Mapping of the fibroblast growth factors in human white adipose tissue.

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Genetic Association and Gene Expression Analysis Identify *FGFR1* as a New Susceptibility Gene for Human Obesity

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

Objective: Our objective was to investigate whether *FGFR1* is an obesity gene by genetic association and functional studies.

Design: The study was designed to genotype common *FGFR1* single-nucleotide polymorphisms (SNP) in large cohorts, confirm significant results in additional cohorts, and measure *FGFR1* expression in human adipose tissue and in rodent hypothalamus.

Setting: General community and referral centers for specialized care was the setting for the study.

Participants: We genotyped *FGFR1* SNP 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 sc adipose tissue was investigated in 202 subjects. We also investigated diet-induced, obese fasting, and fed rats.

Main Outcome Measures: We analyzed the association between *FGFR1* SNP and obesity. In secondary analyses, we related adipose *FGFR1* expression to genotype, obesity, and degree of fat cell differentiation and related hypothalamic *FGFR1* to energy balance.

Results: *FGFR1* rs7012413*T was nominally associated with obesity in all four cohorts; metaanalysis odds ratio = 1.17 (95% confidence interval = 1.10–1.25), and $P = 1.8 \times 10^{-6}$, which was $P = 7.0 \times 10^{-8}$ 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 after diet-induced obesity ($P < 0.05$).

Conclusions: *FGFR1* is a novel obesity gene that may promote obesity by influencing adipose tissue and the hypothalamic control of appetite. **(*J Clin Endocrinol Metab* 96: E962–E966, 2011)**

Fibroblast growth factor receptor 1 (FGFR1) is activated by several fibroblast growth factors, and previous studies suggest a role for FGFR1 signaling in the regulation of energy balance. We have shown that human sc 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 signaling in rodents decreases food intake (for details, see Supplemental Data, pub-

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Abbreviations: BMI, Body mass index; CI, confidence interval; FGFR1, fibroblast growth factor receptor 1; LD, linkage disequilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism.

lished on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) (5–7).

Against this background, we have investigated common single-nucleotide polymorphisms (SNP) 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.

Subjects and 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 Supplemental Table 1 and Supplemental Methods. Cohort 1 comprised obese adults with body mass index (BMI) of 30.0 kg/m^2 or higher and lean with BMI under 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 of 3 or higher. 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 $\pm$ 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 sc adipose tissue were from cohort 1 (see above). In these studies, obesity was defined as BMI over 30 kg/m^2 and leanness as BMI under 25 kg/m^2. These subjects are described in Supplemental Methods. All subjects were healthy according to self-report. An abdominal sc 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 (five men and five women) and age-matched obese subjects with normal glucose tolerance (two men and six 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 d after which half of the rats were subjected to an overnight (16 h) fast. In studies of diet-induced obesity, 4-wk-old male Wistar rats (Harlan, Blackthorne, UK; n = 16) were exposed to a cafeteria-style Western diet or normal chow for 16 wk (n = 8 per group). At the end of the study, the body weight of the cafeteria-fed group (mean $\pm$ SEM = 484 $\pm$ 15 g) was significantly higher than the chow group (mean $\pm$ SEM = 398 $\pm$ 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 that tagged the common (frequency > 10%) haplotypes as well as a number of markers in the region with low LD. See Supplemental Methods for details.

Quantitative real-time PCR

FGFR1 mRNA was quantified by quantitative real-time PCR as described in Supplemental Methods. We calculated relative changes of the target gene employing the comparative method (User Bulletin no. 2; Applied Biosystems, Foster City, CA).

Western blot

We performed Western blot as described (16) with commercial FGFR1 (catalog item Sc-121; Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (catalog item A2066; Sigma Chemical Co., St. Louis, MO) antibodies.

Statistical analysis

We used Haploview (17) to test for Hardy-Weinberg equilibrium, and to evaluate association between single SNP or haplotypes and obesity. The χ^2 test was used to test for association between alleles and obesity. For metaanalysis, 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 (CI) were calculated using the random-effects estimate method implemented in the R package. Model-based tests were carried out to evaluate association of

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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 analysis of covariance 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* SNP in cohorts 1 and 2 (Supplemental Table 2). Two SNP were not in Hardy-Weinberg equilibrium 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, respectively (Table 1). Three more SNP were nominally associated with obesity in one cohort only: rs4733930 and rs6983315 in cohort 1 and rs10958700 in cohort 2 (Supplemental 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 cohort 3 ($P = 0.049$) and in cohort 4 ($P = 0.05$). In a metaanalysis of all four cohorts, rs7012413*T was associated with obesity with $P = 1.8 \times 10^{-6}$, and OR = 1.17 (95% CI = 1.10–1.25). There was no statistical evidence for heterogeneity in impact on obesity between cohorts. Body fat in kilograms was measured in 1484 subjects from cohort 1 with bioimpedance. 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 [$P = 7.0 \times 10^{-8}$ (OR = 1.43; 95% CI = 1.26–1.63) *vs.* $P = 0.003$ (OR = 1.13; 95% CI = 1.04–1.22)] (Supplemental Table 3). rs7012413 was associated with obesity in both women and men (Supplemental 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 nuclear transcription factor Y and CCAAT box binding proteins compared with rs7012413*C (Supplemental Fig. 1).

FGFR1 mRNA 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 ($P < 0.0001$) (Fig. 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 toward increased expression in obese, but the results were nonsignificant ($P = 0.10$) (one-sided test gives $P = 0.05$; because the aim of this analysis was to confirm the results from intact adipose tissue, we think the one-sided test is appropriate to use) (Fig. 1B). Furthermore, FGFR1 protein in adipose tissue was increased 2-fold ($P < 0.05$) in obese women (Fig. 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 ($P < 0.01$) (Fig. 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 genotype subjects showed higher *FGFR1* mRNA levels than CC subjects (Supplemental 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 (16 h) fast and

TABLE 1. Association of *FGFR1* SNP rs7012413 with obesity

Nationality	Cohort	Obese cases (female/male)	Controls[a] (female/male)	Call rate (%)	Cases[b] T (n)	Cases[b] C (n)	Controls[b] T (n)	Controls[b] 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[b]	289/243[c]	95.8	721	1155	331	683	38.4	32.6	0.002
French	3	682/246	1630/1108[c]	96	521	1035	1690	3786	34	31	0.049
German	4	278/209[b]	271/171	100	306	668	240	640	32	27	0.05
Total		3127/1711	3353/2474								

[a] Lean and population-based controls.

[b] Cohorts comprising children in which BMI Z-scores were used to define obesity status as defined in *Materials and Methods*.

[c] Population-based controls. Cohort 2 population-based controls include 29 obese children, and cohort 3 population-based controls include five morbidly obese adults.

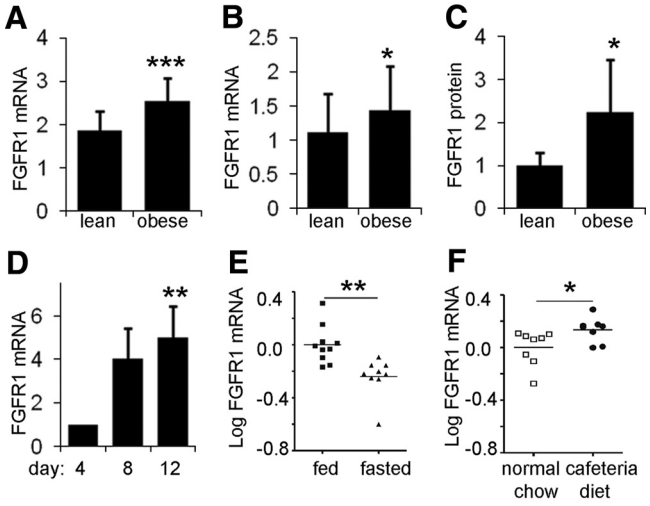

FIG. 1. Expression of *FGFR1* in human abdominal sc adipose tissue and rat hypothalamus. A and B, *FGFR1* mRNA expression in intact adipose tissue of lean (n = 15) and obese (n = 81) women (A) and isolated fat cells of lean (five women and two men) and obese (six women and one man) subjects (B); C, FGFR1 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-fed (n = 8) rats. *FGFR1* mRNA = $2^{(CtFGFR1calibrator-CtFGFR1sample)}/2^{(Ctreferencegenecalibrator-Ctreferencegenesample)}$. 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. ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$.

increased ($P < 0.05$) in diet-induced obese rats (Fig. 1, E and F).

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 sc adipose tissue is associated with rs7012413 genotype and 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* SNP 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.* (20) 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 because many genes have multiple transcriptional regulatory regions. *In vitro* experiments will be necessary to test the significance of the predicted binding sites associated with one allele of 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 after 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 contributes 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 a short time of fasting and increases during long-time overfeeding.

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

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