CLINICAL STUDY

Genetic variation in receptor protein tyrosine phosphatase σ is associated with type 2 diabetes in Swedish Caucasians

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Abstract

Objective: Previously, it has been demonstrated that receptor protein tyrosine phosphatase σ (RPTP σ) is involved in glucose homeostasis and insulin signaling in several animal models. The aim of this study was to evaluate whether polymorphisms in this gene influence the development of type 2 diabetes (T2D) in humans.

Design: We investigated how genetic variations in the *RPTP σ* gene influence susceptibility to impaired glucose tolerance (IGT) and T2D, in Swedish men and women.

Methods: Genotyping of single nucleotide polymorphisms (SNPs) was performed by dynamic allele-specific hybridization in a total of 1057 Swedish Caucasians including 497 subjects with normal glucose tolerance (NGT), 262 subjects with IGT, and 298 patients with T2D.

Results: SNPs rs1143699, rs4807015, and rs1978237 were found to be associated with T2D. SNP rs1143699 was associated with male T2D patients when compared with NGT controls (odds ratio; OR = 1.57; $P = 0.029$). SNP rs4807015 showed association with T2D patients when compared with NGT controls (OR = 1.32; $P = 0.025$). Finally, SNP rs1978237 was associated with T2D patients when compared with NGT controls (OR = 1.59; $P = 0.002$). Logistic regression analysis demonstrated that for SNP rs1143699 in men, C/C homozygosity conveys an increased risk of T2D (OR = 2.19; $P = 0.035$), while SNP rs4807015 was associated with an increased risk of T2D in both men and women (OR = 1.74; $P = 0.029$). SNP rs1978237 also demonstrated a risk of T2D in men and women (OR = 1.59; $P = 0.026$).

Conclusions: This study provides evidence for association of SNPs in the *RPTP σ* gene with T2D in Swedish Caucasians. SNPs rs1143699, rs4807015, and rs1978237 confer an increased risk of developing T2D.

European Journal of Endocrinology 157 459–464

Introduction

Type 2 diabetes (T2D) is a multifactorial disease that involves insulin resistance and impaired glucose-induced insulin release (1). In most patients, T2D results from alterations of several genes, where each of them has a partial and additive effect (2). T2D is also influenced by lifestyle, such as a high calorie intake and sedentary behavior leading to overweight (3). Identification of genes influencing genetic variation in disease offers a better understanding of molecular mechanisms underlying T2D pathogenesis, which is a prerequisite for development of therapeutic methods (4).

We have previously demonstrated that the receptor protein tyrosine phosphatase σ (*RPTP σ*) gene, also known as PTP σ , is overexpressed by ~60% in pancreatic islets and liver of spontaneously diabetic Goto-Kakizaki (GK) rats that have impaired insulin secretion. When islet RPTP σ was inhibited by antisense,

improved glucose-induced insulin secretion was seen in GK islets (5). In addition, *RPTP σ* knockout ($-/-$) mice have shown decreased plasma glucose and insulin levels in the fasted state when compared with wild-type controls. The mice also had increased whole-body insulin sensitivity, suggesting that RPTP σ affects insulin signaling in insulin-sensitive tissues (6).

RPTP σ has a function in the cellular receptor signaling, which is critical for signal transduction in both normal and pathophysiological conditions (7). It is a member of the leukocyte antigen-related (LAR) RPTP family, which has been suggested to act in key steps of neural development and also in diabetes and cancer (8). LAR, a protein closely related to RPTP σ , has negative regulatory effects in the insulin signaling pathway when it is overexpressed (9). The *RPTP σ* gene gives rise to several different distinct isoforms (10). PTPs are key regulators of the insulin receptor signal transduction pathway and the *RPTP σ* gene has been shown to be highly expressed in insulin target tissues, such as liver,

adipose tissue, skeletal muscle, and endothelial cells (11). In humans, the *RPTPσ* gene is located on chromosome 19p13.3, a region that may influence traits underlying lipid abnormalities associated with T2D (12).

Until now, there has not been a genetic report of *RPTPσ* in T2D patients. We have selected SNPs in the *RPTPσ* gene to study in a Swedish cohort including T2D patients, subjects with impaired glucose tolerance (IGT), and normal glucose tolerance (NGT) control subjects. Our study hypothesis was to evaluate the genetic influence of polymorphisms of the *RPTPσ* gene in development of T2D among Swedish men and women.

Subjects and methods

Subjects

The study includes 497 unrelated subjects with NGT (246 men/251 women), 262 subjects with IGT (107/155), and 298 patients with T2D (241/57). All individuals were Swedish Caucasians collected from the Stockholm Diabetes Prevention Program with a baseline study in men 1992–1994 and in women 1996–1998. A follow-up study in about 70% of the baseline participants was conducted 10 years later (13–15). Patients were diagnosed according to the World Health Organization criteria (1998) (16). NGT control subjects had a mean birth body weight of 3742 g (3000–5800), body mass index ≤ 26 kg/m², and no relatives diagnosed with T2D. All clinical data in T2D patients were used from the baseline study (those diagnosed at baseline and incident cases between baseline and follow-up studies) or at follow-up study (diagnosed at follow-up). The data were not influenced by use of anti-diabetic medication. Clinical parameters for each study group are given in Table 1. All participants gave

their informed consent to take part in the study. The procedures followed were in accordance with the declaration of Helsinki II and approved by the ethics committee of Karolinska Hospital. DNA was extracted from peripheral blood using Puregene DNA purification kit (Gentra Systems, Minneapolis, MN, USA).

SNP validation and genotyping

We selected a total of 28 SNPs within the *RPTPσ* gene from the National Center for Biotechnology Information (USA; <http://www.ncbi.nlm.nih.gov/SNP/>) database based on validation status, region, and function for our study. SNPs chosen were picked to cover the whole gene. The Tagger program from the International HapMap project (release no. 21) was also used to select and evaluate tagSNPs from genotype data in HapMap (17). Here, pairwise tagging was used together with an r^2 cutoff of 0.8 and a minor allele frequency of 5%. The majority of the chosen SNPs were captured by tagger.

Validation of 28 chosen SNPs in 32 Swedish DNA samples including 16 T2D patients and 16 NGT controls was performed. Twelve SNPs representing at least 4% allele frequencies were used for further genotyping. Genotyping was performed by dynamic allele-specific hybridization (DASH) (18). PCR-DASH assay design and SNP genotyping protocol were used as described previously (19). Information on primer and probe sequences used in PCR-DASH assays are available, upon request. All PCRs were run in 20 µl volumes using 10–20 ng genomic DNA. Negative controls (water blanks) were included on each plate. For genotyping quality control, the subjects were distributed randomly across plates with the same number of cases and controls per plate. Successful genotype calls were $\geq 95\%$ and plates were randomly genotyped twice for duplication accuracy, which was calculated to be 98%.

Table 1 Clinical data of Swedish normal glucose tolerance (NGT) control subjects, impaired glucose tolerance (IGT) subjects, and type 2 diabetes (T2D) patients.

	NGT		IGT		T2D	
	Male	Female	Male	Female	Male	Female
<i>n</i>	186	252	107	155	232	57
Age (years)	45±5	47±5	47±5	49±4	52±6	50±4
BMI (kg/m ²)	23.7±1.8	22.6±1.7	29.1±4.7	29.2±6.0	29.5±4.7	31.1±6.5
Waist circumference (cm)	86.1±5.9	73.9±4.7	99.1±9.8	89.3±12.7	100.7±10.9	94.8±13.3
Waist-hip ratio	0.87±0.04	0.77±0.04	0.93±0.04	0.84±0.06	0.95±0.05	0.87±0.07
Systolic BP (mmHg)	121±12	115±13	136±19	133±18	138±17	137±17
Diastolic BP (mmHg)	77±8	72±9	85±11	82±10	86±11	83±9
Fasting plasma glucose (mmol/l)	4.5 (4.0–5.0)	4.5±0.4	5.2 (4.6–5.9)	5.2±0.6	6.6 (4.9–8.8)	7.6±2.2
2 h plasma glucose (mmol/l)	4.0 (3.1–5.1)	4.1±1.0	8.7 (8.0–9.5)	8.8±0.8	10.5 (6.8–16.2)	13.6±4.0
Fasting plasma insulin (mU/l)	15.5 (11.1–21.7)	9.2±3.6	24.0 (15.8–36.5)	15.5±7.5	25.8 (15.6–42.7)	21.8±13.6
2 h plasma insulin (mU/l)	35.6 (22.2–56.9)	28.5±12.6	117.4 (70.9–194.2)	88.3±69.4	84.8 (42.9–167.6)	73.8±62.4
HOMA-IR index ^a	3.3±1.2	1.9±0.8	6.2±2.7	3.7±2.0	9.1±6.4	7.4±5.3

All data are means ± s.d. or geometric mean (s.d. range).

^aInsulin resistance index was calculated by HOMA.

Calculations and statistical analysis

The homeostasis model assessment (HOMA) was used to assess insulin resistance. Based on fasting glucose and insulin levels, it was calculated according to the following equation: fasting plasma glucose (mmol/l) $\times$ fasting plasma insulin (mU/ml)/22.5 (20).

Normal probability plots were created and parameter distributions were transformed to natural logarithm as required to improve skewness and to obtain a normal distribution before performing statistical analysis. Homogeneity of variances was tested by Levene's test. Genotype and allele distribution were compared between cases (IGT subjects and/or T2D patients) and NGT control subjects using χ^2 -test. OR and 95% confidence intervals (CI) were calculated to test for relative risk for association. Testing for differences in clinical parameters between genotype groups was performed using ANOVA and covariance analysis adjusting for age. *P* values less than 0.05 were interpreted as statistically significant. Multivariate logistic regression analysis was used to predict the susceptibility to T2D between case and control subjects. Adjustments for age, sex, BMI, and blood pressure (where appropriate) were applied. The effects of the SNPs were tested individually and/or as haplotypes. Linkage disequilibrium (LD) and haplotype frequencies were calculated using Arlequin (21). LD between different markers/SNPs was summarized using $|D'|$. Haplotypes prevalent at 5% were used for further haplotype analysis. All statistical analyses were performed using Statistica version 7.1 (Statsoft Inc., Tulsa, OK, USA) and/or Biomedical package (BMDP) version 1.1 (BMDP Statistical Software Inc., Los Angeles, CA, USA).

Results

A total of 12 valid SNPs were genotyped in the *RPTPσ* gene and all of them were in Hardy-Weinberg equilibrium ($P > 0.05$). Genotype and allele frequencies of all valid

SNPs in NGT and IGT subjects and T2D patients are presented in Table 2. The results are focused on SNPs rs1143699, rs4807015, and rs1978237, since the remaining SNPs studied did not show significant allelic association between study groups.

SNP rs1143699

SNP rs1143699 showed significant allelic association with male T2D patients when compared with NGT controls (0.89 vs 0.84), where the major allele C was present at a higher frequency in T2D ($P = 0.029$; OR = 1.57; 95% CI = 1.05–2.36; Table 3). No allelic association could be noted when comparing male IGT subjects and controls, or in female, IGT and T2D, when compared with controls. Comparison of genotypes and quantitative traits within groups was performed by ANOVA and covariance analysis adjusting for age. No significant associations with quantitative traits were detected (data not shown). Multivariate logistic regression analysis of SNP rs1143699 with adjustments for age, BMI, and blood pressure indicated that in men the C/C genotype was significantly associated with T2D when compared with C/T + T/T ($P = 0.035$; OR = 2.19; 95% CI = 1.055–4.523; Table 4).

SNP rs4807015

A significant difference in allele frequency was found in SNP rs4807015 comparing all T2D patients with NGT controls (0.49 vs 0.56), C being the major allele present at a lower frequency in T2D ($P = 0.025$; OR = 1.32; 95% CI = 1.034–1.678; Table 3). Multiple logistic regression analysis showed that this SNP was associated with increased risk of T2D in men and women carrying the C/C genotype ($P = 0.029$; OR = 1.74; 95% CI = 1.058–2.865) adjusted for age and blood pressure (Table 5). When adding sex as a covariate to the model, this association was no longer seen. In IGT

Table 2 Genotype and allele frequencies of 12 single nucleotide polymorphisms (SNPs) in the *RPTPσ* gene in Swedish normal glucose tolerance (NGT), impaired glucose tolerance (IGT) subjects, and type 2 diabetes (T2D) patients.

SNP ID	Contig position ^a	SNP type	Region	Function	Genotype frequencies (%)			Allele frequencies (%)		
					NGT	IGT	T2D	NGT	IGT	T2D
rs1017092	5 214 986	R = G/A	Intron 1	Intronic	47/45/8	49/41/10	49/45/6	69/31	70/30	72/28
rs1034917	5 214 384	Y = C/T	Intron 1	Intronic	69/28/3	67/28/5	72/25/3	83/17	81/19	85/15
rs1141371	5 213 571	M = A/C	Exon 3	Synonymous	64/32/4	63/33/4	60/36/4	80/20	80/20	78/22
rs1034863	5 207 751	Y = C/T	Intron 3	Intronic	81/18/1	79/21/0	80/19/1	90/10	89/11	90/10
rs886936	5 193 432	R = G/A	Intron 8	Intronic	31/48/21	36/42/22	30/54/16	55/45	57/43	57/43
rs10518243	5 175 958	R = G/A	Intron 12	Intronic	83/15/2	78/20/2	81/18/1	91/09	88/12	90/10
rs4807015	5 171 135	Y = C/T	Intron 13	Intronic	31/50/19	30/45/25	27/44/29	56/44	52/48	49/51
rs2230611	5 152 482	M = C/A	Exon 30	Synonymous	80/18/2	80/19/1	79/20/1	89/11	89/11	89/11
rs1143698	5 150 782	Y = C/T	Exon 33	Synonymous	63/30/7	66/27/7	6/30/9	78/22	80/20	76/24
rs1143699	5 159 762	Y = C/T	Exon 33	Synonymous	75/24/1	77/22/1	80/19/1	87/13	88/12	89/11
rs1978237	5 149 641	S = G/C	Intron 34	Intronic	51.5/41.5/7	49/43/8	65/31/4	72/28	70/30	80/20
rs1143700	5 146 775	Y = C/T	Exon 37	3'-UTR	69/28/3	66/30/4	67/29/4	83/17	81/19	81/19

Genotype frequencies are presented as homozygous major allele/heterozygotes/homozygous minor allele. Allele frequencies are presented as major allele first.

^aThe contig ID number at NCBI is NT_011255/NW_927173.

Table 3 Allelic association of single nucleotide polymorphism (SNP) rs1143699, rs4807015 and rs1978237 in normal glucose tolerance (NGT), impaired glucose tolerance (IGT) subjects, and type 2 diabetes (T2D) patients.

SNP ID	Sex	Allele frequencies (%)			P value T2D	OR (95% CI) T2D
		NGT	IGT	T2D		
rs1143699	M	C84/T16	C86/T14	C89/T11	0.029	1.57 (1.05–2.36)
	F	C89/T11	C89/T11	C89/T11	NS	–
	M+F	C87/T13	C88/T12	C89/T11	NS	–
rs4807015	M	C51/T49	C49/T51	C47/T53	NS	–
	F	C59/T41	C55/T45	C53/T47	NS	–
	M+F	C56/T44	C52/T48	C49/T51	0.025	1.32 (1.03–1.68)
rs1978237	M	G71/C29	G65/C35	G79/C31	NS	–
	F	G73/C27	G74/C26	G78/C22	NS	–
	M+F	G72/C28	G70/C30	G80/C20	0.002	1.59 (1.18–2.13)

P value represents allele frequency difference between NGT and T2D. NS, not significant.

men and women subjects, the association was not significant (data not shown).

SNP rs1978237

SNP rs1978237 showed significant allelic association with all T2D patients when compared with NGT controls (0.80 vs 0.72), where the major allele G was present at a higher frequency in T2D ($P=0.002$; OR = 1.59; 95% CI = 1.184–2.126; Table 3). Multiple logistic regression analysis showed that this SNP was associated with increased risk of T2D in men and women carrying the G/G genotype ($P=0.026$; OR = 1.591; 95% CI = 1.056–2.398) adjusted for age, BMI, and blood pressure (Table 6). A similar result was retrieved when adding the covariate sex to the analysis. In IGT men and women subjects, the association was not significant.

LD

LD between SNPs was found to be in concordance with HapMap data (release no. 21). We found three common haplotypes for SNP rs1143699, rs4807015, and rs1978237 with a frequency above 5%. However, there was no significant difference in haplotype distribution between groups for SNP rs1143699, rs4807015, and rs1978237 (data not shown).

Discussion

We investigated the genetic influence of SNPs for RPTP σ in Swedish NGT subjects, IGT subjects, and T2D patients, and found that three polymorphisms confer susceptibility to development of T2D. SNP rs1143699 was associated with an increased risk of T2D in male patients carrying the C/C genotype. SNP rs4807015 and rs1978237 was associated with an increased risk of T2D in both male and female patients. The moderate OR for these three SNPs, which were around 1.3–1.6, support the hypothesis that there is restricted influence of a single SNP in T2D. However, when comparing

sample groups and adjusting for clinical features separating them (age, BMI, and blood pressure), the relative risk to develop T2D was even more increased for SNP rs1143699 (OR 2.2), but still the same for SNP rs1978237 (OR 1.6). SNP rs4807015 showed the same trend in results. This is a truer reflection of how the different SNPs studied contribute to susceptibility of T2D, since the data have been adjusted for covariates. In all three SNPs, the allele being responsible for the increased risk was the major allele.

SNP rs1143699 is located in exon 33, which is within the PTP catalytic (PTPc) domain, an important functional part of the RPTP σ gene being involved in phosphorylation of interacting proteins. SNP rs4807015 is located in intron 13 and rs1978237 in intron 34. In other genes, intronic SNPs have been suggested to contribute to gene expression, and therefore, this is a possible explanation for the role of SNP rs4807015 and rs1978237 (22–24). Finally, susceptibility is often a result of the combined action of many different variants in a gene, one example being calpain-10 in T2D (25). These considerations need to be emphasized when interpreting association data of RPTP σ .

We did not detect any association between genotype groups and clinical phenotypes for the SNPs in our study. This is most probably because our male patient group contains clinical data at baseline also for patients

Table 4 Association of single nucleotide polymorphism (SNP) rs1143699 C allele in type 2 diabetes (T2D) men.

Variable	OR	95% CI	P value
Age	1.17	1.10–1.25	<0.001
BMI	2.12	1.74–2.57	<0.001
Systolic BP	1.04	1.00–1.08	<0.001
Diastolic BP	0.99	0.94–1.03	0.533
SNP rs1143699 C/C versus C/T + T/T	2.19	1.06–4.52	0.035

Multivariate logistic regression analysis was used to examine independent variables associated with T2D in Swedish T2D men when compared with NGT controls. Odds ratio (95% confidence interval) and P values for multiple logistic analyses including age, BMI, and blood pressure in the model are shown.

Table 5 Association of single nucleotide polymorphism (SNP) rs4807015 G allele in type 2 diabetes (T2D) men and women.

Variable	OR	95% CI	P value
Age	1.15	1.10–1.21	<0.001
Systolic BP	1.06	1.04–1.09	<0.001
Diastolic BP	1.03	1.00–1.06	0.093
SNP rs4807015 G/G versus C/G + C/C	1.74	1.06–2.86	0.029

Multivariate logistic regression analysis was used to examine independent variables associated with T2D in Swedish T2D women and men when compared with NGT controls. Odds ratio (95% confidence interval) and *P* values for multiple logistic analyses including age and blood pressure in the model are shown.

who were diagnosed with T2D during the 10 years between baseline and follow-up studies. This subgroup of T2D men did not have diabetes at baseline, which explains the low fasting plasma glucose in the diabetes group (6.6 mmol/l). However, we chose not to include follow-up data of these patients, since anti-diabetic drugs and lifestyle changes would then be affecting the quantitative traits of the patients.

LAR is a member of the same PTP family as *RPTPσ* and has a role in negative modulation of insulin receptor signaling (26). Increased activity of LAR or related PTPs in insulin target tissues could contribute to development of insulin resistance (27). The fact that *RPTPσ* shows similarity to LAR, 85% at amino acid level, and is expressed to a higher level than LAR in insulin-sensitive tissues, makes this protein a possible candidate for regulating insulin signaling and for playing a role in glucose homeostasis (28). To our knowledge, the present study is the first genetic investigation of *RPTPσ* in T2D. However, there is a genetic association study of an SNP in the promoter region of *LAR* in two non-diabetic populations in Italy (29), showing association with diabetes-related characteristics (29). Thus, *LAR* and *RPTPσ* seem to be important genes in the development of T2D.

SNP rs1143699 was found to be associated with T2D in men but not in women, suggesting that gender differences may be an important factor in the genetic influence of *RPTPσ* in T2D. Our group and others have previously shown that there are sex differences in

Table 6 Association of single nucleotide polymorphism (SNP) rs1978237 G allele in type 2 diabetes (T2D) men and women.

Variable	OR	95% CI	P value
Age	1.09	1.05–1.14	<0.001
BMI	1.92	1.74–2.13	<0.001
Systolic BP	1.04	1.01–1.06	0.001
Diastolic BP	1.01	0.98–1.05	0.436
SNP rs1978237 G/G versus C/G + G/G	1.59	1.06–2.40	0.026

Multivariate logistic regression analysis was used to examine independent variables associated with T2D in Swedish T2D women and men when compared with NGT controls. Odds ratio (95% confidence interval) and *P* values for multiple logistic analyses including age, BMI and blood pressure in the model are shown.

genetic association with T2D. Polymorphisms in the insulin-degrading enzyme gene are associated with increased BMI and insulin resistance in men but not in women (30). Also, a polymorphism in neuropeptide Y, a gene involved in satiety and body weight, is related to IGT and T2D in men (31). Thus, sex specificity could be an important confounding factor in analyzing genetics of T2D and related disorders (30–34).

In conclusion, this study provides evidence that three polymorphisms in the *RPTPσ* gene are associated with the development of T2D in Swedish Caucasians, which support our hypothesis that the *RPTPσ* gene may be important for the development of T2D in humans. Further investigation in other populations and determination of the functional influence of variation in *RPTPσ* are necessary to make conclusive implications.

Acknowledgements

This study was supported by the Swedish Research Council, the Swedish Diabetes Association, Novo Nordisk Scandinavia, GlaxoSmithKline, Novo Nordisk Consortium, and the Loo and Hans Osterman foundation. The authors thank all subjects for participating in the present study. The excellent assistance of Camilla Lagerberg, Yvonne Strömberg, Elisabeth Norén-Krog, and Agneta Hilding is greatly acknowledged.

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Received 22 February 2007

Accepted 26 July 2007