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-
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/m² or higher and lean with BMI under 25.0 kg/m², 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 ≥ 40.0 kg/m²) 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 sc adipose tissue were from cohort 1 (see above). In these studies, obesity was defined as BMI over 30 kg/m² and leanness as BMI under 25 kg/m². 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 in Supplemental Methods. 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 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 χ² 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 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 ± SEM = 484 ± 15 g) was significantly higher than the chow group (mean ± SEM = 398 ± 14 g; P < 0.001).
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 (Supplemental Table 2). No SNP was associated with obesity in both cohorts, nominal \( P > 0.05 \). 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) \( \text{vs.} \ P = 0.003 \) (OR = 1.13; 95% CI = 1.04–1.22] (Supplemental Table 3).

**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

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

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Cohort</th>
<th>Casesa (female/male)</th>
<th>Controlsa (female/male)</th>
<th>Calls rate (%)</th>
<th>Casesb</th>
<th>Controlsb</th>
<th>Allele T in</th>
<th>P</th>
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<tr>
<td>Swedish</td>
<td>1</td>
<td>1526/912</td>
<td>1163/952</td>
<td>96.9</td>
<td>1449</td>
<td>3337</td>
<td>1109</td>
<td>2923</td>
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<td>641/344b</td>
<td>289/243c</td>
<td>95.8</td>
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<td>331</td>
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<td>682/246</td>
<td>1630/1108d</td>
<td>96</td>
<td>521</td>
<td>1035</td>
<td>1690</td>
<td>3786</td>
</tr>
<tr>
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<td>4</td>
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<td>271/171</td>
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<td>3353/2474</td>
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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.
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 genotype and obesity status as well as fat cell differentiation and the hypothalamic control of appetite. 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 and in rats (8) and normal chow-fed (n = 8) rats. 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^(-ΔΔCt) where ΔΔCt = ΔCtFGFR1sample - ΔCtreferencegene and ΔCtreferencegene = ΔCtreferencegenecalibrator - ΔCtreferencegenetissue.

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 ± se except for D, where values are mean ± se. *** P < 0.0001; **, P < 0.01; *, P < 0.05.

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 genotype and obesity status as well as fat cell differentiation and the hypothalamic control of appetite. Furthermore, in rodent studies, we observe that hypothalamic expression of FGFR1 is correlated with energy balance.

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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.

Acknowledgments

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Address all correspondence and requests for reprints to: Ingrid Dahlman, Karolinska Institute for performing genotyping and for excellent technical support by Gaby Astrom, Eva Sjolin, Elisabeth Dungner, and Kerstin Wahlen. We are indebted to Veronique Pelloux and Rohia Alili for DNA preparation.

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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 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^(-ΔΔCt) where ΔΔCt = ΔCtFGFR1sample - ΔCtreferencegene and ΔCtreferencegene = ΔCtreferencegenecalibrator - ΔCtreferencegenetissue.

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increased (P < 0.05) in diet-induced obese rats (Fig. 1, E and F).
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18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC 2007 PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575.
