Original Article
EPIDEMIOLOGY/GENETICS

Obesity

QTLs Influencing Carbohydrate and Fat Choice in a LOU/CxFischer 344 F2 Rat Population

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Objective: Individual differences in macronutrient selection, particularly fat and carbohydrate, and associated body weight gain are partly inherited as polygenic traits, but the potential genetic pathways are unknown. To give an overview of the Quantitative Trait Loci (QTLs) and candidate gene pathways influencing these differences in rat was aimed in this study.

Design and Methods: To that end, F2 rats obtained from the crossbreeding between LOU/C and Fischer 344 rat strains to diet self-selection during 3 weeks were submitted. A genome scan was conducted with microsatellite markers covering evenly the whole genome. Genotypes and phenotypes were analyzed separately in male and female F2 rats by multiple interval mapping. Then, lists of candidate genes were treated by the Ingenuity® Pathway software to propose gene pathways involved in our phenotypes.

Results: Among numerous others, a QTL on chromosome 12 that influences body weight gain, and fat and carbohydrate choices in the LOU/C x Fischer 344 F2 rat population was found. This locus contains notably the acyl-co-A dehydrogenase gene.

Conclusion: A strong genetic determinism and complex pathways involving numerous candidate genes and processes, notably in accordance with the metabolic theory of feeding behavior control were found.

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Introduction

The health consequences of diets with varying macronutrient content are of great interest to design a strategy of nutritional balance combined with a preservation of body weight. The respective quantity of carbohydrate and fat, the most palatable and also the most caloric nutrients, which should be combined in an ideal diet is still under debate. The individual variation of macronutrient selection in the diet appears to be partly heritable both in humans (twin and familial studies (1)) and in laboratory rodents [strain comparisons (2,3), QTL analysis in mice (4)]. Approximately 20% of variation in carbohydrate and fat preferences in humans are related to genetic difference, although a large proportion of the variation in total energy intake can be attributed to environmental influence (5). A lower heritability for protein intake was also estimated in humans (5).

The possible biological factors contributing to macronutrient diet selection are still unknown, however the orosensory and postingestive effects of food seem to be involved (6), and these factors are both susceptible to genetic variation. To explore further the biological pathways involved in macronutrient selection, we aimed to find the genomic regions influencing the carbohydrate or fat choices in rat, and to propose candidate genes and potential metabolic pathways involved in diet self-selection and the associated body weight gain. The experiment was done in a F2 population obtained from the crossbreeding between LOU/C and Fischer 344 (F344) rat strains and the data analyzed by multiple interval mapping (MIM) of quantitative trait loci (QTLs). We showed previously that LOU/C rats are resistant to obesity development, whereas F344 rats develop visceral obesity with age or in response to high-calorie diets (2,7). We also showed previously a tendency for a greater preference for fat in LOU/C than in F344 male rats (8). This behavior was reversed in females (8). This preference for fat diet in males was shown to increase with age when LOU/C rats were compared to Wistar rats (9). Even if no difference had been shown for the carbohydrate intake between LOU/C and F344 inbred strains, the combination of genes was different from parental strains in each F2 rat, in relation

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with a large panel of phenotypes, which allowed us to make a QTL analysis on this trait also.

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 24, 1986, European Community) legislation. They adhered to protocols approved by Région Aquitaine Veterinary Services (Direction Départementale de la Protection des Animaux, 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. All the rats were housed in a temperature-controlled room (23°C ± 1°C) with a light/dark cycle of 12/12 h (lights on at 0700 h). At 16 weeks, rats were housed individually and were given the self-selection diet for 3 weeks. Food and tap water were provided *ad libitum*.

Self-selection diet

The three macronutrients (protein, carbohydrate and fat) were presented separately to rats. The protein diet (3.9 kcal g^{-1}) was composed of 90.5% total milk proteins (Nutrinov, Rennes, France), which is a mixture of casein (85%), albumins and globulins. The carbohydrate diet (3.7 kcal g^{-1}) consisted of 80.5% pregelatinized cornstarch and 10% sucrose. The fat diet (7.6 kcal g^{-1}) contained 36% lard and 54.5% soybean oil. Each diet contained 3.5% minerals (AIN 93-Mx; ICN Pharmaceuticals, Orsay, France), 1% vitamins (AIN 93-Vx; ICN Pharmaceuticals), and 5% α-cellulose (Alphacel; ICN Pharmaceuticals). The protein and carbohydrate diets were in powdered form, whereas the fat diet was semisolid (10). The three food cups containing macronutrients were weighed and refilled three times per week. Spillage was minimal. When it did occur, the food lost was collected and added to the total not consumed. Rats were also weighed three times per week. QTL analyses were done on body weight gain during the 3 weeks of the regimen, and on the percentages of macronutrients that were calculated as the relative amount of calories provided by each macronutrient against the total calories ingested.

DNA extraction and PCR

DNA was isolated from lungs of inbred LOU/C and F344 rats and F2 rats as classically described, by digestion overnight 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^{-1} proteinase K) followed by phenol-chloroform extraction and ethanol precipitation.

Study protocol

F1 hybrids were obtained by crossbreeding LOU/C with F344 rats in both directions, and then F1 heterogygous rats were intercrossed to generate a F2 population of which 93 males and 94 females were studied. The genome scan of the F2 population was done using 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 (autosomes and X chromosome, approximately every 20 cM). 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 1x PCR buffer. Alleles were visualized on ethidium bromide-stained 3% agarose gel.

Trait values were corrected for the effects of experimental batch and direction of the initial cross (LOU/CxF344 vs. F344xLOU/C, the first strain being the dam). For each marker, a two-way ANOVA with sex and allele as two between-subject factors was conducted followed by Newman–Keuls *post hoc* comparisons of the means using a Bonferroni correction, and P values < 0.05 were considered statistically significant. Correlation analyses were done using the Prism® software. Phenotypic distributions were assessed to follow a normal distribution. As ANOVA showed numerous significant sex × allele interactions ($P < 0.05$), QTL analyses were conducted separately by sex. QTL analyses were done using the MultiQTL software (version 2.6), using the simple interval mapping, and the MIM method combining QTL mapping analysis with the analysis of genetic architecture of quantitative traits. Instead of the IM that analyzes chromosome by chromosome, the MIM analyzes the most significant chromosome and fixes its variance, and then reanalyzes the whole genome, sorts the second most significant chromosome and so on. We followed the Korol et al. recommendations (MultiQTL.com (11,12)) for the search strategy to select the "best" genetic model defining the genomic regions influencing the trait, i.e. for each chromosome the hypothesis of one QTL vs. none or two QTLs was kept when the H1 hypothesis only was significant, and the two QTLs model was chosen when the H2 hypothesis vs. the H1 and H0 hypothesis was significant. More than the simple IM, the MIM allowed us to obtain narrow and exhaustive QTLs determining each trait. The hypothesis of one QTL vs. none or two QTLs was kept when the H1 hypothesis only was significant, and the two QTLs model was chosen when the H2 hypothesis vs. the H1 and H0 hypothesis was significant. Permutation analysis (1000 replications) was used to determine the significance level for the LOD score. The QTL effects were estimated as the proportion of the phenotypic variance they explained (PEV). The location of the QTL peaks and their 95% confidence intervals were calculated using 1,000 bootstraps, a technique of statistical inference based on successive resampling (13).

Pathway analysis

Chromosome segments carrying significant QTLs (95% confidence intervals) were investigated for relevant genes and QTLs using http://www.ensembl.org. On the site http://www.ncbi.nlm.nih.gov, we searched any link between genes positioned in the QTLs and feeding behavior or body weight gain respectively. Then, all candidate genes were treated by the Ingenuity® Pathway software (14) to describe gene pathways involved in body weight gain, carbohydrate intake and fat intake, and potentially link the different QTLs determining each trait.

Results

Correlations

The body weight gain was positively correlated to the total calorie intake ($r = 0.44$, $P < 0.0001$, Figure 1a). As expected, carbohydrate

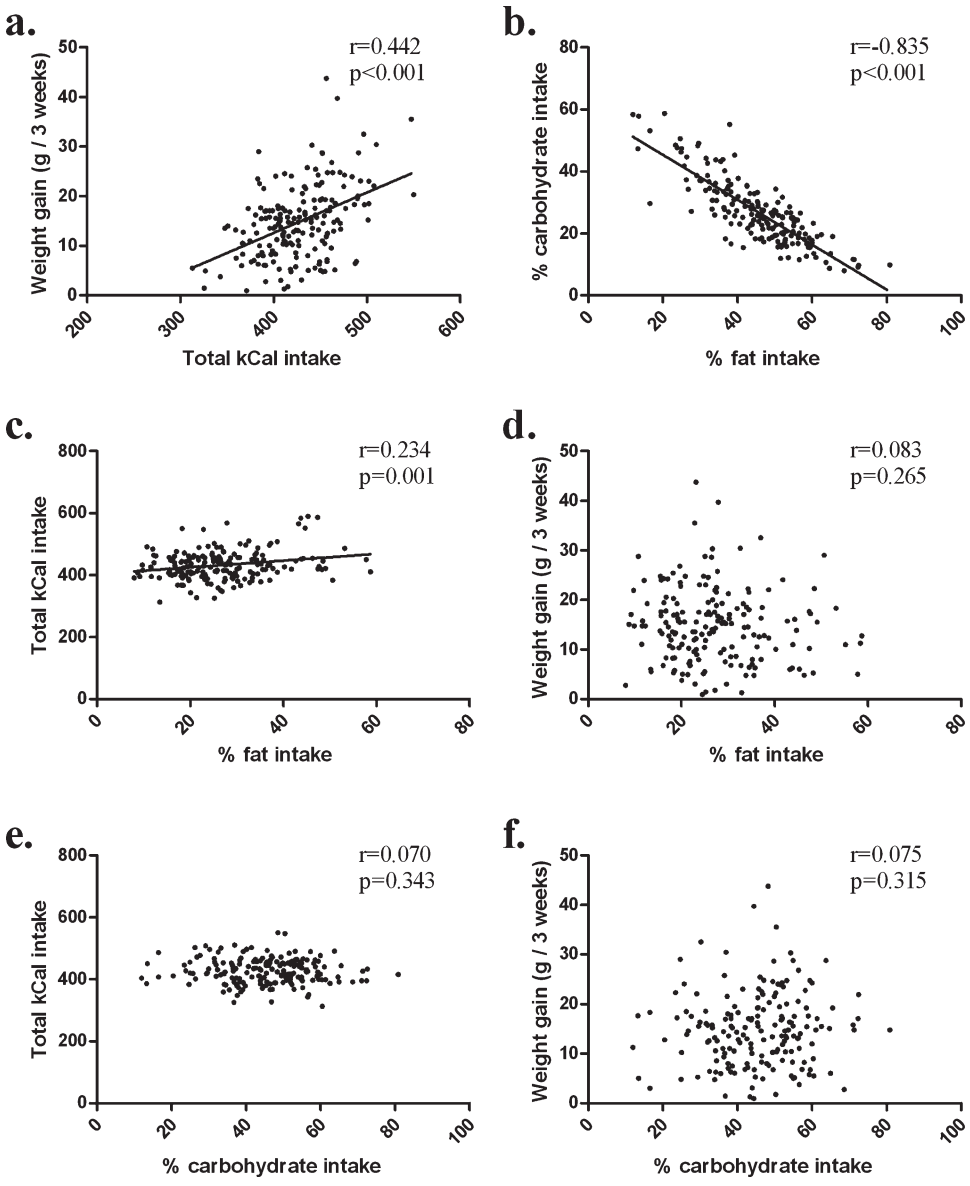

FIGURE 1 Correlations between phenotypes.

and fat intakes were highly correlated ($r = -0.84$, $P < 0.001$, Figure 1b). We can see that the weight gain was not correlated to the percentage of fat intake (Figure 1d) probably because the total calorie intake is maintained despite a large range of preference for the most caloric nutrient, i.e., fat (Figure 1c). The same is true for carbohydrate intake (Figure 1e,f). For all these phenotypes, there was no difference in the results for the correlations between males and females.

QTL analysis

All our QTL were significant. Logarithm-of-odds scores (LODs) and *P* values were calculated for all trait-by-chromosome combinations with the significance of the QTL estimated after 1,000 chromosome wise permutations tests. QTLs were declared significant using a type I error rate of 1% at the chromosome level. Because the permutations tests were calculated at the chromosome level, we further computed the corresponding Type I error rate at the whole genome level. The relationship between Type I error rate at the genome level (αg) and Type I error rate at the chromosome level (αchr) is as follows (15): $\alpha chr = 1 - \{1 - [1 - (1 - \alpha g)^{1/M}]\}^m$, where M is the total number of markers used for the QTL detection on the map and m the number of markers in each linkage group. Finally, QTLs were declared significant using a Type I error rate of 5% at the genome level.

Body weight gain during the macronutrient self-selection diet. In males, the analysis by simple interval mapping (considering

chromosome by chromosome) found only one QTL on chromosome 19 (LOD 3.52, PEV 29.8%). This QTL was confirmed by MIM analysis (considering chromosomes in their global nature) with the highest percentage of explained variance (PEV) of 25.3% and a significant difference between F2 rats homozygous LOU/C/LOU/C and F344/F344 for the closest marker, D19Mgh2 ($P < 0.05$, Figure 2a). Three other QTLs were revealed by MIM on chromosomes 6 (D6Rat105, LOD 5.5, PEV 14.3%) and 12 (near D12Mit2 and D12Mgh5, LOD 5.2, PEV 22.0%). These data are summarized in Table 1.

In females, the analysis by simple interval mapping found two QTLs on chromosome 17 (LOD 4.22, PEV 36.2%), which were confirmed as the strongest QTLs by MIM, H2 vs. H1 being significant ($P < 0.05$). ANOVA showed a significant difference between F2 homozygous LOU/C/LOU/C and F344/F344 for the closest QTL to the centromere (D17Rat43, $P < 0.05$, Figure 3a). The MIM revealed the involvement of 10 other QTLs influencing body weight gain in females. These data are summarized in Table 2.

Percentage of carbohydrate intake. In males, the simple interval mapping did not reveal any QTL. The analysis by MIM found 5 QTLs (Table 1), the strongest being on chromosome 19 (close to D19Rat34, LOD 6.9; PEV 19.6%). Nevertheless the differences between genotypes for this marker did not reach statistical significance (Figure 2b). The lack of ANOVA significance between homozygous groups at the strongest QTL illustrates the fact that other QTLs, with an opposite effect for instance, mask the genetic variation of the trait at this QTL, which gives a high interest of MIM analysis to reveal such QTLs.

In females, no QTL was found by simple interval mapping, but MIM revealed the implication of 14 genomic regions in the regulation of carbohydrate intake (Table 2). The strongest QTL localized on chromosome 7 showed a significant difference between genotypes LOU/C/LOU/C and F344/F344 (D7Rat134, $P < 0.05$, Figure 3b).

Percentage of fat intake. In males, the simple interval mapping revealed a strong QTL on chromosome 9 (D9Rat132, LOD 6.50, PEV 47.7%). The analysis by MIM confirmed this QTL (LOD 7.4, PEV 45.6%, Table 1) and the ANOVA showed a significant difference between homozygous genotypes ($P < 0.05$, Figure 2c). Two other QTLs were found on chromosome 12 (near D12Mit2 and D12Mgh5, LOD 4.0 and PEV 20.2%).

In females, the analysis by simple interval mapping revealed no QTL for the percentage of fat intake. On the other hand, the MIM revealed the involvement of 8 QTLs (Table 2), the strongest being localized on chromosome 13. The difference between genotypes LOU/C/LOU/C and F344/F344 did not reach statistical significance for the closest marker (Figure 3c).

Candidate genes and pathway analysis

We searched for candidate genes in our QTLs and present here those we found the most relevant based by a crossed selection between various criteria: on their detection with both simple and multiple interval mapping, the LOD score level, their location in an overlapping QTL, their proximity to the QTL peak, and their role in food intake or metabolism (Table 3). We compared our QTLs with those found in mouse by Smith Richards et al. (4), and other QTLs already identified and described on www.ensembl.org. The genome tool VC map from http://www.rgd.mcw.edu was also used and

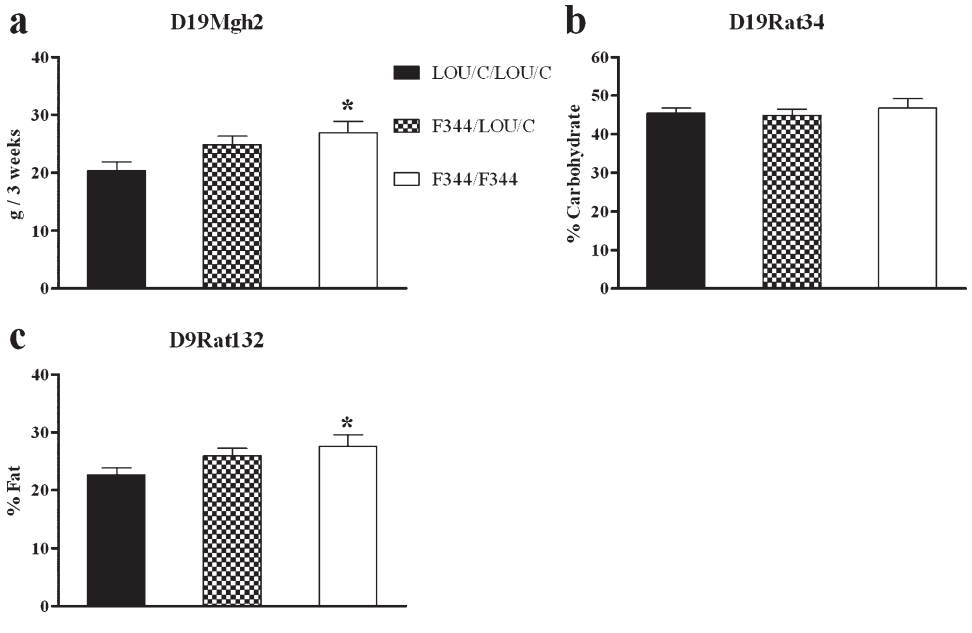

FIGURE 2 Phenotypes of the F2 male rats grouped according to their genotype at the marker placed near the strongest QTL influencing body weight gain during the macronutrient self-selection diet (**a**), carbohydrate intake (**b**) and fat intake (**c**). Different from the homozygous rats for the LOU/C allele: *$P < 0.05$.

TABLE 1 MIM QTLs determining body weight gain and feeding behavior in males

Chromosome	Closest marker and tendencies	Confidence interval (Mb)	Peak (Mb)	LOD	PEV (%)
	Body weight gain				
6	D6Rat105; LOU/C/LOU/C>LOU/C/F344=F344/F344	18.6-29.1	19.5	5.5	14.3
12	D12Mit2; F344/F344>LOU/C/F344=LOU/C/LOU/C	20.9-44.9	32.9	5.2	22.0
	D12Mgh5; F344/F344>LOU/C/F344>LOU/C/LOU/C	40.6-46.8	46.8		
19	D19Mgh2[a]; F344/F344>LOU/C/F344>LOU/C/LOU/C	32.9-51.9	42.4	5.0	25.3
	Percentage of carbohydrate intake				
5	D5Rat71; F344/F344>LOU/C/LOU/C>LOU/C/F344	114.4-139.6	127.0	7.5	13.8
6	D6Rat39; F344/F344>LOU/C/LOU/C>LOU/C/F344	32.2-56.2	44.2	4.3	6.0
12	D12Mit2; LOU/C/LOU/C>LOU/C/F344>f344/F344	20.9-26.9	21.4	5.4	19.0
	D12Mgh5; LOU/C/LOU/C>LOU/C/F344>f344/F344	28.9-51.8	40.6		
19	D19Rat34; F344/F344>LOU/C/F344=LOU/C/LOU/C	0.0-2.3	2.3	6.8	19.6
	Percentage of lipid intake				
9	D9Rat132[a]; F344/F344>LOU/C/F344>LOU/C/LOU/C	11.1-42.3	16.5	7.4	45.6
12	D12Mit2; LOU/C/LOU/C>LOU/C/F344>f344/F344	20.9-43.3	26.6	4.0	20.2
	D12Mgh5; LOU/C/LOU/C>LOU/C/F344>f344/F344	29.3-51.9	41.9		

All QTLs were strongly significant ($P < 0.001$). Last columns present LOD and PEV (percentage of explained variance) at the peak of the QTL.

[a]represents the QTLs also found by simple interval mapping. In the column of the marker name appear the tendencies for the direction of differences between homozygous F344/F344 and LOU/C/LOU/C and heterozygous.

provided the same results. Figure 4 shows the gene pathways obtained from our data lists and proposed by Ingenuity® Pathway software.

Body weight gain during the macronutrient self-selection diet. Among other genes, we found in our QTL on chromosome 12 Acads, acyl-co-A dehydrogenase, and Acacb, acyl-co-A carboxylase beta (Table 3), which both catalyze fatty acid oxidation and insulin secretion signaling. In the strong QTL on chromosome 19, we found other candidate genes, placed a little further from the peak, but known to be involved in the regulation of metabolism, such as actors of the HPA axis, Nr3c2, mineralocorticoid receptor (MR) involved in food efficiency, body weight and fat deposition (2), Hsd11b2, hydroxysteroid 11-beta dehydrogenase 2 involved in glucocorticoid bioavailability, and Agrp, Agouti-related protein involved in appetite and energy homeostasis (16).

In females, we found in our QTLs genes described to be involved in metabolism such as on chromosome 2, in the first QTL, Fabp4 (fatty acid binding protein 4) involved in lipolysis, and in the second QTL Negr1 (Neuronal growth regulator 1), SNP associated with weight, BMI and macronutrient-specific food intake (17).

Percentage of carbohydrate intake. In males, we found on www.ensembl.org QTLs influencing a trait that could be related to selection of sugar, the alcohol consumption, i.e., on chromosome 12, QTL6 and QTL10 (Table 3).

In females, we also found QTLs influencing alcohol consumption on chromosome 10: QTL5, QTL9, and QTL12; and on chromosome 12, QTL6, and QTL10. We compared our results with a previous QTL study of macronutrient selection performed in mice (4) and found some homologous regions with the large QTL found on chromosome 17 influencing carbohydrate intake in mice: our QTL on chromosome 6, and the first QTLs on chromosome 10 and 20. Among other genes, actors of feeding behavior were found, such as numerous olfactory receptors (Olr) on chromosome 20; on chromosome 10, Npw (neuropeptide W) implicated in the central control of feeding (18); and on chromosome 12, Acads and Acacb, in accordance with the theory of the metabolic control of feeding (see discussion) (6).

Percentage of fat intake. In males, we found on chromosome 12 Acacb, and Acads whose KO was shown to alter preference for dietary fat in mice (19). This QTL region was common to the three phenotypes and the tendencies for homozygous differences were the same as in parental strains.

In females, on chromosome 20, we found an orthologous QTL influencing food intake composition in mouse (20), and syntenic regions with QTLs influencing fat intake found by Smith Richards et al. (4) on rat chromosome 16 (chromosome 8 in mice) and rat chromosome 17 (chromosome 18 in mice). Acads and Acacb were found again on rat chromosome 12.

Discussion

This study aimed to find the genomic regions influencing the carbohydrate or fat choices and associated body weight gain in rat, and

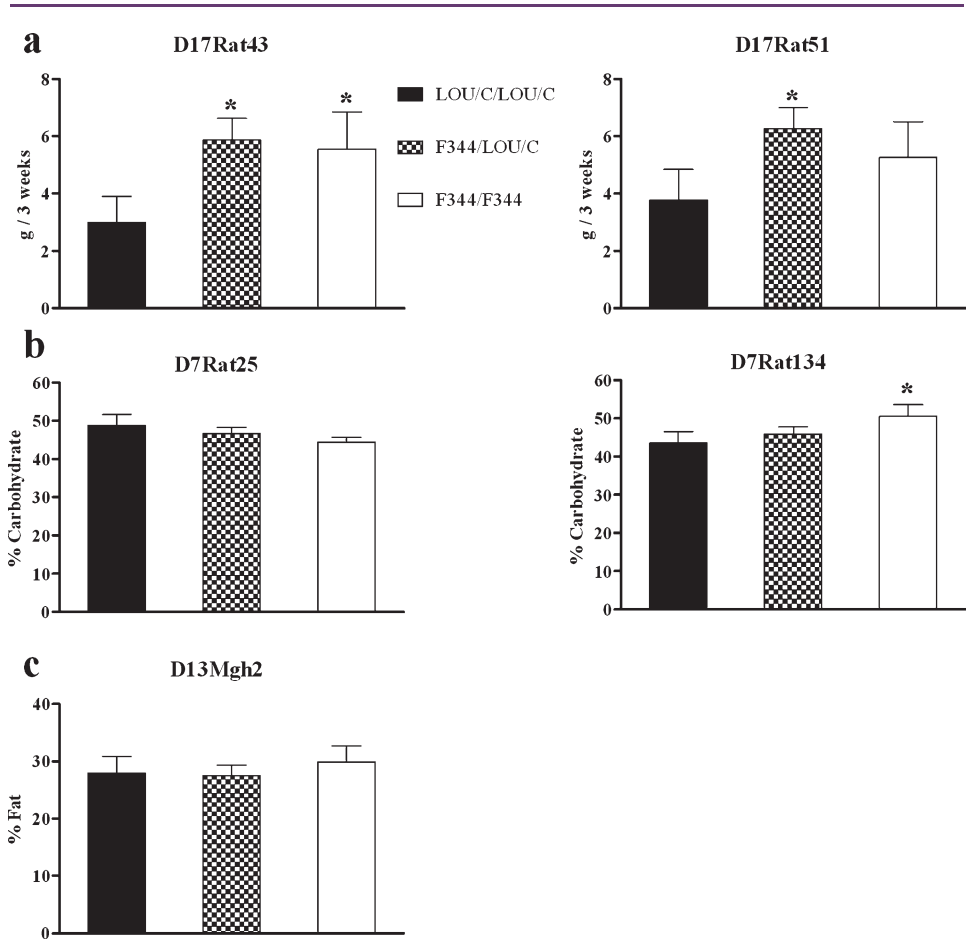

FIGURE 3 Phenotypes of the F2 females grouped according to their genotype at the marker placed near the strongest QTL influencing body weight gain during the macronutrient self-selection diet (**a**), carbohydrate intake (**b**) and fat intake (**c**). Different from the homozygous rats for the LOU/C allele: *$P < 0.05$.

also to propose candidate genes and potential metabolic pathways involved in diet self-selection. A F2 population was obtained from the crossbreeding between LOU/C and F344 rat strains that were previously shown to differ on their body weight and food behavior (8), and the data were analyzed by simple and multiple interval mapping of QTLs. As expected, body weight gain was positively correlated to the total intake of calories. It is interesting to note that the level of total ingested calories is maintained across the high genetic variability of the F2 population, and within a large range of preference for fat, the most caloric nutrient. This process, achieved by eating less when the intake is high in calorie, is classically found in rodent studies (21). An inverse correlation between fat and carbohydrate intakes was already reported in Wistar or Long-Evans rats, mainly involving plasma ghrelin, leptin, and insulin (22,23).

Our study is the first to show an overview of the QTLs influencing self-selection diet and the associated body weight gain in rat. Numerous genomic regions are involved, especially in females, and reveal a strong genetic determinism. No QTLs were found on chromosome X. We show in Figure 4 putative genetic pathways involved in feeding behavior and body weight phenotypes obtained by synthesizing the pathways proposed by Ingenuity® software, with the aim to find common biological systems in males and females, and to connect our QTLs. Actually, strong biological systems emerge from pathways involving candidate genes from QTLs. Nevertheless this representation stays hypothetical and virtual, and real involvement of candidate genes would require functional investigations. Additionally, a gene of unknown function, not listed as candidate, may be the causative gene of the QTL.

During the self-selection diet, body weight gain involves genes that are known for their implication in central and peripheral pathways governing energy homeostasis, like Ins1 (24), Igf1 (25), Fabp4 (26), Pomc (16), or Nr3c2 (coding for MR (2)). The fact that insulin and some of its targets are revealed in the general pathway support the involvement of insulin in the phenotype of F344 parental strain described for their leptin- and insulin-resistance and their

TABLE 2 MIM QTLs determining body weight gain and feeding behavior in females

Chromosome	Closest marker and tendencies	Confidence interval (Mb)	Peak (Mb)	LOD	PEV (%)
Body weight gain					
2	D2Rat145; F344/F344=LOU/C/F344>LOU/C/LOU/C	92.4-95.3	93.2	29.6	23.7
	D2Rat70; LOU/C/F344>f344/F344>LOU/C/LOU/C	249.4-258.2	258.2		
4	D4Rat141; F344/F344>LOU/C/F344>LOU/C/LOU/C	157.0-159.6	159.6	13.84	5.4
5	D5Mit17; F344/F344>LOU/C/F344>LOU/C/LOU/C	67.2-74.2	70.7	24.0	22.8
	D5Rat71; F344/F344>LOU/C/F344>LOU/C/LOU/C	99.2-121.3	110.3		
7	D7Rat113; F344/F344>LOU/C/F344>LOU/C/LOU/C	0.0-11.0	10.9	10.4	7.1
	D7Rat107; LOU/C/F344>LOU/C/LOU/C>f344/F344	6.9-68.7	37.8		
9	D9Rat22; LOU/C/LOU/C>LOU/C/F344>f344/F344	53.5-65.6	59.5	14.3	5.8
14	D14Rat77; F344/F344>LOU/C/F344>LOU/C/LOU/C	1.7-49.2	14.0	4.7	1.8
17	D17Rat43[a]; F344/F344=LOU/C/F344>LOU/C/LOU/C	60.7-62.1	61.4	30.2	26.3
	D17Rat51[a]; LOU/C/F344>f344/F344>LOU/C/LOU/C	87.9-93.3	90.6		
19	D19Rat4; LOU/C/LOU/C>LOU/C/F344>f344/F344	58.0-59.2	59.2	12.7	6.6
Percentage of carbohydrate intake					
1	D1Mit4; LOU/C/LOU/C>LOU/C/F344>f344/F344	179.3-180.8	180.0	18.5	6.5
5	D5Rat71; LOU/C/LOU/C>LOU/C/F344>f344/F344	48.4-123.5	85.9	19.7	8.5
6	D6Rat105; LOU/C/F344>f344/F344>LOU/C/LOU/C	18.6-51.5	33.1	5.5	1.6
7	D7Rat25; LOU/C/LOU/C>LOU/C/F344>f344/F344	51.1-67.0	59.1	24.1	22.4
	D7Rat134; F344/F344>LOU/C/F344=LOU/C/LOU/C	79.5-109.2	94.3		
9	D9Mgh6; LOU/C/F344>LOU/C/LOU/C>f344/F344	30.1-31.1	30.6	25.6	9.5
10	D10Rat45; LOU/C/F344>LOU/C/LOU/C=F344/F344	6.0-41.2	23.7	18.7	9.1
	D10Rat98; F344/F344=LOU/C/F344>LOU/C/LOU/C	66.0-85.8	75.9		
12	D12Mgh5; F344/F344>LOU/C/F344>LOU/C/LOU/C	26.3-34.5	30.4	27.7	13.2
	D12Rat53; LOU/C/F344>f344/F344>LOU/C/LOU/C	43.5-46.8	46.8		
15	D15Rat29; LOU/C/LOU/C=LOU/C/F344>f344/F344	95.4-96.9	96.1	23.7	7.5
16	D16Rat15; LOU/C/LOU/C>LOU/C/F344>f344/F344	65.6-76.6	76.5	15.0	5.5
20	D20Mgh4; LOU/C/F344>f344/F344=LOU/C/LOU/C	0.0-1.0	0.9	20.7	6.7
	D20Rat10; LOU/C/LOU/C>f344/F344>LOU/C/F344	30.3-36.0	34.3		
Percentage of lipid intake					
7	D7Rat134; F344/F344>LOU/C/LOU/C>LOU/C/F344	92.9-108.3	100.5	9.3	11.5
9	D9Rat22; LOU/C/F344>LOU/C/LOU/C>f344/F344	46.7-65.5	56.1	9.8	7.4
11	D11Rat46; LOU/C/LOU/C=LOU/C/F344>f344/F344	101.4-102.6	102.0	16.2	15.1
12	D12Mit2; F344/F344>LOU/C/F344>LOU/C/LOU/C	24.9-53.8	39.3	10.9	11.9
13	D13Mgh2; F344/F344>LOU/C/F344=LOU/C/LOU/C	30.6-31.4	31.0	20.8	17.4
16	D16Rat73; LOU/C/LOU/C>LOU/C/F344>f344/F344	25.7-28.8	27.3	11.0	5.8
17	D17Rat118; F344/F344>LOU/C/F344>LOU/C/LOU/C	46.7-58.6	52.7	11.4	9.9
20	D20Rat60; F344/F344=LOU/C/F344>LOU/C/LOU/C	6.4-16.5	11.5	8.0	7.0

All QTLs were strongly significant ($P < 0.001$). Last columns present LOD and PEV at the peak of the QTL.

[a]represents the QTLs also found by simple interval mapping. In the column of the marker name appear the tendencies for the direction of differences between homozygous F344/F344 and LOU/C/LOU/C and heterozygous.

vulnerability to weight gain. Circulating IGF-1 concentration has been shown to be different between LOU/C and F344 parental strains (27) and it could be involved in their different weight gain in our paradigm (2). We described previously higher MR efficiency in LOU/C than in F344 rats (2,7), which we suggested to be involved in their different vulnerability to fat deposition (2).

For the percentage of carbohydrate intake, a part of the molecular interactions passes by c-Jun-N-terminal Kinase (Jnk). JNK is a serine kinase, known to be activated by metabolic stimuli such as glucocorticoids, cytokines, and free fatty acids. Several studies suggest a strong involvement of JNK in the regulation of food intake (28), obesity or insulin resistance (29). Its inhibition increases insulin

TABLE 3 Overlapping QTLs and relevant candidate genes for body weight gain and nutrient self-selection in male and female F2 LOU/C x F344 rats

QTL interval/chromosome	Sex	Overlapping or syntenic QTLs	Relevant candidate genes
Body weight gain			
(20.9-44.9)/Chr 12	♂	NIDDM QTL 27 (29.1-39.3)	Acads (42.8), Acacb (43.4-43.5)
(32.9-51.9)/Chr 19	♂	NIDDM QTL 38 (24.7-39.9)	Nr3c2 (32.5-32.9), Hsd11b2 (35.3), Agrp (35.4)
(92.4-95.3)/Chr 2; (249.4-258.2)/Chr 2	♀	No replication of QTL	Fabp4 (93.5); Negr1 (254.7-255.4)
Percentage of carbohydrate intake			
(28.9-51.8)/Chr 12	♂♀	Alcohol consumption QTL 6 (29.7-39.0) and 10 (20.9-35.9)	Acads (42.8), Acacb (43.4-43.5)
(6.0-41.2)/Chr 10	♀	Alcohol consumption QTL 5 (5.1-17.5), 9 (5.1-19.6) and 12 (17.5-19.8) Syntenic region with a QTL on chromosome 17 in mice (4)	Npw (13.9), cluster of Olr (34.9-37.0)
(0.0-1.0)/Chr 20	♀	Syntenic region with a QTL on chromosome 17 in mice (4)	Cluster of Olr (0.1-0.9)
Percentage of fat intake			
(24.9-53.8)/Chr 12	♂♀	No replication of QTL	Acads (42.8), Acacb (43.4-43.5)

Positions of QTLs and genes appear in brackets (Mb). NIDDM: noninsulin-dependent diabetes mellitus.

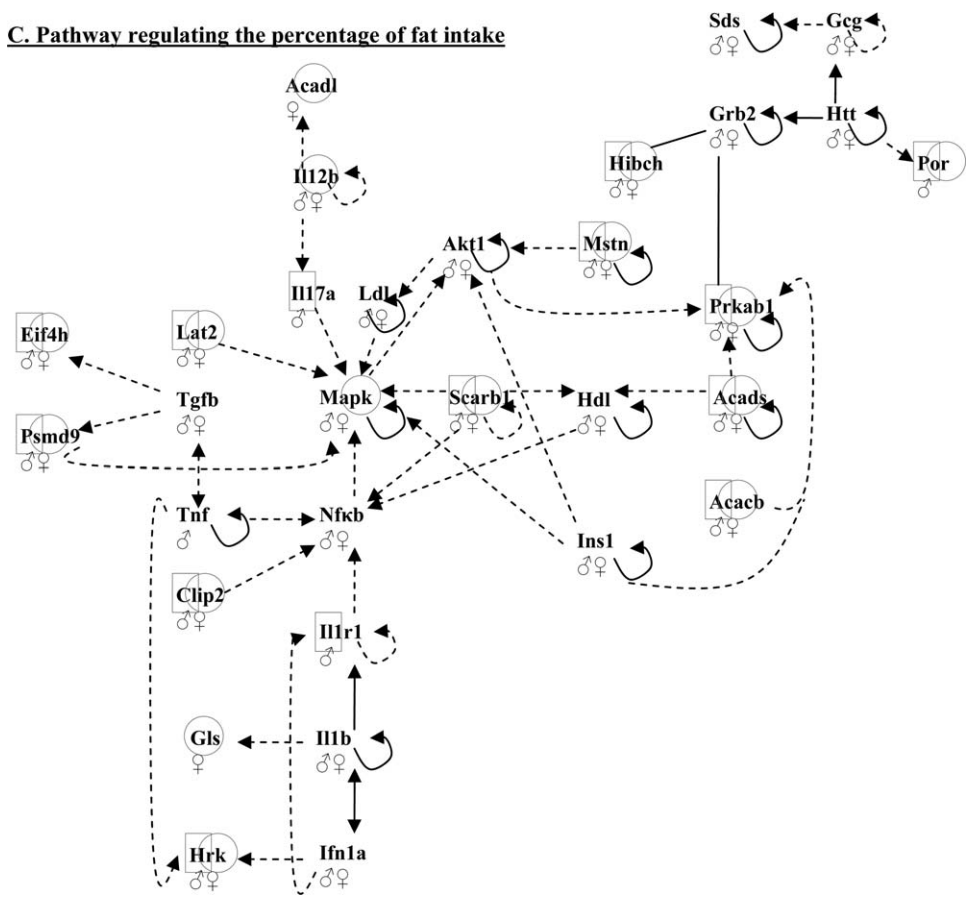

FIGURE 4 Synthesis of pathways proposed by Ingenuity. Full black arrows represent direct interactions and those in dotted line indirect interactions. Genes with under their name ♂/♀ were implicated in pathways proposed by Ingenuity in males or females or both. Squares (males)/circles (females) represent genes whose involvement in the phenotype (body weight gain or regulation of food behavior) has been described in literature, and that were extracted from QTL regions of the present study.

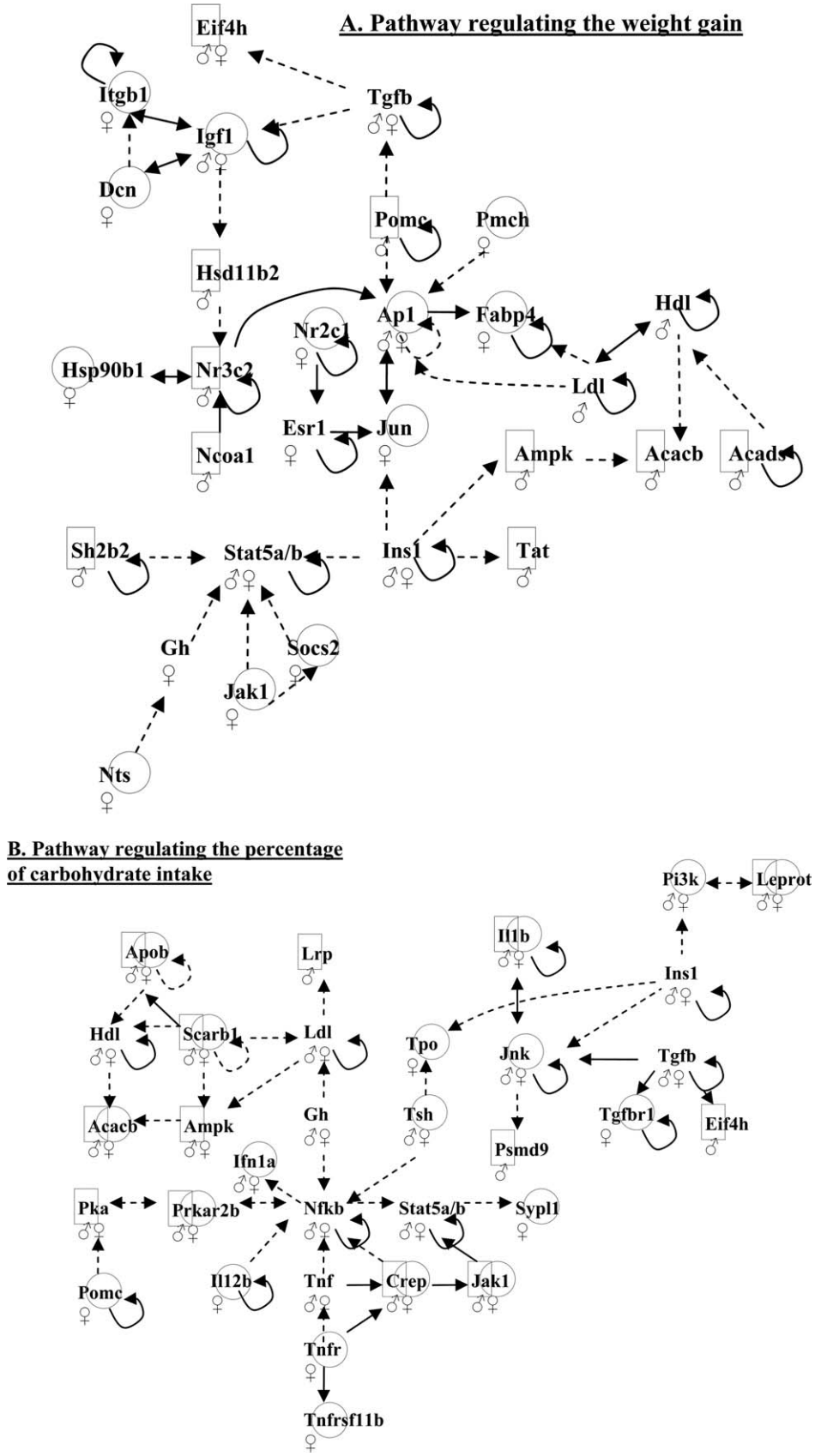

FIGURE 4 Continued

sensitivity in mice, which could be an intermediate mechanism to regulate carbohydrate intake. Unger et al. (28) showed that the central inhibition of JNK increased the orexigenic effects of glucocorticoids on food intake in mice, notably by activating Agouti-related peptide and neuropeptide Y neurons in hypothalamus. A previous study showed a higher expression of Agouti-related peptide and neuropeptide Y in hypothalamus in LOU/C than in Wistar rats (30). Nfκb as a target or as an actor also appears to play a critical role in our general pathway influencing carbohydrate intake. Its involvement in inflammation could be at the origin of this implication since sickness behavior is characterized, besides anorexia, by an increase of preference for carbohydrates in rodents (31). We also found QTLs that overlap with some QTLs influencing alcohol consumption in rat. In both humans and rodents, appetite for sugar has been positively associated with preference for alcohol (32,33). In females, we found syntenic regions with a QTL influencing carbohydrate intake previously found on chromosome 17 in mice (4): on chromosome 6, and on the first QTLs on chromosome 10 and 20 in our F2 rats.

We also found regions on rat chromosomes 16, 17, and 20 that are syntenic with QTLs influencing fat intake measured in mice (4,20). A candidate IPA pathway for fat preference was found, with Nfκb and Mapk as hub molecules. Some cytokines like Tnf, Il1b, Il17a, or Il12b are also involved, and are proinflammatory factors that could participate to the metabolic syndrome, primarily by counteracting insulin action, and secondarily by inferring with ventromedial hypothalamic function (34), which mediates food preferences (35). In the same way, the lipoproteins appearing in the pathway could act as brain sensors to control energy intake (35). As seen in the table of the candidate genes suggested for carbohydrate intake, the Acads gene seems to be involved in the regulation of fat intake. This gene encodes for the short-chain acyl-CoA dehydrogenase (SCAD) which catalyses the first reaction in the β-oxidation of C_4-C_6 fatty acids. Fatty acid oxidation is thought to be determinant in the metabolic control of food intake (6). The dynamic balance between nutrient intake and utilization of the metabolic fuels modulates nutrient appetite in rodents. Smith Richards et al. showed that KO Acads mice suppress their appetite for dietary fat, without any alteration of the acute orosensory response to this fat stimulus (19). It is interesting to note that the role of Acads is well described in literature in the control of fat intake, but not in that of carbohydrate intake. Because Acads is presented as a candidate gene on chromosome 12 for our three phenotypes, we consider it as the best candidate in our study. Many genes involved in our pathway play a role in metabolic processes (Acacb, Acadl, Gcg, Hibch, Por, Sds…) and support the hypothesis of a metabolic control of feeding behavior (6).

Conclusion

Our study is innovative in the way that it gives an overview of the QTLs and potential pathways determining carbohydrate and fat preferences and the associated body weight gain in rat. We found a strong genetic determinism, and complex pathways involving numerous candidate genes and processes, notably in accordance with the metabolic theory of feeding behavior control. We want to test the dynamic of the processes by modeling bioinformatically these interactions, to identify the critical points that could be studied more deeply and potentially targeted to correct tendencies to food disequilibrium. O

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