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QTLs influencing IGF-1 levels in a LOU/CxFischer 344 F2 rat population. Tracks towards the metabolic theory of Ageing

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ABSTRACT

Objective: Since a reduction of the insulin/IGF-1 signaling cascade extends life span in many species and IGF-1 signaling might partly mediate the effects of caloric restriction (CR), an experimental intervention for increasing longevity, the purpose of the present study was to use quantitative trait loci (QTL) analysis, an unbiased genetic approach, to identify particular regions of the genome influencing plasma IGF-1 levels in an F2 intercross between F344 and LOU/C rats; the latter being an inbred strain of Wistar origin, considered as a model of healthy aging since it resists to age (and diet)-induced obesity.
Design: F1 hybrids were obtained by crossbreeding 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. Total plasma IGF-1 levels were determined by radioimmunoassay. A genome scan of the F2 population was made with 100 microsatellite markers) selected for their polymorphism between LOU/C and F344 strains (and by covering evenly the whole genome.
Results: By simple interval mapping sex-dependent QTLs were found on chromosome 17 in males and on chromosome 18 in females. By multiple interval mapping, additional QTLs were found on chromosomes 1, 4, 5, 6, 12, 15 and 19 in males and on chromosomes 3, 5, 6, 12 and 17 in females. Only the markers D1Rat196 and D12Mgh5 were found in both males and females. The majority of QTLs corresponded to metabolic syndrome (cardiac function: $n = 45$ (30%), obesity/diabetes: $n = 22$ (15%), inflammation: $n = 19$ (13%) and only a limited number to body weight: $n = 13$ (9%), proliferation ($n = 10$ (7%) or ossification: $n = 7$ (5%). Ninety-six candidate genes were located on the different QTLs. A significant proportion of these genes are connected to IGF-1 production and receptor pathways ($n = 18$) or metabolic syndrome ($n = 11$).
Conclusions: Subsequent studies are necessary to determine whether the genetic networks underscored are also involved in age-associated obesity, diabetes and inflammation as well as cardiovascular impairments.

1. Introduction

Genetic factors and biological mechanisms appear to influence the ageing process and longevity in general. Specifically, reduction of the insulin/IGF-1 signaling cascade extends life span in diverse species and IGF-1 signaling might partly mediate effects of caloric restriction (CR), an experimental intervention for increasing longevity in mammals [1]. Genetic alteration of mice for life extension [2] and comparison of inbred mouse strains housed in the same conditions [3] indicate correlation to serum IGF-1 levels, and IGF-1 levels have been associated with frailty in elderly women [4].

The LOU/C rat, an inbred strain of Wistar origin, has been proposed both as an obesity-resistant rat and as a relevant model of successful ageing [5]. LOU/C rats present a higher median life span than most commonly used rat strains and they do not develop insulin resistance with ageing [6]. They also appear as a valuable model of spontaneous resistance to diet induced obesity [7], and they seem to channel nutrients toward utilization rather than storage [7,8]. Concerning neuroendocrine parameters, LOU/C rats exhibit an hypoactive/hyporeactive hypothalamo-pituitary-adrenals axis as compared to the Fischer 344 (F344) strain [9], and comparatively lower plasma leptin, TSH and T4 levels, and high ghrelin levels when compared to Wistar rats [10]. For the Growth Hormone (GH) axis, the amplitude of GH ultradian pulses is initially lower in 3 months-old LOU/C rats as compared to age-matched Wistar rats [11]. However, age-associated GH decrease occurs later in LOU/C than Wistar rats [11], in keeping with a maintenance of functional pituitary GHRHR receptors [12]. Remarkably, IGF-1 levels decrease more steeply with ageing in LOU/C than in Wistar rats [11].

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Quantitative trait loci (QTL) analysis is an unbiased genetic approach to identify particular regions of the genome associated with the trait of interest. It has been applied several times to identify 25 QTL for IGF-1 levels in mice (see for review Leduc et al. 2010). In the present study, we followed an equivalent strategy with multiple interval mapping analysis to reveal QTL influencing plasma IGF-1 in an F2 intercross between F344 and LOU/C rats. Some of the candidate genes found in the QTL could be connected in genetic networks, providing a global vision of the biological systems involved in the determination of this trait.

2. Materials and methods

2.1. 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 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 animal used. All rats were born and raised in the laboratory from LOU/C (Harlan, Gannat, France) and F344 breeders (IFFA Credo (L'Arbresle, France). F1 hybrids were obtained by crossbreeding 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 (animals who died during the experimental period were removed from the paradigm). Rats were housed in standard individual cages, in a temperature-controlled room (23 ± 1 °C) with a 12:12 h light:dark cycle (lights on at 0700 h). In standard conditions, they were fed with SAFE-A03 chow (Scientific Animal Food & Engineering, Villemoisson-sur-Orge, France) until weaning at 28 days of age, and subsequently with standard SAFE-A04 (2.9 kcal/g ME). Water was available ad libitum. At the age of 18-weeks, parental and F2 rats were euthanized by decapitation in the morning (0900–1100 AM) after an overnight fast. Blood samples were collected in chilled tubes coated with a 10% EDTA solution, and centrifuged at 4000 g for 15 min at 4 °C. Plasma aliquots were stored at −80 °C for subsequent measurements.

2.2. IGF-1 determination

Total plasma IGF-1 levels were determined by radioimmunoassay (Rat IGF-1 DSL 2900; Diagnostic System Laboratories, Webster, Tex., USA). The sensitivity was 160 ng/ml and the intra- and interassay coefficients of variation were <10%.

2.3. Genome scan

To localize the QTL implicated in IGF-1 plasma concentration, a genome scan of the F2 population was made with 100 microsatellite markers (Eurogentec, Angers, France) selected for their polymorphism between LOU/C and F344 strains (www.rgd.mcw.edu) and by covering evenly the whole genome (approximately every 20 cM). PCR were performed in a 20-µl volume by combining 50 ng of genomic DNA with 5 pmol of each primer, 200 µm dNTP, and 0.4 unit of TaqDNA polymerase (Promega) in 1× PCR buffer. The alleles were visualized on ethidium bromide-stained 3% agarose gel.

Table 1
Plasma concentration of IGF-1 (ng/ml) in the parental strains, LOU/C and F344. Strain effect: *** $p < 0.001$, * $p < 0.05$. Sex effect: °°° $p < 0.001$.

Strain	Males	Females
LOU/C	869 ± 93 °°°	675 ± 31 *
F344	1381 ± 95	775 ± 125 ***

Table 2
QTL influencing IGF-1 levels in a LOU/CxFischer 344 F2 revealed by MIM in males. All QTLs were strongly significant ($p < 0.001$).

Chromosome	Closest marker	Confidence interval (Mb)	LOD	PEV (%)
1	D1Rat196	3.25 – 89.90	7.4	14.5
4	D4Rat142	4.83 – 17.77	7.3	17.7
	D4Rat151	33.80 – 60.87		
5	D5Mgh22	160.00 – end	6.6	11.3
6	D6Rat33	41.99 – 88.13	4.3	9.1
12	D12Mit12	20.93 – 42.54	5.0	9.1
	D12Mgh5	40.58 – 51.88		
15	D15Rat110	13.79 – 71.32	3.0	4.5
17	D17Mgh5	80.73 – 92.90	9.0	15.4
19	D19Rat15	14.05 – 35.61	5.7	9.7

2.4. QTL analysis

For each marker, the data were submitted to a two-way ANOVA with sex and allele as two between subject factors with the SAS© software. The threshold of significativity was submitted to a Bonferroni correction for all the comparisons. Linkage analysis was made separately on males and females since ANOVA showed many significant sex × allele interactions ($p < 0.05$). Linkage analysis was performed in simple interval mapping and then in Multiple Interval Mapping (MIM) with the MultiQTL© Software (http://www.multiqtl.com). The possible involvement of each genomic region was first tested by the probability of one QTL vs. no QTL (hypotheses H1 vs. H0), then by two QTLs vs. no QTL (H2 vs. H0), and then two QTLs vs. one QTL (H2 vs. H1). Then, the MIM model was run according to these results. The 95% confidence interval for each QTL was calculated by bootstrap analysis. Candidate genes localized in the QTL regions were searched on the www.ensembl.org web site. They were treated with the Ingenuity© software in order to emerge the potential genetic networks involved in the determination of IGF-1 plasma concentration.

3. Results

3.1. Plasma IGF-1 in parental inbred strains (Table 1)

Strain and sex effects ($P < 0.001$), and the sex × strain interaction ($P < 0.01$) were significant. Higher plasma levels of IGF-1 were found in F344 than in LOU/C rats ($P < 0.001$). IGF-1 levels were higher in males, and the sex difference was less prominent in LOU/C rats.

3.2. QTL analysis

Sex-dependent QTLs were found by simple interval mapping on chromosome 17 (closest marker D17Rat118, LOD = 2.76, percentage of explained variance PEV = 13.8% at the peak) in males and on chromosome 18 (D18Rat111, LOD = 4.97, PEV = 28.5) in females. Both QTL were confirmed by MIM. Tables 2 and 3 present the results of the MIM analysis, i.e. confidence intervals (95%), LOD and PEV of the nu-

Table 3
QTL influencing IGF-1 levels in a LOU/CxFischer 344 F2 revealed by MIM in females. All QTLs were strongly significant ($p < 0.001$).

Chromosome	Closest marker	Confidence interval (Mb)	LOD	PEV (%)
1	D1Rat196	24.25 – 61.06	4.7	7.7
3	D3Rat66	61.00 – 67.96	7.3	21.8
	D3Rat59	104.65 – 105.45		
5	D5Mgh5	38.17 – 47.00	9.0	14.2
	D5Mgh20	76.51– 100.19		
6	D6Rat105	51.27 – 54.89	9.4	10.4
12	D12Mgh5	57.00 – 60.45	3.5	2.6
17	D17Rat118	43.27 – 46.10	8.7	11.7
18	D18Rat111	0.00 – 3.61	11.4	29.5

merous QTL determining plasma IGF-1 in males (Table 2) and females (Table 3). As expected, the LOD scores obtained with the MIM analysis did not necessarily follow the F values given by the ANOVA (Fig. 1), according to the number of iterations occurring during the multivariate scan analysis [13]. Some QTLs overlapped between males and females and Fig. 2 shows IGF-1 data at the peak of the QTL in both sexes (closest marker). Contrary to the other chromosomes, the linkage on chromosome 1 was transgressive.

List of ID of the microsatellite markers used in genotyping (from Rat Genome Database, http://rgd.mcw.edu) is provided on supplementary table 1 and QTLs found in males ($n = 89$), females ($n = 38$) and both sexes ($n = 21$) are listed on supplementary table 2. The majority of QTLs corresponded to metabolic syndrome (cardiac function: $n = 45$ (30%), obesity/diabetes: $n = 22$ (15%), inflammation: $n = 19$ (13%) and only a limited number to body weight: $n = 13$ (9%), proliferation ($n = 10$ (7%) or ossification: $n = 7$ (5%).

QTLs previously showed to influence cardiac function corresponded to 37% of total in males, 21% in females and 19% in both sexes (Fig. 3). Kidney function came first in sex independent QTLs (29%) and represented 18% in males as compared to 13% in females. Obesity/diabetes/inflammation were prominent in female QTLs (32%) and important in males (27%) and common to both sex QTls (19%). Body weight QTLs were similarly represented in females (16%) and sex independent QTLs (14%) but less present in males (3%). Ossification QTLs appeared in sex independent QTLs (14%) but were less important in males (5%) and absent in females. Opposite proportion was observed for oestrogen-dependent QTLs (13% in female, 5% in sex independent and none in male QTLs). Finally ossification and proliferation QTLs were also represented but at lower proportion (<10% but for ossification in sex-independent QTLs (14%).

4. Pathway analysis and discussion

Some interesting gene networks emerged from the analysis with Ingenuity© software. They are illustrated in Figs. 4 and 5 for male and female respectively. We searched for candidate genes in the QTLs and present the most relevant ones, based on 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 IGF-1 related phenotypes. Strong biological systems emerge from pathways involving candidate genes from QTLs. Nevertheless this representation remains

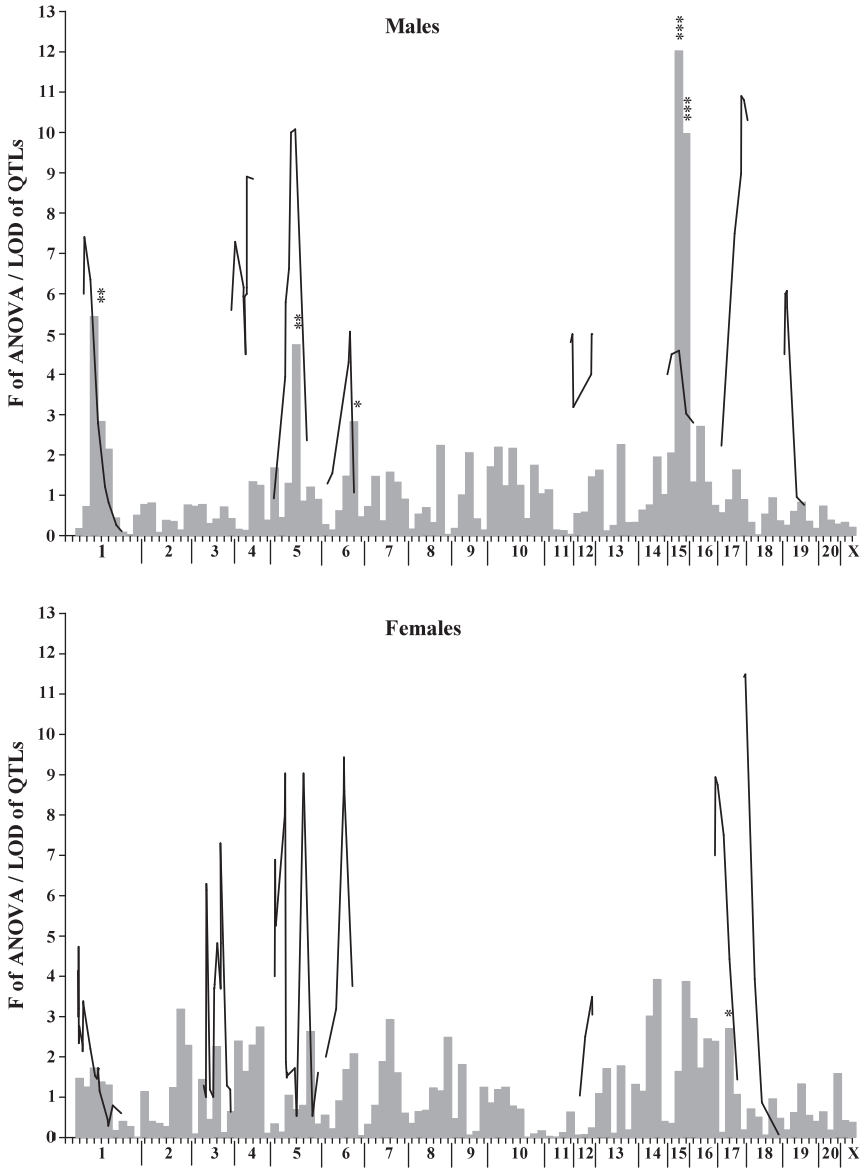

Fig. 1. ANOVA F values (in grey) and LODs of the MIM QTL analysis (in black) according to the genetic markers. Numbers under the X axis represent the chromosomes.

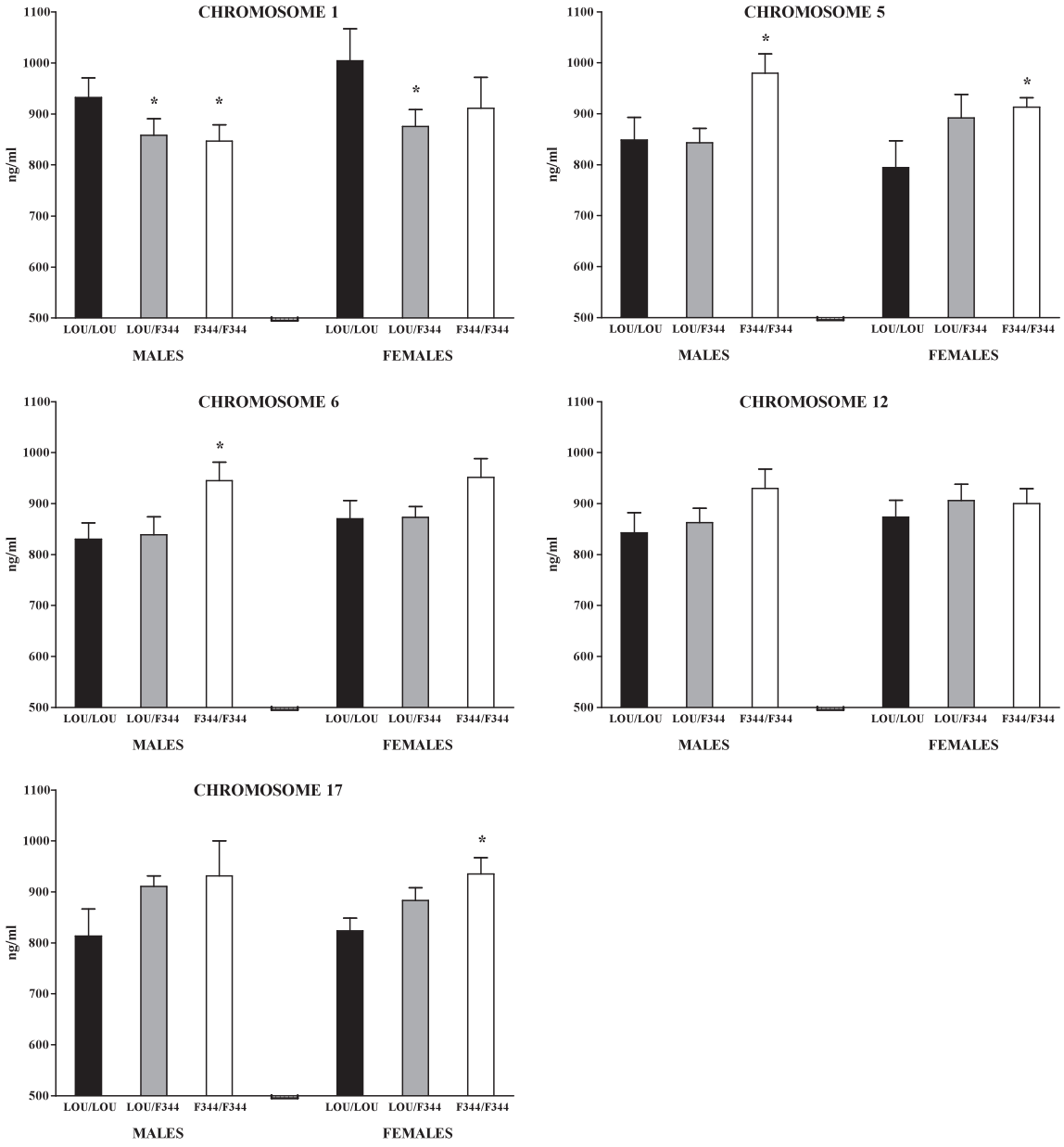

Fig. 2. Plasma IGF-1 of the F2 rats grouped according their genotype (homozygous LOU/C/LOU/C, heterozygous LOU/C/F344, homozygous F344/F344) at the level of the closest marker inside the QTL overlapping between males and females of the F2 LOU/CxF344 population.

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.

Given that a single IGF1 haplotype substantially contributes to size variation in the domestic dog (Sutter et al., 2007) and that for mouse IGF1 levels the most significant locus (with a LOD score of 31.8) is a QTL on Chr 10, which contains the Igf1 gene (Leduc et al., 2010), it is rather unexpected that the Igf1 gene is not detected in a QTL in the Rat species. However several candidate genes in the genetic networks are linked to IGF-1 production, circulating levels or IGF-1 receptor pathways.

4.1. IGF-1 production and levels

Cebpa, CCAAT/enhancer binding protein alpha (C/EBPalpha), plays an important role in the liver-specific expression of the hIGF-I gene [14]. In livers of old mice, it represses the GSK3beta promoter and this can be antagonized by GH treatment [15].

Cftr cystic fibrosis transmembrane conductance regulator homolog (human). Serum IGF-1 level is lower in a CFTR mouse mutant reproducing growth retardation [16] as observed in cystic fibrosis [17].

Per3, period homolog 3 (Drosophila). The longer PER3 repeat is associated with higher serum levels of IGF-I [18].

IL-11, interleukin 11, and TGF-beta, transforming growth factor, beta 1, regulate positively and negatively GH production in pituitary GH [3] cell line by regulating GH gene promoter activity [19]. IL-11 also stimulates bone formation and prevents bone loss with advancing age in mice [20].

Nfatc3, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3. A NFAT-rich regulatory element in the IGF-I exon 1 promoter flanking region is responsive to calcineurin signaling and NFAT activation in skeletal muscle cells [21]. The identification of a calcineurin/NFAT-responsive element in the IGF-I gene represents a potential mechanism of intersection of these signaling pathways in the

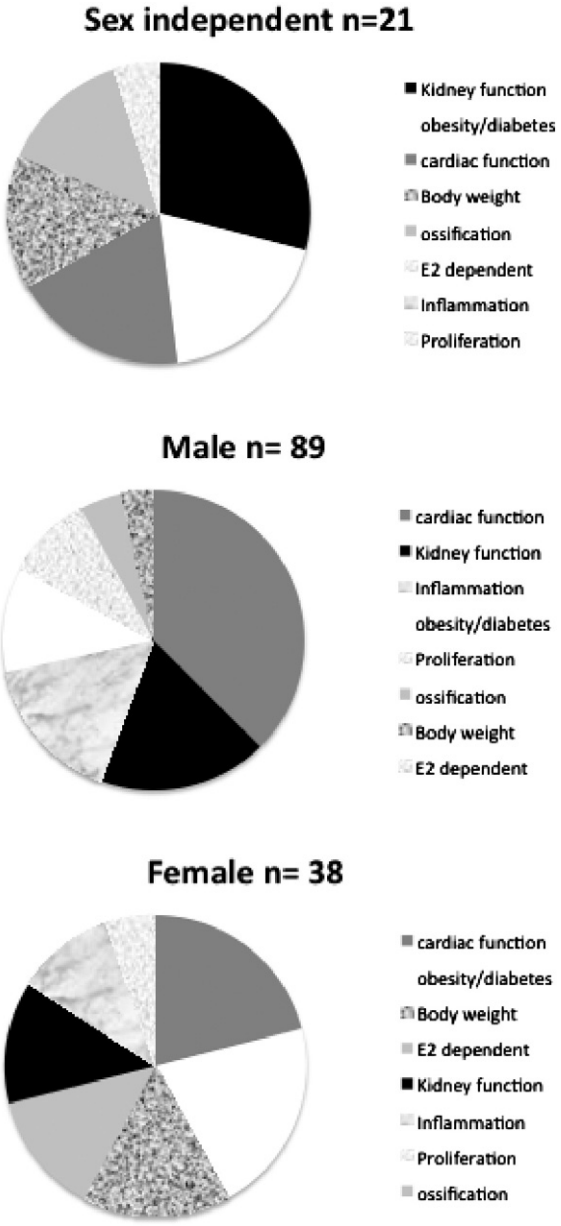

Fig. 3. Functional relevance of QTL overlapping those influencing IGF-1 levels in a LOU/CxFischer 344 F2 according to sex.

control of muscle development and homeostasis. Moreover, NFAT is involved in the depolarization-induced transcriptional activation of GHRH gene in neurons [22].

4.2. IGF-1 levels and longevity

Ezr, Ezrin, Vim, vimentin, and Ywhaq, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide belong to the 14-3-3 family of proteins which mediate signal transduction by binding to phosphoserine-containing proteins and promote a multidrug-resistant phenotype [23]. Ezrin connects the microvillar cytoskeleton to the plasma membrane. Ezrin expression is down-regulated by IGF-1 in the human colon cancer cell line HT29 [24]. IGF-I stimulates the KCl cotransporter 4 (KCC4) recruitment from a presumably inactive cytoplasmic pool of endoplasmic reticulum and Golgi to plasma membrane via binding of KCC4 to ezrin, the latter acting as a membrane cytoskeleton linker [25]. Vim gene was associated with telomere length in an aged human population but this was not replicated in a younger one [26]. Vimentin is specific for mesenchymal tissue and Ywhaq has an essential role in brain function.

Apoe, apolipoprotein E. The ApoE ε4 defining SNP rs429358 has recently been associated with serum levels of IGF-1 in aged women [27].

Sod2, superoxide dismutase 2, is an intramitochondrial free radical scavenging enzyme. In human mammary epithelial cells treated with hydrogen peroxide, its expression is enhanced in the presence of serum from patients carrying mutations in the GHR gene leading to severe IGF-1 deficiency [28]. The same serum reduced DNA breaks and increased apoptosis, promoting cellular protection and life-span extension in model organisms.

4.3. IGF-1 receptor pathway

Akt2, v-akt murine thymoma viral oncogene homolog 2, is a member of the IGF-1 receptor signalling pathway involved in enhancing growth of animals during development but later in life it can potentiate the aging process [29]. Reduced Akt2 phosphorylation in the liver has been related to mechanisms of caloric restriction action on longevity or considered as a potential biomarker of delayed aging [30].

Cav1 & 2, Caveolin-1 is a component of the IGF/PI-3K/Akt pathway regulating cellular proliferation with implications for cancers [31]. Cav2 expression is highly associated with adipose tissue expansion in 10 day-old mice during the dynamic phase of inguinal fat development and in adult animals exposed to an obesogenic environment [32].

Nr3c2 (nuclear receptor subfamily 3, group C, member 2, mineralocorticoid receptor) is involved in the transactivation of the IGF-1R by aldosterone [33].

4.4. IGF-1/IGF-2 interactions

Adam17, ADAM metallopeptidase domain 17, Igf2r, insulin-like growth factor 2 receptor, Plg, plasminogen and Plaur. plasminogen activator, urokinase receptor. ADAM metallopeptidase domain 17 is a GH receptor metalloprotease [34] and it also mediates the release of the ectodomain of IGF2R from human endothelial cells, allowing soluble IGF2R binding to plasminogen (Plg) and thereby preventing Plg from binding to the cell surface and uPA, ultimately inhibiting Plg activation [35]. In this manner, IGF2R controls angiogenesis and restricts the tumourigenicity and invasiveness of carcinoma cells [36]. In osteoblasts, Plaur induces proteolytic activation of IGF-1 from its latent form [37], Targeting of Plaur in human pancreatic carcinoma cells inhibits IGF-I receptor-mediated migration and invasion and orthotopic tumor growth in mice [38]. Plaur also influences the IGF system in tumor progression from benign mucinous cystadenomas to pancreatic ductal adenocarcinoma [39]. Moreover, Plaur binding by the IGF-2 receptor is important for the latter tumor suppressor function via regulation of cell surface proteolytic activities [40].

Glpr, Glucagon-like peptide 1 receptor, is expressed in the gastrointestinal tract, CNS, lung, kidney and heart [41]. In β-cells, GLP-1 increases an IGF-2/IGF-1 receptor autocrine loop by enhancing IGF-1R expression and by stimulating IGF-2 secretion [42]. This mechanism is required for GLP-1 to protect β-cells against apoptosis.

4.5. Diabetes/obesity/inflammation/cardiovascular impairments

Lep, Leptin, a candidate gene not kept in our networks. This adipokine regulates fat metabolism, skeletal growth, and puberty. A recent study on Lep gene polymorphisms in children with idiopathic short stature and growth hormone deficiency suggests that the LEP -2548A polymorphism may associate with the weight and BMI of these patients [43]. However, leptin levels did not correlate with IGF-1 levels in LOU/CxFischer 344 F2 (data not shown). Galp, galanin-like peptide (GALP) is a hypothalamic regulator of energy homeostasis and reproduction and a mediator of leptin action [44]. It increases plasma GH in both male and female rats [45].

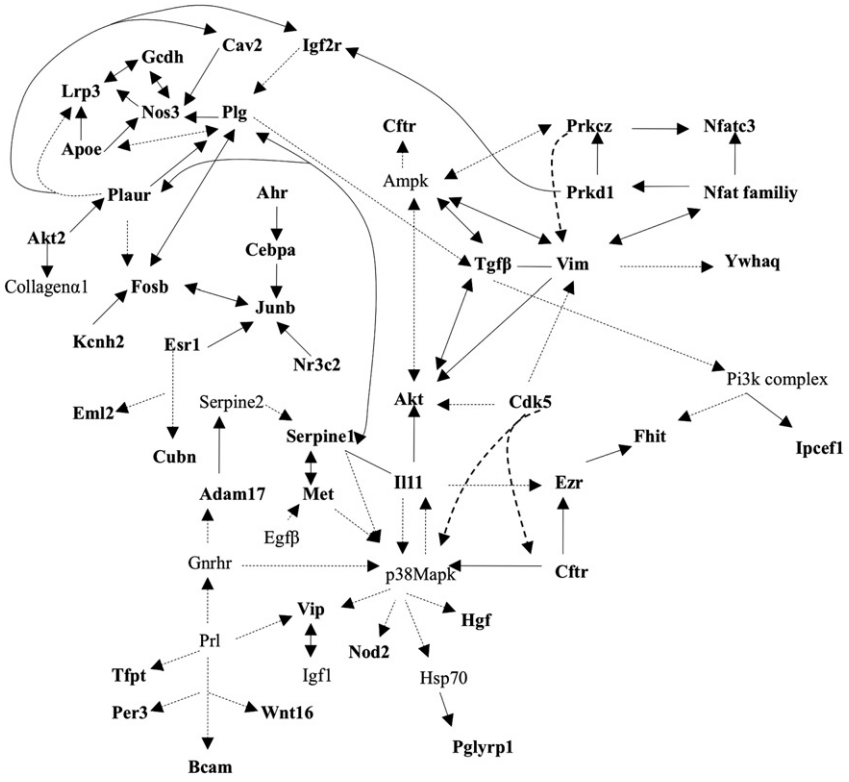

Fig. 4. Genetic pathway involved in the determination of plasma concentration of IGF-1 in males. Full black arrows represent direct interactions and those in dotted line indirect interactions. Genes not in bold are not extracted from the QTL regions but hypothesized by Ingenuity software. **Gene list: Adam17:** chrom 6, ADAM metallopeptidase domain 17; **Ahr:** chrom 6, aryl hydrocarbon receptor**; Akt2:** chrom 1, v-akt murine thymoma viral oncogene homolog 2; **Ampk:** chrom 5, adenosin monophosphate protein-kinase; **Apoe:** chrom 1, apolipoprotein E; **Bcam:** chrom 1, basal cell adhesion molecule; **Cav2:** chrom 4, caveolin 2; **Cdk5:** chrom 4, cyclin-dependent kinase 5; **Cebpa:** chrom 1, CCAAT/enhancer binding protein (C/EBP), alpha**; Cftr**: chrom 4, cystic fibrosis transmembrane conductance regulator homolog; **collagen:** chrom X; **Cubn:** chrom 17, cubilin (intrinsic factor-cobalamin receptor); Egfβ: chrom 2, epidermal growth factor β; **Eml2:** chrom 1, echinoderm microtubule associated protein like 2; **Esr1:** chrom 1, estrogen receptor; **Ezr:** chrom 1, ezrin; **Fhit:** chrom 15, fragile histidine triad gene; **Gcdh**: chrom 19, glutaryl-Coenzyme A dehydrogenase; Gnrhr: chrom 14, gonadotropin-releasing hormone receptor; **Hgf**: chrom 4, hepatocyte growth factor; **Hsp70:** chrom 20, heat shock protein 70; **Igf2r:** chrom 1, insulin-like growth factor 2 receptor; **IL11:** chrom 1, interleukin 11; **Ipcef1:** chrom 1, interactor protein for cytohesin exchange factors 1; **JunB:** chrom 19, jun B proto-oncogene; **Kcnh2**: chrom 4, potassium voltage-gated channel, subfamily H (eag-related), member 2; **Lrp3:** chrom 1, low density lipoprotein receptor-related protein 3; **Met:** chrom 4, met proto-oncogene; **Nfat family:** chrom 19, nuclear factor of activated T-cells, cytoplasmic; **Nfatc3**: chrom 19, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3; **Nod2**: chrom 19, nucleotide-binding oligomerization domain containing 2; **Nos3:** chrom 4, nitric oxide synthase 3, endothelial cell; **Nr3c2:** chrom 19, mineralocorticoid receptor; p38Mapk: chrom1, p38 map kinase; **Per3:** chrom 5, period homolog 3; **Pglyrp1:** chrom 1, peptidoglycan recognition protein 1; **Plaur:** chrom 1, plasminogen activator, urokinase receptor; **Plg**: chrom 1, plasminogen; **Prkcz:** chrom 5, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3; **Prkd1:** chrom 6, protein kinase D1; Prl: prolactin; **Serpine1/2:** chrom 12, serine (or cysteine) peptidase inhibitor, clade E, member 1/2; **Tfpt:** chrom 1, TCF3 (E2A) fusion partner; **Tgfβ:** chrom 1, transforming growth factor, beta; **Vim:** chrom 17, vimentin; **Vip:** chrom 1, vasoactive intestinal peptide; **Wnt16:** chrom 4, wingless-type MMTV integration site family, member 16; **Ywhaq:** chrom 6, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide.

Agrp (Agouti related protein), AGRP-Neuropeptide Y (NPY) arcuate neurons in the mediobasal hypothalamus can be considered as an integrator of different adaptive behaviors in periods of unfavorable metabolic conditions such as diet restriction, extending their action to inhibition of sexual functions and anabolic processes [46–50]. Arcuate AGRP/NPY neurons are a direct target for leptin and they are also key players in GH regulatory autofeedback [51].

Cmklr1 chemokine-like receptor 1. This G protein-coupled receptor binds chemerin, a proinflammatory adipokine often associated with metabolic syndrome [52]. CMKLR1-/- mice display lower food consumption, total body mass, and percent body fat compared with their wild-type controls [53]. CMKLR1-/- mice also exhibit decreased hepatic and white adipose tissue TNFα and IL-6 mRNA levels coincident with decreased hepatic dendritic cell infiltration, decreased adipose CD3+ T cells, and increased adipose natural killer cells. They are glucose intolerant compared with wild-type mice, with decreased glucose stimulated insulin secretion as well as decreased skeletal muscle and white adipose tissue glucose uptake.

Il1, Interleukin-1, an inflammatory cytokine, inhibits the induction of IGF-I by GH in CWSV-1 hepatocytes [54].

Prl, Prolactin, this pituitary hormone is a growth factor for many tissues including cells of the immune system. Differentiation and the proliferation of PRL cells are controlled by IGF-I during the postnatal development, and IGF may be a mediator of E2 action through VIP induction in PRL cells of adults [55].

Vip, vasoactive intestinal polypeptide, appears to play a role in the temporal organization of sleep in humans, a phenomenon markedly dependent on GH secretion [56]. Vasoactive intestinal polypeptide/pituitary adenylate cyclase-activating peptide receptor 2 deficiency in mice results in growth retardation and increased basal metabolic rate [57]. VIP is also an important contributor to age-related female [58] and male [59] reproductive decline.

Tlr4, toll-like receptor 4, is a member of the Toll-like receptor family which plays a fundamental role in pathogen recognition and activation of innate immunity. Down-regulation of Tlr4 signaling is involved in the anti-inflammatory effects of IGF-1 on skeletal muscle [60].

Nos3, nitric oxide synthase 3, Nppa, natriuretic peptide precursor A, and Prkcz, protein kinase C zeta (PRKCZ) are three among several hypertension genes which are genetic markers for insulin sensitivity and resistance [61,62]. Single nucleotide polymorphisms in the first two genes identify subgroups at high and low risk of coronary heart disease among patients receiving antihypertensive treatments [63].

Nr3c2, the mineralocorticoid receptor, and aldosterone mostly active on the kidney to regulate water balance, has recently been implicated in the development of ventricular hypertrophy and cardiac fibrosis [64]. Recent evidence, however, points to cortisol rather than aldosterone

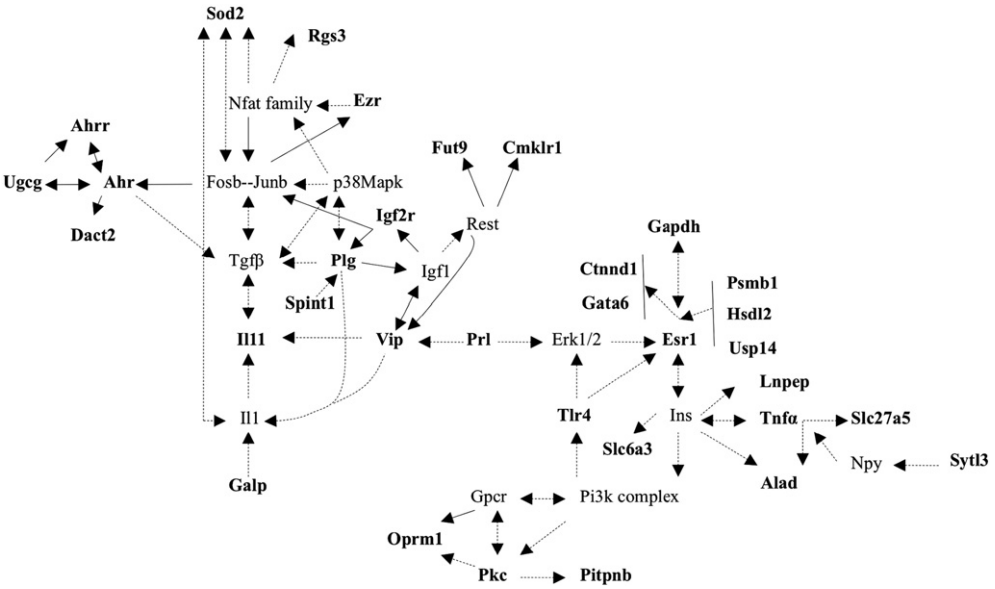

Fig. 5. Genetic pathway involved in the determination of plasma concentration of IGF-1 in females. Full black arrows represent direct interactions and those in dotted line indirect interactions. Genes not in bold are not extracted from the QTL regions but hypothesized by Ingenuity software. **Gene list: Ahr:** chrom 6, aryl hydrocarbon receptor; **Ahrr:** chrom 1, aryl-hydrocarbon receptor repressor; **Alad:** chrom 5, aminolevulinate, delta-, dehydratase; **Cmklr1:** chrom 12, chemokine-like receptor 1; **Ctnnd1:** chrom 3, catenin (cadherin associated protein), delta 1; **Dact2:** chrom 1, dapper, antagonist of beta-catenin, homolog 2; Erk1/2: mitogen activated protein kinase kinase 1/2; **Esr1:** chrom 1, estrogen receptor; **Ezr:** chrom 1, ezrin; **FosB-JunB:** AP1 complex; **Fut9:** chrom 5, fucosyltransferase 9 (alpha (1,3) fucosyltransferase); **Galp:** chrom 1, galanin-like peptide; **Gapdh:** chrom 1, glyceraldehyde-3-phosphate dehydrogenase; **Gata6:** chrom 18, transcription factor GATA-6; Gpcr: chrom 12, relaxin/insulin-like family peptide receptor 2; **Hsdl2:** chrom 5, hydroxysteroid dehydrogenase like 2; Igf1: chrom 7, insulin-like growth factor 1; **Igf2r:** chrom 1, insulin-like growth factor 2 receptor; IL1: chrom3, interleukin 1; **IL11:** chrom 1, interleukin 11; Ins: chrom 1, insulin; **Lnpep:** chrom 1, leucyl/cystinyl aminopeptidase; Nfat family: chrom 19, nuclear factor of activated T-cells, cytoplasmic; Npy: chrom 4, neuropeptide Y; **Oprm1:** chrom 1, Mu-type opioid receptor; p38Mapk: chrom 1, p38 map kinase; **Pkc:** chrom 1, protein kinase C; Pi3k complex: phosphatidyl inositol 3 kinase complex; **Pitpnb:** chrom 12, phosphatidylinositol transfer protein, beta; **Plg:** chrom 1, plasminogen; **Prl:** chrom 17, prolactin; **Psmb1:** chrom 1, proteasome (prosome, macropain) subunit, beta type 1; Rest: chrom 14, RE1-silencing transcription factor; **Rgs3:** chrom 5, regulator of G-protein signaling 3; **Slc6a3:** chrom 1, solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; **Slc27a5:** chrom 1, solute carrier family 27 (fatty acid transporter), member 5; **Sod2:** chrom 1, superoxide dismutase 2, mitochondrial; **Spint1:** chrom 3, serine peptidase inhibitor, Kunitz type 1; **Sytl3:** chrom 1, synaptotagmin-like 3; Tgfβ: chrom 1, transforming growth factor, beta; **Tlr4:** chrom 5, toll-like receptor 4; **Tnfα:** chrom 5, tumor necrosis factor α; **Ugcg:** chrom 5, UDP-glucose ceramide glucosyltransferase; **Usp14:** chrom 18, ubiquitin specific peptidase 14; **Vip:** chrom 1, vasoactive intestinal peptide.

as the hormone activating cardiac mineralocorticoid receptors, under conditions of tissue damage [65].

4.6. E2-dependent regulations

Ahr. Aryl hydrocarbon receptor. Activation of the aryl hydrocarbon pathway by 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) accelerates the transition to reproductive senescence [66]. Ahr mRNA is present in 75% of pituitary lactotropes and 45% of gonadotropes and most Ahr-containing cells are estrogen receptor 1 (Esr1)-positive [67].

Ahrr, aryl hydrocarbon receptor repressor, is a member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of transcription factors [68]. It provides a negative feedback loop with a xenobiotic or endogenous ligand-dependent signal transduction mediated by the AhR. Ahrr might play a role of co-repressor on the transcriptional activity of the ERalpha homodimer [69].

Esr1, the estrogen receptor, ESR1 is significantly associated with longevity and could exert an antagonistic pleiotropic effect on longevity and fertility [70].

4.7. Proliferation and renal function

Hgf, hepatocyte growth factor/scatter factor (HGF/SF) as well as its receptor, the tyrosine kinase MET, have a causal role in uncontrolled cell survival, growth, angiogenesis and metastasis which are essential hallmarks of cancer [71]. HGF/c-MET and Wnt/β-catenin have recently emerged as important to renal cell carcinoma pathogenesis [72].

5. Conclusion

LOU/C and F344 rat strains were described previously for their phenotypic differences concerning ageing, neuroendocrine regulation (mainly GH and HPA axis), fat deposition and its metabolic consequences. This study on a F2 rat population obtained by crossbreeding the two strains permitted to identify the genomic regions influencing IGF-1 circulating levels, and allowed to hypothesize on some genetic networks, involved in determining them in the Rat species. Subsequent studies will reveal whether these genetic networks are really also involved in the phenotypic differences concerning age-associated obesity, diabetes and inflammation as well as cardiovascular impairments.

Conflict of interest

There is no conflict of interest with any of the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ghir.2013.08.002.

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