Leucine 7 to Proline 7 Polymorphism in the Preproneuropeptide Y Is Associated With Proteinuria, Coronary Heart Disease, and Glycemic Control in Type 1 Diabetic Patients

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OBJECTIVE — Neuropeptide Y is a potent vasoconstrictor thought to enhance the development of atherosclerosis. The leucine 7 to proline 7 (Leu7Pro) polymorphism, located in the signal peptide part of the human preproneuropeptide Y, has been associated with serum lipid levels, intima-media thickness of the common carotid arteries, and diabetic retinopathy in type 2 diabetic patients. Therefore, we investigated the impact of the Leu7Pro polymorphism on diabetic nephropathy, cardiovascular risk factors, and cardiovascular disease in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — A total of 996 patients from the Finnish Diabetic Nephropathy study were studied in a case-control, cross-sectional study. The carrier frequency of the Pro7 substitution was 13% in the entire study population.

RESULTS — The Pro7 substitution was more common in patients with proteinuria than in those with a normal albumin excretion rate (16 vs. 11%, $P < 0.05$). Patients with the Pro7 allele had worse glycemic control (HbA_{1c} 8.8 vs. 8.5%, $P < 0.005$), more coronary heart disease (CHD) (14 vs. 8%, $P < 0.05$), and higher serum triglycerides (1.65 vs. 1.35 mmol/l, $P < 0.005$) than patients with the wild-type genotype. There were no differences in the plasma neuropeptide Y levels between the patients with Pro7 compared with those with the wild-type genotype. The Leu7Pro polymorphism was independently associated with HbA_{1c} ($P < 0.001$), proteinuria ($P < 0.01$), and CHD ($P < 0.01$) in multiple regression analyses.

CONCLUSIONS — We conclude that the Leu7Pro polymorphism may contribute to the genetic susceptibility to diabetic nephropathy and CHD in type 1 diabetic patients, possibly by influencing glycemic control and triglycerides.

Diabetes Care 27:503–509, 2004

Diabetic nephropathy is associated with a high risk for coronary heart disease (CHD) and early mortality. Although risk factors such as poor glycemic control, hypertension, and dyslipidemia contribute to nephropathy, they cannot entirely explain why it develops in only a subset of patients. Evidence suggests that a genetic predisposition is involved in the pathogenesis of diabetic nephropathy, but the genetic factors are still unknown (1).

Neuropeptide Y (NPY) is a 36–amino acid polypeptide mainly present as an intact peptide in the central and peripheral nervous system, several peripheral organs, and plasma. NPY has been shown to regulate renal blood flow and to alter renal hormone levels such as renin (2). At least two of five known NPY receptors are expressed in the kidney. Stimulation of these receptors has resulted in a decrease in glomerular filtration rate, aldosterone concentration, plasma renin activity, and increase in sodium excretion (3). Furthermore, since interventions that block the renin-angiotensin-aldosterone system alter the renal function in diabetic nephropathy (4), it could be hypothesized that NPY is involved in the pathogenesis of the disease.

The gene that codes NPY is located on chromosome 7 p15.1. A functional leucine 7 to proline 7 (Leu7Pro) polymorphism located in the signal peptide region of the human preproneuropeptide Y (prepro-NPY) was recently observed. This signal peptide region, which is cleaved off during the peptide maturation process, is important for the proper folding and packaging of the NPY peptide, and a substitution can result in a loss of directing activity of the signal sequence (5). This Leu7Pro polymorphism has been associated with variation in blood pressure (6), serum total and LDL cholesterol (7), serum triglycerides (8), and accelerated progression of carotid atherosclerosis in both obese healthy subjects (6) and patients with type 2 diabetes (9). Type 2 diabetic patients with the Pro7 substitution had a higher prevalence of diabetic retinopathy (10). Whether the Leu7Pro polymorphism is associated with diabetic nephropathy is not known. Therefore, the

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Received for publication 27 June 2003 and accepted in revised form 4 November 2003.

M.K.K., M.K., and U.P. hold stock in Hormos Medical (Finland).

Abbreviations: AER, albumin excretion rate; CHD, coronary heart disease; ESRD, end-stage renal disease; Leu7Pro, leucine 7 to proline 7; NPY, neuropeptide Y; prepro-NPY, preproneuropeptide Y; RIA, radioimmunoassay.

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present study was undertaken in order to evaluate whether the Leu7Pro polymorphism of the prepro-NPY is associated with diabetic nephropathy, cardiovascular risk factors, or cardiovascular disease in a cohort of Finnish type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

— This study is part of the ongoing Finnish Diabetic Nephropathy (FinnDiane) study, a comprehensive, multicenter, nationwide project with the aim to characterize 25% ($n = 8,000$) of all adult patients with type 1 diabetes in Finland. In 1999, there were 1,700 patients in the FinnDiane database representing ~5% of all type 1 diabetic patients in Finland. All patients with an ascertained renal status ($n = 1,006$) were included in the present study. These patients had been recruited from 20 referral centers. The patients were required to be C-peptide negative (<0.3 nmol/l) and to have permanent insulin treatment initiated before the age of 35 years and within 1 year of the diabetes diagnosis. Six patients with residual C-peptide secretion and four with evidence of nondiabetic kidney disease were excluded. Renal status was based on the albumin excretion rate (AER) in at least two of three consecutive overnight or 24-h urine collections or the presence of end-stage renal disease (ESRD). A total of 321 patients had normal AER (<20 μg/min or <30 mg/24 h), 166 patients had microalbuminuria (AER 20–200 μg/min or 30–300 mg/24 h), and 325 patients overt proteinuria (AER >200 μg/min or >300 mg/24 h). A total of 184 patients were classified as having ESRD and were either on dialysis ($n = 44$) or had had a kidney transplant ($n = 140$). Patients with normal AER were required to have had diabetes for >15 years to qualify for the study. Plasma NPY was measured in all normoalbuminuric and proteinuric patients ($n = 76$) with the Pro7/− genotype and in 164 age- and BMI-matched normoalbuminuric and proteinuric patients with the wild-type genotype. Information about antihypertensive treatment, lipid medication, and cardiovascular disease, including CHD, acute myocardial infarction, and stroke, was also obtained from medical records. The patient had CHD when there was a verified acute myocardial infarction, previous coronary artery bypass surgery or angioplasty, or permanent nitroglycerine treatment. Informed consent was obtained from all subjects participating in the study. The protocol followed the principles expressed in the Declaration of Helsinki and was approved by the local ethics committees of each center.

Assays

Serum and urine creatinine levels for calculation of creatinine clearance were measured at a central laboratory by a modified Jaffé reaction (Boehringer Mannheim, Mannheim, Germany). Urinary albumin concentration was measured with radioimmunoassay (RIA) (Pharmacia, Uppsala, Sweden) and serum C-peptide concentrations with a Human C-peptide RIA kit (Linco Research, St. Charles, MO). HbA_{1c}, serum cholesterol, and serum triglycerides were locally measured using standardized assays in each referral center. The normal range for HbA_{1c} was 4.0–6.0% in 75% of the local laboratories and the upper normal value was <7.0% in all centers, whereas the normal range for triglycerides was 0.4–1.7 mmol/l in 55% of the centers, the upper normal range was <2.0 mmol/l in 90% of local laboratories and <3.0 mmol/l in all centers. Serum cholesterol and lipoproteins were analyzed using routine standardized laboratory methods at each center. All local laboratories participated in a national quality assessment program. The estimated glucose disposal rate, which measures insulin sensitivity, was calculated according to a formula as suggested by Williams et al. (11). Plasma NPY was measured using a commercial RIA kit (EURIA-NPY; Euro-Diagnostica, Malmö, Sweden).

Genotyping

Prepro-NPY genotypes were determined by an investigator unaware of the phenotypes using the previously described method (7). Due to failure of the PCR, one patient remained ungenotyped.

Statistical analysis

Data are means ±SE or median (range) value unless otherwise indicated. Categorical variables were compared using the χ^2 test or Fisher's exact test. Normally distributed continuous variables were tested with Student's t test, and non-normally distributed variables were logarithmically transformed or assessed with the Kruskal-Wallis or Mann-Whitney tests. Data were adjusted for HbA_{1c} and BMI when appropriate. Pearson correlation coefficient was used to evaluate the correlation between the plasma NPY level and a continuous variable. To evaluate the independent association between categorical or continuous variables, a multiple logistic or linear regression model was used with a means substitution of absent data. Analyses were carried out using a Statistica 4.1 statistical package (Tulsa, OK). A P value <0.05 was considered statistically significant.

RESULTS

— The clinical characteristics of the study population are shown in Table 1. A more detailed description has been previously reported (12).

Patients homo- and heterozygous for the Leu7Pro genotype were pooled in all further analyses because there were only four patients homozygous for the Pro7 allele. The frequency of the Pro7 substitution was 10.9% in the patients with normal AER, 10.2% with microalbuminuria, 16.2% with proteinuria, and 13.0% with ESRD ($\chi^2 = 5.48$ and df = 3, $P = 0.14$). The Pro7 substitution was thus more common in patients with overt nephropathy compared with the patients with normal AER ($\chi^2 = 4.00$ and df = 1, $P < 0.05$). The genotype frequencies in all four groups were in Hardy-Weinberg equilibrium.

Patients with the Pro7 substitution had higher values of HbA_{1c}, triglycerides, and BMI and a higher prevalence of CHD (Table 2). Since a majority of patients with ESRD had a renal transplant and were normoalbuminuric at the time of investigation, and since there may be a survival bias in this group, analyses were also performed without ESRD patients. HbA_{1c}, triglycerides, BMI, and the prevalence of CHD were also significantly higher when ESRD patients were excluded (Table 3). In addition, total cholesterol was also higher in these patients, whereas the estimated glucose disposal rate was lower and insulin dose higher in females with the Pro7 substitution.

The plasma NPY was 111.5 ± 3.0 in those with the wild-type genotype and 110.3 ± 4.4 in those with the Pro7 substitution (P = NS). No differences were detected when males and females were analyzed separately. There were no differences when those with proteinuria and normal AER were analyzed separately or when the patients were divided into quintiles according to their plasma NPY (data not shown). The plasma NPY was 96.7 ±

Table 1—*Clinical characteristics of 996 type 1 diabetic patients included in the study*

	NORMO	MICRO	PROT	ESRD
n	321	166	325	184
M/F (%)	41/59	64/36	59/41	59/41
Age (years)	40.4 ± 0.5	38.1 ± 0.9	40.3 ± 0.5	44.0 ± 0.6
Duration of diabetes (years)	26.7 ± 0.4	25.1 ± 0.7	28.0 ± 0.4	32.0 ± 0.6
BMI (kg/m^2)	24.9 ± 0.2	25.7 ± 0.3	25.9 ± 0.2	23.8 ± 0.3
Waist-to-hip ratio				
Male	0.90 ± 0.01	0.91 ± 0.01	0.94 ± 0.01	0.95 ± 0.01
Female	0.81 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	0.87 ± 0.01
Antihypertensive therapy	47 (15)	99 (62)	304 (96)	154 (91)
Cardiovascular disease	10 (3)	6 (4)	42 (13)	48 (27)
Acute myocardial infarction	5 (2)	4 (2)	21 (7)	23 (13)
CHD	9 (3)	6 (4)	33 (11)	37 (21)
Stroke	2 (1)	0 (0)	17 (5)	18 (10)
Retinal laser treatment	92 (29)	84 (51)	260 (81)	179 (99)
Systolic blood pressure (mmHg)	132 ± 1	138 ± 1	144 ± 1	154 ± 2
Diastolic blood pressure (mmHg)	79 ± 1	82 ± 1	83 ± 1	87 ± 1
HbA_{1c} (%)	8.0 ± 0.1	8.7 ± 0.1	8.9 ± 0.1	8.4 ± 0.1
AER				
(μg/min)*	5 (1–17)	38 (3–198)	—	—
(mg/24 h)*	—	—	1,063 (10–16,600)	—
Creatinine clearance ($ml \cdot s^{-1} \cdot 1.73\ m^{-2}$)	1.62 ± 0.02	1.51 ± 0.04	1.02 ± 0.04	0.81 ± 0.07

Data are means ± SE, median (range), or *n* (%). *Some patients with previously abnormal AER had responded to antihypertensive treatment and showed a regression of AER at the time of investigation. NORMO, normal AER; MICRO, microalbuminuria; PROT, proteinuria; ESRD, end-stage renal disease.

2.7 in those with normal AER compared with 121.3 ± 3.5 in those with proteinuria ($P < 0.001$). There were correlations between plasma NPY and HbA_{1c} ($r = 0.24$, $P < 0.001$), total cholesterol ($r = 0.40$, $P < 0.001$), LDL cholesterol ($r = 0.23$, $P < 0.001$), and serum creatinine ($r = 0.51$, $P < 0.001$). However, the correlation between plasma NPY and these variables were significant only when proteinuric patients were included in the analysis, but nonsignificant when only patients with normal AER were analyzed (data not shown).

In a multiple backward stepwise linear regression analysis, the Leu7Pro polymorphism of the prepro-NPY was independently associated with HbA_{1c} (Table 4). Similarly, the polymorphism was independently associated with proteinuria and CHD in a multiple backward stepwise logistic regression analysis (Table 5).

CONCLUSIONS — In this study, the Pro7 substitution of Leu7Pro polymorphism of the prepro-NPY was associated with HbA_{1c}, triglycerides, BMI, and total cholesterol. Furthermore, the Pro7 substitution was also independently associated with proteinuria and CHD in multiple regression analyses. We cannot exclude the possibility that an unknown polymorphism in close linkage disequilibrium with the Leu7Pro could be responsible for the results. However, experimental studies indicate that the Leu7Pro polymorphism influences the heart rate level, growth hormone, free fatty acid, insulin concentration, and plasma NPY (13,14), indicating that the polymorphism is potentially functional. There is a considerable variance in NPY levels during normal activities, and therefore differences in plasma NPY between genotypes have been observed only during standardized conditions, i.e., during cycle-ergometer exercise test, in healthy young subjects (13,15). This may explain why we could not detect any differences in the plasma NPY levels. The correlation between plasma NPY, serum lipids, and proteinuria is most likely explained by higher plasma NPY levels in patients with impaired renal function (16), since triglyceride-rich lipoproteins are a key feature of diabetic nephropathy (17). It is of note that the half-life of plasma NPY is rather short, only 4 min (18), which can also explain the lack of association between the polymorphism and the plasma levels. Furthermore, since NPY is present in the central and peripheral nervous system, kidney, and endothelium, it is possible that the effect is not mediated via plasma NPY.

Our patients with the Pro7 substitution had higher serum triglycerides, a phenomenon that was previously seen in preschool-aged boys (8). However, in our study the association was observed within the entire cohort, predominantly in females. A suggested effect of the Pro7 substitution on lipolysis has also been studied in healthy nondiabetic control subjects, where subjects with the Pro7 substitution had a lower free fatty acid level (19). This finding does not, however, contradict our results because free fatty acid levels do not appear to be an important determinant of triglyceride levels (20). The differences in triglycerides between the genotypes were observed when analyzed both with and without the patients with ESRD and after adjusting for HbA_{1c}. At this point, how the polymorphism causes a potentially altered NPY effect on serum lipids is not known, although an altered triglyceride metabolism via specific stimulation of lipoprotein lipase has been suggested (21). The Leu7Pro polymorphism has also been suggested to alter the composition of the lipoprotein particles affecting the postprandial lipid metabolism (22).

The observed association between the prepro-NPY Leu7Pro polymorphism and HbA_{1c} is a novel finding and was detected in both sexes, although the association was stronger in women. HbA_{1c} was higher in those with the Pro7 substitution both when patients with and without ESRD were analyzed and after adjusting for BMI. It was also independently associated with HbA_{1c} in a multiple linear regression analysis. The mechanism is unknown but could be due to differences in insulin sensitivity, although a recent study (19) did not show an association with Leu7Pro and insulin sensitivity, insulin secretion, or glucose metabolism. That study was, however, performed in healthy control subjects, whereas our study comprised type 1 diabetic patients with various stages of late complications. The association between HbA_{1c} and the Leu7Pro genotype and its possible connection with insulin sensitivity is also supported by the fact that the estimated glucose disposal rate was lower and insulin dose higher in female patients with the Pro7 substitution. Interestingly, the association between the Leu7Pro genotype

Table 2—*Clinical characteristics according to the Leu7Pro polymorphism of the NPY gene in all patients*

	All			Male			Female		
	Leu7Leu	Pro7/−	*P*	Leu7Leu	Pro7/−	*P*	Leu7Leu	Pro7/−	*P*
n (%)	866 (87)	129 (13)	—	469 (87)	69 (13)	—	397 (87)	60 (13)	—
BMI (kg/m^2)	25.1 ± 0.1	25.8 ± 0.4	0.037	25.3 ± 0.3	25.7 ± 0.2	0.413	24.8 ± 0.2	25.9 ± 0.2	0.029
Waist-to-hip ratio	0.880 ± 0.003	0.887 ± 0.008	NS	0.927 ± 0.004	0.930 ± 0.008	NS	0.824 ± 0.004	0.837 ± 0.010	NS
Coronary heart disease	68 (8)	17 (14)	0.043	47 (11)	9 (14)	0.447	21 (5)	8 (14)	0.017
Acute myocardial infarction	44 (5)	9 (7)	NS	31 (7)	7 (10)	NS	13 (3)	2 (3)	NS*
Stroke	33 (4)	4 (3)	1.000*	29 (6)	1 (1)	0.157*	4 (1)	3 (5)	0.050*
Antihypertensive medication	525 (63)	78 (62)	NS	322 (71)	44 (66)	NS	203 (52)	34 (58)	NS
HbA_{1c} (%)†	8.5 ± 0.0	8.8 ± 0.1	0.004	8.6 ± 0.3	8.9 ± 0.1	0.068	8.3 ± 0.1	8.8 ± 0.2	0.024
Total cholesterol (mmol/l)	5.20 ± 0.01	5.39 ± 0.11	NS	5.17 ± 0.05	5.38 ± 0.15	NS	5.22 ± 0.06	5.40 ± 0.16	NS
HDL cholesterol (mmol/l)	1.51 ± 0.02	1.52 ± 0.05	NS	1.37 ± 0.02	1.34 ± 0.05	NS	1.69 ± 0.03	1.74 ± 0.09	NS
LDL cholesterol (mmol/l)	3.10 ± 0.03	3.07 ± 0.09	NS	3.15 ± 0.05	3.15 ± 0.11	NS	3.03 ± 0.05	2.98 ± 0.15	NS
Triglycerides (mmol/l)‡	1.35 ± 0.03	1.65 ± 0.11	0.003	1.49 ± 0.05	1.77 ± 0.19	0.135	1.19 ± 0.03	1.50 ± 0.11	0.003

Data are means ± SE or *n* (%). *Fisher's exact two-tailed test. †$P = 0.005$, $P = 0.047$, and $P = 0.048$ in all, male, and female, respectively, when adjusted for BMI. ‡$P = 0.029$, $P = 0.275$, and $P = 0.028$ in all, male, and female, respectively, when adjusted for HbA_{1c}.

and insulin dose disappeared when it was adjusted for HbA_{1c}, suggesting a possible link between Leu7Pro, insulin sensitivity, and HbA_{1c}. Our results may indicate a role for NPY in the glucose metabolism, although the mechanisms need to be further investigated.

The lipid profile, BMI, and HbA_{1c} may partly explain why there was more CHD and proteinuria in patients with the Pro7 substitution than in those with the wild-type genotype. This is supported by the outcome in a forward logistic regression model with proteinuria as dependent variable. When the variables most strongly associated with the Leu7Pro in a univariate analysis (HbA_{1c} and serum triglycerides) were added to this model, the association between proteinuria and Leu7Pro was no longer significant (data not shown). However, the Leu7Pro polymorphism was independently associated with proteinuria and CHD in a backward stepwise logistic regression analysis with known cardiovascular risk factors as covariates. In this backwards model, Leu7Pro was independently associated with proteinuria although serum triglycerides and HbA_{1c} were included in the model, thus suggesting that there could also be a direct effect of the Leu7Pro on the renal function. This is further supported by experimental studies showing that higher growth hormone levels increase the AER (23), which is interesting because the Leu7Pro polymorphism has been shown to increase growth hormone levels (14). Furthermore, in vitro studies have shown a possible link between NPY and atherosclerosis because NPY has been shown to stimulate vascular growth (24). NPY is also a potent vasoconstrictor of renal arteries (25) and decreases renal blood flow and glomerular filtration (26), which could indicate a direct influence on the kidneys contributing to the pathogenesis of diabetic nephropathy.

The association between the Leu7Pro and diabetic nephropathy was only seen between those with normoalbuminuria and proteinuria. The fact that there was no association between the Leu7Pro and microalbuminuric patients may be explained by the fact that these patients had a rather long duration of diabetes and, according to recent epidemiological data, there is frequently a regression toward normoalbuminuria in this phenotype (27). Consequently, these patients cannot necessarily be considered to have true di-

Table 3—*Clinical characteristics according to the Leu7Pro polymorphism of the NPY gene excluding ESRD patients*

	All			Male			Female		
	Leu7Leu	Pro7/−	*P*	Leu7Leu	Pro7/−	*P*	Leu7Leu	Pro7/−	*P*
n (%)	706 (87)	105 (13)	—	372 (87)	57 (13)	—	334 (87)	48 (13)	—
Age (years)	40.0 ± 0.4	40.0 ± 1.2	NS	41.5 ± 0.5	41.8 ± 1.3	NS	39.7 ± 0.5	38.7 ± 1.5	NS
Diabetes duration (years)	26.9 ± 0.3	27.0 ± 0.9	NS	26.7 ± 0.4	28.0 ± 1.3	NS	27.0 ± 0.4	25.8 ± 1.2	NS
BMI (kg/m^2)	25.3 ± 0.1	26.1 ± 0.4	0.042	25.6 ± 0.2	25.9 ± 0.6	0.528	25.0 ± 0.2	26.3 ± 0.6	0.021
Waist-to-hip ratio	0.872 ± 0.004	0.882 ± 0.009	NS	0.921 ± 0.004	0.927 ± 0.009	NS	0.817 ± 0.004	0.826 ± 0.011	NS
Cardiovascular disease	46 (7)	12 (12)	0.061	31 (8)	6 (11)	0.567	15 (4)	6 (13)	0.020
CHD	37 (5)	11 (11)	0.033	22 (6)	6 (11)	0.200	15 (5)	5 (11)	0.086*
Acute myocardial infarction	24 (3)	6 (6)	NS	16 (4)	5 (9)	NS*	8 (2)	1 (2)	NS*
Stroke	16 (2)	3 (3)	NS*	13 (4)	1 (2)	NS*	3 (1)	2 (4)	NS*
Antihypertensive medication	393 (57)	56 (55)	NS	236 (65)	33 (60)	NS	157 (47)	13 (49)	NS
Lipid lowering medication	67 (10)	12 (12)	NS	45 (12)	7 (13)	NS	22 (7)	5 (11)	NS*
Systolic blood pressure (mmHg)	138 ± 1	136 ± 2	NS	141 ± 1	138 ± 2	NS	135 ± 1	134 ± 3	NS
Diastolic blood pressure (mmHg)	81 ± 1	80 ± 1	NS	83 ± 1	81 ± 1	NS	79 ± 1	79 ± 2	NS
HbA_{1c} (%)†	8.5 ± 0.1	9.0 ± 0.1	0.0006	8.6 ± 0.1	9.0 ± 0.2	0.0613	8.3 ± 0.1	9.0 ± 0.2	0.0027
eGDR (mg · kg^{-1} · min^{-1})	6.13 ± 0.10	5.61 ± 0.26	0.067	5.11 ± 0.13	4.92 ± 0.31	0.60	7.27 ± 0.14	6.43 ± 0.40	0.034
Insulin dose (IU/kg)‡	0.70 ± 0.01	0.73 ± 0.02	0.170	0.72 ± 0.01	0.70 ± 0.03	0.659	0.68 ± 0.01	0.77 ± 0.03	0.010
Total cholesterol (mmol/l)	5.10 ± 0.04	5.38 ± 0.13	0.023	5.10 ± 0.04	5.44 ± 0.17	0.036	5.12 ± 0.06	5.29 ± 0.15	0.315
HDL cholesterol (mmol/l)	1.52 ± 0.02	1.50 ± 0.06	NS	1.37 ± 0.02	1.33 ± 0.05	NS	1.71 ± 0.03	1.70 ± 0.10	NS
LDL cholesterol (mmol/l)	3.04 ± 0.04	3.03 ± 0.10	NS	3.12 ± 0.05	3.18 ± 0.12	NS	2.95 ± 0.05	2.86 ± 0.16	NS
Triglycerides (mmol/l)§	1.27 ± 0.03	1.72 ± 0.13	<0.0001	1.42 ± 0.05	1.82 ± 0.21	0.0521	1.10 ± 0.03	1.60 ± 0.13	<0.0001

Data are means ± SE or *n* (%). *Fisher's exact two-tailed test. †$P = 0.001$, $P = 0.061$, and $P = 0.006$ in all, male, and female, respectively, when adjusted for BMI. ‡$P = 0.630$, $P = 0.375$ and $P = 0.090$ in all, male, and female, respectively, when adjusted for HbA_{1c}. §$P = 0.001$, $P = 0.117$ and, $P = 0.001$ in all, male, and female, respectively, when adjusted for HbA_{1c}. eGDR, estimated glucose disposal rate.

Table 4—*Multiple backward stepwise linear regression analysis with HbA_{1c} as dependent variable when excluding the patients with ESRD*

	Coefficient	SE	*P*
Age	−0.023	0.005	<0.001
Triglycerides	1.205	0.260	<0.001
LDL cholesterol	0.290	0.060	<0.001
Waist-to-hip ratio	1.492	0.617	0.016
NPY Leu7Pro	0.555	0.156	<0.001

Complete data were available from 679 patients. Variables included in the model: sex, age, serum creatinine, triglycerides, LDL cholesterol, waist-to-hip ratio, systolic blood pressure, lipid lowering medication, and NPY Leu7Leu vs. Pro7/− polymorphism. Adjusted $R^2 = 0.14$.

abetic nephropathy. The lack of association between the Leu7Pro polymorphism and those with ESRD could be due to a survival bias, since those with ESRD have a high mortality rate. This is further supported by the fact that the Leu7Pro was also associated with CHD and several cardiovascular risk factors in this study. Furthermore, any population substratification with respect to Finnish ancestral history is unlikely in this study because the prevalence of the Pro7 substitution and the main results did not differ when the southwest and "isolate" eastern regions were analyzed separately (data not shown), as suggested by Perola et al. (28). In conclusion, the Leu7Pro polymorphism of the prepro-NPY may contribute to the genetic susceptibility of diabetic nephropathy and CHD, possibly by influencing the glycemic control and lipid metabolism, in type 1 diabetic patients.

Acknowledgments— This study was supported by the Finnish Medical Society, Samfundet Folkhälsan, Folkhälsan Research Foundation, The Wilhelm and Else Stockmann Foundation, the Perklén Foundation, and the Liv och Hälsa Foundation.

We acknowledge our laboratory technician Tarja Vesisenaho and research fellow Carola Saloranta for their skillful contributions. Finally, we acknowledge the physicians and nurses in each center participating in the collection of patients: Central Finland Central Hospital: A. Halonen, A. Koistinen, P. Koskiaho, M. Laukkanen, J. Saltevo, and M. Tiihonen; Central Hospital of Aland Islands: A-C. Blomqvist, M. Forsen, H. Granlund, and B. Nyroos; Central Hospital of Kanta-Hame: P. Kinnunen, A. Orvola, T. Salonen, and A. Vähänen; Central Hospital of Kymenlaakso: R. Paldanius, M. Riihelä, and L. Ryysy; Central

Table 5—*Multiple backward stepwise logistic regression analyses*

	Coefficient	SE	*P*
Proteinuria versus normal AER as dependent variable*			
Duration of diabetes	−0.031	0.015	0.036
HbA_{1c}	0.207	0.090	0.022
Triglycerides	2.942	0.613	<0.001
LDL cholesterol	0.543	0.168	0.001
Antihypertensive medication	5.287	0.598	<0.001
NPY Leu7Pro	1.250	0.457	0.006
Coronary heart disease as dependent variable in all patients†			
Age	0.092	0.010	<0.001
HbA_{1c}	0.160	0.055	<0.005
Antihypertensive medication	1.645	0.365	<0.001
NPY Leu7Pro	0.634	0.229	0.006

*Complete data were available from 679 patients. Variables included in the model were sex, duration of diabetes, BMI, HbA_{1c}, triglycerides, LDL cholesterol, current smoking, antihypertensive medication, and NPY Leu7Leu vs. Pro7/− polymorphism. $R^2 = 0.69$. †Complete data were available from 957 patients. Variables included in the model were sex, age, HbA_{1c}, LDL cholesterol, waist-to-hip ratio, current smoking, antihypertensive medication, and NPY Leu7Leu vs. Pro7/− polymorphism. $R^2 = 0.14$.

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