Diabetologia (2001) 44: 2220–2226

Diabetologia

Mutation analysis of peroxisome proliferator-activated receptor-γ coactivator-1 (*PGC-1*) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus

J. Ek[1], **G. Andersen**[1], **S. A. Urhammer**[1], **P. H. Gæde**[1], **T. Drivsholm**[2], **K. Borch-Johnsen**[1], **T. Hansen**[1], **O. Pedersen**[1]

[1] Steno Diabetes Center and Hagedorn Research Institute, Gentofte, Copenhagen, Denmark
[2] Centre of Preventive Medicine, Glostrup University Hospital, Denmark

Abstract

Aim/hypothesis. This study aimed to investigate if variability in the peroxisome proliferator-activated receptor-γ coactivator-1 (*PGC-1*) gene is associated with Type II (non-insulin-dependent) diabetes mellitus.
Methods. The *PGC-1* gene was examined in 53 Type II diabetic patients applying single strand conformational polymorphism analysis followed by nucleotide sequencing. Identified variants were genotyped in an association study comprising 483 Type II diabetic patients and 216 glucose-tolerant control subjects. A replication study was done in an additional 201 Type II diabetic patients and 293 glucose-tolerant subjects. Furthermore, a potential interaction between the *Pro12Ala* polymorphism of *PPAR-γ2* and the *PGC-1 Gly482Ser* variant on risk of Type II diabetes was investigated.
Results. A total of seven variants *(Ser74Leu*, *IVS2 + 52C→A*, *Thr394Thr*, *Asp475Asp*, *Gly482Ser*, *Thr528Thr*, and *Thr612Met*) were identified and investigated in an association study. Six of the variants showed no association with Type II diabetes in the initial study. However, the *Gly482Ser* polymorphism, was more frequent among Type II diabetic patients (37.0 %) than among glucose-tolerant subjects (30.8 %) ($p = 0.032$). In a replication study the difference in allele frequencies of the *Gly482Ser* variant remained significant ($p = 0.0135$). The combined study yielded an allele frequency of 37.3 % (34.7–39.9) for Type II diabetic patients and 30.5 % (27.7–33.4) for glucose-tolerant subjects ($p = 0.0007$). No interaction between this variant and the *Pro12Ala* polymorphism of *PPAR-γ2* was observed.
Conclusion/interpretation. A widespread *Gly482Ser* polymorphism of *PGC-1* is associated with a 1.34 genotype relative risk of Type II diabetes. [Diabetologia (2001) 44: 2220–2226]

Keywords PPAR-γ coactivator-1, mutations, Type II diabetes, genetic epidemiology, association study.

Peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) is a novel transcriptional co-activator of a series of nuclear receptors including peroxisome proliferator-activated receptor-γ (PPAR-γ), a transcription factor involved in adipogenesis and a functional receptor for thiazolidinediones [1, 2]. Similarly, *PGC-1* is a coactivator of peroxisome proliferator-activated receptor-α (PPAR-α), which plays a key role in the transcriptional control of genes encoding mitochondrial fatty acid beta-oxidation enzymes [3]. Studies in cultured muscle-cell lines show that PGC-1 stimulates mitochondrial biogenesis and respiration through an induction of uncoupling protein 2 and through regulation of the nuclear respiratory factors [2]. Thus, PGC-1 is a key factor in the stimulation of adaptive thermogenesis, e.g. during high caloric diets or cold exposure. It has recently

Received: 4 April 2001 and in revised form: 19 July 2001

Corresponding author: Oluf Pedersen, Professor, M.D., D.M.Sc., Steno Diabetes Center, Niels Steensens Vej 2, DK-2820 Gentofte, Copenhagen, Denmark,
E-mail: oluf.p@dadlnet.dk
Abbreviations: PPAR, peroxisome proliferator-activated receptor; PGC-1, PPAR-γ coactivator-1; MEF2C, myocyte enhancer factor 2C; OHA, oral hypoglycaemic agent; SSCP, single strand conformational polymorphism; GRR, genotype relative risk.

been shown that PGC-1 by binding to myocyte enhancer factor 2C (MEF2C) also controls the expression of the endogenous glucose transporter (*GLUT4*) gene in muscle cells [4]. In humans PGC-1 is expressed in high quantities in liver, heart, kidney, and skeletal muscle and to a lesser extent in white adipose tissue, pancreas, and brain [5, 6]. Given the critical roles of PGC-1 in several aspects of adipogenesis, oxidative metabolism, thermogenesis and glucose uptake and the fact that human *PGC-1* is mapped to a chromosomal region (4p15.1) that in Pima Indians has been shown to be linked to fasting serum insulin concentrations [7] we hypothesized that variability in the *PGC-1* gene confers susceptibility to Type II diabetes. This study reports the results of the mutation analysis of the entire coding region of the *PGC-1* gene and the identification of a widespread amino acid polymorphism, which is reproducibly associated with Type II diabetes among Danish Caucasians.

Subjects and methods

Subjects. Mutation analysis was completed in 53 Type II diabetic patients (30 men, 23 women) recruited from the outpatient clinic at Steno Diabetes Center. The age of the patients was 64 ± 9 years, age of diagnosis 57 ± 9 years, body mass index (BMI) 29.7 ± 4.9 kg/m^2, and HbA_{1C} 8.3 ± 1.7 % (means ± SD). More than 70 % of the patients fulfilled the 1998 WHO criteria for the metabolic syndrome [8], 31 % of the patients were treated with diet alone, 65 % with oral hypoglycaemic agents (OHA), and 4 % with insulin alone or in combination with OHA.

The initial association studies were done in a group of unrelated Type II diabetic patients recruited from the outpatient clinic at Steno Diabetes Center during 1994–1997 and a group of unrelated glucose-tolerant subjects without a known family history of diabetes sampled at random during 1994–1997 from the Danish Central Population Register and all living in the same area of Copenhagen as the Type II diabetic patients. In the group of Type II diabetic patients (n = 483, 278 men, 205 women) the age was 61 ± 11 years, age of diagnosis 55 ± 11 years, BMI 29.0 ± 5.3 kg/m^2, and HbA_{1C} 8.1 ± 1.6 %. The patients were treated with diet alone (27 %), with OHA (58 %), or with insulin in combination with OHA (15 %). In the group of glucose tolerant subjects (n = 216, 105 men, 111 women) the age was 52 ± 14 years, and BMI 25.3 ± 3.8 kg/m^2.

The association study used for replication comprised unrelated Type II diabetic patients recruited from the outpatient clinic at Steno Diabetes Center during 1992–1993 and a population based sample of unrelated glucose-tolerant subjects without a known family history of diabetes born in 1936 and examined during 1996–1997 at the Copenhagen County Center of Preventive Medicine. In the group of Type II diabetic patients (n = 201, 152 men, 49 women) the age was 55 ± 7 years, age of diagnosis 48 ± 8 years, BMI 29.8 ± 4.4 kg/m^2, and HbA_{1C} 8.6 ± 1.7 %. The patients were treated with diet alone (29 %), with OHA (60 %), or with insulin in combination with OHA (11 %). In the group of glucose-tolerant subjects (n = 293, 134 men, 159 women) the age was 60.5 ± 0.4 years and BMI 26.2 ± 3.7 kg/m^2.

Diabetes was diagnosed according to 1998 WHO criteria [8]. All glucose-tolerant subjects underwent a 75 g oral glucose tolerance test (OGTT). All participants were Danish Caucasians by self-report. Informed written consent was obtained from all subjects before participation. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II.

Biochemical assays. Blood samples for measurement of serum concentrations of insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and plasma glucose and non-esterified fatty acids (NEFA) were drawn after a 12-h overnight fast. Serum triglycerides, total serum cholesterol, serum HDL-cholesterol, and plasma NEFA were analysed using enzymatic colorimetric methods (GPO-PAP and CHOD-PAP, Roche Molecular Biochemicals, Germany and NEFA C, Wako, Neuss, Germany). The plasma glucose concentration was analysed by a glucose oxidase method (Granutest, Merck, Darmstadt, Germany) and serum specific insulin (excluding des(31,32)- and intact proinsulin) was measured by ELISA (Dako insulin kit K6219, Dako Diagnostics, Ely, UK). HbA_{1C} was measured by ion-exchange high performance liquid chromatography (non-diabetic reference range: 4.1–6.4 %).

Mutation analysis and genotyping. The genetic analyses were done on genomic DNA isolated from human leukocytes. The coding region of the *PGC-1* gene (EMBL AF106698) including intron-exon boundaries (in total 3357 bp) was divided into 17 segments (sized 145–273 nucleotides) for SSCP and heteroduplex analysis. In our laboratory, this method has an estimated sensitivity of more than 95 % for detecting a variety of known mutations. The segments also included the 5' untranslated sequence of 90 bp. Primer sequences are listed in Table 1. PCR amplification was carried out in a volume of 25 µl containing 100 ng genomic DNA, 1 × PCR-buffer, 0.2 µmol/l of each primer, 0.2 mmol/l dNTP, 10 mCi/ml α-^{32}P-dCTP, 0.6 units AmpliTaq Gold polymerase (Perkin Elmer, Foster City, Calif., USA) and $MgCl_2$ concentration as shown in Table 1. The cycling programme was a denaturation step at 95 °C for 15 min followed by 40 cycles of 94 °C for 30 s, annealing at T_{anneal} for 30 s, and elongation at 72 °C for 60 s with a final elongation step at 72 °C for 9 min using a GeneAmp 9600 thermal cycler (Perkin Elmer). The annealing temperatures are listed in Table 1. SSCP was carried out at two different experimental settings as reported [9] and aberrantly migrating samples were sequenced using fluorescent chemistry (Dye Primer Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Calif., USA). The *Ser74Leu* and *IVS2 + 52C→A* variants were genotyped by PCR with primers PC2F-PC2RNY followed by digestion with *Dra*I and *Apa*I, respectively. The *Thr394Thr* variant was genotyped employing restriction site generating (RG) PCR (2 mmol/l $MgCl_2$, T_{anneal} 55 °C) with upstream RG-primer 5'-GCC AGT CAA TTA ATT CCA AAC C-3' (mismatched nucleotide is underlined) and downstream primer PC15R followed by digestion with *Hpa*II. The *Asp475Asp* variant was genotyped using RG-PCR (2 mmol/l $MgCl_2$, T_{anneal} 59 °C) with upstream RG-primer 5'-ATC CCA GTC AAG CTG TTT TTC T-3' and downstream RG-primer 5'-GAA GAA CAA GAA GGA GAC ACA TCG-3' followed by digestion with *Taq*I. The *Gly482Ser* variant was amplified with primers PC15F-PC17R and digested with *Hpa*II. The *Thr528Thr* variant was genotyped using RG-PCR (2 mmol/l $MgCl_2$, T_{anneal} 60 °C, upstream RG-primer 5'-GAC GAC GAA GCA GGC AAG-3', downstream primer 5'-GAT TTG GGT GGT GAC ACA GA-3') with subsequent digestion with *Cac*8I. The *Thr612Met* variant was amplified with primers PC8F-PC8R and digested with *Nla*III. The *Pro12Ala* polymorphism of *PPAR-γ2* was genotyped by RG-PCR (3 mmol/l $MgCl_2$, T_{anneal}

Table 1. Primer sequences and PCR conditions for mutational analysis of *PGC-1*

Primer	Sequence, 5'→ 3'	Location	T_{anneal}	$MgCl_2$
PC1F	CTG GGG ACT GTA GTA AGA C	5'UTR +		
PC1R	AGG GAA GCG TCA GTT GTG G	Exon 1	55 °C	1 mmol/l
PC2F	CCT GTG GTT AAT GGA AGC			
PC2RNY	GCC CAA GCC AAA CTC AAT G	Exon 2	50 °C	2 mmol/l
PC3F	CTG CCT CCC AGG GTC AAC			
PC3R	CAA CTC CAA TTC CTG CTA AAC	Exon 3	55 °C	1 mmol/l
PC4F	GAT GCA TAA CTT TAC TTG			
PC4R	CTG CTT CAA GCC AAA ATC	Exon 4	50 °C	2 mmol/l
PC5F	CTG ATA AGG TTC AGT TCA C			
PC5R	CCT CAC CAA CAG CTC GT	Exon 5	50 °C	2 mmol/l
PC6F	CCA ACT TGA CTG TTG TGG AG			
PC6R	ACA AAC TGA AAT GGA GTT GC	Exon 6	55 °C	2 mmol/l
PC7F	GGG TTC TAA TAC ATT TGG C			
PC7R	CAC ATA GAC AGT ACA TCT	Exon 7	50 °C	2 mmol/l
PC8F	GTT AAG TGG CAG TTG CAA ATG			
PC8R	GGG AGC TAA AGG AAA ATG AC	Exon 9	55 °C	2 mmol/l
PC9F	GGT GGT TGA CTT AGT GAT AAA G			
PC9R	CAC AGA AAA AGA AGA AAC CCT AC	Exon 10	55 °C	3 mmol/l
PC10F	CCA CTC CAG AAC TCT CTC C			
PC10R	CAA CTC CCA TCC CAG TAA TC	Exon 11	55 °C	1 mmol/l
PC11F	GGT TAC AGT CCC ATA TAC T			
PC11R	GAT TCC TCA TTC CAC GTA C	Exon 12	50 °C	3 mmol/l
PC12F	GCC ATC AGC AAA GTG TGT			
PC12R	TGA GGT ATT CGC CAT CCC	Exon 13	50 °C	2 mmol/l
PC13F	GAA ACA TGT GTC TTC GCA			
PC14R	CGC TTG GTC TTC CTT TCC TCG	Exon 8	55 °C	2 mmol/l
PC15F	CAA GTC CTC AGT CCT CAC			
PC15R	CTT GCC TCC AAA GTC TCT C	Exon 8	50 °C	2 mmol/l
PC16F	CAG ATT CAG ACC AGT G			
PC16R	CAT AGG TAG TTT GGA G	Exon 8	45 °C	1 mmol/l
PC17F	GGG ACA GTG ATT TCA GTA ATG			
PC17R	GGG GTC TTT GAG AAA ATA AGG	Exon 8	55 °C	1 mmol/l
PC18F	GTA GAG ATT CTG TGT CAC			
PC18R	CTT TTG TGT TAT TTA GGG	Exon 8	45 °C	2 mmol/l

All forward primers were extended with a 21M13 tail for sequencing (TGT AAA ACG ACG GCC AGT) and all reverse primers with an M13 tail (CAG GAA ACA GCT AGT ACC)

53 °C) with upstream primer 5'-CAA GCC CAG TCC TTT CTG TG-3' and downstream RG-primer 5'-AGT GAA GGA ATC GCT TTC *C*G-3' (derived from EMBL AB005520) followed by digestion with *Hpa*II. All restriction enzyme digests were separated on 4 % agarose gels.

Statistical analysis. Fisher's exact test was applied to examine differences in allele frequencies between diabetic and non-diabetic subjects. A general linear model was used to test variables (or transformed variables) for differences between genotype groups. Genotype and gender were considered as fixed factors and age and BMI as covariates. A p-value of less than 0.05 was considered significant. All analyses were done using Statistical Package for Social Science (SPSS, Chicago. Ill., USA) version 10.0. The genotype relative risk (GRR) was estimated by logistic regression from the genotype data using a log-additive model for the risk. Test for additivity gave a likelihood ratio statistic of 0.285 on 1 df ($p = 0.593$). Interaction between the *Pro12Ala* polymorphism of *PPAR-γ2* and the *Gly482Ser* polymorphism of *PGC-1* was tested using logistic regression.

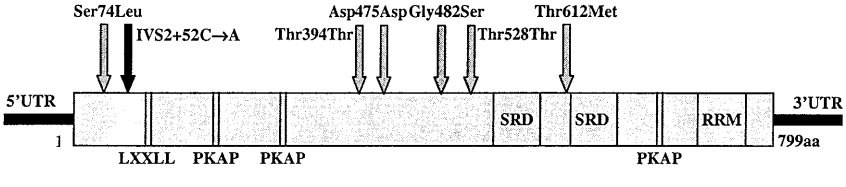

Fig. 1. Schematic presentation of identified PGC-1 variants and approximate positions relative to known functional domains. LXXLL, recognition site (LXXLL motif); PKAP, protein kinase A phosphorylation consensus site; SRD, serine and arginine rich domain; RRM, RNA recognition motif

Table 2. Genotype and allele frequencies of the examined variants in the *PGC-1* gene in Type II diabetic patients and glucose tolerant subjects

	Initial association study			Replication study			Combined study		
	Type II diabetic patients	Glucose-tolerant subjects	*p*	Type II diabetic patients	Glucose-tolerant subjects	*p*	Type II diabetic subjects	Glucose-tolerant subjects	*p*
Ser 74Leu									
Ser/Ser	466 (99)	197 (99)		223 (99)	290 (99)		689 (99)	487 (99)	
Ser/Leu	3 (1)	1 (1)		2 (1)	2 (1)		5 (1)	3 (1)	
Leu/Leu	0 (0)	0 (0)	1.0[a]	0 (0)	0 (0)	1.0[a]	0 (0)	0 (0)	1.0[a]
Allele frequency	0.3 (0–0.7)	0.3 (0–0.7)	1.0	0.4 (0–1.0)	0.3 (0–0.8)	1.0	0.4 (0–0.7)	0.3 (0–0.7)	1.0
IVS2 + 52C→A									
C/C	178 (37)	62 (30)		74 (33)	107 (37)		252 (36)	169 (34)	
C/A	221 (46)	102 (50)		110 (49)	142 (49)		331 (47)	244 (49)	
A/A	79 (17)	40 (20)	0.2[a]	39 (17)	42 (14)	0.5[a]	118 (17)	82 (17)	0.8[a]
Allele frequency	39.6 (36.6–42.7)	44.6 (39.8–49.4)	0.09	42.2 (37.6–46.7)	38.8 (34.9–42.8)	0.3	40.4 (37.9–43.0)	41.2 (38. 1–44.3)	0.7
Gly482Ser									
Gly/Gly	186 (41)	97 (49)		76 (38)	146 (50)		262 (40)	243 (49)	
Gly/Ser	200 (44)	80 (40)		97 (48)	116 (40)		297 (45)	196 (40)	
Ser/Ser	68 (15)	21 (11)	0.11[a]	28 (14)	31 (10)	0.03[a]	96 (15)	52 (11)	0.0035[a]
Allele frequency	37.0 (33.8–40.1)	30.8 (26.2–35.3)	0.032	38. 1 (33.3–42.8)	30.4 (26.7–34.0)	0.0135	37.3 (34.7–39.9)	30.5 (27.7–33.4)	0.0007
Thr612Met									
Thr/Thr	443 (93)	183 (90)		211 (96)	260 (89)		654 (94)	443 (90)	
Thr/Met	31 (7)	20 (10)		9 (4)	31 (11)		40 (6)	51 (10)	
Met/Met	1 (0)	0 (0)	0.2[a]	0 (0)	0 (0)	0.007[a]	1 (0)	0 (0)	0.005[a]
Allele frequency	3.5 (2.3–4.6)	4.9 (2.8–7.0)	0.2	2.1 (0.7–3.0)	5.3 (3.5–7.2)	0.009	3.0 (2.1–3.9)	5.2 (3.8–6.5)	0.01

Data are number of subjects with each genotype (% of each group) and allele frequencies of minor allele in % (95%-CI). The *p* values compare genotype distribution ([a]) and allele frequencies between Type II diabetic patients and glucose tolerant subjects

Results

The mutation screening covered the coding region of *PGC-1*. In the 53 Type II diabetic patients, we identified a total of 7 different variants (Fig. 1): *Ser74Leu* (identified in 2 out of 53 patients, nucleotide position 341: TCA→TTA), *IVS2 + 52C→A* (19 patients), *Thr394Thr* (11 patients, 1302: ACG→ACA), *Asp475Asp* (13 patients, 1545: GAC→GAT), *Gly482Ser* (24 patients, 1564: GGT→AGT), *Thr528Thr* (37 patients, 1704: ACA→ACG), and *Thr612Met* (3 patients, 1955: ACG→ATG). The variants were further examined in an association study comprising 483 Type II diabetic patients and 216 glucose-tolerant control subjects and in a replication study comprising 201 Type II diabetic patients and 293 glucose-tolerant control subjects. All variants were in Hardy-Weinberg equilibrium. The allele frequencies of the *Ser74Leu*, *IVS2 + 52C→A*, *Asp475Asp*, and *Thr528Thr* variants did not differ significantly between diabetic and non-diabetic subjects (Table 2 and data not shown). The common Gly-allele of codon 482 and the common C-allele of the intronic variant segregated together (disequilibrium coefficient D = 0.04). The allele frequencies of the *Ser74Leu* and *Thr612Met* variants were not sufficiently high to provide reliable estimates of linkage disequilibrium with the *Gly482Ser* polymorphism. The minor *Ser*-allele of codon 482 and the minor Asp-allele of codon 475 (allele frequencies: NGT subjects, 5.6%; diabetic patients, 8.6%) segregated together (D = –0.02). The common *Thr528Thr* substitution allele (NGT subjects, 38.5%; diabetic patients, 45.3%) segregated with the *Ser*-allele of codon 482 (D = 0.21). The minor Ser-allele of codon 482 and the minor *Thr*-allele of codon 394 segregated together (D = –0.07).

The allele frequency of the *Gly482Ser* variant was higher among Type II diabetic patients compared to glucose-tolerant subjects (37.0% vs 30.8%, $p = 0.032$) (Table 2). In a replication study the differences in allele frequencies remained significant (38.1% vs 30.4%, $p = 0.0135$). The combined study yielded an allelic frequency of 37.3% for the Type II diabetic patients and 30.5% for the glucose-tolerant subjects ($p = 0.0007$). The genotype relative risk for diabetes was estimated to 1.34 (95%-CI: 1.13–1.59) corresponding to a population attributable risk of 18%. In the combined group of diabetic subjects, carriers of the *Gly482Ser* polymorphism did not differ significantly from wildtype carriers in clinical or biochemical values including age of diabetes onset, BMI, waist circumference, treatment, degree and prevalence of micro- and macrovascular complications, HbA_{1C} or fasting serum lipids (data not shown). Moreover, in the glucose-tolerant subjects there was no evidence of a relation between

Table 3. Clinical and biochemical characteristics of two normal glucose-tolerant Danish Caucasian study samples classified according to *PGC-1 Gly482Ser* genotype

	Gly/Gly	*Gly/Ser*	*Ser/Ser*	*p*
Glucose-tolerant subjects (n = 198)				
n(men/ women)	97 (45/52)	80 (38/42)	21 (10/11)	
Age (years)	51 ± 14	53 ± 13	50 ± 14	
BMI (kg/m^2)	25.4 ± 4.0	25.3 ± 3.7	24.2 ± 3.6	0.5
Fasting-plasma-glucose (mmol/l)	5.1 ± 0.4	5.1 ± 0.5	5.1 ± 0.5	0.8
Fasting-serum-insulin (pmol/l)	42.1 ± 21.2	37.1 ± 18.0	44.1 ± 21.3	0.06
Fasting-serum-triglyceride (mmol/l)	1.2 ± 0.7	1.2 ± 0.7	1.0 ± 0.5	0.7
Fasting-serum-total cholesterol (mmol/l)	5.4 ± 1.0	5.7 ± 1.1	4.9 ± 1.3	0.015
Fasting-serum-HDL-cholesterol (mmol/l)	1.4 ± 0.4	1.5 ± 0.4	1.3 ± 0.3	0.006
Glucose-tolerant subjects (n = 293)				
n (men/women)	146 (68/78)	116 (49/67)	31 (17/14)	
Age (years)	61 ± 0.5	61 ± 0.4	60 ± 0.4	
BMI (kg/m^2)	25.9 ± 3.2	26.5 ± 4.2	26.0 ± 3.6	0.4
Fasting-plasma-glucose (mmol/l)	5.2 ± 0.5	5.1 ± 0.5	5.1 ± 0.5	0.08
Fasting-serum-insulin (pmol/l)	39.1 ± 19.2	39.9 ± 25.1	37.6 ± 16.0	0.9
Fasting-serum-triglyceride (mmol/l)	1.3 ± 0.7	1.4 ± 0.9	1.3 ± 0.6	0.3
Fasting-serum-total cholesterol (mmol/l)	6.3 ± 0.9	6.2 ± 1.0	6.2 ± 1.3	0.5
Fasting-serum-HDL-cholesterol (mmol/l)	1.5 ± 0.4	1.5 ± 0.5	1.4 ± 0.4	0.3

Data are means ± standard deviation. Values of insulin were logarithmically transformed. p values were adjusted for age, gender, and BMI

Table 4. Distribution of genotypes defined by the *Gly482Ser* variant of *PGC-1* and the *Pro12Ala* variant of *PPAR-γ2* among Type II diabetic patients and glucose tolerant subjects

PPAR-γ2 Pro12Ala genotype	Protective *PPAR-γ2* variant *Ala/X*			Diabetogenic *PPAR-γ2* variant *Pro/Pro*		
PGC-1 Gly482Ser genotype	*Gly/Gly*	*Gly/Ser*	*Ser/Ser*	*Gly/Gly*	*Gly/Ser*	*Ser/Ser*
Type II diabetic patients	43 (41)	48 (46)	13 (13)	134 (42)	140 (43)	48 (15)
Glucose-tolerant subjects	56 (46)	55 (45)	10 (8)	183 (50)	139 (38)	42 (12)

Data are number of subjects with each genotype combination (% of each group). A test for synergistic effect for risk of Type II diabetes using logistic regression was not significant (p = 0.7)

the codon 482 variant and estimates of BMI, waist circumference, fasting serum triglycerides, plasma free fatty acids or plasma glucose, serum insulin and serum C-peptide in the fasting state or during an OGTT (Table 3 and data not given). There was a strong association of the *Gly482Ser* variant with fasting serum total and HDL-cholesterol (p = 0.015 and p = 0.006, respectively) in the initial study. However, this association was not observed in the replication study (Table 3).

When stratifying the examined study population according to the *Gly482Ser* genotype for *Pro12Ala* genotype of *PPAR-γ2* the genotype distribution and allele frequencies of the Ser-allele in relation to Type II diabetes remained unchanged (Table 4). No interaction of the two polymorphisms on risk of Type II diabetes was observed (p = 0.7).

The allele frequency of the *Thr612Met* variant was lower among Type II diabetic patients than among normal glucose-tolerant control subjects in the replication study (2.1 % vs 5.3 %, p = 0.009) but not in the initial study (3.5 % vs 4.9 %, p = 0.2) (Table 2). The combined study yielded an allele frequency of 3.0 % among Type II diabetic patients and 5.2 % among glucose-tolerant control subjects (p = 0.01). The genotype relative risk for diabetes was estimated to 0.57 (95 %-CI: 0.37 – 0.86). In the combined group of diabetic subjects, carriers of the *Thr612Met* variant did not differ significantly from wildtype carriers in clinical or biochemical values (data not shown). Moreover, in the glucose-tolerant subjects there was no evidence of a relation between the codon 612 variant and clinical or biochemical estimates (data not shown).

The allele frequency of the *Ser74Leu* and the *IVS2 + 52C→A* variants were not statistically different between diabetic and control subjects (Table 2). The allele frequencies of the *Thr394Thr* variant did not differ significantly between diabetic and non-diabetic subjects in the initial study (19.8 % vs 23.4 %, p = 0.1) or in the replication study (19.7 % vs 24.3 %, p = 0.08). However, in the combined study the allele frequency was lower among Type II diabetic patients compared to glucose-tolerant control subjects (19.7 % vs. 23.9 %, p = 0.02) (data not shown).

Discussion

There is extensive circumstantial evidence from family investigations including studies in twins and from studies of hybrid populations descended from high-risk and low-risk ancestral populations in favour of genetic determinants for the common late onset form of Type II diabetes. It is also likely that Type II diabetes in many cases is polygenic and it is suggested that subsets of patients display changes in various diabetes susceptibility genes thereby adding to the heterogeneity of Type II diabetes.

Among the few Type II diabetes susceptibility gene variants, which have been reproducibly reported to be associated with Type II diabetes are the *Pro12Ala* polymorphism of *PPAR-γ* and polymorphisms in the *CAPN10* gene, the *SUR* gene, and the *KIR6.2* gene [10–20]. In a study of more than 3000 subjects the common *Pro*-allele of *PPAR-γ2* has been shown to confer a 1.25-fold increase in risk of Type II diabetes [12]. The diabetogenic effect of this variant appears to be mediated through a weak impairment of whole body insulin sensitivity [10, 11]. These findings prompted us to examine the *PGC-1* gene for variability, which might be associated with Type II diabetes because PGC-1, besides being a co-activator of PPAR-γ and -α, has a critical role in glucose uptake and adaptive thermogenesis [1]. Only one out of seven tested gene variants – the *Gly482Ser* polymorphism – showed nominal allelic association in the initial association study. Testing of this variant in a replication sample confirmed the association of the polymorphism to Type II diabetes. Combining the initial and the replication samples showed a 1.34-fold increase in diabetes risk associated with the *Ser*-allele of *Gly482Ser*. Although this diabetes-susceptibility effect seems to be small, it translates into a considerable population attributable risk of 18% due to the high frequency of the risk allele.

In this study, we failed to relate the *Gly482Ser* polymorphism to subphenotypes like BMI, waist circumference, plasma glucose, serum insulin and serum C-peptide during an OGTT in the glucose tolerant subjects. Similarly, we were not able to associate the *PGC-1* variant with biochemical and anthropometric characteristics or age of clinical disease onset in the two groups of diabetic subjects. However, in the initial study of glucose-tolerant subjects we did observe a significant association of the *Gly482Ser* polymorphism with fasting serum cholesterol levels, which could be consistent with the role of PGC-1 as a coactivator of PPAR-α, in which genetic variability confers alterations in circulating cholesterol levels [21].

Even though the *PGC-1* Gly482Ser variant is associated with Type II diabetes in the examined populations this variant might not be the causative polymorphism but could be in linkage disequilibrium with an as yet unidentified aetiological variant. *Gly482Ser* is located in a part of the protein whose function is not known and glycine at residue 482 is not conserved between human beings and mice. However, a recent study showed that residues 403–570 of PGC-1 are critical for its interaction with MEF2C and thereby the ability of PGC-1 to restore insulin sensitive GLUT4 expression [4].

Intriguingly, homozygosity for the intronic variant *IVS2 + 52C→A* of the *PGC-1* gene has been reported to be associated with a decrease in age-adjusted and sex-adjusted BMI in a study of 964 American Caucasian subjects suggesting that this noncoding variant could act protectively against fat accumulation [22]. Because stimulation of the β-3 adrenergic receptor (β3AR) has been shown to induce PGC-1 expression [2], the same authors also tested the interaction between the intronic *PGC-1* variant and the *Trp64Arg* variant in *β3AR* [22], which has been shown to be associated with features of the metabolic syndrome [23–25]. They found that the *IVS2 + 52C→A* variant of *PGC-1* decreased the odds of obesity in the absence of the Arg-allele but not in the presence of the Arg-allele. In this study, the *IVS2 + 52C→A* variant was not associated with Type II diabetes nor was homozygosity of the variant associated with a higher BMI in the diabetic or the normal glucose-tolerant subjects.

In the initial association study, the *Thr612Met* variant was not associated with Type II diabetes. In contrast, the variant was found to be associated with Type II diabetes both in the replication study and in the combined study. It is not clear whether this observation is due to linkage disequilibrium with the *Gly482Ser* variant or whether it is a true functional variant providing a reduced risk of developing Type II diabetes or a chance finding due to multiple testing of various gene variants. *Thr612Met* is located in a part of the protein whose function is not known and is conserved between human beings and mice.

In this study a test for interaction between the *Gly482Ser* variant and the *Pro12Ala* variant in *PPAR-γ2* gave no indication for additive effects on diabetes status. However, due to the polygenic nature of the common forms of Type II diabetes, future studies should examine the potential interactions of the *PGC-1 Gly482Ser*, *PPAR-γ Pro12Ala* and *β3AR Trp64Arg* polymorphisms to see if they have additive or synergistic impact on the susceptibility to common subsets of Type II diabetes mellitus.

Acknowledgements. The authors would like to thank A. Forman, L. Aabo, I. L. Wantzin, and C. B. P. Søholm for their dedicated technical assistance, and G. Lademann for secretarial support. The study was supported by the Danish Medical Research Council, the Danish Diabetes Association, the Danish Heart Foundation, the Velux Foundation, and EEC (BMH4-CT98–3084 and QLRT-CT-1999–00546).

References

1. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92: 829–839
2. Wu Z, Puigserver P, Andersson U et al. (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98: 115–124
3. Vega RB, Huss JM, Kelly DP (2000) The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor-α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20: 1868–1876
4. Michael LF, Wu Z, Cheatham B et al. (2001) Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. Proc Natl Acad Sci USA 98: 3820–3825
5. Larrouy D, Vidal H, Andreelli F, Laville M, Langin D (1999) Cloning and mRNA tissue distribution of human PPAR-γ coactivator-1. Int J Obes 23: 1327–1332
6. Esterbauer H, Oberkofler H, Krempler F, Patsch W (1999) Human peroxisome proliferator activated receptor gamma coactivator 1 (PPARGC1) gene: cDNA sequence, genomic organization, chromosomal localization, and tissue expression. Genomics 62: 98–102
7. Pratley RE, Thompson DB, Prochazka M et al. (1998) An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. J Clin Invest 101: 1757–1764
8. Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15: 539–553
9. Hansen T, Andersen CB, Echwald SM et al. (1997) Identification of a common amino acid polymorphism in the p85-α regulatory subunit of phosphatidylinositol 3-kinase: effects on glucose disappearance constant, glucose effectiveness, and the insulin sensitivity index. Diabetes 46: 494–501
10. Deeb SS, Fajas L, Nemoto M et al. (1998) A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 20: 284–287
11. Hara K, Okada T, Tobe K et al. (2000) The Pro12Ala polymorphism in PPAR γ2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun 271: 212–216
12. Altshuler D, Hirschhorn JN, Klannemark M et al. (2000) The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet 26: 76–80
13. Douglas JA, Erdos MR, Watanabe RM et al. (2001) The peroxisome proliferator-activated receptor-γ2 Pro12Ala variant. Association With Type II Diabetes and Trait Differences. Diabetes 50: 886–890
14. Mori H, Ikegami H, Kawaguchi Y et al. (2001) The $Pro^{12}\rightarrow$Ala substitution in PPAR-γ is associated with resistance to development of diabetes in the general population. Diabetes 50: 891–894
15. Horikawa Y, Oda N, Cox NJ et al. (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26: 163–175
16. Inoue H, Ferrer J, Welling CM et al. (1996) Sequence variants in the sulfonylurea receptor (SUR) gene are associated with NIDDM in Caucasians. Diabetes 45: 825–831
17. Hani EH, Clement K, Velho G et al. (1997) Genetic studies of the sulfonylurea receptor gene locus in NIDDM and in morbid obesity among French Caucasians. Diabetes 46: 688–694
18. Hansen T, Echwald SM, Hansen L et al. (1998) Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene. Diabetes 47: 598–605
19. Hart LM, de Knijff P, Dekker JM et al. (1999) Variants in the sulphonylurea receptor gene: association of the exon 16–3t variant with Type II diabetes mellitus in Dutch Caucasians. Diabetologia 42: 617–620
20. Hani EH, Boutin P, Durand E et al. (1998) Missense mutations in the pancreatic islet beta cell inwardly rectifying K^+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. Diabetologia 41: 1511–1515
21. Flavell DM, Torra IP, Jamshidi Y et al. (2000) Variation in the PPAR alpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. Diabetologia 43: 673–680
22. Fan Z, Pollin TI, Gong D et al. (2000) Association of an intronic variant of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) with decreased body mass index (BMI) in Caucasians and interaction with the Trp64Arg variant of the β-3 adrenergic receptor. Am J Hum Genet 67: 234 (Abstract)
23. Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC (1995) Association of a polymorphism in the β_3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333: 348–351
24. Clement K, Vaisse C, Manning BS et al. (1995) Genetic variation in the β_3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 333: 352–354
25. Kadowaki H, Yasuda K, Iwamoto K et al. (1995) A mutation in the β_3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 215: 555–560