

Common polymorphisms of the *PPAR-γ2* (*Pro12Ala*) and *PGC-1α* (*Gly482Ser*) genes are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial

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Abstract

Aim/hypothesis. We investigated the effects of the common polymorphisms in the peroxisome proliferator-activated receptor $\gamma 2$ (*PPAR-γ2*; *Pro12Ala*) and in *PPAR-γ* coactivator 1 α (*PGC-1α*; *Gly482Ser*) genes on the conversion from impaired glucose tolerance to type 2 diabetes in participants in the STOP-NIDDM trial. This trial aimed to study the effect of acarbose in the prevention of type 2 diabetes.

Methods. Genotyping was performed in 770 study subjects whose DNA was available. The *Gly482Ser* variant in the *PGC-1α* gene was determined with the polymerase chain reaction amplification, Hpa II enzyme digestion, and gel electrophoresis. The *Pro12Ala* polymorphism of the *PPAR-γ2* gene was determined by the polymerase chain reaction–single-strand conformation polymorphism analysis.

Results. The *Pro12Pro* genotype of the *PPAR-γ2* gene predicted the conversion to diabetes in women in the

acarbose group (odds ratio 2.89, 95% CI 1.20 to 6.96; $p=0.018$). The *482Ser* allele of the *PGC-1α* gene had a significant interaction with the mode of treatment ($p=0.012$), and in the placebo group the *482Ser* allele was associated with a 1.6-fold higher risk for type 2 diabetes compared to the *Gly482Gly* genotype (95% CI 1.06 to 2.33; $p=0.023$). Acarbose prevented the development of diabetes independently of the genotype of the *PPAR-γ2* gene, but only the carriers of the *482Ser* allele of the *PGC-1α* gene were responsive to acarbose treatment.

Conclusion/interpretation. We conclude that the *Pro12Pro* genotype of the *PPAR-γ2* gene and the *482Ser* allele of the *PGC-1α* gene are associated with the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial.

Keywords Impaired glucose tolerance · PGC-1 alpha · Polymorphism · PPAR gamma · Type 2 diabetes.

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Abbreviations: FPG, fasting plasma glucose · HOMA-IR, homeostasis model assessment for insulin resistance · PGC-1, peroxisome proliferator-activated receptor γ coactivator-1 · PPAR, peroxisome proliferator-activated receptor · SSCP, single-strand conformation polymorphism · STOP-NIDDM, Study To Prevent Non Insulin Dependent Diabetes Mellitus

The members of the STOP-NIDDM Study Group are listed in a previous article published in the *Lancet* (2002; 359:2072–2077)

Introduction

Type 2 diabetes is a common disease due to both genetic and environmental factors [1, 2, 3, 4]. Although up to 40 genes have been reported to be associated with this disease, the *Pro12Ala* polymorphism of the peroxisome proliferator-activated receptor $\gamma 2$ (*PPAR-γ2*) gene has been identified as the most widely reproduced genetic variation for the risk of type 2 diabetes [4]. Another important association with type 2 diabetes has been reported for a missense variant (*Gly482Ser*) in the peroxisome proliferator-activated receptor γ coactivator-1 (*PGC-1α*) gene [4].

PPARγ is a ligand-activated transcription factor, a member of the nuclear hormone receptor superfamily,

that functions as a heterodimer with a retinoid X receptor (RXR) to promote transcription of numerous target genes [5, 6]. PPAR- γ 2, an isoform of PPAR- γ with 28 additional amino acids at its N-terminus, is expressed almost exclusively in adipose tissue [7]. It plays a key role in adipogenesis [8, 9, 10, 11], is involved in the regulation of insulin sensitivity [12, 13], and is the major functional receptor for the thiazolidinedione class of insulin-sensitising drugs [11, 14]. Therefore, the PPAR- γ gene has been viewed as a “thrifty gene”, with an important role in the development of type 2 diabetes and diabetes-related traits [7, 15]. Additionally, the *Pro12Ala* substitution in exon B of the PPAR- γ 2 gene, first reported in Caucasians [16], has been associated with diabetes mellitus [17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28]. Although not all associations have been consistent, a meta-analysis of published studies has confirmed a modest (1.25-fold), but statistically significant, increase in diabetes risk for the *Pro12Pro* genotype [4, 19].

PGC-1 co-activates a series of nuclear receptors including PPAR- γ , and controls transcription of genes involved in adaptive thermogenesis, adipogenesis and oxidative metabolism [29, 30, 31]. Moreover, PGC-1 α regulates the determination of muscle fibre type [32], enhances glucose uptake by controlling insulin-sensitive glucose transporter (GLUT-4) expression in muscle cells [33] and is implicated in hepatic gluconeogenesis by increasing gene transcription of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [34]. Genes involved in oxidative metabolism and responsive to PGC-1 α , as well as PGC-1 α itself, have been showed to be co-ordinately down-regulated in diabetic subjects [35, 36] and even in insulin-resistant individuals [37]. The *Gly482Ser* variant in the PGC-1 α gene has been reported to be associated with type 2 diabetes in Danish [38] and Japanese [39] subjects. In contrast, no association was found in French Caucasians [40] or in Pima Indians [41] and in addition, no interaction was observed between this variant and the *Pro12Ala* polymorphism of the PPAR- γ 2 gene [38].

We investigated the association of the *Pro12Ala* polymorphism of the PPAR- γ 2 gene, and the *Gly482Ser* variant of the PGC-1 α gene with the risk of type 2 diabetes among the participants in the Study To Prevent Non Insulin Dependent Diabetes Mellitus (STOP-NIDDM).

Materials and methods

Study population and design. The STOP-NIDDM trial design and rationale have previously been described in detail [42]. The aim of this study was to investigate the effect of α -glucosidase inhibitor acarbose on the prevention of type 2 diabetes in a population with IGT. This international, multicentre, double-blind, placebo-controlled trial, conducted in Canada, Germany, Austria, Finland, Sweden, Norway, Denmark, Israel and

Spain randomised 1429 subjects with fasting plasma glucose 5.6–7.7 mmol/l and 2-h plasma glucose 7.8–11.0 mmol/l to either the acarbose or placebo group.

All patients met with a dietician before randomisation and then on a yearly basis, and were recommended to follow a weight-reduction or weight-maintenance diet and encouraged to exercise regularly. Fasting plasma glucose (FPG) was measured every 3 months and a 2-h glucose tolerance test was performed if FPG levels were ≥ 7 mmol/l. All subjects underwent annual OGTT to evaluate the conversion to diabetes according to the World Health Organization criteria [43]. Improvement of glucose tolerance (i.e. return to normal glucose tolerance (NGT), either from IGT or from diabetes) was also assessed. Mean follow-up time for the whole population was 3.3 ± 1.2 years.

DNA was available from 770 subjects (387 men and 383 women), 356 of whom were initially allocated to acarbose and 414 to placebo. Their mean body mass index (BMI) was 30.8 ± 4.1 kg/m² and their mean age was 54.7 ± 7.9 years. DNA was not available for subjects recruited in Israel and Sweden. Subjects who were included in the study did not differ from those whose DNA was not available with respect to sex and age, baseline characteristics such as weight, BMI, waist circumference and waist-to-hip ratio and laboratory measures such as fasting and 2-h glucose and insulin. However, those subjects whose DNA was available, had a higher systolic blood pressure and a significantly higher incidence of diabetes (40.3 vs 32.8% , $p=0.004$) compared to those whose DNA was not available. The incidence of diabetes was higher among subjects whose DNA was available compared to subjects whose DNA was not available for all countries with the exception of Denmark and Germany. All participants signed an informed consent form, approved by appropriate Institutional Review Boards.

Measurements. Anthropometric measurements, nutritional evaluations and laboratory analyses were carried out at baseline, at annual visits and at the end of the study, as previously reported [42, 44]. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated according to the formula: fasting plasma glucose (mmol/l) \times fasting serum insulin (mU/l)/22.5 [45].

Genomic DNA was isolated from human leukocytes by the salt-precipitation method. Genotyping of the *Gly482Ser* polymorphism of the PGC-1 α gene was performed with the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. The sequences of the primers were 5'-TGC TAC CTG AGA GAG ACT TTG-3' (forward primer) and 5'-CTT TCA TCT TCG CTG TCA TC-3' (reverse primer). PCR amplification was carried out in a volume of 10 μ l, that contained 50 ng of genomic DNA, 5 pmol of each primer, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of MgCl₂, 0.1% Triton X-100, 112 μ mol/l dNTP and 0.25 U of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland). Reaction mixtures were incubated at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s, with the final extension at 72°C for 6 min, and 260-bp length PCR products were then digested with Hpa II at 37°C overnight, resolved by electrophoresis on 9% polyacrylamide gel, and visualised by staining with ethidium bromide.

The *Pro12Ala* polymorphism of the PPAR- γ 2 gene was detected by polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis as previously described [17]. The 167-bp length product, encompassing the polymorphic site was amplified by PCR with the sense primer 5'-GAC AAA ATA TCA GTG TGA ATT ACA GC-3' and the

Table 1. Clinical and biochemical characteristics of the subjects in the STOP-NIDDM study in relation to the *Pro12Ala* polymorphism of the *PPAR-γ2* gene and the *Gly482Ser* polymorphism of the *PGC-1α* gene

	<i>PPAR-γ2</i> genotypes		<i>PGC-1α</i> genotypes	
	12Ala	Pro12Pro	Gly482Gly	482Ser
Baseline study				
<i>n</i>	178	592	334	436
Sex (men/women)	88/90	299/293	168/166	219/217
Age (years)	54.9±7.8	54.7±7.9	54.6±7.9	54.9±7.9
Systolic blood pressure (mmHg)	132.1±18.3	132.3±15.9	132.4±16.8	132.1±16.2
Diastolic blood pressure (mmHg)	82.5±9.9	82.9±9.1	82.8±9.2	82.8±9.4
Body mass index (kg/m ²)	31.11±4.45	30.65±4.00	30.79±3.98	30.74±4.21
Waist-to-hip ratio	0.93±0.08	0.92±0.08	0.93±0.08	0.92±0.08
Fasting plasma glucose (mmol/l)	6.23±0.57	6.27±0.53	6.25±0.52	6.27±0.54
2-h plasma glucose (mmol/l)	9.25±1.10	9.26±1.04	9.28±1.10	9.24±1.01
Fasting insulin (pmol/l)	97.4±55.6	97.9±53.2	95.9±47.1	99.2±58.3
2-h insulin (pmol/l)	592.2±427.7	606.5±449.5	585.0±403.3	617.2±472.6
HOMA-IR (mmol/l·mU/l)	4.50±2.55	4.55±2.49	4.43±2.19	4.62±2.71
HbA _{1c} (%)	5.10±0.73	5.15±0.79	5.21±0.75	5.08±0.79 ^b
Total cholesterol (mmol/l)	5.71±1.08	5.72±1.00	5.73±1.07	5.64±0.97
HDL cholesterol (mmol/l)	1.23±0.34	1.18±0.32	1.19±0.31	1.20±0.33
LDL cholesterol (mmol/l)	3.61±0.96	3.61±0.88	3.64±0.94	3.58±0.87
Total triglycerides (mmol/l)	2.01±1.23	2.00±1.07	2.07±1.09	1.95±1.12
Follow-up values ^a				
Change in weight (kg)	-0.31±4.56	0.06±4.59	0.02±4.59	-0.05±4.58
Change in waist circumference (cm)	-0.03±6.16	-0.21±5.87	-0.01±5.87	-0.29±5.99
Change in fasting plasma glucose (mmol/l)	-0.07±0.74	-0.06±0.85	-0.04±0.79	-0.07±0.86
Change in 2-h plasma glucose (mmol/l)	-0.35±2.67	0.12±2.70	-0.03±2.57	0.05±2.79
Change in fasting insulin (pmol/l)	-1.8±72.2	-0.7±52.5	2.7±50.6	-3.9±62.5
Change in 2-h insulin (pmol/l)	-102.8±360.6	-15.1±405.6 ^b	-17.0±357.8	-49.1±425.2

Data are *n* or means ± SD; ^a the non-parametric Mann–Whitney test was used to compare these variables; ^b *p* value <0.05, while comparing the two genotype groups

antisense primer 5'-CCC AAT AGC CGT ATC TGG AAG G-3'. Amplification was performed using 50 ng of DNA in a total volume of 6 µl containing 2.8 pmol of each primer, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of MgCl₂, 0.1% Triton X-100, 95 µmol/l dNTP, 0.14 U DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland), and 0.25 µCi [α -³³]dCTP. The cycling programme was denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing and extension at 66°C for 1 min, with the final extension at 72°C for 6 min. Thereafter, PCR products were diluted 4-fold with 0.1% SDS and 10 mmol/l EDTA and the loading mix (95% formamide, 20 mmol/l EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) was then added (1:1). After denaturation at 94°C for 4 min, the samples were placed on ice and 4 µl of each was loaded onto non-denaturing 6% acrylamide gels. Electrophoresis was carried out at 38°C for approximately 5 h, the gels were subsequently dried and autoradiographed overnight.

Statistical analysis. All statistical analyses were undertaken using the Statistical Package for Social Sciences (SPSS, Chicago, Ill., USA) version 11.0 for Windows. Data are expressed as either mean ± SD or percentages, unless indicated otherwise. The normality of the distribution of variables was tested with the Kolmogorov–Smirnov test, and parameters that did not have a normal distribution were logarithmically transformed (systolic and diastolic blood pressure, weight, BMI, fasting and 2-h plasma glucose and insulin). Comparison of variables between the genotype groups was carried out with the two-

tailed Student's *t* test or the Mann–Whitney non-parametric test, when appropriate. Categorical variables were compared using the chi square test. In order to evaluate whether the *PGC-1α* and *PPAR-γ2* polymorphisms and other variables predicted the development of type 2 diabetes, odds ratios and 95% CIs (non-adjusted and adjusted for confounding variables) were calculated by logistic regression analysis. A *p*-value equal to or less than 0.05 was considered to be statistically significant.

Results

In 770 study subjects the frequencies of genotypes were as follows: 592 (76.9%) *Pro12Pro*, 164 (21.3%) *Pro12Ala* and 14 (1.8%) *Ala12Ala* for the *PPAR-γ2* gene and 334 (43.4%) *Gly482Gly*, 339 (44.0%) *Gly482Ser* and 97 (12.6%) *Ser482Ser* for the *PGC-1α* gene. The frequencies of the *12Ala* allele ranged from 0.186 to 0.307 and of the *482Ser* allele from 0.498 to 0.780 in different countries (Austria was excluded because of a low number of cases). No differences in the frequencies of these polymorphisms were found between the acarbose and placebo groups and all genotypic distributions were in Hardy–Weinberg equilibrium.

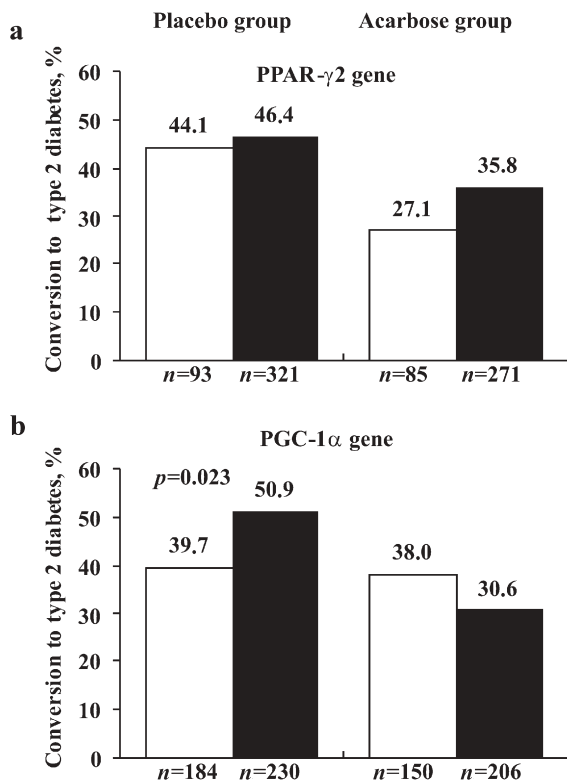


Fig. 1. Conversion (%) to type 2 diabetes in the placebo group and in the acarbose group in relation to the presence of the *12Ala* allele (white bars) and the *Pro12Pro* genotype (black bars) of the *PPAR- γ 2* gene (a). Conversion (%) to type 2 diabetes in the placebo group and in the acarbose group in relation to the presence of the *Gly482Gly* genotype (white bars) and the *482Ser* allele (black bars) of the *PGC-1 α* gene (b)

Clinical characteristics, fasting and 2-h plasma glucose, fasting and 2-h serum insulin, HOMA IR and lipid levels at baseline did not differ significantly in relation to the *Pro12Ala* polymorphism of the *PPAR- γ 2* gene or the *Gly482Ser* polymorphism of the *PGC-1 α* gene either in the entire study population (Table 1) or within the acarbose and placebo groups (data not shown). Carriers of the *12Ala* allele showed a significantly higher reduction in 2-h serum insulin levels ($p=0.035$) compared to subjects with the *Pro12Pro* genotype. In the placebo group, carriers of the *482Ser* allele, compared to carriers of the *Gly482Gly* genotype, showed a significant increase in their 2-h glucose levels during the 3-year follow-up (0.66 ± 2.81 vs 0.15 ± 2.56 mmol/l, $p=0.048$) whereas carriers of the *482Ser* allele in the acarbose group showed a more pronounced decrease in their 2-h glucose levels compared to the 2-h glucose levels among carriers of the *Gly482Gly* genotype (-0.64 ± 2.60 vs -0.25 ± 2.58 mmol/l, $p=0.131$).

During the follow-up of 3.3 years, a total of 310 genotyped subjects (190 in the placebo group and 120 in the acarbose group) developed type 2 diabetes. In the placebo group four of eight subjects (50.0%) having the *Ala 12Ala* genotype, 37 of 85 subjects (43.5%)

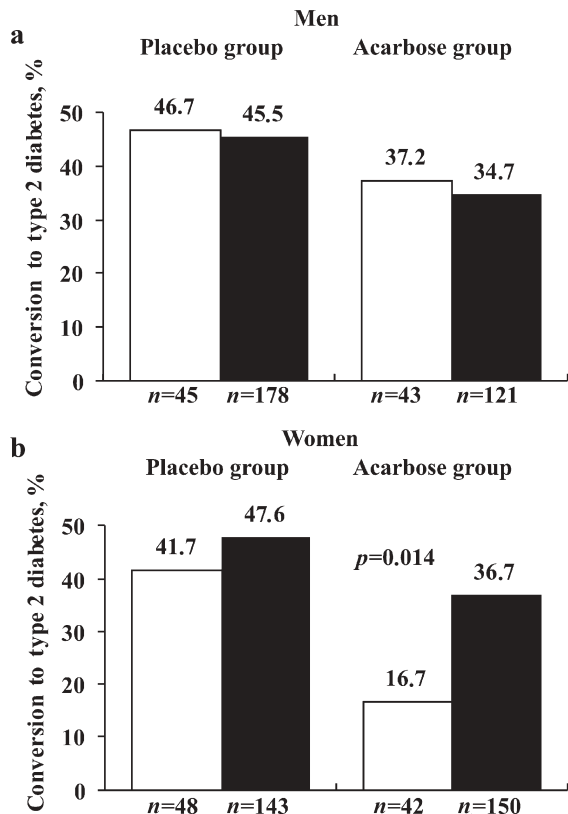


Fig. 2. Conversion (%) to type 2 diabetes in the placebo group and in the acarbose group in men (a), and conversion (%) to type 2 diabetes in the placebo group and in the acarbose group in women (b) according to the presence of the *12Ala* allele (white bars) and the *Pro12Pro* genotype (black bars) of the *PPAR- γ 2* gene

having the *Pro12Ala* genotype and 149 of 321 subjects (46.4%) having the *Pro12Pro* genotype of the *PPAR- γ 2* gene developed diabetes. In the acarbose group the corresponding numbers were one of six (16.7%), 22 of 79 (27.8%) and 97 of 271 (35.8%). In both treatment groups there was a trend for subjects with the *Pro12Pro* genotype to have a higher incidence of type 2 diabetes, but the difference was not statistically significant ($p=0.673$ in the placebo group and $p=0.131$ in the acarbose group; Fig. 1a). When the results were analysed by sex, the *Pro12Pro* genotype was associated with the conversion to diabetes in women treated with acarbose ($p=0.014$; Fig. 2).

In the placebo group 73 of 184 subjects (39.7%) with the *Gly482Gly* genotype, 96 of 178 subjects (53.9%) with the *Gly482Ser* genotype and the 21 of 52 subjects (40.4%) with the *Ser482Ser* genotype of the *PGC-1 α* gene developed diabetes ($p=0.017$ over the three genotype groups). In the acarbose group the corresponding numbers were 57 of 150 subjects (38.0%), 51 of 161 subjects (31.7%), and 12 of 45 subjects (26.7%). In the placebo group the *482Ser* allele of the *PGC-1 α* gene, compared to the *Gly482Gly* genotype, was associated with a higher incidence of diabetes ($p=0.023$; Fig. 1b). The associa-

Table 2. The effect of treatment on the conversion to diabetes (%) according to the genotypes of the *PPAR-γ2* and the *PGC-1α* genes

	Placebo	Acarbose	<i>p</i>	Placebo	Acarbose	<i>p</i>
PPAR-γ2 gene	<i>12Ala</i> allele			<i>Pro12Pro</i> genotype		
Whole population	44.1	27.1	0.018	46.4	35.8	0.008
Men	46.7	37.2	0.369	45.5	34.7	0.057
Women	41.7	16.7	0.010	47.6	36.7	0.059
PGC-1α gene	<i>Gly482Gly</i> genotype			<i>482Ser</i> allele		
Whole population	39.7	38.0	0.755	50.9	30.6	<0.001
Men	36.4	39.1	0.716	53.2	32.6	0.002
Women	43.5	37.0	0.394	48.1	28.8	0.003

Table 3. The *Pro12Ala* polymorphism of the *PPAR-γ2* gene and the *Gly482Ser* polymorphism of the *PGC-1α* gene as predictors for the development of type 2 diabetes by treatment group (logistic regression analysis)

	Placebo group			Acarbose group			Gene/treatment interaction <i>p</i>
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	
Model 1 (univariate)							
PPAR-γ2/ <i>Pro12Pro</i>	1.11	0.69–1.76	0.673	1.50	0.88–2.58	0.139	0.649
Model 2 (univariate)							
PGC-1α/ <i>482Ser</i> allele	1.57	1.06–2.33	0.023	0.72	0.46–1.12	0.144	0.012
Model 3 (multivariate)							
PGC-1α/ <i>482Ser</i> allele	1.59	1.07–2.36	0.021	0.71	0.45–1.12	0.138	
Age	0.99	0.97–1.02	0.541	0.97	0.94–0.99	0.016	
Sex	1.00	0.68–1.48	0.999	0.89	0.57–1.39	0.600	
Smoking	1.61	0.91–2.84	0.101	0.58	0.28–1.22	0.154	
Model 4 (multivariate)							
PGC-1α/ <i>482Ser</i> allele	1.56	1.04–2.34	0.031	0.73	0.46–1.15	0.175	
Age	1.00	0.98–1.03	0.882	0.97	0.95–1.00	0.973	
Sex	1.07	0.70–1.64	0.743	0.89	0.55–1.43	0.629	
Weight at baseline	1.01	0.99–1.03	0.242	1.01	0.99–1.02	0.574	
Weight change	1.11	1.05–1.16	0.000	1.07	1.02–1.13	0.011	

OR=odds ratio. Smoking was coded as 0 = never smokers and ex-smokers and 1 = current smokers at baseline. *PPAR-γ2* genotypes were coded as 0 = the *12Ala* allele and 1 = the

Pro12Pro genotype. *PGC-1α* genotypes were coded as 0 = the *Gly482Gly* genotype and 1 = the *482Ser* allele

tion in the placebo group was even more marked among men ($p=0.012$), as 36.4% of those (36 of 99) having the *Gly482Gly* genotype and 53.2% of those (66 of 124) with the *482Ser* allele converted to diabetes. As shown in Table 2, acarbose was effective in the prevention of diabetes independently of the genotypes of the *PPAR-γ2* gene in all subjects, but if analysed by sex, women with the *12Ala* allele were more responsive to treatment with acarbose than men. In contrast, acarbose treatment prevented the development of diabetes only among carriers of the *482Ser* allele of the *PGC-1α* gene in both men and women. There was no difference in weight change with respect to the polymorphisms of the *PPAR-γ2* or *PGC-1α* genes among the different treatment groups or according to sex (data not shown).

Univariate logistic regression analysis showed that the *Pro12Pro* genotype of the *PPAR-γ2* gene was not associated with a higher risk of developing type 2 diabetes in the placebo group or in the acarbose group (Table 3, Model 1). However, in women the presence of the *Pro12Pro* genotype was related to a 2.9-fold higher risk (95% confidence intervals (CI) 1.20 to 6.96; $p=0.018$) of developing diabetes in the acarbose group (Table 4). Adjustment for age and smoking (odds ratio (OR) 2.87, 95% CI 1.19 to 6.93; $p=0.019$), or for age, smoking and the country of origin (OR 2.90, 95% CI 1.17 to 7.20; $p=0.022$) or for age, weight at baseline and weight change (Table 4, Model 4, OR 3.55, 95% CI 1.38 to 9.13; $p=0.009$) did not essentially change the results.

No statistically significant gene–gene interaction was found between the *PPAR-γ2* and *PGC-1α* genes

Table 4. The *Pro12Ala* polymorphism of the *PPAR-γ2* gene as a predictor for the development of type 2 diabetes in the treatment groups by sex (logistic regression analysis)

	Placebo group			Acarbose group			Gene/treatment interaction <i>p</i>
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>	
Men							
Model 1 (univariate)							
PPAR-γ2/Pro12Pro	0.96	0.50–1.86	0.913	0.90	0.44–1.85	0.767	0.662
Women							
Model 2 (univariate)							
PPAR-γ2/Pro12Pro	1.27	0.66–2.46	0.479	2.89	1.20–6.96	0.018	0.289
Model 3 (multivariate)							
PPAR-γ2/Pro12Pro	1.23	0.63–2.40	0.544	2.87	1.19–6.93	0.019	
Age	0.99	0.95–1.02	0.422	0.97	0.93–1.01	0.110	
Smoking	1.01	0.44–2.33	0.975	0.60	0.20–1.77	0.352	
Model 4 (multivariate)							
PPAR-γ2/Pro12Pro	1.38	0.69–2.76	0.359	3.55	1.38–9.13	0.009	
Age	0.99	0.96–1.04	0.837	0.98	0.94–1.02	0.378	
Weight at baseline	1.01	0.98–1.04	0.353	1.01	0.99–1.04	0.315	
Weight change	1.10	1.02–1.17	0.005	1.03	0.96–1.10	0.469	

OR=odds ratio. Smoking was coded as 0 = never smokers and ex-smokers and 1 = current smokers at baseline. *PPAR-γ2* genotypes were coded as 0 = the *12Ala* allele and 1 = the *Pro12Pro* genotype

($p=0.562$). However, the *482Ser* allele of the *PGC-1α* had a significant interaction (interaction term: *PGC-1α/482Ser* allele × treatment group) with the mode of the treatment ($p=0.012$). In the placebo group subjects with the *482Ser* allele had an approximate 1.6-fold increase (95% CI 1.06 to 2.33, $p=0.023$) in the risk of developing diabetes (Table 3, Model 2). The results remained essentially unchanged after adjustment for age, sex and smoking (OR 1.59, 95% CI 1.07 to 2.36; $p=0.021$), or for age, sex, smoking and the country of origin (OR 1.53, 95% CI 1.01 to 2.32; $p=0.043$), or for age, sex, weight at baseline and weight change (odds ratio 1.56, 95% CI 1.04 to 2.34; $p=0.031$).

The *12Ala* allele of the *PPAR-γ2* tended to be related to an increase in conversion to normal glucose tolerance (NGT) in both sexes, but the results were not statistically significant among the different treatment groups (data not shown).

Discussion

We have shown that the *Pro12Pro* genotype of the *PPAR-γ2* gene and the *482Ser* allele of the *PGC-1α* gene predicts the conversion from IGT to diabetes in the STOP-NIDDM trial. The *Pro12Pro* genotype of the *PPAR-γ2* gene was associated with a 2.9-fold higher risk of developing diabetes compared to the *12Ala* allele in women in the acarbose group ($p=0.018$), whereas the *482Ser* allele of the *PGC-1α* gene predicted the conversion to diabetes in the placebo group (OR 1.57, $p=0.023$).

There is substantial evidence that *PPAR-γ* contributes to the risk for type 2 diabetes by influencing insulin sensitivity, insulin secretion and susceptibility to obesity [6]. The *12Ala* allele of the *PPAR-γ2* gene, that has been shown to have a decreased transcriptional activity, is also associated with improved insulin sensitivity and lower risk for diabetes [17]. This finding is in agreement with results from heterozygous *PPAR-γ* null mice exhibiting increased insulin sensitivity compared with wild-type mice [46]. The *12Ala* allele was associated with a higher reduction in the 2-h serum insulin level, probably indicating an increase in insulin sensitivity. However, it cannot be excluded that a decrease in insulin levels could also be due to impaired insulin secretion, because the *12Ala* allele has been associated with reduced insulin secretion capacity in previous studies [20, 47].

PPAR-γ plays a key role in adipocyte differentiation [10, 11], and can therefore influence body fat mass and obesity. In our study subjects, those with the *12Ala* allele had a somewhat higher BMI at baseline, and tended to lose more weight. This finding is in accordance with our results from the Finnish Diabetes Prevention Study [48]. In that study, subjects belonging to the intervention group (lifestyle changes) and who had the *Ala12* allele lost significantly more weight (and were protected from type 2 diabetes) than subjects with the *Pro12Pro* genotype, although in the control group the *12Ala* allele did not confer protection against diabetes. In the present study, the effect of the *Pro12Pro* genotype in increasing the risk for diabetes was independent of baseline weight change and other

confounding factors in women in the acarbose group, indicating that women possessing the *Pro12Pro* genotype were less responsive to acarbose treatment. This implies that the effect of acarbose treatment was modified by the *Pro12Ala* polymorphism.

Several mechanisms could explain why the *Gly482Ser* polymorphism of the *PGC-1 α* gene regulates the conversion from IGT to diabetes. *PGC-1 α* increases and coordinates the expression of different genes that stimulate mitochondrial biogenesis, adaptive thermogenesis, fibre-type switching [32], expression of GLUT-4 in skeletal muscle [33], and regulation of gluconeogenesis in the liver [34]. Thus, a reduction in the activity of *PGC-1 α* , possibly also as a result of the *Gly482Ser* mutation, might lead to insulin resistance in skeletal muscle. Additionally, a quantitative trait linkage analysis in Pima Indians suggested a link between basal insulin concentrations and chromosome 4p15.1 [49] in cases where the *PGC-1 α* gene has been mapped [50]. In the present study the *Gly482Ser* variant was not related to fasting and 2-h plasma glucose, serum insulin, or their changes, or to BMI and weight change. However, compared to the *Gly482Gly* genotype, the *482Ser* allele was associated with a 1.6-fold higher risk for diabetes in the placebo group but not in the acarbose group. The *482Ser* allele had a significant interaction with treatment and acarbose treatment was able to reduce the risk of diabetes particularly among carriers of the *482Ser* allele.

Previous attempts to relate the *Gly482Ser* polymorphism to type 2 diabetes have shown an 1.34-fold increase in risk among Danish Caucasians [38] and a significant association among Japanese subjects [39]. In contrast, the *482Ser* allele did not predict diabetes in French Caucasians or Pima Indians [40, 41]. These studies were carried out in single populations. Because several different populations were used in the STOP-NIDDM trial, our data provides strong evidence that the *Gly482Ser* polymorphism of the *PGC-1 α* gene contributes to the risk of type 2 diabetes. In agreement with this, the reduced expression of *PGC-1 α* in adipose tissue has been associated with insulin resistance [51]. Moreover, recent studies have reported that down-regulation of the *PGC-1 α* gene and coordinated changes in other genes involved in oxidative phosphorylation in man are associated with IGT, diabetes mellitus [35] and insulin resistance [37].

The regulation of many important metabolic processes and energy homeostasis by the *PGC-1 α* gene is achieved through a specific interaction with a variety of transcription factors and nuclear hormone receptors, including *PPAR- γ 2*. Moreover, *PGC-1 α* was first identified as a transcriptional co-activator for *PPAR- γ* [29]. Therefore, it is possible that polymorphisms in both of these genes might interact. In our study no statistically significant interaction which may lead to an increase in the conversion to type 2 diabetes was found between the polymorphisms of the *PPAR- γ 2* and

PGC-1 α genes, this is in accord with the results of a previous study among Danish subjects [38].

In summary, we have demonstrated that the *Pro12Pro* genotype of the *PPAR- γ 2* gene and the *482Ser* allele of the *PGC-1 α* gene predict the conversion from IGT to type 2 diabetes. Our study also shows that the interaction between drug treatment (acarbose) and genetic variation may be significant with respect to the conversion from impaired glucose tolerance to type 2 diabetes.

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