Genetic association and gene expression analysis identify *FGFR1* as a new susceptibility gene for human obesity

Hong Jiao[1,2] , Peter Arner[3] , Suzanne L Dickson[4], Hubert Vidal[5], Niklas Mejhert[3], Corneliu Henegar[6], Magdalena Taube[4], Caroline Hansson[4], Anke Hinney[7], Pilar Galan[8], Chantal Simon[5,9], Angela Silveira[10], Anna Benrick[4], John-Olov Jansson[4], Anne Bouloumié[11], Dominique Langin[11], Martine Laville[5], Cyrille Debard[5], Tomas Axelsson[12], Mikael Rydén[3], Juha Kere[1,2], Karin Dahlman-Wright[1], Anders Hamsten[10], Karine Clement[6], Ingrid Dahlman[3]

[1]Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 57 Stockholm, Sweden
[2]Clinical Research Centre, Karolinska University Hospital, SE-141 57 Stockholm, Sweden
[3]Department of Medicine at Karolinska Institutet and Karolinska University Hospital, SE-141 86 Stockholm, Sweden
[4]Department of Physiology/Endocrinology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, SE-405 30 Gothenburg, Sweden
[5]University of Lyon, INSERM U-870, INRA U-1235, Human Nutrition Research Center, Hospices Civils de Lyon, F-69600, Oullins, France
[6]INSERM, U-872, Nutriomique (team 7) 75006 Paris, France; University Pierre and Marie Curie-Paris 6, Cordeliers Research Center, 75006 Paris, France; AP-HP, Pitié-Salpétrière Hospital, 75013 Paris, France
[7]Department of Child and Adolescent Psychiatry of the University of Duisburg-Essen, Essen, Germany
[8]INSERM, U-557/INRA U-1125, CNAM, UP13, CRNH-IdF, 93017 Bobigny, France; University Paris 13, 93017, Bobigny, France; AP-HP, Avicenne Hospital, 93017 Bobigny, France
[9]University of Strasbourg, EA 1801, F-67000, Strasbourg, France
[10]Cardiovascular Genetics Group, Atherosclerosis Research Unit, Department of Medicine Solna, Karolinska Institutet, SE-17176 Stockholm, Sweden
[11]Rangueil Institute of Molecular Medicine, Inserm U858, Paul Sabatier University, BP 84225, 31432 Toulouse Cedex 4, France
[12]Department of Medical Sciences, Molecular Medicine, Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[13]Steno Diabetes Center, Copenhagen, Denmark
[14]Faculty of Health Science, University of Aarhus, Aarhus, Denmark
[15]Institute for Preventive Medicine, Copenhagen University Hospital, Center for Health and Society, Copenhagen, Denmark

Abbreviated title: The *FGFR1* gene is associated with obesity

Precis: *FGFR1* is a novel susceptibility gene for obesity, which may promote obesity by influencing adipose tissue and the hypothalamic control of appetite.

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Correspondance to:

Ingrid Dahlman, Karolinska University Hospital in Huddinge, Department of Medicine, M63, SE 141 86 Stockholm, Sweden

Phone: +46 8 58580000, fax: +46 8 58582407, e-mail: ingrid.dahlman@ki.se

Juha Kere, Department of Biosciences and Nutrition, Karolinska Institutet, SE-141 57 Stockholm, Sweden

Phone: +46 8 6089158, fax: +46 8 7745538, e-mail: juha.kere@ki.se

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Abstract

Context: Previous studies suggest a role for Fibroblast growth factor receptor 1 (*FGFR1)* in the regulation of energy balance.

Objective: To investigate if *FGFR1* is an obesity gene by genetic association and functional studies.

Design: Genotype common *FGFR1* single nucleotide polymorphisms (SNPs) in large cohorts. Confirm significant results in additional cohorts. Measure *FGFR1* expression in human adipose tissue and in rodent hypothalamus.

Setting: General community and referral centers for specialized care.

Participants: We genotyped *FGFR1* SNPs in 2438 obese and 2115 lean adults, and 985 obese and 532 population-based children. Results were confirmed in 928 obese and 2738 population-based adults, and 487 obese and 441 lean children. Abdominal subcutaneous adipose tissue was investigated in 202 subjects. We also investigated diet induced obese, fasting and fed rats.

Main Outcome Measures: Association between *FGFR1* SNPs and obesity. In secondary analyses, relate adipose *FGFR1* expression to genotype, obesity, and degree of fat cell differentiation, and relate hypothalamic *FGFR1* to energy balance.

Results. *FGFR1* rs7012413*T was nominally associated with obesity in all four cohorts; meta-analysis OR 1.17 [95% C.I. 1.10-1.25] and $P=1.8\text{x}10^{-6}$, which was $P=7.0\text{x}10^{-8}$ in the recessive model. rs7012413*T was associated with *FGFR1* expression in adipose tissue ($P<0.0001$). In this organ, but not in skeletal muscle, *FGFR1* mRNA ($P<0.0001$) and protein ($P<0.05$) were increased in obesity. In rats, hypothalamic expression of *FGFR1* declined after fasting ($P<0.001$) and increased following diet-induced obesity ($P<0.05$).

Conclusions. *FGFR1* is a novel obesity gene which may promote obesity by influencing adipose tissue and the hypothalamic control of appetite.

Introduction

Fibroblast Growth Factor Receptor 1 (FGFR1) is activated by several Fibroblast growth factors (FGFs) and previous studies suggest a role for FGFR1-signaling in the regulation of energy balance. We have shown that human subcutaneous adipose tissue secretes the FGFR1 ligand FGF1 (1). Silencing of *FGFR1* inhibits differentiation (adipogenesis) in human precursor cells (2, 3). Furthermore, adipocyte number is a major determinant for the fat mass in adults and fat cells are continuously being renewed in adult humans (4). In addition, modulation of hypothalamic FGFR1 signalling in rodents decreases food intake (see supplement for detail) (5-7).

Against this background, we have investigated common single nucleotide polymoprhisms (SNPs) in the *FGFR1* gene for association with obesity. To further strengthen the notion of *FGFR1* as an obesity gene, we studied the expression of *FGFR1* in human adipose tissue, and also in the hypothalamic region of the rat brain, in relation to energy balance. Finally, we investigated the influence of *FGFR1* genotype on adipose gene expression.

Methods

The study was approved by the local Ethics Committees. All adults gave their informed consent to participation. For subjects under age 18, written authorization was obtained from the parents.

Cohorts

The cohorts for genetic studies are described in Table 1 and Supplementary methods. Cohort 1 comprised obese adults with BMI $\geq$30.0 kg/m^2 and lean with BMI <25.0 kg/m^2, all having European ancestry and living in the greater Stockholm area. Cohort 2 comprised French obese and population-based control children (8). The obese population had BMI Z-score $\geq$3. In this case, in the obese population, we used the Rolland and Cachera methodology who defined BMI curve and evolution in the French population (9). The control children participated in a population-based physical activity study (10). Phenotypes were collected before the intervention. Cohort 3 comprised adult French morbidly obese (BMI $\geq$40.0 kg/m^2) cases and population-based control subjects. The adults in the

control group were participants of SU.VI.MAX (11). Phenotypes were collected at study entry. Cohort 4 encompassed German extremely obese children and adolescents (BMI Z-score 4.6±2.3) and adult lean controls (BMI Z-score: -1.4±0.4) (12). The BMI of the obese patients was above the 90th BMI percentile for German children and adolescents (see www.mybmi.de).

Subjects included in analysis of human abdominal subcutaneous adipose tissue were from Cohort 1 (see above). In these studies obesity was defined as BMI >30 kg/m^2 and leanness as BMI <25kg/m^2. These subjects are described in Supplementary methods. All subjects were healthy according to self-report. An abdominal subcutaneous fat biopsy was obtained under local anesthesia in the morning after an overnight fast (13). Fat cells were isolated as described (14). Cells from the stroma fraction were used for in vitro differentiation of preadipocytes as described (15). Adipose tissue pieces or 200 µl of isolated adipocytes were immediately frozen in liquid nitrogen.

Percutaneous biopsies of the vastus lateralis muscle were obtained after an overnight fast from healthy never-obese lean controls (5 men and 5 women) and age-matched obese subjects with normal glucose tolerance (2 men and 6 women). All subjects had a stable body weight over the last 3 months and were not involved in heavy exercise programs.

Studies in rodents

For fasting studies, Sprague–Dawley rats (Charles River, Frankfurt, Germany; n=19) were handled daily for 10 days following which half of the rats were subjected to an overnight (16 h) fast. In studies of diet-induced obesity, 4-week-old male Wistar rats (Harlan, Blackthorne, UK; n=16) were exposed to a cafeteria-style Western diet or normal chow for 16 weeks (n=8 per group). At the end of the study, the body weight of the cafeteria-fed group (mean±SEM = 484±15 g) was significantly higher than the chow group (mean±SEM = 398±14 g, $p<0.001$).

Genotyping

The *FGFR1*gene is encoded on chromosome 8 and is in Caucasian samples composed of two haploblocks separated by a region with low LD (www.hapmap.org). We genotyped markers which

tagged the common (frequency >10%) haplotypes, as well as a number of markers in the region with low LD. Se supplementary methods for details.

Quantitative real-time PCR

*FGFR1*mRNA was quantifed by quantitative real-time PCR as described in Supplementary methods. We calculated relative changes of the target genes employing the comparative method (User Bulletin no. 2, Applied Biosystems).

Western blot

We performed Western blot as described (16) with commercial FGFR1 (cat. nr. Sc-121, Santa Cruz Biotechnology, CA, USA) and β-actin (cat. nr. A2066, Sigma, St Louis, USA) antibodies.

Statistical analysis

We used Haploview (17) to test for Hardy Weinberg Equilibrium, and to evaluate association between single SNPs or haplotypes and obesity. The χ^2 test was used to test for association between alleles and obesity. For meta-analysis, the inverse variance method was used for pooling of cohort results. The combination of data and the combined value of the odds ratio (OR) and 95% confidence interval (C.I.) were calculated using the random effects estimate method implemented in the R package. Model-based tests were carried out to evaluate association of genotype with obesity using logistic regression implemented in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) (18).

Differences in specific quantitative phenotypes between genotypes were evaluated by ANCOVA with age and BMI as covariates. Gender did not affect gene expression. The influence of genotype on specific mRNA according to the additive model was tested by Spearman Rank correlation. Student's t test was used for two-group comparisons. Values are mean±SD unless otherwise indicated.

Results

FGFR1 rs7012413 is associated with obesity

We genotyped nine *FGFR1* SNPs in cohort 1 and 2 (Supplementary Table 2). Two SNPs were not in HWE and were therefore excluded from analysis. One SNP in intron 1 of *FGFR1*, rs7012413, was

associated with obesity in both cohorts, nominal *P*=0.0043 and 0.002 respective (Table 1). Three more SNPs were nominally associated with obesity in one cohort only; rs4733930 and rs6983315 in cohort 1; rs10958700 in cohort 2 (Supplentary Table 2). No haplotype was associated with obesity. To confirm the association of rs7012413 with obesity two more cohorts were investigated, Table 1. rs7012413 was associated with obesity in a cohort 3 (*P*=0.049) and in cohort 4 (*P*= 0.05). In a meta-analysis of all four cohorts rs7012413*T was associated with obesity with $P=1.8x10^{-6}$ and OR 1.17 [95% C.I. 1.10-1.25]. There was no statistical evidence for heterogeneity in impact on obesity between cohorts. Body fat in kg was measured in n=1484 subjects from cohort 1 with Bioimpendance. In this cohort rs7012413*C allele was associated with lower body fat (*P*=0.019) using a generalized linear model and adjusting for height squared, gender, and age.

The impact of rs7012413 on obesity under different genetic models was tested next in a joint analysis of all cohorts. The recessive but not the dominant model reached genome-wide significance, $P=7.0x10^{-8}$ (OR 1.43 [95% C.I. 1.26-1.63]) versus *P*=0.003 (1.13 [95% C.I. 1.04-1.22)] (Supplementary Table 3). rs7012413 was associated with obesity in both women and men (Supplementary Table 3). We performed bioinformatic analysis to explore a potential function of rs7012413. According to TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html), rs7012413*T is predicted to cause two extra transcription factor binding sites for NF-Y and CCAAT as compared to rs7012413*C (Supplementary Figure 1).

FGFR1mRNA in human adipose tissue is associated with rs7012413 genotype and obesity

We next studied *FGFR1* expression. *FGFR1* mRNA in intact adipose tissue was increased by about one-third in obese women (*P*<0.0001) (Figure 1A). Smaller cohorts were used to explore in more detail the pattern of expression of *FGFR1*. *FGFR1* mRNA in isolated fat cells showed a trend towards increased expression in obese, but the results were non-significant, *P*=0.10 (1 sided test gives *P*=0.05; since aim of this analysis was to confirm the results from intact adipose tissue we think 1-sided test is appropriate to use.) (Figure 1B). Furthermore, FGFR1 protein in adipose tissue was increased twofold (*P*<0.05) in obese women (Figure 1C). By contrast, *FGFR1* mRNA in human skeletal muscle was not influenced by obesity (results not shown). Finally, *FGFR1* mRNA was increased during differentiation

in vitro of precursor cells to adipocytes, $P<0.01$ (Figure 1E). There was a significant overall effect of rs7012413 genotype on adipose *FGFR1* expression in all subjects combined ($P<0.001$) and in the obese ($P=0.005$). TT and CT subjects showed higher *FGFR1* mRNA levels than CC subjects (Supplementary Table 4). CT subjects had slightly higher expression levels of *FGFR1* than TT subjects; this may be caused by the small number of TT subjects (n=6). An additive model was significant ($P=0.018$).

Hypothalamic FGFR1 mRNA expression is regulated by energy balance in rodents.

The hypothalamic expression of *FGFR1* was significantly decreased ($P<0.01$) by an overnight (16h) fast and increased ($P<0.05$) in diet-induced obese rats (Figure 2A and 2B).

Discussion

We report a common SNP, rs7012413, in the first intron of the *FGFR1* gene that is associated with obesity in four cohorts, together comprising 4838 obese cases and 5827 lean or population-based controls. We show that *FGFR1* mRNA in subcutaneous adipose tissue is associated with rs7012413 genotype, obesity status, as well as fat cell differentiation. Furthermore, in rodent studies we observe that hypothalamic expression of *FGFR1* is correlated with energy balance.

Association of rs7012413 with obesity was observed in both adults and children. This is in agreement with the recent report that most obesity-susceptibility loci are already associated with anthropometric traits in children/adolescents (19). *FGFR1* SNPs have previously been examined for association with BMI in 629 individuals from 207 families who were not ascertained based on obesity (20). The lack of association between *FGFR1* and obesity in the study by Kaess et al is not surprising given the limited power of the sample, and does not exclude an impact of *FGFR1* on obesity.

rs7012413 could hypothetically affect gene expression since many genes have multiple transcriptional regulatory regions. *In vitro* experiments are necessary to test the significance of the predicted binding sites introduced by the SNP. Of note, we cannot rule out that rs7012413 is in close LD with another SNP that mediates the impact on obesity and mRNA levels. However, rs7012413 is

located in a region spanning intron 1 to 2 that displays low LD between markers and among other markers genotyped in the region none is associated with obesity in both cohorts 1 and 2.

Previous studies have shown that *FGFR1* regulates human preadipocyte differentiation *in vitro* (2, 3). We here report that *FGFR1* genotype is associated with adipose tissue mRNA levels, and *FGFR1* mRNA is up-regulated following differentiation of human adipose tissue precursor cells to adipocytes. Together, these results together are consistent with the hypothesis that *FGFR1* could be a regulator of adipogenesis that contribute to obesity by regulating fat cell number. Fat cell number is a major determinant for fat mass (4).

FGFR1 gene variants may also influence obesity by other independent mechanisms e.g. modulating central regulation of food intake. We demonstrate the novel finding that *FGFR1* expression in the rat hypothalamus decreases during short time fasting and increases during long-time over-feeding.

In summary, we identified *FGFR1* is a novel obesity gene which may promote obesity by influencing adipose tissue and the hypothalamic control of appetite.

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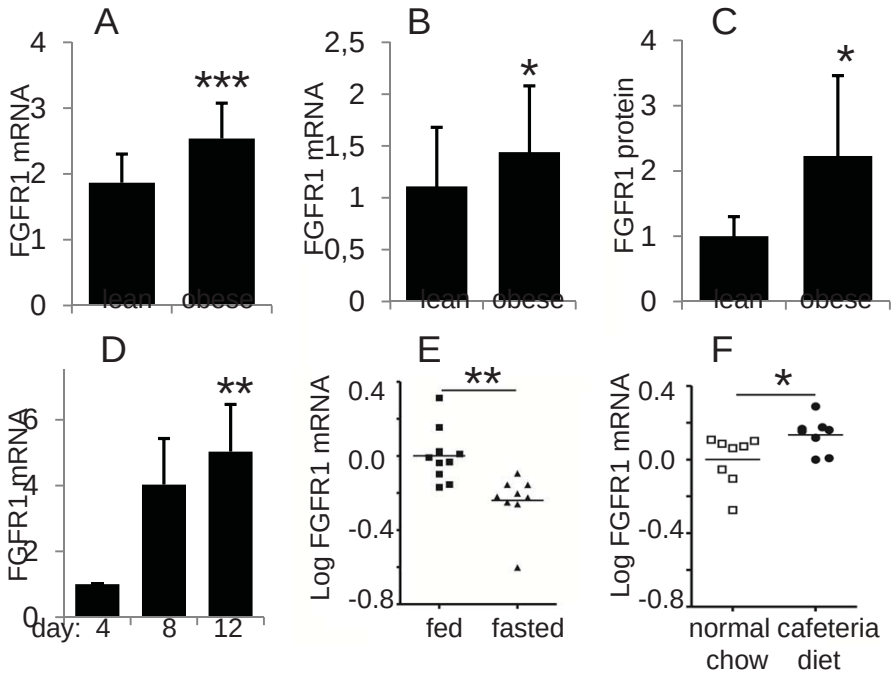
Table 1. Association of *FGFR1* SNP rs7012413with obesity

nationality	cohort	obese cases female/male	controls* female/male	call rate %	cases** T (n)	cases** C (n)	controls** T (n)	controls** C (n)	allele T in cases (%)	allele T in controls (%)	*P*
Swedish	1	1526/912	1163/952	96.9	1449	3337	1109	2923	30.3	27.5	0.0043
French	2	641/344**	289/243#	95.8	721	1155	331	683	38.4	32.6	0.002
French	3	682/246	1630/1108#	96	521	1035	1690	3786	34	31	0.049
German	4	278/209**	271/171	100	306	668	240	640	32	27	0.05
Total		**3127/1711**	**3353/2474**								

* **lean and population-based controls**; ** Cohorts comprising children in which BMI Z-scores were used to define obesity status as defined in Methods. # population-based controls. Cohort 2 population-based controls include 29 obese children and cohort 3 population-based controls 5 morbidly obese adults.

Figure 1. Expression of *FGFR1* in human abdominal subcutaneous adipose tissue and rat hypothalamus. (A) *FGFR1* mRNA expression in intact adipose tissue of lean (n=15) and obese (n=81) women, and (B) isolated fat cells of lean (n=5 women and 2 men) and obese (n=6 women and 1 man) subjects. (C) FGFR1 protein levels in adipose tissue of lean (n=6) and obese (n=6) women. (D) *FGFR1* mRNA expression in progenitor cells during differentiation to fat cells (n=11) as judged by ANOVA. (E) *FGFR1* mRNA levels in hypothalamus of fasted (n=9) and fed (n=10) rats. (F) *FGFR1* mRNA levels in hypothalamus of diet-induced obese (n=8), and normal chow (n=8) rats. *FGFR1* mRNA $=2^{(\text{Ct FGFR1 calibrator- Ct FGFR1 sample})} / 2^{(\text{Ct refrerence gene calibrator- Ct reference gene sample})}$. As reference gene we used in human experiments *18S* and in rats *HPRT* and *Actb.* Two group comparisons were performed with Student's t-test. Values are mean±SD except for (D) where values are mean±SE. *** $P<0.0001$; ** $P<0.01$, * $P<0.05$

Figure 1


A
B
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FGFR1 mRNA
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lean
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fasted
normal chow
cafeteria diet

Supplements

Introduction

Central administration of the FGFR1 agonists FGF1 (previously called acidic FGF) or of FGF2 (previously called basic FGF) inhibits food intake (1, 2). Administration of an antibody that blocks FGFR1 signaling also leads to inhibition of food intake (3). These seemingly opposing observations could be due to species differences, i.e. the FGF1 and FGF2 studies(1, 2)were performed in rats and the antibody study in mice (3), or FGFR1-independent effects of FGF1, FGF2 or the FGFR1-antibody.

Methods

Cohorts for genetic association study

Cohort 1 was selected according to the above BMI inclusion criteria amongst subjects recruited by local advertisement or amongst participants in population-based surveys or case-control studies of myocardial infarction. 282 subjects had myocardial infarction, of which 89 were obese. Some subjects in cohort 1 were diagnosed with type 2 diabetes (n=301), hypertension (n=810) or dyslipidemia (n=385). Patients with chronic inflammatory diseases other than cardiovascular disease, type 1 diabetes mellitus, renal insufficiency (serum creatinine >200 micromol/L), drug addiction or psychiatric disease were excluded. The obese and lean groups were sex-matched.

Cohorts and clinical evaluation – adipose tissue studies

FGFR1 mRNA levels in relation to obesity in pieces of adipose tissue were investigated in 96 women (15 lean with BMI 23±1 kg/m^2 and age 40±9 years; 81 obese with BMI 36±7 kg/m^2 and age 38±9 years), and in isolated fat cells in seven lean (5 women and 2 men with BMI 23.3±1.7 kg/m^2 and age 33.0±9.8 years) and seven obese subjects (6 women and 1 man with BMI 34.4±5.9 kg/m^2 and age 48.6±12.2 years). FGFR1 protein levels were analyzed in the same biopsy for a smaller cohort of women (6 lean with BMI 22±1 kg/m^2 and age 38±6 years; 6 obese with BMI 36±6 kg/m^2 and age 38±5 years). The association between *FGFR1* genotype and adipose tissue mRNA levels was investigated in 61 women and 19 men [BMI 34±10 (range 20 to 52) kg/m^2 and age 40±10 years], who were not selected on the basis of BMI. Abdominal subcutaneous biopsies for isolation of the fat cell and stroma vascular fraction of adipose fraction were obtained from 14 subjects during elective surgery for non-malignant disorders (4).

Studies in rodents

Rats were kept in a temperature controlled environment on a 12 h light/dark cycle with free access to water and, unless otherwise stated, standard food (R3, Lactamin AB, Vadstena, Sweden) *ad libitum*.

The highly palatable cafeteria-style diet consisted of soft chocolate/cocoa-type cakes and fatty cheese together with standard chow.

Genotyping

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SEQUENOM) iPLEX Gold chemistry was used to genotype cohorts 1, 2 and the obese cases in cohort 3. Primers for these assays are provided on request. The controls in cohort 3 (SU.VI.MAX) were genotyped by TaqMan (Applied Biosystems, Foster City, CA). Affymetrix 6.0 GWA genotypes were available for cohort 4. Approximately 1,000 subjects in cohort 1 were genotyped twice for *r*s7012413 with a different method (Illumina Golden Gate) and all genotypes were concordant between platforms.

RNA extraction and cDNA synthesis

Total RNA was extracted from adipose tissue samples and transcribed to cDNA as described previously (5). Skeletal muscle total RNA was prepared using TRIZOL reagent. First-strand cDNAs were synthesized from 500 ng of total RNA in the presence of 100 units of Superscript II (Invitrogen, Eragny, France) using a mixture of random hexamers and oligo (dT) primers (Promega, Charbonnières, France). Hypothalami were dissected and total RNA was purified using RNeasy$^{@}$ Mini Lipid tissue Kit (Qiagen GmbH, Hilden, Germany) with additional DNase treatment (Qiagen) as described(6). For cDNA synthesis total RNA (1 µg) was reverse transcribed using Superscript III reverse transcriptase (Invitrogen Life Technologies, Paisley, UK) and random hexamers according to the manufacturer's instructions. Recombinant RNaseout® Recombinant Ribonuclease Inhibitor (Invitrogen Life Technologies, Paisley, UK) was added to prevent RNase-mediated degradation.

Quantitative real-time PCR

Adipose tissue *FGFR1* and the reference gene *18S* were quantified using SYBR Green-based quantitative real-time PCR (qRT-PCR). Primers were for *FGFR1:* 5'-CATCACGGCTCTCCTCCAGT -3' and 5'- AGGGGTTTGCCTAAGACCAG -3', and for 18S: 5'-CACATGGCCTCCAAGGAGTAAG -3' and 5'- CCAGCAGTGAGGGTCTCTCT -3'. All reactions were run in duplicate. In muscle, mRNA levels of *FGFR1* and the reference gene Hypoxanthine phosphoribosyltransferase (*HPRT*) were quantified using a SYBR Green qRT-PCR on a Light-Cycler (Roche-Diagnostics, Meylan, France) as described (7). The PCR primer sequences are available on request (vidal@sante.univ-lyon1.fr). For rat hypothalamic samples, qRT-PCR was performed with TaqMan®Low Density Arrays (LDA) (Applied Biosystems). A custom array was designed with the following assays that were amplified according to the manufacturer's instructions: *FGFR1* (Rn00577234_m1) and as endogenous controls *18S* (Hs99999901_s1), *Actb* (Rn00667869_m1), *Gapdh* (Rn99999916_s1), *Hprt* (Rn01527840_m1) and *Ppia* (Rn00690933_m1). Duplicates of cDNA were run on separate LDA cards and analyzed using the 7900HT system with a TaqMan LDA Upgrade. *HPRT* and *Actb* displayed the most stable ct values according to the NormFinder algorithm

(http://www.mdl.dk/publicationsnormfinder.htm) and were used as controls in the calculations of relative gene expression. We calculated relative changes of the target genes employing the comparative method (User Bulletin no. 2, Applied Biosystems) using the house-keeping genes as reference genes.

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Supplementary Table 1. Cohorts

		obese cases			lean and population-based controls		
nationality	**cohort**	**female/male**	**BMI* (kg/m^2)**	**age* (years)**	**female/male**	**BMI* (kg/m^2)**	**age* (years)**
Swedish	1	1526/912	39.0±6.2	45.6±12.0	1163/952	22.7±1.7	49.3±10.1
French	2	641/344**	33.7±8.2	14.1±5.0	289/243#	18.7±3.3	11.8±1.6
French	3	682/246	48.5±7.6	43.0±12.1	1630/1108#	23.8±3.5	49.7±6.3
German	4	278/209**	33.4±6.8	14.4±3.7	271/171	18.3±1.1	16.1±5.8
Total		**3127/1711**			**3353/2474**		

* Values are mean±SD; ** Cohorts comprising children in which BMI Z-scores were used to define obesity status as defined in Methods. # population-based controls

Supplementary Table 2. Association of *FGFR1* SNPs with obesity*

SNP	position	region	cohort	call rate (%)	alleles A	alleles B	cases** A (n)	cases** B (n)	controls** A (n)	controls** B (n)	allele A in cases (%)	allele A in controls (%)	$P^{\#}$
rs2467531	38444952	5' UTR	1	99.1	T	C	15	4815	20	4174	0.3	0.5	0.20
			2	failed									
rs17182134	38443438	intron 1	1	99.1	A	C	337	4481	303	3899	7	7.2	0.69
			2	98	A	C	179	1727	81	969	9.4	7.7	0.12
rs6996321	38441503	intron 1	1	95.4	A	G	1787	2915	1531	2455	38	38.4	0.70
			2	95.4	A	G	717	1125	386	650	38.9	37.3	0.38
rs4733946	38438506	intron 1	1	97.3	T	G	373	4365	328	3796	7.9	8	0.89
			2	84.2	T	G	120	1426	78	916	7.8	7.8	0.94
rs7012413	38436555	intron 1	1	96.9	T	C	1449	3337	1109	2923	30.3	27.5	0.0043
			2	95.8	T	C	721	1155	331	683	38.4	32.6	0.002
			3	96	T	C	521	1035	1690	3786	34	31	0.049
			4	100	T	C	306	668	240	640	32	27	0.05
rs3758102	38436006	intron 1	1	99.1	T	C	1300	3524	1136	3064	26.9	27	0.92
			2	failed									
rs4733930	38430158	intron 2	1	94	T	C	1874	2732	1503	2453	40.7	38	0.011
			2	94.5	T	C	708	1094	440	608	39.3	42	0.16
rs10958700	38430067	intron 2	1	91.1	G	T	993	3453	899	2945	22.3	23.4	0.25
			2	93.7	G	T	306	1496	223	801	17	21.8	0.0017
rs6983315	38418576	intron 2	1	96.6	A	G	2138	2650	1880	2130	44.7	46.9	0.036
			2	96.2	A	G	788	1094	434	586	41.9	42.5	0.72

*Two SNPs displayed HWE P <0.001 in cohort 2. Those SNPs are indicated as failed in the Table. All other SNPs displayed HWE P >0.05 in each cohort. ** Numbers of alleles A and B, respectively. # Allele frequencies were compared between cases and controls with Chi^2 test.

Supplementary Table 3.
Association of *FGFR1* rs7012413 with obesity under different genetic models

Gender	Test	Obese (n)	Control (n)	OR (95% C.I.)	P value§§
All	Dominant*	2466/2130	2888/2812	1.13 (1.04,1.22)	0.003
	Recessive§	533/4063	479/5221	1.43 (1.26-1.63)	$7.0x10^{-08}$
Male	Dominant	874/753	1222/1188	1.13 (0.99,1.28)	0.06
	Recessive	186/1441	208/2202	1.37 (1.11,1.68)	0.003
Female	Dominant	1590/1377	1666/1624	1.13 (1.02,1.24)	0.02
	Recessive	346/2621	271/3019	1.47 (1.24,1.74)	$6.3x10^{-06}$

* Numbers of subject with genotype TT or CT versus number of subjects with genotype CC and, § numbers of subject with genotype TT versus CT and CC where T is the risk allele; §§Model based analysis was carried out by logistic regression, see Statistical analysis.

Supplementary Table 4.
***FGFR1* mRNA levels in adipose tissue in relation to rs7012413**

Group	CC	CT	TT	*P***
Obese*	4.2 ± 0.9 (21)	5.4 ± 1.1 (23)	5.0 ± 1.0 (5)	0.005
All	4.0 ± 1.1 (43)	5.3 ± 12 (31)	4.8 ± 1.0 (6)	<0.001

FGFR1 values are expressed according to the relative CT method with 18S as contol, see methods. Number of subject per genotype is shown in paranthesis. * Obesity is defined as BMI >30 kg/m^2. ** mRNA levels were compared by ANCOVA with age and BMI as cofactors since these parameters influenced mRNA values in both obese and nonobese; overall P value across three genotype groups; Values are mean±SD

Supplementary Figure 1