Brief Genetics Report

Evidence of an Association Between the Arg72 Allele of the Peptide YY and Increased Risk of Type 2 Diabetes

Signe S. Torekov,[1,2] Lesli H. Larsen,[1,3] Charlotte Glümer,[1,4] Knut Borch-Johnsen,[1,4,5] Torben Jørgensen,[4] Jens J. Holst,[6] Ole D. Madsen,[1,2] Torben Hansen,[1] and Oluf Pedersen[1,5]

We tested the hypothesis that variants in the gene encoding the prepropeptide YY (*PYY*) associate with type 2 diabetes and/or obesity. Mutation analyses of DNA from 84 patients with obesity and familial type 2 diabetes identified two polymorphisms, IVS3 + 68C>T and Arg72Thr, and one rare variant, +151C>A of *PYY*. The common allele of the Arg72Thr variant associated with type 2 diabetes with an allele frequency of the Arg allele of 0.667 (95% CI 0.658–0.677) among 4,639 glucose-tolerant subjects and 0.692 (0.674–0.710) among 1,326 patients with type 2 diabetes ($P = 0.005$, odds ratio 1.19 [95% CI 1.05–1.35]). The same polymorphism associated with overweight ($25 \leq$ BMI < 30 kg/m^2) ($P = 0.018$, 1.15 [1.02–1.28]). In quantitative trait analyses of a population-based sample of 6,022 subjects, the Arg allele was associated with an increased plasma glucose level 2 h after an oral glucose tolerance test (OGTT) ($P = 0.03$), an increased area under the curve for the post-OGTT plasma glucose level ($P = 0.03$), and a lower insulinogenic index ($P = 0.01$). In conclusion, the common Arg allele of the *PYY* Arg72Thr variant modestly associates with type 2 diabetes and with type 2 diabetes–related quantitative traits. *Diabetes* 54:2261–2265, 2005

Peptide YY (PYY) is released from L-cells, primarily located in the distal part of the intestine, in the postprandial state in proportion to the energy content of a meal. The PYY prohormone is cleaved extracellularly to the active form of PYY, $PYY_{3–36}$ (1,2).

In 2002, Batterham et al. (3) reported that $PYY_{3–36}$ reduces food intake in rodents as well as in humans by affecting appetite-regulating centers in the hypothalamus. The following year, the same group reported the finding of a relatively lower plasma $PYY_{3–36}$ level in obese subjects, as well as a lower increase in the plasma $PYY_{3–36}$ concentration after food intake, compared with lean subjects (4). Subsequently, there has been an ongoing debate regarding the actual effect of $PYY_{3–36}$ on appetite regulation, as several other investigators have failed to reproduce evidence of the anorexic effect in rodents (5). Batterham et al. (6) argue, however, that to demonstrate an anorexic effect of $PYY_{3–36}$, thorough acclimatization of the rodents is needed, and interestingly, a recent study in rhesus monkeys supports Batterham et al.'s original finding (7).

$PYY_{3–36}$ is an agonist for the hypothalamic neuropeptide Y (NPY) receptor Y2 (8). $PYY_{3–36}$ inhibits the neuronal NPY activity and thereby activates the neuronal proopiomelanocortin pathway (3). Besides involvement in appetite regulation, $PYY_{3–36}$ also plays a role in insulin action. Acute infusion of $PYY_{3–36}$ in mice during a hyperinsulinemic-euglycemic clamp caused an increase in glucose uptake in both muscle and fat tissue (9), while chronic infusion of the peptide reduced the HbA_{1c} (A1C) level in diabetic fatty Zucker rats (10). Early studies of PYY have shown that the peptide directly inhibits glucose-mediated insulin secretion (11), and PYY has been reported to be coexpressed with glucagon in pancreatic α-cells (12). Therefore, $PYY_{3–36}$ may affect both appetite control and regulation of glucose metabolism.

Consequently, we hypothesize that variants in the gene encoding $PYY_{3–36}$ (*PYY*) are associated with common subsets of type 2 diabetes and/or obesity. Two studies have examined variants in *PYY* for association with metabolic disorders. An intronic variant, IVS3 + 68C>T, was reported to associate with an increased risk of type 2 diabetes among patients from the U.K. (13), while a recent study in 101 obese children showed no association with *PYY* gene variants and early-onset morbid obesity (14). In the current study, we give the results of a mutation analysis of the coding region of *PYY* in subjects with obesity and familial type 2 diabetes, providing evidence that the common Arg72 allele confers a modestly increased risk of type 2 diabetes in Danish whites.

RESEARCH DESIGN AND METHODS

The screening for genetic variants was performed on genomic DNA extracted from human leukocytes from 84 unrelated obese subjects (40 men, 44 women,

From the [1]Steno Diabetes Center and Hagedorn Research Institute, Gentofte, Denmark; the [2]Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark; the [3]Danish Epidemiology Science Centre and Research Unit for Dietary Studies at the Institute of Preventive Medicine, Copenhagen University Hospital, Copenhagen, Denmark; the [4]Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; the [5]Faculty of Health Science, University of Aarhus, Aarhus, Denmark; the [6]Department of Medical Physiology, University of Copenhagen, Denmark.

Address correspondence and reprint requests to Signe S. Torekov, MSc, Steno Diabetes Center, Niels Steensens Vej 2, DK-2820, Gentofte, Denmark. E-mail: skto@steno.dk.

Received for publication 22 December 2004 and accepted in revised form 21 April 2005.

O.D.M. is employed by, holds stock in, and has received grant support from Novo Nordisk.

HPLC, high-performance liquid chromatography; OGTT, oral glucose tolerance test; NPY, neuropeptide Y; PYY, peptide YY; UTR, untranslated region.

TABLE 1
Association studies of the IVS3 + 68C>T variant of *PYY* with overweight, obesity, and type 2 diabetes in Danish whites

Group	*n*	CC	CT	TT	T allele frequency (95% CI)	*$P_{allele\ frequency}$	*$P_{genotype\ distribution}$
Subjects with BMI <25 kg/m^2	2,676	2,220 (83.0)	432 (16.1)	24 (0.9)	9.0 (8.2–9.7)		
Subjects with 25 ≤ BMI < 30 kg/m^2	2,366	1,968 (83.2)	372 (15.7)	26 (1.1)	9.0 (8.1–9.8)	0.99	0.72
Subjects with BMI ≥30 kg/m^2	1,048	865 (82.5)	172 (16.4)	11 (1.0)	9.3 (8.0–10.5)	0.70	0.89
Glucose-tolerant subjects	4,675	3,911 (83.7)	716 (15.3)	48 (1.0)	8.7 (8.1–9.3)		
Type 2 diabetic patients	1,381	1,167 (84.5)	206 (14.9)	8 (0.6)	8.0 (7.0–9.1)	0.29	0.25

Data are *n* (%), unless otherwise indicated. Top three rows: **P* values are calculated with χ^2 test and describe the significance levels comparing the lean and overweight group and the lean and obese group. Bottom two rows: **P* values are calculated with χ^2 test and describe the significance levels comparing the glucose-tolerant subjects and the type 2 diabetic patients.

age 50 ± 15 years [means ± SD], and BMI 36 ± 5 kg/m^2). All subjects were recruited through an ongoing family study of type 2 diabetes at Steno Diabetes Center, and 62 of the 84 participants were diagnosed with type 2 diabetes.

Overweight and obesity. The Inter99 cohort is a population-based randomized nonpharmacological intervention study for the prevention of cardiovascular disease and is performed at the Research Centre for Prevention and Health (15). Genomic DNA from 6,365 Danish whites from this cohort was available for genotyping. The Arg72Thr variant was successfully genotyped in 6,022 subjects from the Inter99 sample involved in the case-control study of overweight and obesity. These subjects were divided into three groups: *1*) 2,635 lean subjects with BMI <25 kg/m^2 (BMI 22.4 ± 1.8 kg/m^2, age 45.1 ± 7.9 years), *2*) 2,352 overweight subjects with 25 ≤ BMI < 30 kg/m^2 (27.2 ± 1.4 kg/m^2, 46.6 ± 7.9 years), and *3*) 1,035 obese subjects with BMI ≥30 kg/m^2 (33.9 ± 3.9 kg/m^2, 47.7 ± 7.9 years). The number of subjects refers to the subjects included in the Arg72Thr analyses. The IVS3 + 68C>T and Arg72Thr variants had a slightly different genotype success rate, which resulted in slightly different study subject numbers. Subject numbers included in the IVS3 + 68C>T variant analyses are given in Table 1. The average characteristics of the subjects genotyped for the IVS3 + 68C>T variant are essentially the same as those for the Arg72Thr variant analyses.

Type 2 diabetes. The diabetes study enrolled a total of 1,326 cases with type 2 diabetes and 4,639 glucose-tolerant control subjects. Of the 1,326 patients, 992 were recruited from the outpatient clinic at Steno Diabetes Center and 334 were recruited from the Inter99 study sample. Diabetes was diagnosed in accordance with the 1999 World Health Organization criteria. The basic characteristics of the type 2 diabetic patients were age 57 ± 11 years, age at clinical onset 52 ± 10 years, BMI 29.7 ± 5.3 kg/m^2, and A1C 7.8 ± 1.7%. Patients with diabetes due to known chronic pancreatitis, hemochromatosis, severe insulin resistance, maturity-onset diabetes of the young, maternally inherited diabetes and deafness; patients with a family history of first-degree relatives with type 1 diabetes; patients with insulin requirement within the 1st year after diabetes diagnosis; patients with a fasting serum C-peptide level ≤150 pmol/l at the time of recruitment; or patients with diabetes onset before the age of 25 were excluded from the present study from the category of clinically defined type 2 diabetes.

The control group was a population-based sample of middle-aged individuals from the Inter99 cohort comprising 4,276 glucose-tolerant control subjects and 363 glucose-tolerant control subjects recruited from the Steno Diabetes Center (age 46 ± 8 years, BMI 25.5 ± 4.0 kg/m^2).

Only glucose-tolerant subjects and type 2 diabetic patients were included in the case-control study of diabetes, while the entire Inter99 cohort was enrolled in the obesity case-control studies in which subjects were divided into the following three groups: BMI <25 kg/m^2, 25 ≤ BMI < 30 kg/m^2, and BMI ≥30 kg/m^2. The larger subject number in the case-control study of obesity compared with the case-control study of diabetes accounts for the subjects with impaired glucose tolerance and impaired fasting glucose.

Quantitative traits. The genotype–quantitative trait studies were performed in the 6,022 subjects from the Inter99 cohort who were successfully genotyped for the Arg72Thr variant. In addition, fasting plasma PYY levels were measured in 12 glucose-tolerant subjects recruited at Steno Diabetes Center who were genotyped for the *PYY* Arg72Thr variant. Six subjects were Arg/Arg carriers (three women and three men, BMI 23.6 ± 1.0 kg/m^2, age 68.1 ± 11.8 years) and six matched subjects were Thr/Thr carriers (three women and three men, BMI 23.0 ± 2.3 kg/m^2, age 69.3 ± 11.0 years).

All study participants were Danish whites by self-report. Informed written and oral consent were obtained from all study participants. The studies were in accordance with the Helsinki Declaration II and were approved by the Ethical Committee of Copenhagen County.

Biochemical and anthropometric measurements. Blood samples for analyses of biochemical variables were drawn in the morning after an overnight fast. Plasma glucose and serum-specific insulin, excluding des(31,32) and intact proinsulin, were analyzed at Steno Diabetes Center (15). A1C was analyzed by principles of ion-exchange high-performance liquid chromatography (HPLC) using Bio-Rad VARIANT Hemoglobin A_{1C} (Bio-Rad, Richmond, CA) (normal range 4.1–6.4%). The radioimmunoassay of PYY in plasma was performed using antiserum code no. 8412-5 (Euro-Diagnostica, Malmö, Sweden), as described (16). The antiserum cross-reacts 100% with human $PYY_{1–36}$ and with $PYY_{3–36}$. Synthetic human $PYY_{1–36}$ (Peninsula, Merseyside, U.K.) was used for standards, and porcine ^{125}I-PYY (code no. IM259) was purchased from Amersham Biosciences, (Buckinghamshire, U.K.). Detection limit of the assay was <2 pmol/l and 50% inhibition was obtained with 40 pmol/l PYY. Recovery of PYY added to plasma in concentrations between 5 and 50 pmol/l deviated <15% from expected values. Intra-assay coefficient of variation was <0.05. The antiserum showed no crossreaction with human NPY or human pancreatic polypeptide in concentrations up to 500 pmol/l.

Height and weight were measured in light indoor clothes and without shoes, and BMI was calculated as reported (15).

Mutation analysis and genotyping. The examination for mutations in *PYY* was conducted by applying denatured HPLC and direct sequencing (MWG, Ebersberg, Germany) on genomic DNA extracted from human leukocytes. In our laboratory, the denatured HPLC method has a sensitivity >95%. The genotyping method used for detection of the IVS3 + 68C>T polymorphism was a chip-based matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (DNA MassARRAY) analysis of PCR-generated primer extension products as previously described (17). The genotyping success rate for the IVS3 + 68C>T variant was 96%, and there were no mismatches among 89 replicate samples. The genotyping method used for detection of the Arg72Thr polymorphism in *PYY* was Taqman allelic discrimination (KBioscience, Herts, U.K.). The genotyping success rate for the Arg72Thr variant was 95%, and among 851 replicate samples there were three mismatches, giving a discrepancy rate of 0.35%.

Statistical analysis. Linkage disequilibrium was estimated as R^2, and the χ^2 test was applied to test for significant differences in allele frequencies and genotype distribution in the obesity and type 2 diabetes case-control studies. Phenotypic differences between the genotype groups were tested with a general linear model that included genotype and sex as fixed factors and age and BMI (if appropriate) as covariate factors. Differences in plasma PYY level between the genotype groups were tested with a paired *t* test, where subjects were matched in pairs for age and BMI. A *P* value <0.05 was considered significant. All analyses were done using SPSS version 12.0.

RESULTS

Eighty-four unrelated obese individuals, most of whom had a family history of type 2 diabetes, were examined for variants in the coding region of *PYY*. One rare variant, +151C>A, and two polymorphisms, IVS3 + 68C>T (rs162430) and Arg72Thr (rs1058046), were identified. The two polymorphisms were in Hardy-Weinberg equilibrium. Linkage disequilibrium studies revealed an R^2 value of 0.20. The IVS3 + 68C>T variant in intron 3 was not significantly associated with overweight, obesity, or type 2 diabetes (Table 1). The Arg72Thr in *preproPYY* was examined for association with overweight, obesity, and type 2 diabetes. In the total Inter99 cohort, homozygozity of the 72Arg allele was associated with an increased risk of overweight (P = 0.018, odds ratio [OR] 1.15 [95% CI 1.02–1.28]) and overweight and

obesity combined ($P = 0.019$, 1.14 [1.02–1.25]) when compared with hetero- and homozygozity of the Thr allele (Table 2). Investigations of association with overweight omitting the 334 patients diagnosed with type 2 diabetes in the Inter99 cohort gave similar and statistically significant values (data not shown).

In the study of 1,326 cases with type 2 diabetes and 4,639 glucose-tolerant control subjects, the Arg allele was associated with an increased risk of type 2 diabetes. Homozygozity of the Arg allele was associated with an estimated OR of 1.19 (95% CI 1.05–1.35, $P = 0.005$) compared with hetero- and homozygozity of the Thr allele (Table 2). The association with type 2 diabetes remained significant in the scenario where only the 992 diabetic patients recruited from the diabetes clinic were included in the analysis.

In the population-based studies of relationships between pertinent quantitative traits among 6,022 subjects in the Inter99 cohort and the codon 72 polymorphism, we found that homozygous carriers of the Arg allele had a significantly increased plasma glucose level 2 h after an OGTT ($P = 0.03$), as well as an increased area under the curve for plasma glucose during an OGTT ($P = 0.03$). Carriers of the Arg/Arg alleles also had a significantly lower insulinogenic index ($P = 0.01$) (Table 3). Investigations of the quantitative traits for circulating levels of insulin and glucose in the Inter99 cohort omitting the 334 patients in the cohort with known type 2 diabetes gave similar and statistically significant values (data not shown). Measurements of fasting plasma PYY levels in 12 subjects genotyped for the Arg72Thr variant showed that Arg/Arg carriers had on average a 20% lower fasting PYY plasma level than matched Thr/Thr carriers (7.50 ± 0.43 vs. 9.33 ± 1.0 pmol/l [means ± SE], $P = 0.048$).

The +151C>A variant in the 3′ untranslated region (UTR) was identified in its heterozygous form in one obese woman with type 2 diabetes (BMI 36 kg/m^2, age 74 years). Her two sisters and one of the sisters' two children were also heterozygous carriers of the variant. The variant does not, however, cosegregate with obesity, though the variant might confer an increased risk of type 2 diabetes since all mutation carriers except one had type 2 diabetes (Fig. 1).

DISCUSSION

We present the results of a mutation analysis of the coding region of *PYY* and a subsequent relatively large-scale epidemiological study of identified polymorphisms in whites. Our major novel finding relates to the common Arg allele of a missense variant at codon 72 in *preproPYY*, which is associated with a modestly increased risk of type 2 diabetes. The additional studies of population physiology show that the same amino acid polymorphism is associated with an elevated plasma glucose level after an OGTT as well as a lower glucose-stimulated insulin release. It should be noted, however, that the control subjects in the case-control study of diabetes were recruited from the same Inter99 cohort as the subjects enrolled in the genotype-quantitative trait analyses. Therefore, the two study results are to a certain extent interdependent.

The Arg72Thr variant is located five amino acids downstream of the dibasic cleavage site of preproPYY. Alteration of the helical structure in the proximity of the dibasic cleavage site in prohormones has been shown to impair

TABLE 2
Association studies of the Arg72Thr variant of *PYY* with overweight, obesity, and type 2 diabetes in Danish whites

Group	*n*	Arg/Arg	Arg/Thr	Thr/Thr	Arg allele frequency (95% CI)	$*P_{\text{allele frequency}}$	$*P_{\text{genotype distribution}}$	$*P_{\text{Arg/Arg versus Arg/Thr + Thr/Thr}}$ (OR [95% CI])
Subjects with BMI <25 kg/m^2	2,635	1,149 (43.6)	1,196 (45.4)	290 (11.0)	66.3 (65.0–67.6)			
Subjects with 25 ≤ BMI < 30 kg/m^2	2,352	1,104 (46.9)	985 (41.9)	263 (11.2)	67.9 (66.5–69.2)	0.096	0.037	0.018 (1.15 [1.02–1.28])
Subjects with BMI ≥30 kg/m^2	1,035	476 (46.0)	433 (41.8)	126 (12.2)	66.9 (64.9–68.9)	0.62	0.14	
Subjects with BMI ≥25 kg/m^2	3,387	1,580 (46.6)	1,418 (41.9)	389 (11.5)	67.6 (66.5–68.7)	0.14	0.023	0.019 (1.14 [1.02–1.25])
Glucose-tolerant subjects	4,639	2,077 (44.8)	2,037 (43.9)	525 (11.3)	66.7 (65.8–67.7)			
Type 2 diabetic patients	1,326	652 (49.2)	531 (40.0)	143 (10.8)	69.2 (67.4–71.0)	0.018	0.017	0.005 (1.19 [1.05–1.35])

Data are *n* (%), unless otherwise indicated. Top four rows: $*P$ values are calculated with χ^2 test and describe the significance levels comparing the lean and the overweight, the lean and the obese, and the lean and the overweight + obese groups. Bottom two rows: $*P$ values are calculated with χ^2 test and describe the significance levels comparing the glucose-tolerant subjects and the type 2 diabetic patients.

TABLE 3
Association studies of the Arg72Thr variant of *PYY* with diabetes-related quantitative traits in a population-based cohort of Danish whites

Response	Arg/Arg	Arg/Thr	Thr/Thr	$P_{Arg/Arg}$ vs. Arg/Thr vs. Thr/Thr	$P_{Arg/Arg}$ + Arg/Thr vs. Thr/Thr	$P_{Arg/Arg}$ vs. Arg/Thr + Thr/Thr
n (men/women)	2,729 (50/50)	2,614 (47/53)	679 (51/49)			
Age (years)	45.3 (45.0–46.6)	45.9 (45.6–46.2)	46.3 (45.7–46.9)			
BMI (kg/m^2)	26.4 (26.2–26.6)	26.2 (26.0–26.3)	26.4 (26.0–26.7)	0.14	0.69	0.09
Fasting plasma insulin (pmol/l)*	43 (42–44)	43 (42–44)	43 (41–45)	0.67	0.61	0.39
Fasting plasma glucose (mmol/l)*	5.6 (5.6–5.7)	5.6 (5.6–5.7)	5.6 (5.5–5.7)	0.90	0.65	0.96
Plasma glucose 2 h after OGTT (mmol/l)*	6.3 (6.2–6.4)	6.2 (6.1–6.3)	6.1 (5.9–6.2)	0.02	0.01	0.03
Area under the curve for post-OGTT plasma glucose (mmol/l)*	892 (885–898)	885 (878–892)	875 (862–889)	0.06	0.09	0.03
Insulinogenic index (insulin)*	28.7 (28.0–30.0)	30.0 (29.2–30.7)	30.5 (29.0–32.0)	0.04	0.16	0.01

Data are means (95% CI), unless otherwise indicated. Means were adjusted for the effect of age, sex, and BMI. *Kubic-transformed before analysis. The insulinogenic index was calculated as fasting serum insulin (pmol/l) subtracted from 30 min post-OGTT serum insulin (pmol/l) and divided by 30 min post-OGTT plasma glucose (mmol/l). The area under the curve for glucose was calculated using the trapezoidal method.

FIG. 1. Family with the rare +151C>A mutation of *PYY*. Circles, women; squares, men; slashes, deceased family members; open symbols, nondiabetic family member; filled symbols, family member with type 2 diabetes. He, heterozygous; Wt, wild type

the efficiency of the proteolytic processing by prohormone convertase 1/3 (18). Indeed, in silico analysis predicts a secondary structure change when substituting the arginine residue for the threonine residue (analyzed at www.cmpharm.ucsf.edu/~nomi/nnpredict.html, data not shown). Interestingly, the Thr allele is conserved among species, e.g., rat (*Rat norvegicus*), chicken (*Gallus gallus*), and African clawed frog (*Xenopus laevis*) (analyzed at www.ncbi.nlm.nih.gov/blast/Blast.cgi, data not shown). Furthermore, several studies have shown that substitution of arginine in propeptides alters the cleavage process (19,20). Thus, the introduction of an arginine residue instead of a threonine residue might affect the cleavage efficiency and thus lead to reduced levels of PYY and thereby give rise to altered glucose regulation. Another possibility is that the cleavage product in itself may have a biological function, like with glucagon and glucagon-like peptide-1 and -2, which are encoded by the same prepropeptide.

Interestingly, in a pilot study, Arg/Arg carriers had lower fasting plasma PYY levels compared with matched Thr/Thr carriers. If this finding is replicated in a larger group of individuals, it may, in accordance with experiments in rodents where infusion of high levels of PYY improves glucose regulation (9,10), suggest that a lower plasma PYY level in Arg/Arg carriers is involved in their impaired glucose regulation.

Still, the Arg72Thr variant may also be in linkage disequilibrium with unknown variants located outside the coding region, explaining the functionality and the demonstrated association with metabolic disorders. The rare +151C>A variant in the 3′ UTR of *PYY* might also be associated with type 2 diabetes, since all mutation carriers except one had type 2 diabetes. The carrier without type 2 diabetes is still young and not obese, whereas the only noncarrier in the family does not have type 2 diabetes although he is obese.

In contrast to the findings by Barroso et al. (13), we did not show an association with type 2 diabetes for the IVS3 + 68C>T variant, despite enrolling three times as many subjects compared with the first study reported.

To our knowledge, the present study is the largest association study investigating the potential effect of variants in *PYY* on risk of overweight and obesity. Neither the rare +151C>A variant in the 3′ UTR nor the intron variant were associated with obesity in the present study. The

missense variant in *preproPYY* Arg72Thr shows, however, a weak association with overweight and the combined phenotypes of overweight and obesity for Arg/Arg carriers. This finding is not supported by association with any obesity-related quantitative traits, such as BMI, waist-to-hip ratio, or waist measurements (data not shown). In conclusion, the Arg/Arg alleles at codon 72 of the prepro-PYY associate modestly with type 2 diabetes and overweight in Danish whites.

ACKNOWLEDGMENTS

The study was supported by the Danish Medical Research Council, the Danish Centre for Evaluation and Health Technology Assessment, Novo Nordisk, Copenhagen County, the Danish Heart Foundation, the Danish Diabetes Association, the Danish Pharmaceutical Association, the Augustinus Foundation, the Ib Henriksen Foundation, the Becket Foundation, and EEC grants (BMH4-CT98-3084 and QLRT-CT-1999-00546).

We thank Annemette Forman, Inge Lise Wantzin, and Marianne Stendal for dedicated technical assistance; Grete Lademann for secretarial support; and the staff engaged in data collection at Research Centre for Prevention and Health, Glostrup University Hospital.

REFERENCES

1. Grandt D, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE, Reeve JR: Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1–36 and PYY 3–36. *Regul Pept* 51:151–159, 1994
2. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR: Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 89:1070–1077, 1985
3. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR: Gut hormone PYY3–36 physiologically inhibits food intake. *Nature* 418:650–654, 2002
4. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR: Inhibition of food intake in obese subjects by peptide YY3–36. *N Engl J Med* 349:941–948, 2003
5. Tschop M, Castaneda TR, Joost HG, Thone-Reineke C, Ortmann S, Klaus S, Hagan MM, Chandler PC, Oswald KD, Benoit SC, Seeley RJ, Kinzig KP, Moran TH, Beck-Sickinger AG, Koglin N, Rodgers RJ, Blundell JE, Ishii Y, Beattie AH, Holch P, Allison DB, Raun K, Madsen K, Wulff BS, Stidsen CE, Birringer M, Kreuzer OJ, Schindler M, Arndt K, Rudolf K, Mark M, Deng XY, Whitcomb DC, Halem H, Taylor J, Dong J, Datta R, Culler M, Craney S, Flora D, Smiley D, Heiman ML: Physiology: does gut hormone PYY3–36 decrease food intake in rodents? *Nature* 430:165, 2004
6. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR: Physiology: does gut hormone PYY3–36 decrease food intake in rodents? *Nature* 430:166, 2004
7. Moran TH, Smedh U, Kinzig KP, Scott KA, Knipp S, Ladenheim EE: Peptide YY (3–36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. *Am J Physiol Regul Integr Comp Physiol* 288:384–388, 2004 (Epub 23 September 2004)
8. Grandt D, Teyssen S, Schimiczek M, Reeve JR, Feth F, Rascher W, Hirche H, Singer MV, Layer P, Goebell H, Ho FJ, Eysselein VE: Novel generation of hormone receptor specificity by amino terminal processing of peptide YY. *Biochem Biophys Res Communs* 186:1299–1306, 1992
9. van den Hoek AM, Heijboer AC, Corssmit EPM, Voshol PJ, Romijn JA, Havekes LM, Pijl H: PYY3–36 reinforces insulin action on glucose disposal in mice fed a high-fat diet. *Diabetes* 53:1949–1952, 2004
10. Pittner RA, Moore CX, Bhavsar SP, Gedulin BR, Smith PA, Jodka CM, Parkes DG, Paterniti JR, Srivastava VP, Young AA: Effects of PYY[3–36] in rodent models of diabetes and obesity. *Int J Obes Relat Metab Disord* 28:963–971, 2004
11. Bertrand G: Evidence for a direct inhibitory effect of PYY on insulin secretion in rats. *Pancreas* 7:595–600, 1992
12. Bottcher G: Peptide YY: intrapancreatic localization and effects on insulin and glucagon secretion in the mouse. *Pancreas* 4:282–288, 1989
13. Barroso I, Luan J, Middelberg RPS, Harding AH, Franks PW, Jakes RW, Clayton D, Schafer AJ, O'Rahilly S, Wareham NJ: Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. *PLoS Biology* 1:41–55, 2003
14. Connie Hung CC, Pirie F, Luan J, Lank E, Motala A, Yeo GSH, 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
15. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. *Eur J Cardiovasc Prev Rehabil* 10:377–386, 2003
16. Schmidt PT, Ljung T, Hartmann B, Hare KJ, Holst JJ, Hellstrom PM: Tissue levels and post-prandial secretion of the intestinal growth factor, glucagon-like peptide-2, in controls and inflammatory bowel disease: comparison with peptide YY. *Eur J Gastroenterol Hepatol* 17:207–212, 2005
17. Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A: High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci U S A* 98:581–584, 2001
18. Brakch N, Rholam M, Simonetti M, Cohen P: Favourable side-chain orientation of cleavage site dibasic residues of prohormone in proteolytic processing by prohormone convertase 1/3. *Eur J Biochem* 267:1626–1633, 2000
19. Casonato A, Sartorello F, Cattini MG, Pontara E, Soldera C, Bertomoro A, Girolami A: An Arg760Cys mutation in the consensus sequence of the von Willebrand factor propeptide cleavage site is responsible for a new von Willebrand disease variant. *Blood* 101:151–156, 2003
20. Lind B, Johnsen AH, Thorsen S: Naturally occurring Arg-1 to his mutation in human protein C leads to aberrant propeptide processing and secretion of dysfunctional protein C. *Blood* 89:2807–2816, 1997