

Association studies of body mass index and type 2 diabetes in the Neuropeptide Y pathway: a possible role for NPY2R as a candidate gene for type 2 diabetes in men

Received for publication 27 July 2006 and accepted in revised form 13 February 2007.

Catarina D. Campbell^{1,2}, Helen N. Lyon^{1,2}, James Nemesh^{1,3}, Jared A. Drake^{1,3}, Tiinamaija Tuomi^{4,5}, Daniel Gaudet⁶, Xiaofeng Zhu⁷, Richard S. Cooper⁷, Kristin G. Ardlie¹⁰, Leif C. Groop^{4,9}, and Joel N. Hirschhorn^{1,2,3}

¹Program in Genomics and Division of Endocrinology, Children's Hospital, Boston, Massachusetts

²Department of Genetics, Harvard Medical School, Boston, Massachusetts

³Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, Massachusetts

⁴Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland

⁵Folkhalsan Genetic Institute, Folkhalsan Research Center; and Research Program for Molecular Medicine, University of Helsinki, Helsinki, Finland

⁶University of Montreal Community Genomic Center, Chicoutimi Hospital, Quebec, Canada

⁷Department of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, Illinois

⁸Genomics Collaborative/Seracare LifeSciences, Cambridge, Massachusetts

⁹Department of Clinical Sciences, Diabetes and Endocrinology, University Hospital, Lund University, Malmö, Sweden

¹⁰Current affiliation: Biological Samples Platform, Broad Institute of Harvard and MIT, Cambridge, Massachusetts

Some samples used in this study are from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. This work was supported by the National Heart, Lung and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195)

Running Title: Association studies of NPY pathway genes

Address correspondence to:

Joel N. Hirschhorn

Enders 561, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115

joelh@broad.mit.edu

ABSTRACT:

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 (T2D). We genotyped a set of 71 single nucleotide polymorphisms (SNPs) that capture most common variation in NPY, PPY, PYY, NPY1R, NPY2R, and NPY5R in 2800 individuals of recent European ancestry drawn from the near-extremes of the body mass index (BMI) distribution. Five SNPs located upstream of NPY2R were nominally associated with BMI in men (p values 0.001-0.009, odds ratios 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 to BMI in 2,500 men, and tested these SNPs for association to T2D 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 T2D in men (p=1.73x10⁻⁴, OR=1.36) and not in women. Variation in this pathway likely does not have a major influence on BMI, although small effects can not be ruled out; NPY2R should be considered a candidate gene for T2D in men.

Obesity, as measured by body mass index (BMI), is an important predictor of type 2 diabetes (T2D), 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 approximately 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 T2D.

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 and J.N.H., personal communication). A few exceptions are rare variation in the MC4R receptor in early onset obesity (10-13) and an association with 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). PYY₃₋₃₆ 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), 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 T2D 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 common variation in the genes in the NPY pathway for association to 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 to T2D.

RESEARCH DESIGN AND METHODS:

Design overview. First, we used reference panels to determine the patterns of linkage disequilibrium (LD) and choose haplotype tagging SNPs. Second, tag SNPs and multimarker haplotypes comprised of tag SNPs were tested in two screening panels: European American and Polish. 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 gender dimorphic. We tested SNPs and haplotypes for nominal association with BMI in the European American panel (2-tailed $p < 0.05$), and then tested these SNPs and haplotypes for replication in the Poland sample (1-tailed $p < 0.05$). SNPs that met these criteria or that comprised haplotypes that met these criteria were genotyped in the replication samples (Framingham unrelated, Scandinavian unrelated, Scandinavian trios, and two

African American samples). 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 to T2D.

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

Reference panels: The European-derived reference sample consists of 93 individuals in 12 multi-generational pedigrees (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 (1218 cases and 624 controls) and Polish (700 cases and 330 controls) panels were obtained from Genomics Collaborative, Inc. (Table 1) as described(14). These individuals were selected from a collection of over 60,000 subjects, and include healthy controls plus patients with osteoarthritis, rheumatoid arthritis, asthma, hypertension, coronary artery disease, myocardial infarction, hyperlipidemia, stroke, T2D, or osteoporosis. We determined the BMI distribution in healthy individuals for each decade of life, gender, 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 cases, and designated any subjects with a BMI between the 5th and 12th percentiles as potential lean controls. From this set of obese and lean individuals, a subset of obese and lean

individuals was selected by matching for age, gender 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 by limiting the analysis to subjects from the healthy controls.

BMI replication panels: The panel from the Framingham Heart Study (FHS) contains 1739 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 1971-1998, 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 non-diabetic individuals with waist-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, non-overlapping sample of 977 unrelated men to test for association with BMI, consisting of the male controls, non-diabetic fathers in parent-offspring trio panels, and a single non-diabetic male sibling from each sibship.

Two African American panels were studied. The first consists self-described African Americans born in the United States (192 obese men; 71 lean men). This panel is identical in design to the European American and Polish case-control panels described above, and was also collected by Genomics Collaborative, Inc. The second panel consists of African Americans from Maywood, IL, 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 men; 111 lean men) from this 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).

T2D panels: The panels used for association studies of T2D (Table 2) have been described elsewhere (32; 33) and include a panel of 321 Scandinavian trios with offspring with T2D, impaired glucose tolerance, or impaired fasting glucose levels (166 male offspring) and 1189 Scandinavian discordant sib-pairs (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 1028 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 2452 subjects (644 diabetic men and 644 men with normal glucose tolerance). The Polish panel includes 2018 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 μ l with 5 ng of DNA, 0.6 pmol of each primer, 1.2 nmole of dNTP and 0.2 units of Taq DNA polymerase (Qiagen) in 1.5X PCR buffer (Qiagen) and 1mM MgCl₂.

PCR conditions are as previously described (27). Extra dNTPs were inactivated using 0.3 U 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 (LD) and select tag SNPs because this study was begun before the release of HapMap(34). The density of coverage and extent of LD in these samples is similar to that 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 approximately 20kb upstream and 10kb downstream (Supplementary Tables 1 and 2). The density of attempted SNPs was one per 1kb. 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 LD 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 1.3kb for NPY, 1.5kb for PPY and PYY, 560bp for NPY2R in CEPH, 490bp for NPY2R in the African American reference panel, and 1.9kb 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 greater than 0.8 for all SNPs with frequencies greater than 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 greater than 5% frequency in the African American panel were captured with a minimum $r^2 > 0.8$. We selected all tag SNPs in the region of LD with the BMI associated SNPs in either European Americans or African Americans, and genotyped them in the African American study samples.

Data analysis: In the screening panels, all analyses were performed in the total sample as well as 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 chi-squared test (1 df). For haplotype analyses, fully phased data was generated using PHASE 2.1 (38; 39) for each haplotype block, defined using Haploview specifying the “solid spine of LD” option (37). Within each block, we tested all haplotypes with frequency $> 5\%$ for association against all others using a chi-square test (1 df).

To assess significance of our initial results, we permuted the case/control labels

within the screening panels 1000 times. For each permutation, we did Mantel-Haenszel tests of the screening panels for men, women, and the two genders combined. For the 127 SNPs and haplotypes tested across the 6 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 was available as a continuous measure. To increase the normality of the BMI distributions, we analyzed $\log(\text{BMI})$. We created a z-score for $\log(\text{BMI})$ based on age and, in the FHS cohort, adjusted for smoking status. Specifically, z-scores (difference from mean divided by standard deviation) were calculated for each individual based on the means and standard deviations of the distributions of $\log(\text{BMI})$ within in each decade of life and gender 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 gender and 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 FBAT-GEE test, 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 $\log(\text{BMI})$ score as obese and lean, and used a chi-square 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 sub-samples of the European-American (238 cases, 130 controls) and Polish (254 cases, 114 controls) BMI case-control studies. From the 105 SNPs that passed QC in the European-American panel we estimate a mean r^2 value of 1.22 and median r^2 value of 0.63 (a mean r^2 of 1 and a median r^2 of 0.45 are expected when there is no stratification). Comparing the observed distribution of r^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 (mean r^2 =0.99; median r^2 =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 multi-generational pedigrees (CEPH) (27) and 95 SNPs in NPY2R in a panel of 50 unrelated African Americans (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 Table 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 Methods for criteria). The LD between these five SNPs is high (r^2 =0.46-0.95). The association

was observed in men but not in women (Table 3, Supplementary Table 3). Meta-analysis by Mantel-Haenszel test (44) of the two samples yielded p values between 0.001 and 0.009 and odds ratios between 1.27 and 1.34. A multi-marker haplotype comprised of the associated alleles of these SNPs was similarly associated (frequency 26-32%; p = 0.002; OR = 1.34 95% confidence interval, 1.11-1.61) (Table 3). We have assessed the significance of the original association by permuting the affected status 1000 times; we calculated an experiment-wide p-value of 0.22 for our data (see Methods).

Because we observed mild evidence for stratification in the European American sample (see Methods), we assessed the allele frequencies of one associated SNP, rs11099992, across multiple European populations. Allele frequencies ranged from 0.25-0.44 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 1739 unrelated individuals from the Framingham Heart Study (FHS), 1018 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 controls and men in the top quartile as cases 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 $p=0.27$, odds ratio=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). But, we are unable to compare these results to the haplotype-based tests in the European-derived samples because the LD 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 T2D. We genotyped the same seven SNPs upstream of NPY2R in samples discordant for T2D including over 3000 previously described Scandinavian subjects, 2400 European Americans, 2000 Polish subjects, and a panel of 250 French Canadians (32; 33) (Table 2). Surprisingly, a 9% haplotype was associated with T2D 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 Methods), we obtained a gene-wide corrected p -value of 0.02 for this association.

DISCUSSION:

We performed an extensive survey of common genetic variation in the peptides and receptors of the NPY pathway for association to BMI. We observed no reproducible association in NPY, PPY, PYY, NPY1R, and 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 T2D. 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 population (100 obese men; 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 LD relationship between these SNPs could be different in the Pima population. A recent study motivated by a pre-publication abstract of our results also found an association ($p=0.02$, OR=1.24) with BMI upstream of NPY2R in 6000 Danish men and women; a stronger result was observed in men compared to women(45). However, this study did not test the SNP or haplotype most strongly associated with T2D in our samples. The Ma et al. study and another study by Hung et al. (420 men) have reported that a silent SNP in NPY2R, rs1047214, is associated with BMI in men (22; 23), although Lavebratt et al. (500 men) observed an association of BMI with the alternate allele (46). In our samples, this SNP is not significantly associated with BMI ($p=0.657$, OR=0.97) or T2D ($p=0.128$, OR=0.93).

We have attempted to estimate the likelihood that the associations to BMI and T2D 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 T2D, 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 to BMI is valid range from 0.02 to 0.19, assuming the odds ratio observed is close to the true genetic effect. These probabilities suggest that this association to BMI is likely spurious. For T2D, 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 to 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 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 T2D. We have presented data that genetic variation in NPY pathway is not an important contributor to BMI, although small effects can not be excluded. Association of NPY2R to T2D in men should be tested in further studies. Our study is the first to observe an association between NPY2R and T2D 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.

ACKNOWLEDGEMENTS:

We thank Mark J. Daly and David Altshuler for insightful discussions and advice about this project and for helpful comments on the manuscript. We thank T. Bersaglieri for genotype data and analysis of PPYR1. We thank J. Butler for technical assistance and members of the laboratories of J.N.H., M.J.D., and D.A. for helpful discussions. We thank the Framingham Heart Study (NHLBI) and the Botnia Study group for providing DNA samples. This work was supported by the American Diabetes Association/Smith Family Pinnacle Program Project grant. The Botnia study has been funded by grants from the Sigrid Juselius Foundation, Academy of Finland, Folkhälsan Research Foundation, Swedish Research Council and Finnish and Swedish Diabetes Research Foundations. X.Z. and R.S.C. are supported in part by a grant, R01HL074166, from National Heart,

Lung and Blood Institute. The Framingham Heart Study is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with Boston University. This manuscript has been reviewed by Boston University and NHLBI

for scientific content and consistency of data interpretation with previous Framingham publications and significant comments have been incorporated prior to submission for publication.

REFERENCES:

1. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ: Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 348:1625-1638, 2003
2. Flegal KM, Graubard BI, Williamson DF, Gail MH: Excess deaths associated with underweight, overweight, and obesity. *Jama* 293:1861-1867, 2005
3. Ajani UA, Lotufo PA, Gaziano JM, Lee IM, Spelsberg A, Buring JE, Willett WC, Manson JE: Body mass index and mortality among US male physicians. *Ann Epidemiol* 14:731-739, 2004
4. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC: Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 17:961-969, 1994
5. Colditz GA, Willett WC, Rotnitzky A, Manson JE: Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 122:481-486, 1995
6. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K: The heritability of body mass index among an international sample of monozygotic twins reared apart. *Int J Obes Relat Metab Disord* 20:501-506, 1996
7. Rice T, Sjostrom CD, Perusse L, Rao DC, Sjostrom L, Bouchard C: Segregation analysis of body mass index in a large sample selected for obesity: the Swedish Obese Subjects study. *Obes Res* 7:246-255, 1999
8. Maes HH, Neale MC, Eaves LJ: Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 27:325-351, 1997
9. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Perusse L, Bouchard C: The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* 14:529-644, 2006
10. Vaisse C, Clement K, Guy-Grand B, Froguel P: A frameshift mutation in human MC4R is associated with a dominant form of obesity. *Nat Genet* 20:113-114, 1998
11. Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S: A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* 20:111-112, 1998
12. Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P: Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest* 106:253-262, 2000
13. Hirschhorn JN, Altshuler D: Once and again-issues surrounding replication in genetic association studies. *J Clin Endocrinol Metab* 87:4438-4441, 2002
14. Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G, Hinney A, Hebebrand J, Koberwitz K, Zhu X, Cooper R, Ardlie K, Lyon H, Hirschhorn JN, Laird NM, Lenburg ME, Lange C, Christman MF: A common genetic variant is associated with adult and childhood obesity. *Science* 312:279-283, 2006
15. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW: Central nervous system control of food intake and body weight. *Nature* 443:289-295, 2006
16. Berglund MM, Hipkind PA, Gehlert DR: Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. *Exp Biol Med (Maywood)* 228:217-244, 2003
17. Mattevi VS, Zembruiski VM, Hutz MH: Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord* 26:1179-1185, 2002

18. Nordman S, Ding B, Ostenson CG, Karvestedt L, Brismar K, Efendic S, Gu HF: Leu7Pro polymorphism in the neuropeptide Y (NPY) gene is associated with impaired glucose tolerance and type 2 diabetes in Swedish men. *Exp Clin Endocrinol Diabetes* 113:282-287, 2005
19. Bray MS, Boerwinkle E, Hanis CL: Sequence variation within the neuropeptide Y gene and obesity in Mexican Americans. *Obes Res* 8:219-226, 2000
20. Torekov SS, Larsen LH, Glumer C, Borch-Johnsen K, Jorgensen T, Holst JJ, Madsen OD, Hansen T, Pedersen O: Evidence of an association between the Arg72 allele of the peptide YY and increased risk of type 2 diabetes. *Diabetes* 54:2261-2265, 2005
21. Jenkinson CP, Cray K, Walder K, Herzog H, Hanson, Ravussin E: Novel polymorphisms in the neuropeptide-Y Y5 receptor associated with obesity in Pima Indians. *Int J Obes Relat Metab Disord* 24:580-584, 2000
22. Hung CC, Pirie F, Luan J, Lank E, Motala A, Yeo GS, Keogh JM, Wareham NJ, O'Rahilly S, Farooqi IS: Studies of the peptide YY and neuropeptide Y2 receptor genes in relation to human obesity and obesity-related traits. *Diabetes* 53:2461-2466, 2004
23. Ma L, Tataranni PA, Hanson RL, Infante AM, Kobes S, Bogardus C, Baier LJ: Variations in Peptide YY and Y2 Receptor Genes Are Associated With Severe Obesity in Pima Indian Men. *Diabetes* 54:1598-1602, 2005
24. Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC: Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered* 55:27-36, 2003
25. Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA: Haplotype tagging for the identification of common disease genes. *Nat Genet* 29:233-237, 2001
26. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D: Efficiency and power in genetic association studies. *Nat Genet* 37:1217-1223, 2005
27. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225-2229, 2002
28. Bersaglieri T, Sabeti PC, Patterson N, Vanderploeg T, Schaffner SF, Drake JA, Rhodes M, Reich DE, Hirschhorn JN: Genetic signatures of strong recent positive selection at the lactase gene. *Am J Hum Genet* 74:1111-1120, 2004
29. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP: An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol* 110:281-290, 1979
30. Atwood LD, Heard-Costa NL, Cupples LA, Jaquish CE, Wilson PW, D'Agostino RB: Genomewide linkage analysis of body mass index across 28 years of the Framingham Heart Study. *Am J Hum Genet* 71:1044-1050, 2002
31. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76-80, 2000
32. Florez JC, Burt N, de Bakker PI, Almgren P, Tuomi T, Holmkvist J, Gaudet D, Hudson TJ, Schaffner SF, Daly MJ, Hirschhorn JN, Groop L, Altshuler D: Haplotype structure and

- genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. *Diabetes* 53:1360-1368, 2004
33. Winckler W, Burt NP, Holmkvist J, Cervin C, de Bakker PI, Sun M, Almgren P, Tuomi T, Gaudet D, Hudson TJ, Ardlie KG, Daly MJ, Hirschhorn JN, Altshuler D, Groop L: Association of common variation in the HNF1alpha gene region with risk of type 2 diabetes. *Diabetes* 54:2336-2342, 2005
 34. The International HapMap Consortium: A haplotype map of the human genome. *Nature* 437:1299-1320, 2005
 35. Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C: Detection of large-scale variation in the human genome. *Nat Genet* 36:949-951, 2004
 36. Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M: Large-scale copy number polymorphism in the human genome. *Science* 305:525-528, 2004
 37. Barrett JC, Fry B, Maller J, Daly MJ: Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263-265, 2005
 38. Stephens M, Smith NJ, Donnelly P: A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978-989, 2001
 39. Stephens M, Donnelly P: A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 73:1162-1169, 2003
 40. Lange C, Silverman EK, Xu X, Weiss ST, Laird NM: A multivariate family-based association test using generalized estimating equations: FBAT-GEE. *Biostatistics* 4:195-206, 2003
 41. Allison DB: Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet* 60:676-690, 1997
 42. Campbell CD, Ogburn EL, Lunetta KL, Lyon HN, Freedman ML, Groop LC, Altshuler D, Ardlie KG, Hirschhorn JN: Demonstrating stratification in a European American population. *Nat Genet* 37:868-872, 2005
 43. Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D: Assessing the impact of population stratification on genetic association studies. *Nat Genet* 36:388-393, 2004
 44. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177-182, 2003
 45. Torekov SS, Larsen LH, Andersen G, Albrechtsen A, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O: Variants in the 5' region of the neuropeptide Y receptor Y2 gene (NPY2R) are associated with obesity in 5,971 white subjects. *Diabetologia* 49:2653-2658, 2006
 46. Lavebratt C, Alpman A, Persson B, Arner P, Hoffstedt J: Common neuropeptide Y2 receptor gene variant is protective against obesity among Swedish men. *Int J Obes (Lond)* 30:453-459, 2006
 47. Newton-Cheh C, Hirschhorn JN: Genetic association studies of complex traits: design and analysis issues. *Mutat Res* 573:54-69, 2005

48. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N: Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 96:434-442, 2004
49. Hanson RL, Looker HC, Ma L, Muller YL, Baier LJ, Knowler WC: Design and analysis of genetic association studies to finely map a locus identified by linkage analysis: sample size and power calculations. *Ann Hum Genet* 70:332-349, 2006
50. Van Gestel S, Houwing-Duistermaat JJ, Adolfsson R, van Duijn CM, Van Broeckhoven C: Power of selective genotyping in genetic association analyses of quantitative traits. *Behav Genet* 30:141-146, 2000
51. Sainsbury A, Schwarzer C, Couzens M, Herzog H: Y2 receptor deletion attenuates the type 2 diabetic syndrome of ob/ob mice. *Diabetes* 51:3420-3427, 2002

Panel	Type	Gender	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 American [†]	African	Replication	Male	Lean	111	39 ± 11	20.6 ± 1.2
				Obese	108	38 ± 10	38.1 ± 6.2

Table 1: Panels for BMI association studies

For age and BMI, means ± standard deviation are given. *Lean subjects are in the 5th – 12th percentile in BMI and Obese subjects in the 90th – 97th percentile in BMI †Lean subjects are in the bottom quartile and obese subjects are in the top quartile of age adjusted logBMI 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).

Panel	Gender	Affection status	N	Age (years)	BMI (kg/m ²)	
Scandinavian Trios	Male	Diabetes/severe			27.3	±
		IGT	168	38 ± 9	4.5	
	Female	Diabetes/severe			27.5	±
		IGT	153	39 ± 9	5.9	
Scandinavian Discordant Sibs	Male	Diabetes/severe			28.7	±
		IGT	280	62 ± 11	4.1	
	Female	NGT	275	60 ± 9	3.4	
		Diabetes/severe			29.4	±
		IGT	329	66 ± 10	5.6	
		NGT	305	64 ± 10	3.5	
Scandinavian Case/Control	Male	Diabetes/severe			28.2	±
		IGT	252	61 ± 10	4.1	
	Female	NGT	254	60 ± 11	3.0	
		Diabetes/severe			28.4	±
		IGT	219	61 ± 10	5.0	
		NGT	217	60 ± 10	4.3	
Swedish Case/Control	Male	Diabetes	267	66 ± 12	3.5	
		NGT	267	66 ± 12	3.4	
	Female	Diabetes	247	67 ± 12	4.6	
		NGT	247	67 ± 12	4.6	
French Canadian Case/Control	Male	Diabetes	70	52 ± 8	4.1	

European American Case/Control	Female	NGT	70	51 ± 8	28.8 ± 3.2
		Diabetes	57	54 ± 7	29.0 ± 4.8
	Male	NGT	57	53 ± 7	28.5 ± 4.9
		Diabetes	644	62 ± 11	32.0 ± 6.3
Polish Case/Control	Female	NGT	644	61 ± 10	27.7 ± 4.5
		Diabetes	582	63 ± 11	34.0 ± 7.5
	Male	NGT	582	61 ± 9	27.0 ± 5.8
		Diabetes	422	60 ± 10	28.7 ± 4.5
	Female	NGT	422	58 ± 7	25.4 ± 2.9
		Diabetes	587	63 ± 9	30.3 ± 5.0
		NGT	587	59 ± 7	26.6 ± 4.0

Table 2: Patient Samples used to test association to type 2 diabetes. Age and BMI data are mean ± standard deviation. IGT – impaired glucose tolerance. NGT – normal glucose tolerance.

	Eu. American		Polish			Combined Data*				permuted p**
	MAF [†]	p [‡]	OR [§]	MAF	p	OR	p	OR	95% CI	
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
hCV152699										
5	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
CCAGAA										
G [#]	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

Table 3: An initial association of NPY2R with obesity in men from the screening panels.

*Results combined using a Mantel-Haenszel test (44). [†]Minor allele frequency. [‡]P-value from χ^2 test using an allelic model. [§]Odds ratio for the effect of the minor allele of the SNP or the effect of the indicated haplotype versus all others. ^{||}95% confidence intervals around the combined odds ratio. [¶]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 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 T2D in further samples. **Using 1000 permutations, see Methods for details.

	FHS men			Scandinavian men			Unrelated men combined		Scandinavian Trios		
	MAF*	p [†]	β [‡]	MA F	p	β	p [§]	β	MA F	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	-
hCV152699											
5	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	-

Table 4: Association of NPY2R SNPs to BMI analyzed as a continuous trait.

BMI was transformed as described in the Methods into an age-adjusted z-score. *Minor allele frequency. [†]p-value for the linear regression of age-adjusted score against genotype coded as 0, 1, 2 denoting the number of minor allele or haplotype copies carried by an individual. [‡]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 family based sample was analyzed using a FBAT-GEE test as implemented in PBAT (40). The direction is given a “+” if the minor allele is associated with higher age-adjusted score.

	Eu. derived replication*		African replication [†]	American		All replication samples [‡]			All Samples [§]		
	p	OR	Freq	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	0.93-1.28	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-

hCV152699 5	0.32	1.10	0.10	0.12	0.69	0.73	1.03	0.87- 1.22	0.01	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	0.01	1.15	1.03- 1.29
CCAGAA G [#]	0.54	1.06	–	–	–	–	–	–	0.00	1.19	1.05- 1.36
CTCAGAA #	0.23	0.90	–	–	–	–	–	–	0.05	0.89	0.79- 1.00
GTCAGAA #	0.18	0.81	–	–	–	–	–	–	0.27	0.89	0.72- 1.10

Table 5: Association of SNPs in NPY2R with BMI in men in all samples.

The tagSNPs were tested in men from three European-derived and two African American panels. In these panels, BMI was dichotomized as described in the Methods. 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. [†]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 to BMI: screening and replication. ^{||}Frequency of allele tested. [¶]Minor alleles are different in the African American panels compared to the European-derived samples. Data for the minor allele in the European-derived samples is reported. [#]The obesity risk, protective, and diabetes risk haplotypes were only evaluated in the European-derived samples due to differences in LD in the African American population.

	Scandinavian related			Scandinavian Case-Control			Swedish Case-Control			French Canadian Case-Control			European American Case-Control			Polish Case-Control			Combined Data		
	MAF*	p	OR†	MAF	p	OR	MAF	p	OR	MAF	p	OR	MAF	p	OR	MAF	p	OR	p	OR	95% CI
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	5.75x10⁻⁴	1.22	1.09-1.36
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
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
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
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
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
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
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
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
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	1.73x10⁻⁴	1.36	1.16-1.59

Table 6: Association of SNPs and haplotypes in *NPY2R* with type 2 diabetes in men.

The minor allele of each SNP was tested. Data for the obesity risk haplotype, the protective haplotype, and the haplotype associated to T2D are shown. *Minor allele frequency. †Odds ratio for developing T2D with the minor allele. ‡Calculated using 1000 permutations.