as the percentage of decrease in glycemia in each F2 rat.

Assay techniques

Corticosterone: Plasma Cort was measured after steroid extraction with absolute ethanol with an in-house RIA [14] using a highly specific anti-Cort antibody provided by Prof H. Vaudry (University of Rouen, France).

Na^+/K^+ ratio: Urine samples were 5-fold diluted with distilled water to fit the detection limits of the measuring devices. Na^+/K^+ determinations were made with a flame photometer (M410, VWR international) and osmolarity on an Osmomat (Gonotec, Berlin, Germany). Urinary electrolyte values were expressed as mmol/osmol to correct for urinary volume variations.

Plasma glucose and FFAs were measured in duplicate with colorimetric kits (Biolabo, Maizy, France, and Wako Chemicals, Neuss, Deutschland, respectively). Plasma insulin and leptin were measured in duplicate with RIA kits (Insik 5, DiaSorin SA, Antony, France, and Linco Research, St Charles, MO, USA, respectively).

For body composition, 3 depots of adipose tissue were carefully removed and weighed: epididymal (around testis and ductus deferens), retroperitoneal (along the posterior wall, from the kidney to the hip region), and subcutaneous fat (estimated by the weight of skin with subcutaneous fat).

DNA extraction and PCR

DNA was isolated from the lungs of parental strains and F2 rats as classically described, by overnight digestion at 55 °C in lysis buffer (10 mM Tris pH 8.0, 100 mM NaCl, 50 mM EDTA, 0.5 % SDS and 0.2 mg/ml proteinase K) followed by phenol-chloroform extraction and ethanol precipitation [15].

The genome scan of the F2 population was made with 108 microsatellite markers (Eurogentec, Angers, France) selected for their polymorphism between LOU/C and F344 strains (http://www.rgd.mcw.edu), and covering evenly the whole genome. PCR reactions were performed in a 20 µl reaction volume by combining 50 ng of genomic DNA with 5 pmol of each primer, 200 µM dNTP and 0.4 U of Taq DNA polymerase (Promega) in 1 × PCR buffer (Promega). Alleles were visualized on ethidium bromide-stained 3 % agarose gel.

QTL analysis

Trait values were corrected for the effects of experimental batch, sex, and directions of the initial cross (LOU/C × F344 vs. F344 × LOU/C, the first strain being the dam). QTL analyses were performed with the MultiQTL software, using the simple interval mapping, and the MIM method combining QTL mapping analysis with the analysis of genetic architecture of quantitative traits. The Korol et al. recommendations (MultiQTL.com, [16, 17]) were followed for the search strategy to select the "best" genetic model defining the genomic regions influencing the trait, that is, the hypothesis of one QTL vs. none or 2 QTL were kept when the "one QTL hypothesis" only was significant, and the 2 QTL model was chosen when the "2 QTL hypothesis" vs. the "one QTL hypothesis" was significant. Permutation analysis (1 000 replications) was used to determine the significance level for the LOD score. All QTL were strongly significant ($p < 0.001$) unless notified in **Table 1–4**. The QTL effects were estimated as the proportion of the observed F2 phenotypic variance explained by each QTL (PEV). The location of the QTL peak and its 95 % confidence interval were estimated after 1 000 bootstraps.

Genetic analysis

Overlapping chromosome segments carrying significant QTL (95 % confidence intervals) determining both HPA axis and metabolic parameters were investigated for relevant genes and QTL already identified by using http://www.ensembl.org. On the site

Table 1 QTL influencing body composition.

Chromosome	Marker	Confidence interval (Mb)	LOD	PEV (%)
Body weight 18 weeks				
1	D1Mit4	106.3–192.4	6.83	12.2
5	D5Mgh22	72.1–157.6	5.00	9.7
10	D10Rat98	28.7–110.7	4.38	6.5
12	D12Mit2	42.7–46.8	2.90	4.4
17	D17Rat43	71.6–97.3	4.36	7.9
19	D19Rat13	4.7–56.4	3.34	6.0
20	D20Mgh4	0.0–19.0	3.13	4.6
Percentage of total visceral fat				
2	D2Rat240	222.8–254.0	11.70	14.3
3	D3Rat66	46.0–57.8	16.58	14.1
5	D5Mgh20	112.9–132.3	16.13	21.0
8	D8Rat16	95.0–102.0	9.07	9.1
10	D10Mgh1	90.6–101.3	14.99	8.0
15	D15Rat110	10.2–24.6	10.20	6.3
16	D16Rat15	73.4–80.0	12.22	7.1
18	D18Rat8	55.0–82.2	6.38	3.4
Percentage of lean mass				
1	D1Rat200	68.9–229.8	8.91	31.0
	D1Rat49	117.0–250.8		
4	D4Rat151	24.0–63.1	3.39	7.5
11	D11Rat68	12.6–87.8	2.25	4.5
18	D18Rat57	12.5–85.7	3.59	7.5
Ratio WAT tissue/lean mass				
2	D2Rat240	207.9–249.5	7.69	12.8
3	D3Rat66	33.1–75.8	4.52	5.6
5	D5Mgh20	99.6–152.3	6.17	9.6
8	D8Rat16	78.5–112.9	8.06	18.2
11	D11Rat68	22.8–73.5	3.80	3.7
15	D15Rat52	12.3–56.6	4.82	5.3
16	D16Rat15	53.0–80.1	5.00	6.9
18	D18Rat8	27.8–82.2	3.81	4.5
Percentage of retroperitoneal fat				
2	D2Rat240	215.7–243.9	14.08	14.6
3	D3Mit12	105.0–112.6	22.46	12.4
4	D4Arb10	56.9–64.9	9.57	4.5
5	D5Mgh20	112.5–135.4	17.61	20.7
8	D8Rat16	94.8–101.5	12.07	12.4
10	D10Mgh1	90.1–91.0	16.22	7.4
16	D16Rat67	52.8–83.2	8.33	9.4
Percentage of epididymal fat				
2	D2Rat70	192.6–255.0	7.47	4.9
5	D5Mit17	57.1–83.0	20.66	12.1
	D5Mgh20	124.7–152.9		
8	D8Rat29	55.2–97.1	11.78	22.5
	D8Rat16	80.2–109.1		
10	D10Rat98	69.6–70.9	19.24	7.8
11	D11Rat68	20.0–70.6	9.29	13.3
	D11Rat51	34.9–87.8		
14	D14Rat91	1.9–107.7	6.00	8.8
	D14Rat95	68.9–112.2		
18	D18Rat8	70.8–74.5	17.08	7.9
Percentage of subcutaneous fat				
2	D2Rat240	155.8–258.2	5.80	9.0
4	D4Rat142	4.8–52.6	5.54	6.4
6	D6Rat105	18.6–33.1	4.57	4.8
8	D8Rat29	23.4–122.7	3.37	3.9
9	D9Rat132	11.1–28.5	5.55	5.6
17	D17Rat118	47.5–82.3	8.62	27.1
	D17Rat43	57.7–97.3		

All QTL were strongly significant ($p<0.001$)

Table 2 QTL influencing biochemical parameters related to metabolism.

Chromosome	Marker	Confidence interval (Mb)	LOD	PEV (%)
Plasma leptin				
2	D2Mit30	10.2–38.1	3.84	6.1 ($p<0.01$)
4	D4Rat142	4.8–116.2	5.09	22.2
	D4Rat151	8.2–63.8		
5	D5Mgh2	17.5–173.1	5.20	8.0
19	D19Rat13	10.2–38.1	4.63	7.9
Plasma insulin				
3	D3Mgh19	15.0–36.6	13.86	12.1
	D3Rat59	120.0–163.4		
6	D6Rat105	20.0–25.3	19.06	12.6
8	D8Rat49	28.6–43.4	11.86	10.8
	D8Rat159	43.6–94.2		
9	D9Mgh6	25.9–54.3	17.96	10.6
	D9Rat22	43.6–94.2		
10	D10Rat37	4.4–86.4	10.46	19.0
	D10Rat98	5.5–110.7		
13	D13Mgh2	18.7–46.4	10.82	6.3
	D13Mgh9	23.4–94.8		
15	D15Rat110	13.8–28.9	9.06	4.8
17	D17Rat118	53.8–64.2	8.82	8.4
	D17Rat43	64.5–94.0		
18	D18Rat25	13.8–26.3	7.15	3.9 ($p<0.05$)
20	D20Mgh4	1.8–21.6	6.38	6.3
	D20Rat60	9.2–32.0		
Plasma basal glucose				
9	D9Rat132	11.5–53.5	6.82	16.9
	D9Rat72	66.9–107.1		
13	D13Rat38	27.6–56.0	6.67	8.3
14	D14Rat77	1.7–37.4	4.54	5.6
18	D18Rat57	0.0–87.3	7.25	32.6
	D18Rat8	24.4–82.2		
Insulin resistance (T 30 min)				
1	D1Rat196	23.5–143.8	10.75	16.2
	D1Rat27	29.9–245.7		
2	D2Rat124	23.0–24.7	28.70	23.2
	D2Mit11	170.9–188.8		
8	D8Rat77	0.0–25.1	18.54	6.9
	D8Rat159	42.8–63.7		
12	D12Mit2	20.9–28.1	18.97	12.7
	D12Rat53	45.6–46.8		
17	D17Rat118	83.1–84.3	20.38	8.6
19	D19Rat15	12.0–44.7	10.81	19.7
	D19Rat13	14.3–41.6		
20	D20Rat10	28.2–33.7	18.38	8.1
Plasma FFA				
2	D2Rat201	24.0–132.8	6.78	17.3
	D2Mit30	33.4–258.2		
4	D4Rat142	4.8–111.3	7.36	22.9 ($p<0.05$)
	D4Rat151	18.8–62.3		
7	D7Rat30	10.2–32.7	8.62	11.1
13	D13Mgh9	77.9–94.8	5.81	8.0
17	D17Mgh5	79.4–97.3	4.81	6.7
20	D20Mgh4	0.0–15.2	5.32	19.3 ($p<0.01$)
	D20Rat60	18.8–55.3		

All QTL were strongly significant ($p<0.001$), unless otherwise notified

http://www.ncbi.nlm.nih.gov, we searched any link between genes positioned in the QTL and metabolism and HPA axis function. The presence of a glucocorticoid responsive element (GRE) in the sequences of candidate genes was checked on the site http://rvista.dcode.org/.

Table 3 QTL influencing HPA axis function.

Chromosome	Marker	Confidence interval (Mb)	LOD	PEV (%)
Plasma Cort at basal level				
3	D3Rat66	48.9–77.1	23.37	15.4
	D3Mit12	99.7–121.8		
6	D6Rat50	77.7–84.2	14.49	5.4
7	D7Rat113	28.6–43.4	24.40	10.4
	D7Rat25	57.6–64.5		
8	D8Rat159	44.4–69.2	17.54	13.7
	D8Rat29	63.9–89.2		
12	D12Mit2	20.9–28.2	13.61	10.2
	D12Rat53	42.3–46.8		
16	D16Rat38	8.6–57.4	8.45	4.9
	D16Rat73	4.4–52.1		
17	D17Rat118	43.3–46.9	23.57	8.3
	D17Rat43	80.5–86.7		
18	D18Rat111	0.0–39.7	19.71	24.6
	D18Rat25	13.4–37.8		
19	D19Rat15	13.6–17.3	23.45	11.4
	D19Rat13	23.6–26.0		
20	D20Rat60	9.8–15.5	20.46	9.7
MR-mediated aldosterone effect on urinary electrolytes				
3	D3Rat66	28.1–84.9	3.50	9.4
6	D6Rat50	64.4–106.4	3.96	11.3
8	D8Rat16	1.0–129.0	3.62	8.2
14	D14Rat91	55.9–112.2	5.66	10.9
19	D19Rat15	0.0–44.5	3.59	6.4
Stress effect on Cort and GR-mediated restoration to basal level				
Plasma Cort at the peak in response to restraint stress				
10	D10Rat37	4.4–91.5	3.26	8.5
15	D15Rat52	12.3–82.1	2.78	9.2
20	D20Mgh4	0.0–9.7	3.48	10.5
	D20Rat10	16.4–33.7		
GR-mediated negative feedback				
1	D1Rat200	28.6–231.6	4.39	13.7
	D1Rat49	47.7–250.8		
4	D4Rat151	22.0–56.4	4.96	10.8
6	D6Rat50	40.8–106.5	2.88	3.4
10	D10Rat37	5.5–110.7	6.30	20.2
	D10Rat98	47.7–110.7		
11	D11Rat68	12.6–32.1	6.51	9.6
17	D17Mgh5	75.3–89.1	4.75	6.2
18	D18Rat57	0.0–87.3	7.64	26.3
	D18Rat8	29.7–87.3		

All QTL were strongly significant ($p < 0.001$)

Statistical analysis

For each marker, comparisons of data were submitted to a Bonferroni correction. Data were normalized for sex and direction of cross. Since we did not reveal any sex × allele interaction, comparisons were conducted with one-way ANOVA with allele as the between-subject factor. Correlation analyses were made with the Prism® software.

Results

Correlations

The correlation coefficients between selected variables were computed in F2 rats in order to validate our experiment and explore the potential link between fat deposition and HPA axis function in both sexes (**Fig. 1**). As expected, plasma leptin concentration was positively correlated to the percentage of white adipose tissue (WAT) ($r = 0.261$, $p < 0.05$ in males; $r = 0.295$, $p < 0.05$ in females). The ratio WAT/lean mass was positively correlated ($r = 0.233$, $p < 0.05$ in males; $r = 0.331$, $p < 0.01$ in females), and the subcutaneous fat was negatively correlated ($r = -0.267$, $p < 0.05$ in males; $r = -0.470$, $p < 0.001$ in females), with plasma Cort in basal condition.

QTL analysis

Table 1–3 show the QTL influencing body composition, biochemical parameters related to metabolism, and HPA axis function respectively. The level of significance of the QTL depends on the strength of the connection between the genomic regions with the trait, but is also influenced by the number of iterations involved in the MIM analysis.

A partial overlap was found between the QTL influencing HPA axis function and those determining phenotypes related to metabolic characteristics. **Fig. 2** shows the chromosomal map of the QTL and in grey the genomic regions that we selected to explore for the candidate genes that could be involved in the relationship between HPA axis function and fat deposition process. We searched for QTL already identified and for candidate genes in our QTL. We present here the most relevant candidates based on their presence in an overlapping QTL, and their role in HPA axis function and metabolism (**Table 4**).

Fig. 3 illustrates the differences between the rats grouped according to their genotypes, homozygous LOU/C/LOU/C and F344/F344 and heterozygous LOU/C/F344, at the D18Rat57 marker the closest to the GR gene for phenotypes related to metabolism or HPA axis function. Except for the lean mass, for each trait, we observed significant differences between the genotypes in the same direction as in the parental inbred strains. ANOVA showed a significant difference in basal B between LOU/C/LOU/C genotype and the F344/F344 genotype ($p < 0.001$). ANOVA revealed also significant differences for the B response to DEX (named GR effect, $p < 0.01$), the ratio WAT/lean mass ($p < 0.001$), and glycemia ($p < 0.05$) between LOU/C/LOU/C and F344/F344 genotypes. The difference between genotypes in lean mass did not reach significance.

Discussion

The objective of this study was to identify genomic regions and to hypothesize on candidate genes involved in the complex association between HPA axis function and fat deposition and its metabolic consequences. Towards this aim, we studied the phenotypes related to HPA axis function and metabolism in a F2 rat population obtained by crossbreeding LOU/C and F344 rat strains, described previously for large differences in these phenotypes [4, 6, 18]. Expected relationships were confirmed such as the positive correlations between abdominal fat and leptin and basal plasma Cort, or the negative correlation between basal plasma Cort and subcutaneous fat. Many strong QTL were found for our phenotypes and we chose to focus our explorations on regions where QTL influencing HPA axis and metabolic characteristics overlapped (**Fig. 3**). Within these overlapping QTL we searched for candidate genes underlying the relationships between HPA axis function and fat deposition.

Numerous published QTL influencing body weight and noninsulin-dependent diabetes mellitus (NIDDM) overlap with our QTL (**Table 4**). The presence of a QTL influencing Cort stress response was confirmed on chromosome 10 (QTL5 [19] and

Table 4 Overlapping QTL and relevant candidate genes for QTL influencing both HPA and metabolic phenotypes in F2 LOU/C×F344 rats.

Chr	Interval of interest (Mb)	Our overlapping QTLs	Known overlapping QTLs	Relevant candidate genes
3	48.9–57.8	MR effect Basal Cort WAT WAT/lean mass	Body weight QTLs 24 (36.6–88.0), 31 (30.3–76.6) and 36 (30.3–103.3), NIDDM QTL 46 (36.6–50.6)	Lrp2 (51.6–51.7), Mettl8 (53.2–53.3), Atf2 (56.4–56.5)
	105.0–112.6	Basal Cort Retroperitoneal fat	Body weight QTL 8 (111.8–112.8), NIDDM QTL 39 (93.9–170.3)	
4	24.0–52.6	GR effect Plasma FFA Plasma leptin Subcutaneous fat Lean mass	Body weight QTL 2 (5.4–41.7), glucose level QTL 12 (4.6–26.3), triglyceride level QTLs 10 (17.6–41.7) and 19 (4.6–64.8)	Pon1 (29.9–30.0)
8	78.5–101.5	MR effect Basal Cort Plasma insulin Subcutaneous fat Epididymal fat Retroperitoneal fat WAT WAT/lean mass	Body weight QTLs 3 (73.1–117.4) and 23 (94.5–103.7), triglyceride level QTL 6 (64.7–116.8), NIDDM QTL 61 (38.0–103.7), stress response QTL 11 (100.0–101.0)	Bckdhb (89.0–89.2)
10	90.6–91.5	GR effect Stress Cort Plasma insulin Retroperitoneal fat WAT BW 18 weeks	Body weight QTL 21 (55.9–103.6), stress response QTLs 5 (43.5–108.8) and 7 (82.6–91.5)	
11	22.8–32.1	GR effect Epididymal fat WAT/lean mass Lean mass	NIDDM QTL 51 (22.2–43.2), triglyceride level QTL 7 (29.7–84.6)	
15	13.8–24.6	Stress Cort Plasma insulin WAT/lean mass WAT	Body weight QTL 7 (22.5–39.9), cholesterol level QTL 1 (18.7–56.1)	Acox2 (18.6–18.7)
16	52.8–57.4	Basal Cort Retroperitoneal fat WAT/lean mass	NIDDM QTL 29 (19.5–80.0)	
17	80.5–86.7	GR effect Basal Cort Plasma FFA Insulin resistance Plasma insulin Subcutaneous fat BW 18 weeks	Insulin level QTL 1 (10.0–81.0)	Phyh (84.4), Nmt2 (86.0–86.1)
18	27.8–39.7	GR effect Basal Cort Plasma glucose WAT/lean mass Lean mass		Nr3c1 (32.4–32.5)
	70.8–74.5	GR effect Plasma glucose Epididymal fat WAT WAT/lean mass Lean mass		Pias2 (74.1), Acaa2 (71.6)

The intervals of interest were chosen to overlap the maximum of our QTL and to be the thinnest as possible (**Fig. 2**). Positions of QTL and genes appear in brackets (Mb).
NIDDM: Noninsulin-dependent diabetes mellitus

QTL7 [20]). This QTL overlap with a QTL influencing GR effect in this study. The presence of a QTL influencing plasma insulin level was also confirmed on chromosome 17 in rat (QTL1 [21]).
On chromosome 3, among others, some genes involved in lipid metabolism were found, such as Lrp2 (low-density lipoprotein receptor-related protein 2) exhibiting lipoprotein transporter activity, Mettl8 (methyltransferase like 8) involved in fat cell differentiation and skeletal muscle development, and Atf2 (activating transcription factor 2) involved in adipose tissue development and fat cell differentiation. These genes could be involved in total white adipose tissue deposition.
On chromosome 4, QTL determining body weight (QTL2 [22]), glucose level (QTL12 [23]), and triglyceride level (QTL10 [24] and QTL19 [23]) overlap with our QTL (GR effect, plasma FFA and

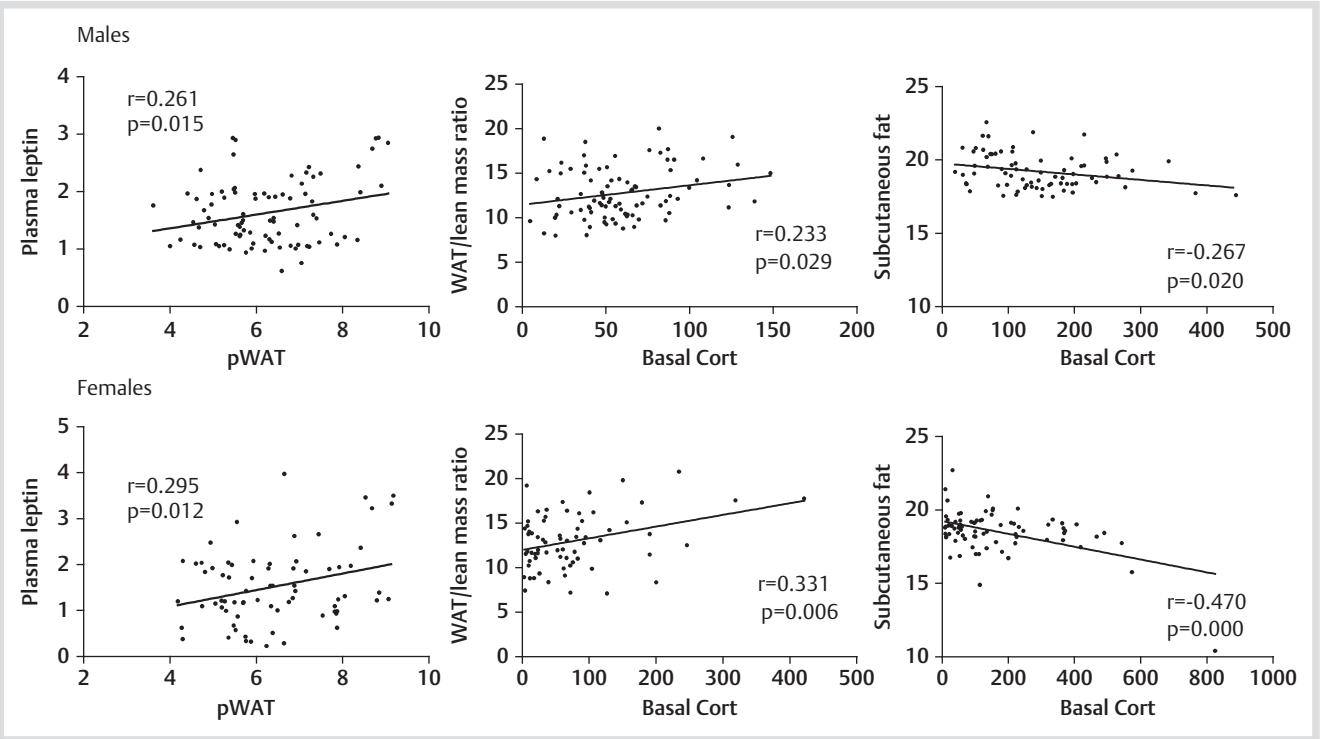

Fig. 1 Correlations between phenotypes in both sexes. Plasma Cort concentration was in control condition.

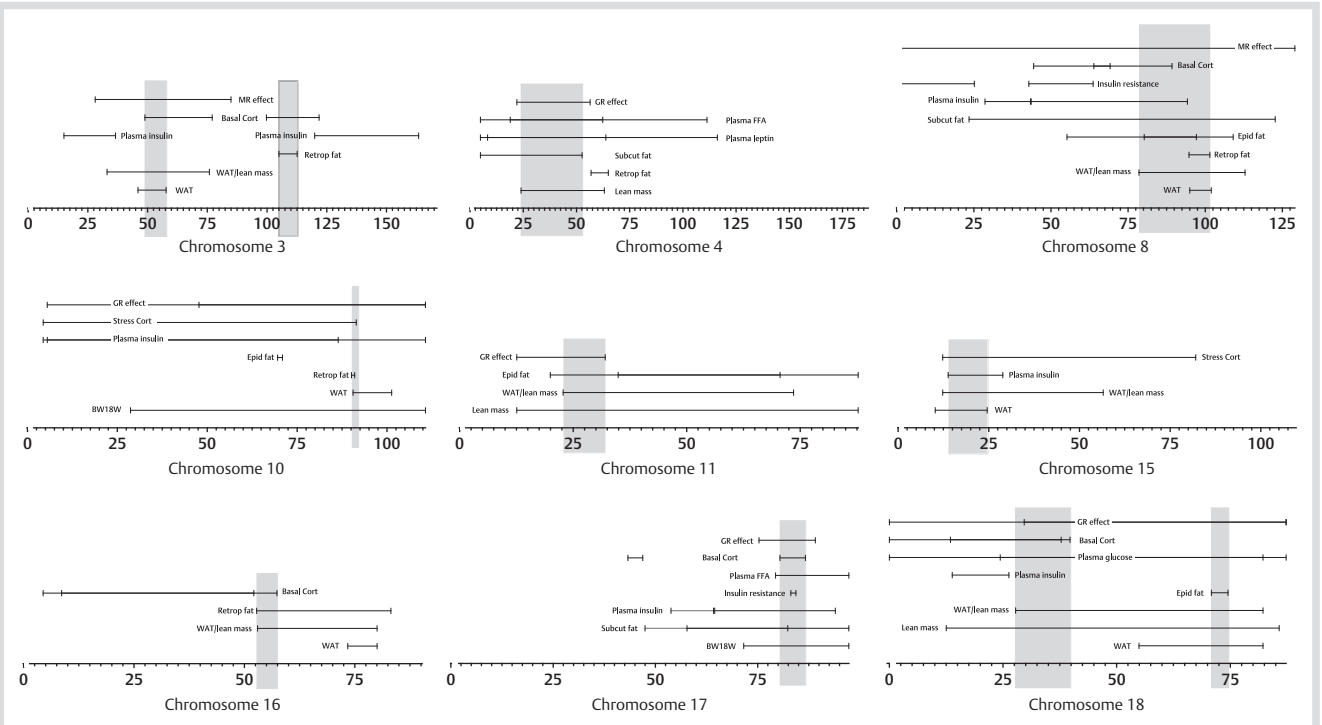

Fig. 2 Genomic regions (in grey) common to metabolic and HPA axis measures that were explored for their candidate genes. QTL regions are represented by their confidence intervals. Abscises are expressed in Mb.

leptin, subcutaneous fat and lean mass). Among other genes, we found Pon1 (paraoxonase 1), involved in lipid metabolic process, associated with hyperlipoproteinemias [25].

On chromosome 8, QTL influencing body weight (QTL3 [22], QTL23 [26]), serum triglyceride (QTL6 [27]), NIDDM (QTL61 [26]), and stress response (QTL11 [28]) overlap with our QTL influencing MR effect, basal concentration of Cort, and body composition. One candidate gene, Bckdhb (branched chain keto acid dehydrogenase E1, β polypeptide) has been involved both in response to glucocorticoid stimulus and in response to nutrient [29]. It was also associated with NIDDM [30].

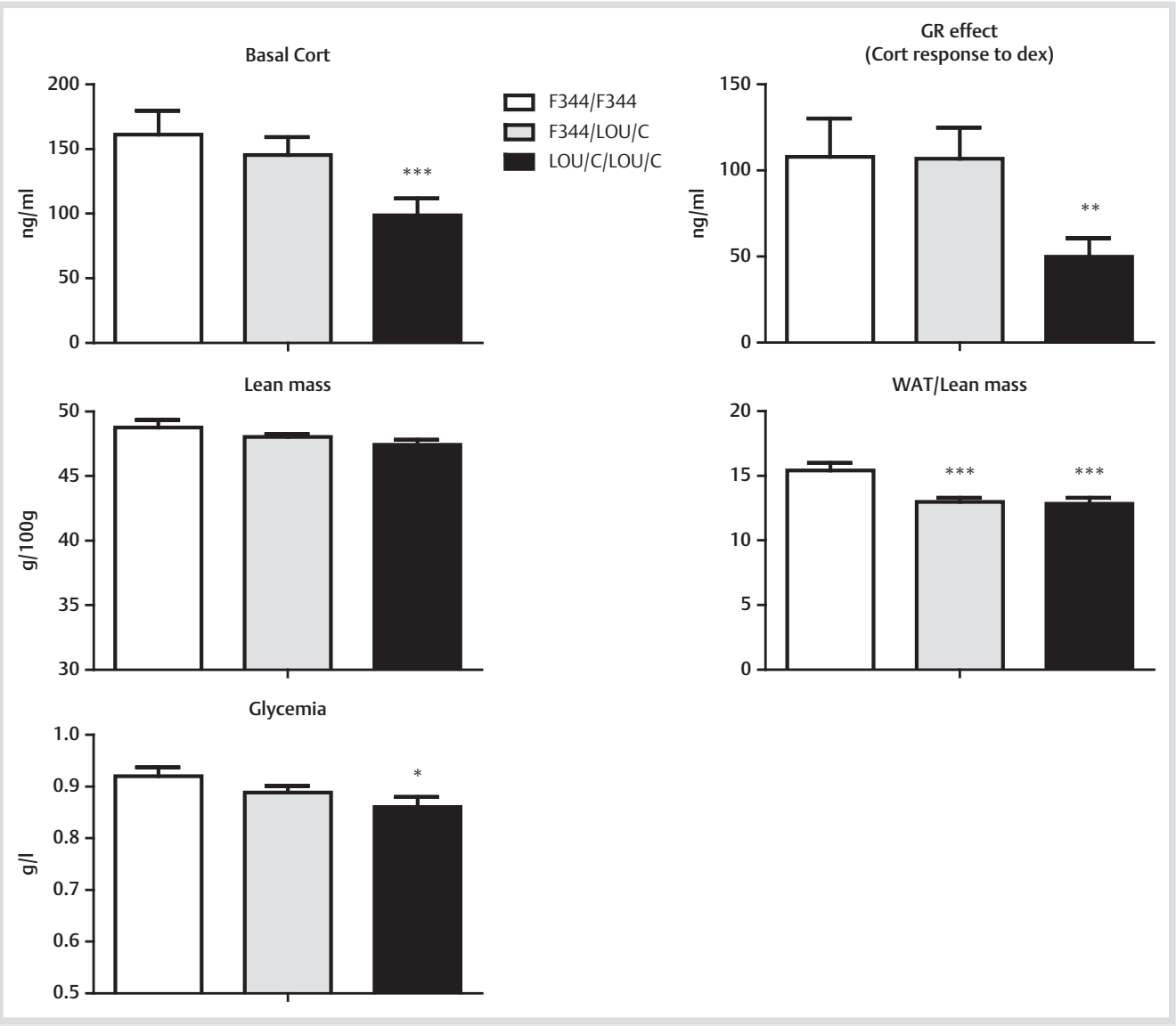

Fig. 3 Phenotypes illustrating metabolic and HPA axis characteristics grouped by genotype at the level of the marker the closest to the GR gene (D18Rat57). Significantly different from F344/F344 genotype: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

QTL determining body weight (QTL7 [31]) and cholesterol level (QTL1 [32]) overlap with our QTL on chromosome 15. Among other genes, some involved in metabolism were found in this region, such as Gapdh (glyceraldehyde-3-phosphate dehydrogenase), and Acox2 (acyl-CoA oxidase 2, branched chain) involved in fatty acid β-oxidation, that may be also involved in WAT deposition (our QTL).

On chromosome 17, genes involved in metabolism were found: Phyh (phytanoyl-CoA 2-hydroxylase), involved in fatty acid α-oxidation, and which could be involved in plasma FFA in this study, and Nmt2 (*N*-myristoyltransferase 2) shown to be involved in preadipocyte differentiation in cow [33], which could be involved in subcutaneous fat deposition (our QTL).

On chromosome 18, the Nr3c1 gene (nuclear receptor subfamily 3, group C, member 1, or GR) is located in our QTL influencing GR effect, basal Cort, plasma glucose, and WAT/lean mass ratio. The Nr3c1 locus has been previously associated with adiposity in mice [34] and in humans [35,36]. GR stimulates preadipocyte differentiation, favors accumulation of fat and drives adipose tissue distribution [3,4]. GR induces insulin resistance directly by perturbing insulin transduction [37] and indirectly by promoting visceral obesity. Except for lean mass, significant differences were shown between F344/F344 and LOU/C/LOU/C genotypes for the phenotypes influenced by this region (basal B, GR effect, WAT/lean mass, and glycemia). In the same locus, we also found Pias2 (protein inhibitor of activated STAT 2), exhibiting GR binding and positive regulation of transcription of GR targets [38], and one gene involved in fatty acid β-oxidation, Acaa2 (acetyl-CoA acystransferase 2). For the other QTL, even if we did not find any candidate gene directly related to HPA axis function, most of the candidate genes contain putative GRE in their sequence according to rvista (Lrp2, Mettl8, Atf2, Pon1, Acox2, Nmt2). In a previous study [4], we showed a decrease of circulating lymphocyte counts exerted via GR activation by dexamethasone that revealed a higher efficiency of GR in LOU/C than in F344 rats. This higher efficiency could be involved in the lower activity and reactivity to stress measured previously in LOU/C rats through a better negative feedback, and a greater lipolysis with low insulin levels under high-fat diet, and higher diet-induced thermogenesis [4].

Conclusion

To conclude, numerous candidate genes were found in our overlapping QTL, and some of them could link HPA axis function and fat deposition, and their metabolic consequences. Beside the presence of Nr3c1, the GR-encoding gene, in a highly significant QTL, most of the sequences of our candidate genes present a GRE, which places this receptor at a central level in a potential network. However, the real involvement of the candidate genes described here requires functional investigations. Additionally, a gene of unknown function, not listed as a candidate, may be a causative gene of the QTL. Further investigations are needed to reveal the biological relevance of each gene, and potentially to connect our different QTL into a more comprehensive network.

Authors' Contributions

Nathalie Marissal-Arvy conducted the genotyping, the analysis and wrote the article. J-M. Heliès phenotyped the animals. C. Tridon took care of the animals and participated in the genotyping. M-P. Moisan and P. Mormède supervised the work and corrected the manuscript.

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Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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