Original Article

Association Studies of BMI and Type 2 Diabetes in the Neuropeptide Y Pathway

A Possible Role for *NPY2R* as a Candidate Gene for Type 2 Diabetes in Men

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The neuropeptide Y (NPY) family of peptides and receptors regulate food intake. Inherited variation in this pathway could influence susceptibility to obesity and its complications, including type 2 diabetes. We genotyped a set of 71 single nucleotide polymorphisms (SNPs) that capture the most common variation in *NPY*, *PPY*, *PYY*, *NPY1R*, *NPY2R*, and *NPY5R* in 2,800 individuals of recent European ancestry drawn from the near extremes of BMI distribution. Five SNPs located upstream of *NPY2R* were nominally associated with BMI in men (P values = 0.001–0.009, odds ratios [ORs] 1.27–1.34). No association with BMI was observed in women, and no consistent associations were observed for other genes in this pathway. We attempted to replicate the association with BMI in 2,500 men and tested these SNPs for association with type 2 diabetes in 8,000 samples. We observed association with BMI in men in only one replication sample and saw no association in the combined replication samples (P = 0.154, OR = 1.09). Finally, a 9% haplotype was associated with type 2 diabetes in men ($P = 1.73 \times 10^{-4}$, OR = 1.36) and not in women. Variation in this pathway likely does not have a major influence on BMI, although small effects cannot be ruled out; *NPY2R* should be considered a candidate gene for type 2 diabetes in men.
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Obesity, as measured by BMI, is an important predictor of type 2 diabetes, cardiovascular disease, cancer, and death (1–5). Although environmental factors influence the rising tide of obesity, genetic factors strongly influence obesity; the heritability of BMI within individual populations is ~30–70% (6–8). Identifying the underlying genetic causes of obesity could provide valuable insights into the pathways that are relevant in patients and help guide the development of more effective preventive measures and therapies. In addition, genes that influence susceptibility to obesity may also contribute to the common sequelae of obesity, such as type 2 diabetes.

Variants in over 120 genes have been reported to be associated with measures of obesity (9), but few of these associations have been repeatedly reproduced with convincing statistical evidence (9) (H.N.L., J.N.H., personal communication). A few exceptions are rare variants in the *MC4R* receptor in early-onset obesity (10–13) and possibly an association with common variation near *INSIG2* (14). As one approach to identify genes contributing to obesity in the general population, we are testing common genetic variation in candidate genes implicated by physiological and genetic studies in humans and model organisms.

Several lines of evidence suggest that the genes encoding the neuropeptide Y (NPY) family of peptides and receptors are good candidates for association studies with obesity (15,16). NPY is a potent stimulus of food intake (15,16), primarily through binding the Y1 and Y5 receptors (16). Two related peptides, pancreatic polypeptide (PP) and polypeptide YY (PYY), inhibit food intake (16). $PPY_{3–36}$ inhibits food intake by binding to Y2 receptor (16). Suggestive evidence for linkage has been seen for the regions containing *NPY* (NPY), *PYY* (PYY), *PPY* (PP),

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CEPH, Centre d'Etude du Polymorphisme Humain; FHS, Framingham Heart Study; NHLBI, National Heart, Lung, and Blood Institute; NPY, neuropeptide Y; PYY, polypeptide YY; SNP, single nucleotide polymorphism.

TABLE 1
Panels for BMI association studies

Panel	Type	Sex	Affection status	n	Age (years)	BMI (kg/m^2)
European American*	Screening	Male	Lean	288	56 ± 10	22.3 ± 0.5
			Obese	552	57 ± 9	35.8 ± 2.2
		Female	Lean	336	57 ± 9	20.8 ± 0.5
			Obese	666	58 ± 9	37.8 ± 2.3
Polish*	Screening	Male	Lean	151	56 ± 10	21.9 ± 0.4
			Obese	334	54 ± 9	30.4 ± 1.6
		Female	Lean	180	57 ± 9	21.2 ± 0.8
			Obese	366	57 ± 8	33 ± 1.1
Framingham†	Replication	Male	Lean	206	60 ± 9	23.8 ± 1.7
			Obese	206	60 ± 9	34.4 ± 3.5
Scandinavian unrelated†	Replication	Male	Lean	247	63 ± 11	22.9 ± 1.6
			Obese	241	64 ± 11	31.0 ± 2.2
Scandinavian trios†	Replication	Male	Lean	51	31 ± 8	21.0 ± 1.7
			Obese	57	35 ± 9	31.3 ± 3.2
GCI African American*	Replication	Male	Lean	71	52 ± 9	20.7 ± 0.6
			Obese	192	53 ± 10	35.8 ± 2.0
Maywood African American†	Replication	Male	Lean	111	39 ± 11	20.6 ± 1.2
			Obese	108	38 ± 10	38.1 ± 6.2

Data are means ± SD unless otherwise indicated. *Lean subjects are in the 5th–12th percentile in BMI, and obese subjects are in the 90th–97th percentile in BMI. †Lean subjects are in the bottom quartile and obese subjects are in the top quartile for age-adjusted log(BMI) z score. BMI was also analyzed as a continuous trait in the FHS (847 men), Scandinavian unrelated (977 men), and Scandinavian trios (218 male offspring).

NPY1R (NPY Y1 receptor), *NPY2R* (NPY Y2 receptor), and *NPY5R* (NPY Y5 receptor), although none of these linkages have been consistently reproduced (9). Finally, genetic association studies have implicated these genes in obesity or type 2 diabetes, with P values in the range of 0.001–0.05 (17–23), suggesting that common variation in these genes may be involved in obesity in humans. Larger sample sizes are needed to determine if these results represent true associations.

Because of the considerable biological connection to appetite control and the suggestive genetic data, we sought to comprehensively study a common variation in the genes in the NPY pathway for association with BMI. We selected tag SNPs that capture the majority of common variation in these genes (24–26). We genotyped these tag SNPs in multiple large samples to survey common variation in these genes for association with BMI, and we tested the most associated variants for association with type 2 diabetes.

RESEARCH DESIGN AND METHODS

First, we used reference panels to determine the patterns of linkage disequilibrium and choose tagging SNPs. Second, tag SNPs and multimarker haplotypes comprised of tag SNPs were tested in two screening panels: European-American and Polish subjects. SNPs and haplotypes were tested in the full panels and in men and women separately, based on an a priori hypothesis that effects on BMI or related traits could be sex dimorphic. We tested SNPs and haplotypes for nominal association with BMI in the European-American panel (two-tailed $P < 0.05$) and then tested these SNPs and haplotypes for replication in the Polish sample (one-tailed $P < 0.05$). SNPs that met these criteria or comprised haplotypes that met these criteria were genotyped in the replication samples (Framingham unrelated, Scandinavian unrelated, Scandinavian trios, GCI African American, and Maywood African American). In the replication samples, only the specific hypotheses suggested by the screening samples were tested. Specifically, we observed an association in men only, so we only analyzed men from the replication studies. The SNPs carried forward into the BMI replication panels were also tested for association with type 2 diabetes.

Consent. All subjects gave informed consent, and the project was approved by the institutional review board of Children's Hospital (Boston, MA).

Reference panels. The European-derived reference sample consists of 93 individuals in 12 multigenerational pedigrees (Centre d'Etude du Polymorphisme Humain [CEPH]) representing 96 independent chromosomes, as previously described (27). The African-American reference panel is comprised of 50 unrelated individuals, as previously described (28).

Screening panels. The European-American (1,218 case and 624 control subjects) and Polish (700 case and 330 control subjects) panels were obtained from Genomics Collaborative (Table 1), as previously described (14). These individuals were selected from a collection of >60,000 subjects and include healthy control subjects plus patients with osteoarthritis, rheumatoid arthritis, asthma, hypertension, coronary artery disease, myocardial infarction, hyperlipidemia, stroke, type 2 diabetes, or osteoporosis. We determined the BMI distribution in healthy individuals for each decade of life, sex, and country of origin (U.S. or Poland). We designated any subjects from the original set of 60,000 with a BMI between the 90th and 97th percentile of the described distribution as potential obese case subjects and designated any subjects with a BMI between the 5th and 12th percentiles as potential lean control subjects. From this set of obese and lean individuals, a subset of obese and lean individuals was selected by matching for age, sex, and grandparental region of origin. Collectively, these samples will be referred to as the screening panels. There was no significant effect on the odds ratios (ORs) by limiting the analysis to those from the healthy control subjects.

BMI replication panels. The panel from the Framingham Heart Study (FHS) contains 1,739 unrelated individuals, from which we only analyzed data for the 847 men (Table 1). These individuals are drawn from the offspring cohort, which is the second generation of a longitudinal study of the general population of Framingham, Massachusetts. Height and weight were measured on six separate occasions from 1971 to 1998, as described elsewhere (29,30). We focused on the exam 6 data because BMI was most heritable in this exam cycle (30).

The Scandinavian parent-offspring trios (218 male offspring) are from the Botnia Study conducted in Finland and Sweden; the offspring were ascertained as nondiabetic individuals with waist-to-hip ratios in the upper quintile or lower decile (31). In addition, from this panel and the Scandinavian panels described below, we constructed a separate, nonoverlapping sample of 977 unrelated men to test for association with BMI, consisting of the male control subjects, nondiabetic fathers in parent-offspring trio panels, and a single nondiabetic male sibling from each sibship.

Two African-American panels were studied. The first consists of self-described African-American men born in the U.S. (192 obese and 71 lean). This panel is identical in design to the European-American and Polish case-control panels described above and was also collected by Genomics Collaborative. The second panel consists of African-American individuals from Maywood, Illinois, and contains 866 individuals in nuclear families and sibships and 186 unrelated individuals, as described elsewhere (14). For analytical purposes, we constructed a panel of unrelated men (108 obese and 111 lean) from this

TABLE 2
Patient samples used to test association to type 2 diabetes

Panel	Sex	Affection status	n	Age (years)	BMI (kg/m^2)
Scandinavian trios	Male	Diabetes/severe IGT	168	38 ± 9	27.3 ± 4.5
	Female	Diabetes/severe IGT	153	39 ± 9	27.5 ± 5.9
Scandinavian discordant sibships	Male	Diabetes/severe IGT	280	62 ± 11	28.7 ± 4.1
		NGT	275	60 ± 9	26.5 ± 3.4
	Female	Diabetes/severe IGT	329	66 ± 10	29.4 ± 5.6
		NGT	305	64 ± 10	26.2 ± 3.5
Scandinavian case-control	Male	Diabetes/severe IGT	252	61 ± 10	28.2 ± 4.1
		NGT	254	60 ± 11	26.3 ± 3.0
	Female	Diabetes/severe IGT	219	61 ± 10	28.4 ± 5.0
		NGT	217	60 ± 10	26.7 ± 4.3
Swedish case-control	Male	Diabetes	267	66 ± 12	27.4 ± 3.5
		NGT	267	66 ± 12	27.3 ± 3.4
	Female	Diabetes	247	67 ± 12	28.2 ± 4.6
		NGT	247	67 ± 12	27.8 ± 4.6
French Canadian case-control	Male	Diabetes	70	52 ± 8	30.0 ± 4.1
		NGT	70	51 ± 8	28.8 ± 3.2
	Female	Diabetes	57	54 ± 7	29.0 ± 4.8
		NGT	57	53 ± 7	28.5 ± 4.9
European-American case-control	Male	Diabetes	644	62 ± 11	32.0 ± 6.3
		NGT	644	61 ± 10	27.7 ± 4.5
	Female	Diabetes	582	63 ± 11	34.0 ± 7.5
		NGT	582	61 ± 9	27.0 ± 5.8
Polish case-control	Male	Diabetes	422	60 ± 10	28.7 ± 4.5
		NGT	422	58 ± 7	25.4 ± 2.9
	Female	Diabetes	587	63 ± 9	30.3 ± 5.0
		NGT	587	59 ± 7	26.6 ± 4.0

Data are means ± SD unless otherwise indicated. IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

sample by taking all the unrelated men and one man from each nuclear family in the top and bottom quartiles of age-adjusted log(BMI).

Type 2 diabetes panels. The panels used for association studies of type 2 diabetes (Table 2) have been described elsewhere (32,33) and include a panel of 321 Scandinavian trios with offspring with type 2 diabetes, impaired glucose tolerance, or impaired fasting glucose levels (166 male offspring) and 1,189 Scandinavian individuals from discordant sibpairs (280 affected men); these two panels are collectively referred to as Scandinavian related. A case-control study with 942 Scandinavian subjects matched on age, BMI, and geographic region (252 affected men and 254 unaffected men); a Swedish case-control study with 1,028 subjects matched on age and BMI (267 affected men and 267 unaffected men); and a case-control study with 254 subjects from the Saguenay Lac-St. Jean region in Quebec, Canada (70 affected men and 54 unaffected men), were also tested. The European-American and Polish diabetes panels were drawn from the same cohorts as the European-American and Polish BMI panels, as described elsewhere (32,33). The European-American panel includes 2,452 subjects (644 diabetic men and 644 men with normal glucose tolerance). The Polish panel includes 2,018 subjects (422 diabetic men and 422 men with normal glucose tolerance).

Genotyping. All genotyping was performed using the mass spectrometry–based MassArray platform (Sequenom) (27,34). Primers and probes were designed using SpectroDesigner (Sequenom). Assays were multiplexed (maximum 7-plex) and PCR performed in 6 μl with 5 ng of DNA, 0.6 pmol of each primer, 1.2 nmol of dNTP, and 0.2 units of Taq DNA polymerase (Qiagen) in 1.5× PCR buffer (Qiagen) and 1 mmol/l $MgCl_2$. PCR conditions are as previously described (27). Extra dNTPs were inactivated using 0.3 units of shrimp alkaline phosphatase. Primer extension was performed with 6 pmol of probe for each assay, 5.2 nmol of appropriate termination mix, and 0.64 units of Thermosequenase (Sequenom).

Tag SNP selection. The European-American CEPH reference panel and African-American reference panels described above were used to assess the patterns of linkage disequilibrium and select tag SNPs because this study began before the release of HapMap (34). The density of coverage and extent of linkage disequilibrium in these samples are similar to those described by HapMap (data not shown).

For each locus, SNPs were chosen from dbSNP and the Celera database to cover the entire gene region, as well as ~20 kb upstream and 10 kb downstream (Supplementary Tables 1 and 2, which can be viewed in an online appendix at http://dx.doi.org/10.2337/db06-1051). The density of attempted SNPs was 1 per 1 kb. *PPY* and *PYY* lie 10 kb apart, so we characterized these genes together. *NPY1R* and *NPY5R* are 14 kb apart, so we also characterized these genes together. After observing an association in *NPY2R*, we genotyped all reported SNPs from dbSNP. SNPs were included in the analysis of linkage disequilibrium if the allele frequency was >5%, the genotyping success rate was >85%, there was a maximum of one apparent inheritance (Mendelian) error, and the genotypes were in Hardy-Weinberg equilibrium ($P > 0.01$). The average spacing of working polymorphic SNPs (frequency >1%) is 1.3 kb for *NPY*, 1.5 kb for *PPY* and *PYY*, 560 bp for *NPY2R* in CEPH, 490 bp for *NPY2R* in the African-American reference panel, and 1.9 kb for *NPY1R* and *NPY5R*. The overall genotype success rate across all panels was 96.4% for polymorphic working markers.

We were unable to analyze the *NPY4R/PPYR1* gene because all SNPs tested failed quality control in a manner strongly suggestive of a polymorphic duplication, the presence of which has been confirmed (35,36).

We selected tag SNPs using Tagger (26). Tag SNPs were chosen so that the minimum r^2 was >0.8 for all SNPs with frequencies >5%. Additional tag SNPs were genotyped because they were previously selected using the algorithm implemented in Haploview (37). Tag SNPs for all genes were initially genotyped in the screening panels. For *NPY2R*, SNPs that were predictive of the associated haplotypes ($r^2 > 0.8$) were also genotyped in the screening panels.

To maximize comparability across populations, tag SNPs in *NPY2R* were chosen for the African-American panels using Tagger by first including all the tag SNPs from the European-American reference panel. Then, we picked extra tag SNPs so that all SNPs >5% frequency in the African-American panel were captured with a minimum $r^2 > 0.8$. We selected all tags SNPs in the region of linkage disequilibrium with the BMI-associated SNPs in either European American or African American subjects and genotyped them in the African-American study samples.

Data analysis. In the screening panels, all analyses were performed in the total sample and in men and women separately. Because of the design of these panels, BMI was treated as a dichotomous trait, and all SNPs and haplotypes were tested under an allelic model using a χ^2 test (1 df). For haplotype analyses, fully phased data were generated using PHASE, version 2.1 (38,39), for each haplotype block, defined using Haploview specifying the "solid spine of LD [linkage disequilibrium]" option (37). Within each block, we tested all

TABLE 3
An initial association of *NPY2R* with obesity in men from the screening panels

	European American			Polish			Combined data*		
	MAF	*P*†	OR‡	MAF	*P*	OR	*P*	OR (95% CI)	Permuted *P*§
rs2880416¶	0.18	0.16	0.83	0.26	0.14	0.79	0.044	0.82 (0.67–0.99)	0.99
rs2342676	0.39	0.05	1.25	0.38	0.08	1.30	0.0087	1.27 (1.06–1.51)	0.76
rs12649641	0.39	0.02	1.30	0.38	0.14	1.24	0.0048	1.28 (1.08–1.51)	0.57
rs11099992	0.31	0.04	1.28	0.33	0.10	1.29	0.0076	1.28 (1.07–1.54)	0.71
hCV1526995	0.30	0.02	1.34	0.32	0.06	1.34	0.0019	1.34 (1.11–1.62)	0.33
rs12507396	0.11	0.11	0.77	0.15	0.29	0.81	0.057	0.79 (0.99–1.63)	1.00
rs6857530	0.39	0.006	1.37	0.39	0.09	1.28	0.0013	1.34 (1.12–1.59)	0.22
CCAGAAG‖	0.38	0.02	1.32	0.41	0.04	1.36	0.002	1.34 (1.11–1.61)	0.33
CTCAGAA	0.41	0.13	0.85	0.35	0.68	0.94	0.140	0.88 (0.74–1.03)	1.00
GTCAGAA	0.06	0.62	1.11	0.04	0.37	0.82	0.815	0.97 (0.73–1.29)	1.00

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*Results combined using a Mantel-Haenszel test. †*P* value from χ^2 test using an allelic model. ‡Odds ratio (OR) for the effect of the minor allele of the SNP or the effect of the indicated haplotype vs. all others. §Using 1,000 permutations. ¶The first seven SNPs are the tag SNPs from the most associated block of *NPY2R*. The haplotypes are denoted by the alleles of the seven SNPs above that which they carry. ‖The first haplotype is associated with increased risk or obesity, the second is slightly protective for obesity, and the third was found be associated with type 2 diabetes in further samples. MAF, minor allele frequency.

haplotypes with frequency >5% for association against all others using a χ^2 test (1 df).

To assess significance of our initial results, we permuted the case-control labels within the screening panels 1,000 times. For each permutation, we did Mantel-Haenszel tests of the screening panels for men, women, and the two sexes combined. For the 127 SNPs and haplotypes tested across the six genes, we recorded the best *P* value for each permutation. We observed an association better than the original result in 221 permutations, giving our data an empirical *P* value of 0.22 within the screening panels.

For the FHS panel, the unrelated Scandinavian men, Scandinavian trios, and Maywood African-American men, BMI data were available as a continuous measure. To increase the normality of the BMI distributions, we analyzed log(BMI). We created a *z* score for log(BMI) based on age and, in the FHS cohort, adjusted for smoking status. Specifically, *z* scores (difference from mean divided by SD) were calculated for each individual based on the means and SDs of the distributions of log(BMI) within each decade of life and sex for each population. In the Scandinavian population, we considered individuals from Botnia, Helsinki, and southern Sweden separately. The *z* scores were further corrected by regressing against age within each decade, with separate regressions for each sex and geographic population. We performed linear regression to test the association of the age- and smoking-adjusted score and genotype using SAS statistical software (SAS Institute, Cary, NC). We used a multivariate family-based association test using generalized estimating equations, as implemented in PBAT (40), to analyze age-adjusted score as a continuous trait in the Scandinavian trios. To analyze BMI as a dichotomous trait in these samples, we defined the top and bottom quartiles of age-adjusted log(BMI) score as obese and lean and used a χ^2 test for association; for the parent-offspring trios, we used the TDTQ4 test (41).

Population stratification. To assess stratification, we genotyped 128 random SNPs (42,43) in subsamples of the European-American (238 case and 130 control subjects) and Polish (254 case and 114 control subjects) BMI case-control studies. From the 105 SNPs that passed quality control in the European-American panel, we estimate a mean χ^2 value of 1.22 and median χ^2 value of 0.63 (a mean χ^2 of 1.0 and a median χ^2 of 0.45 are expected when there is no stratification). Comparing the observed distribution of χ^2 values with the distribution expected with no stratification gives a *P* value = 0.059, suggesting there may be mild stratification in this sample. We saw no evidence for stratification from the 113 SNPs tested in the Polish case-control study (mean χ^2 = 0.99 and 0.36; *P* = 0.50).

RESULTS

To characterize the patterns of common genetic variation in NPY pathway genes, we genotyped 26 SNPs in *NPY*, 28 SNPs in *PPY* and *PYY*, 84 SNPs in *NPY2R*, and 54 SNPs in *NPY1R* and *NPY5R* in a reference sample of 12 European-derived multigenerational pedigrees (CEPH) (27) and 95 SNPs in *NPY2R* in a panel of 50 unrelated African-American subjects (28) (Supplementary Tables 1 and 2). Using these data, we selected 11 tag SNPs in *NPY*, 14 tag SNPs in *PPY* and *PYY*, 26 tag SNPs in *NPY2R*, and 26 tag SNPs in *NPY1R* and *NPY5R* to capture the underlying common variation; polymorphic missense SNPs were also included.

The tag SNPs were genotyped in the European-American and Polish case-control studies (Table 1, screening panels). Although nominal associations were observed in the *NPY*, *PYY*, *PPY*, *NPY1R*, and *NPY5R* genes (Supplementary Tables 3 and 4), only five SNPs in a region upstream of *NPY2R* showed nominal association with BMI with the same allele in both screening panels (see RESEARCH DESIGN AND METHODS for criteria). The linkage disequilibrium between these five SNPs is high (r^2 = 0.46–0.95). The association was observed in men but not in women (Table 3 and Supplementary Table 3). Meta-analysis by Mantel-Haenszel test (44) of the two samples yielded *P* values between 0.001 and 0.009 and ORs between 1.27 and 1.34. A multimarker haplotype comprised of the associated alleles of these SNPs was similarly associated (frequency 26–32%, *P* = 0.002, OR = 1.34, and 95% CI 1.11–1.61) (Table 3). We have assessed the significance of the original association by permuting the affected status 1,000 times; we calculated an experiment-wide *P* value of 0.22 for our data (see RESEARCH DESIGN AND METHODS).

Because we observed mild evidence for stratification in the European-American sample (see RESEARCH DESIGN AND METHODS), we assessed the allele frequencies of one associated SNP, rs11099992, across multiple European populations. Allele frequencies ranged from 0.25 to 0.44, roughly trending from west to east. We rematched our European-American panel along this axis using previously described methods (42) and observed no decrease in association (*P* = 0.016, OR = 1.33 vs. *P* = 0.036, OR = 1.28 in the original sample), suggesting the association is not due to stratification in this sample.

Because we observed the strongest potential association for variation upstream of *NPY2R* and BMI in men, we focused our further replication efforts on this locus. We genotyped the seven SNPs in *NPY2R* that were associated with BMI in the screening panels or comprise the associated haplotype in a set of 1,739 unrelated

TABLE 4
Association of *NPY2R* SNPs to BMI analyzed as a continuous trait

	FHS men			Scandinavian men			Unrelated men combined		Scandinavian trios		
	MAF	*P**	β†	MAF	*P*	β	*P*‡	β	MAF	*P*	Direction§
rs2880416	0.19	0.41	−0.052	0.22	0.26	+0.062	0.80	+0.010	0.24	0.97	+
rs2342676	0.39	0.18	+0.066	0.44	0.72	−0.017	0.58	+0.019	0.47	0.46	−
rs12649641	0.37	0.10	+0.082	0.43	0.24	−0.057	0.82	+0.008	0.47	0.90	−
rs11099992	0.28	0.46	+0.041	0.34	0.06	+0.133	0.07	+0.079	0.38	0.81	−
hCV1526995	0.27	0.59	+0.029	0.34	0.33	+0.048	0.34	+0.035	0.37	0.94	−
rs12507396	0.11	0.58	+0.041	0.13	0.05	+0.134	0.07	+0.090	0.13	0.59	+
rs6857530	0.38	0.18	+0.066	0.42	0.36	−0.043	0.85	+0.006	0.36	0.46	−
CCAGAAG	0.28	0.73	+0.019	0.33	0.53	+0.03	0.54	+0.022	0.37	0.67	−
CTCAGAA	0.43	0.36	−0.044	0.35	0.77	−0.014	0.47	−0.024	0.29	0.85	+
GTCAGAA	0.07	0.08	−0.173	0.09	0.79	−0.021	0.08	−0.108	0.12	0.37	−

**P* value for the linear regression of age-adjusted score against genotype coded as 0, 1, or 2 denoting the number of minor allele or haplotype copies carried by an individual. †For the regression coefficient, β, a positive value indicates that the minor allele is associated with a higher age-adjusted score. ‡The combined results were generated by performing linear regression on the two datasets combined. §The direction is given a "+" if the minor allele is associated with higher age-adjusted score. MAF, minor allele frequency.

individuals from the FHS, 1,018 unrelated Scandinavian men, and 437 Scandinavian parent-offspring trios. Analyzing age-adjusted log(BMI) as a quantitative trait, we found rs11099992 was associated with BMI in the Scandinavian men (one-tailed $P = 0.03$) but not associated in men from FHS or in the Scandinavian trios (Table 4).

To permit a combined analysis across all of the samples, we examined BMI as a dichotomous trait in the Scandinavian and FHS panels, assigning men in the bottom quartile of age-adjusted log BMI as control subjects and men in the top quartile as case subjects for each panel. The combined association in the European-derived replication samples using a Mantel-Haenszel test (44) does not reach significance for the high-BMI haplotype (one-tailed $P = 0.27$, OR = 1.06) (Table 5).

Tag SNPs for *NPY2R* were also tested in two African-American studies. No SNP or haplotype was associated with BMI in these panels (Table 5). However, we are unable to compare these results to the haplotype-based tests in the European-derived samples because the linkage

TABLE 5
Association of SNPs in *NPY2R* with BMI in men in all samples

	European-derived replication*		African-American replication†			All replication samples‡		All samples§	
	P	OR	Frequency¶	*P*	OR	*P*	OR (95% CI)	*P*	OR (95% CI)
rs4467508	—	—	0.11	0.85	0.95	—	—	0.17	0.90 (0.76–1.05)
rs9999820	—	—	0.23	0.03	1.46	—	—	0.72	0.97 (0.84–1.13)
rs10022685	—	—	0.02	0.46	0.69	—	—	0.46	0.69 (0.26–1.83)
rs7673701	—	—	0.13	0.61	1.12	—	—	0.35	0.93 (0.79–1.08)
rs7671213	—	—	0.11	0.63	1.12	—	—	0.38	0.93 (0.79–1.09)
rs12641982	—	—	0.11	0.39	1.22	—	—	0.46	0.94 (0.81–1.10)
rs9307928	—	—	0.16	0.64	1.09	—	—	0.64	1.09 (0.75–1.59)
rs6849115	—	—	0.24	0.90	0.98	—	—	0.90	0.98 (0.71–1.35)
rs2880416	0.34	1.11	0.07	0.68	0.89	0.45	1.08 (0.89–1.31)	0.39	0.94 (0.82–1.08)
rs2342676‖	0.41	1.07	0.67	0.95	0.99	0.50	1.05 (0.91–1.22)	0.026	1.14 (1.02–1.27)
rs2342675	—	—	0.30	0.70	0.94	—	—	0.70	0.94 (0.70–1.28)
rs12649641‖	0.91	1.01	0.64	0.86	1.03	0.84	1.01 (0.88–1.18)	0.044	1.12 (1.00–1.25)
rs11099992	0.47	1.07	0.23	0.42	1.15	0.31	1.09 (90.93–1.28)	0.010	1.17 (1.04–1.32)
rs6850289	—	—	0.10	0.11	0.69	—	—	0.013	1.24 (1.05–1.47)
rs10212938	—	—	0.09	0.50	0.85	—	—	0.50	0.85 (0.52–1.37)
hCV1526995	0.32	1.10	0.10	0.12	0.69	0.73	1.03 (0.87–1.22)	0.017	1.17 (1.03–1.32)
rs12507396	0.02	1.35	0.05	0.32	0.72	0.07	1.25 (0.98–1.58)	0.96	1.00 (0.85–1.19)
rs6857530‖	0.79	1.02	0.74	0.62	0.92	0.64	1.04 (0.89–1.21)	0.014	1.15 (1.03–1.29)
CCAGAAG**	0.54	1.06	—	—	—	—	—	0.008	1.19 (1.05–1.36)
CTCAGAA**	0.23	0.90	—	—	—	—	—	0.057	0.89 (0.79–1.00)
GTCAGAA**	0.18	0.81	—	—	—	—	—	0.27	0.89 (0.72–1.10)

The tag SNPs were tested in men from three European-derived and two African-American panels. The European-derived minor allele for each SNP was tested. *Combined data from a Mantel-Haenszel test in men for the Framingham, Scandinavian-unrelated, and Scandinavian trios panels. †Combined data for GCI and Maywood African-American panels. ‡Combined data for European-derived and African-American replication panels. §Combined data from all panels tested for association with BMI: screening and replication. ¶Frequency of allele tested. ‖Minor alleles are different in the African-American panels compared with the European-derived samples. Data for the minor allele in the European-derived samples are reported. **The obesity-risk, protective, and diabetes-risk haplotypes were only evaluated in the European-derived samples due to differences in linkage disequilibrium in the African-American population.

disequilibrium structure is quite different (data not shown).

Because obesity is an important risk factor for type 2 diabetes, we considered the possibility that variation in this gene also influences the risk of type 2 diabetes. We genotyped the same seven SNPs upstream of *NPY2R* in samples discordant for type 2 diabetes, including >3,000 previously described Scandinavian subjects, 2,400 European Americans, 2,000 Polish subjects, and a panel of 250 French Canadian individuals (32,33) (Table 2). Surprisingly, a 9% haplotype was associated with type 2 diabetes in men only ($P = 1.73 \times 10^{-4}$, OR = 1.36; for women, $P = 0.42$, OR = 1.07) (Table 6). Through permutation testing (see RESEARCH DESIGN AND METHODS), we obtained a gene-wide corrected P value of 0.02 for this association.

TABLE 6
Association of SNPs and haplotypes in *NPY2R* with type 2 diabetes in male case-control subjects

	Scandinavian family based		Scandinavian case-control		Swedish		French Canadian		European American		Polish		Combined data	
	MAF	P	OR*	MAF	P	OR	MAF	P	OR	MAF	P	OR	P	OR (95% CI)	Permuted P†
rs2880416	0.25	0.22	1.21	0.27	0.01	1.45	0.21	0.39	1.15	0.22	0.12	1.57	0.19	0.14	1.17	0.25	0.23	1.15	5.75×10^{-4}	1.22 (1.09–1.36)	0.024
rs2342676	0.46	1.00	1.00	0.47	1.00	1.00	0.39	0.18	1.18	0.42	0.83	0.95	0.40	0.93	0.99	0.38	0.17	0.87	0.812	0.99 (0.90–1.09)	1.00
rs12649641	0.46	0.67	1.06	0.47	0.83	0.97	0.39	0.05	1.30	0.42	0.89	1.04	0.40	0.74	0.97	0.38	0.14	0.86	0.918	1.00 (0.91–1.09)	1.00
rs11099992	0.36	0.78	0.96	0.37	0.63	1.06	0.3	0.02	1.38	0.31	0.73	0.90	0.31	0.40	0.86	0.32	0.46	0.93	0.371	1.05 (0.95–1.15)	0.93
hCV1526995	0.36	0.32	1.17	0.37	0.69	1.06	0.29	0.03	1.35	0.31	0.69	0.90	0.31	0.40	1.06	0.32	0.46	0.93	0.208	1.07 (0.97–1.18)	1.00
rs12507396	0.14	0.67	0.91	0.14	0.49	1.13	0.12	0.48	0.87	0.1	0.84	1.10	0.11	0.59	1.07	0.15	0.24	1.18	0.424	1.06 (0.92–1.22)	1.00
rs6857530	0.45	0.84	0.97	0.46	0.91	1.01	0.37	0.03	1.33	0.43	0.80	0.94	0.40	1.00	0.97	0.38	0.15	0.87	0.992	1.00 (0.91–1.10)	1.00
CCAGAAG	0.35	0.87	1.03	0.37	0.83	1.03	0.29	0.02	1.37	0.30	0.77	0.92	0.31	0.93	1.01	0.32	0.47	1.08	0.177	1.07 (0.97–1.18)	0.84
CTCAGAA	0.29	0.33	0.86	0.26	0.01	0.71	0.41	0.03	0.77	0.35	0.23	0.73	0.40	0.63	0.96	0.37	0.27	0.89	0.002	0.86 (0.78–0.95)	0.061
GTCAGAA	0.10	0.025	1.73	0.12	0.006	1.73	0.09	0.04	1.64	0.12	0.08	1.84	0.08	0.43	1.12	0.09	0.55	1.11	1.73×10^{-4}	1.36 (1.16–1.59)	0.029

*Odds ratio (OR) for developing type 2 diabetes with the minor allele. †Calculated using 1,000 permutations. MAF, minor allele frequency.

DISCUSSION

We performed an extensive survey of common genetic variation in the peptides and receptors of the NPY pathway for association with BMI. We observed no reproducible association in *NPY*, *PPY*, *PYY*, *NPY1R*, or *NPY5R* in two large studies of individuals sampled from the extremes of the BMI distribution, although modest associations at these loci, or associations that are strongly influenced by gene-gene or gene-environment interactions, cannot be ruled out. In the case of *NPY2R*, we observed an association in both studies between variation upstream of this gene and BMI in men, although we were not able to significantly replicate this result in further panels. Because of our initial result with BMI, we examined the association between variation upstream of *NPY2R* and type 2 diabetes. Importantly, the association we observed between *NPY2R* and diabetes is not a replication of our initial BMI result because the phenotype is different and a different haplotype is most strongly associated with diabetes.

Published studies provide a small amount of support for an association between variation upstream of *NPY2R* and BMI in men. A prior study of *NPY2R* and BMI in the Pima Indian population (100 obese and 67 lean men) (23) found a trend ($P = 0.13$, OR = 1.39) toward association with rs2880412 ($r^2 = 0.86$ to rs11099992), although the linkage disequilibrium relationship between these SNPs could be different in the Pima Indian population. A recent study motivated by a prepublication abstract of our results also found an association ($P = 0.02$, OR = 1.24) with BMI upstream of *NPY2R* in 6,000 Danish men and women; a stronger result was observed in men compared with women (45). However, this study did not test the SNP or haplotype most strongly associated with type 2 diabetes in our samples. A study by Ma et al. (23) and another by Hung et al. (22) (420 men) have reported that a silent SNP in *NPY2R*, rs1047214, is associated with BMI in men, although Lavebratt et al. (46) (500 men) observed an association of BMI with the alternate allele. In our samples, this SNP is not significantly associated with BMI ($P = 0.657$, OR = 0.97) or type 2 diabetes ($P = 0.128$, OR = 0.93).

We have attempted to estimate the likelihood that the associations with BMI and type 2 diabetes are valid by setting a prior probability for this pathway. We have previously estimated that appropriate prior probabilities for variants in good candidate genes (such as the NPY pathway genes for BMI) range from 0.0003 to 0.003 (47). For type 2 diabetes, these genes would be not considered as strong candidates, giving a range of prior probabilities

of 8×10^{-5} (considering these genes as no better than random) to 0.001 (considering these genes as interesting candidates). Using these values and methods described previously to estimate false-positive report probabilities (48), the posterior probabilities that the association with BMI is valid range from 0.02 to 0.19, assuming the OR observed is close to the true genetic effect. These probabilities suggest that this association with BMI is likely spurious. For type 2 diabetes, the posterior probability that this association is valid ranges from 0.04 to 0.87. These probabilities suggest that this association could also be spurious, but *NPY2R* should be considered a candidate gene to be tested in further samples for association with diabetes in men.

In theory, the different design of our screening and replication samples could have contributed to the inability to replicate an association with BMI. Our screening samples included subjects from the near extremes of the BMI distribution, whereas our replication samples included subjects from the full BMI distribution. The design of the screening sample is very powerful for detecting variants that shift the trait distribution by a constant amount but can also detect variants that influence whether subjects are above or below a threshold (49,50). By contrast, the replication samples are less well powered on a per-sample basis, which we tried to account for by testing several large replication samples. Also, continuous trait data would be much less powerful if the associated variants were to have a threshold effect. To more closely mimic the design of the screening samples, we analyzed the replication samples with BMI as a dichotomous rather than a continuous trait; however, when we compared the top and bottom quartile or reproduced the exact sampling scheme of the screening panels, we still observed no significant association in the combined replication panels (Table 5 and data not shown).

In summary, we have conducted a comprehensive study to look for variants in the NPY pathway that influence BMI and to test for involvement of associated variants with type 2 diabetes. We have presented data that genetic variation in NPY pathway is not an important contributor to BMI, although small effects cannot be excluded. Association of *NPY2R* with type 2 diabetes in men should be tested in further studies. Our study is the first to observe an association between *NPY2R* and type 2 diabetes in humans. It has been shown that the diabetic phenotype of the obese *ob/ob* mouse is reduced when *Npy2r* has been deleted even though the double-knockout animals show no decrease in body weight (51), lending some biological plausibility to our preliminary finding.

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