MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

276G>T Polymorphism of the ADIPOQ Gene Influences Plasma Adiponectin in Type 2 Diabetes Patients but Is Not Predictive for Presence of Type 2 Diabetes in a Caucasian Cohort from Romania

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This study was possible thanks to the grant idei 1167/208 of CNCSIS Romania.

ABSTRACT

Background: *Previous reports associated* ADIPOQ *276G>T polymorphism with plasma adiponectin levels and diabetes. Our* ***objective*** *was to study this polymorphism in type 2 diabetes (T2D) Romanian patients and to assess its influence on plasma adiponectin levels; possible link to prevalence of T2D was also addressed.*

Design: *Case control study*

Material and Methods: *Consecutive T2D patients, age and sex matched controls were genotyped for the 276* ADIPOQ *locus. Medical history, laboratory evaluation, plasma adiponectin were assessed.*

Outcomes: *105 T2D patients and 48 controls were included. Adiponectin was higher in controls (17.04±3.02 μg/ml) than in T2D patients (10.32±1.16 μg/ml), difference failed to reach significance (p=0.06). Genotype distribution wasn't different between T2D patients and controls. 44 (41.90%) of T2D patients had GG genotype, 51 (48.57%) GT and 10 (9.52%) TT genotype. Adiponectin was higher (19.03±3.46 μg/ml) in diabetic TT allele carriers than in GT (9.96±1.76 μg/ml) or GG patients*

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Article received on the 7th of July 2012. Article accepted on the 21st of October 2012.

(8.71±1.60 μg/ml) p=0.003. In controls, 28 (58.33 %) subjects were carriers of the GG genotype, 16 (33.33%) had GT genotype and 4 (8.33%) had TT genotype. There weren't significant differences in the studied parameters between different genotypes in the control group. Logistic regression disclosed age p=0.0001 (OR 1.086; CI 1.041/1.133), waist circumference p=0.00049 (OR 1.084; CI 1.036/1.135), adiponectinemia p=0.036 (OR 0.963; CI 0.929/0.998) but not genotype as predictors for the presence of diabetes.

Conclusion: *Presence of the TT allele at the 276 locus of the ADIPOQ gene is associated with higher plasma adiponectin levels in type 2 diabetes patients. Plasma adiponectin, and not genotype at the 276 locus is predictive for the presence of T2D.*

Keywords: adiponectin, gene polymorphism, type 2 diabetes

INTRODUCTION

Adiponectin is a cytokine secreted in differentiated adipocytes and credited with insulin-sensitizing, anti-inflammatory and anti-atherogenic properties (1,2). Decreased levels of plasma adiponectin have been associated with insulin resistance and type 2 diabetes (T2D). Plasma levels of this cytokine are influenced by various factors but might also be determined by genotype. Adiponectin is encoded in the 3q27 region, where several quantitative trait loci for the metabolic syndrome and type 2 diabetes were reported (3,4). In the gene encoding for adiponectin-*ADIPOQ,* different single nucleotide polymorphisms (SNP) have been identified (5,6), some of them being associated with plasma adiponectin levels, insulin resistance, metabolic syndrome or diabetes. One of the most studied SNP of the *ADIPOQ* gene is 276 G>T substitution in exon 2.

Our study describes, for the first time to our knowledge, 276G>T polymorphism in the *ADIPOQ* gene T2D patients from Romania; the main objective was to assess influence of genotype on plasma adiponectin. Secondly the link of this polymorphism to the presence of diabetes was addressed. ❑

MATERIAL AND METHODS

Consecutive unrelated type 2 diabetic patients presenting in the ambulatory setting of our clinic between June and December 2010 were screened for the study; inclusion criteria were presence of T2D, exclusion criteria were presence of acute inflammation, infection or other acute clinical condition. Healthy volunteering health-employees recruited at their yearly check-up visit were used as a control group. The *ADIPOQ* 276G>T polymorphism was genotyped by a PCR-RFLP (Polymerase Chain Reaction – Restriction Fragments Length Polymorphism) assay, as previously described, with minor modifications (7). Briefly, a 468-bp fragment of the *ADIPOQ* gene was amplified by PCR, under the following conditions: denaturation 10 min at 95°C, followed by 35 cycles of denaturation 30s at 95°C, annealing 30s at 55°C and elongation 30s at 72°C, and final elongation 7 min at 72°C. The primers used have the following sequences:

Fw_ 5'-TCT CTC CAT GGC TGA CAG TG-3'

Rev_ 5'-AGATGC AGC AAA GCC AAA GT-3'

The amplicon was digested overnight at 37°C with 5U of the restriction enzyme Mva1269I (Fermentas MBI, Vilnius , Lituania). The restriction fragments were submitted to electrophoresis in 3% high resolution agarose (Lonza, Rockland, ME, USA) gels stained with ethidium bromide. In the presence of the G allele, the 468-bp amplicon is cut in two fragments of 320-bp and 148-bp, respectively; however, in the presence of the T allele, the amplicon is resistant to digestion. Thus, the homozygote for the G allele have 2 fragments of 320 and 148-bp, the homozygote for the T allele present after digestion only the 468-bp fragment, whereas heterozygote present all 3 fragments. The electrophoresis gels were documented by the use of a photo plate coupled to an UV- transiluminator (Vilber Lourmat, France).

Baseline evaluation consisted in medical history, physical exam, anthropometric measurements, and standard laboratory evaluation (including lipid profile); in diabetic subjects glycated hemoglobin was also measured. Total plasma adiponectin was determined by an ELISA assay (R&D System), with following precision provided by manufacturer: intra-assay co-

efficient of variation 2.5, 3.4 and 4.7% and inter-assay coefficient of variation 5.8, 5.8 and 6.9% respectively. Presence of metabolic syndrome was established according to ATP3 criteria.

Statistic analysis was performed using SPSS 13.0, StatView 7.0, Statistica 8.0 and Microsoft EXCEL programs. To estimate the relation between a dichotomous dependent variable and several qualitative and quantitative variables, multivariate logistic regression were used (enter method, forward LR). Odds ratio (OR), 95% confidence intervals (CI) and statistical significance of each parameter were presented. For comparison of three or more means of normally distributed continuous variables Anova test was used, followed by a Scheffe post-hoc analysis. If distribution of variables was not normal, Kruskall-Wallis followed by Mann-Whitney test was used. For testing normal distribution Kolmogorov-Smirnov test was applied. Statistic significance threshold was considered $\alpha = 0.05$. Sample size (with main outcome measure plasma adiponectin) was estimated according to genotype-related plasma adiponectin differences and to genotype distribution previously reported in the literature (6). Values are expressed as mean ±standard error of the mean.

The study was approved by the ethical committee of our university; informed and written consent was obtained from each participant. ❑

OUTCOMES

105 diabetic subjects were included in the study; 48 healthy non-diabetic subjects served as controls. The power of the study according to recorded differences in plasma adiponectin was 99%. The distribution of the genotypes for the 276G>T polymorphism in our cohort was in accordance with the Hardy-Weinberg equilibrium.

Characteristics of the patients in comparison to controls are presented in table 1. There were significant differences in body fat and plasma lipids between T2D patients and controls. Plasma adiponectin was higher in controls than in T2D diabetic patients but difference failed to reach statistical significance. Genotype distribution was not significantly different between T2D patients and controls.

44 (41.90%) of the 105 diabetic patients had GG genotype, 51 (48.57%) GT and 10 (9.52%) TT genotype. Comparison of diabetic patients according to genotype is presented in table 2. Adiponectin is higher in TT allele carriers than in GT or GG allele carriers. Also glycated hemoglobin is lower in TT genotype patients when compared to GG genotype. There were no other significant differences between different genotypes.

28 (58.33%) of the subjects in the control group were carriers of the GG genotype, 16 (33.33%) had GT genotype and 4 (8.33%) had TT genotype. Comparison of controls according to genotype did not show any significant differences (Table 3). Although in the control group TT subjects had somewhat higher adiponectin levels than the other subjects, the difference was not statistically significant.

Logistic regression (backward method) revealed as the only variables predictive for the presence or absence of diabetes age $p= 0.0001$ (OR 1.086; CI 1.041/1.133); waist circumference $p=0.00049$ (OR 1.084; CI 1.036/1.135) and plasma adiponectin levels $p=0.036$(OR 0.963; CI 0.929/0.998) but not genotype. ❑

DISCUSSION

Significance of gene polymorphisms seems to be different among various ethnic groups, due to differences in adiponectin gene structure and role of gene–environment interaction. The influence of 276G>T polymorphism on plasma adiponectin levels and on prevalence of type 2 diabetes or its complications remains a subject of debate; moreover approach of this polymorphism in eastern Europe population is limited (8,9). These findings prompted the present study.

Association of the TT allele of the SNP 276G>T with increased adiponectin levels has previously been reported in diabetic (10) or non-diabetic individuals (11-15); however this association is not universally accepted (5,16). Our data for diabetic subjects show that plasma adiponectin levels are significantly higher in TT homozygous T2D patients, in accord to similar findings in the literature. In controls the difference is not statistically significant. One explanation for this lack of significance could be the relatively smaller number of patients in the control group. Nevertheless we could also speculate that TT genotype confers a potential advantage for adiponectin synthesis in both diabetic and non-diabetic subjects; however T2D is characterized by "suppressed" adiponectin

synthesis –reflected by lower adiponectin levels in T2D patients as compared to controls (Table 1). Diabetic subjects carriers of the TT allele are able to overcome this suppressed state and adequately increase adiponectin levels (as would be expected to happen in response to permanent inflammatory state that is characteristic of T2D). Therefore, in the presence of a stimulus for adiponectin synthesis in T2D patients but not in controls, the advantage conferred by the presence of homozygous TT allele at the 276 locus translates into significantly higher adiponectin levels.

One interesting point to emphasize is the better glycemia control of homozygous T allele diabetic patients as compared to the other T2D subjects, suggesting a possible protective effect of this polymorphism; however duration of diabetes is also smaller (although not significantly) in these patients.

In our study diabetic patients have the tendency for lower adiponectin levels than controls. This is in agreement with most data in the literature, although some authors did not find significant differences in plasma adiponectin between controls and type 2 diabetic patients (17). As expected there are also significant differences in anthropometric characteristics (body mass index, abdominal circumference, prevalence of metabolic syndrome) between diabetics and controls. However we found similar genotype distribution between T2D patients and controls. Logistic regression was performed to further investigate factors that might account for the presence of diabetes. Adiponectinemia but not genotype at the 276 locus was predictive for diabetes in our study. This is in contradiction with previous findings that have linked 276G>T polymorphisms to insulin resistance (8,13,18-20) and prevalence or development of type 2 diabetes (6,21-23) but goes along with other, negative findings (5,16, 24-28). Furthermore, a recent meta-analysis concluded that 276 G>T polymorphism is not

Parameter		Diabetes n=105	Controls n=48	p	
Age (years)		63.17±0.96	60.02±2.41	0.07	
Sex n (% male)		57(54.28)	19(39.58)	0.09	
BMI (kg/m2)		31.30±0.67	24.88±0.76	<0.001	
Waist circumference (cm)		108.73±1.46	90.28±2.20	<0.001	
Metabolic syndrome -n(%)		86(81.90)	12(25.00)	0.001	
SBP (mm Hg)		142.42±1.91	132.22±4.87	0.21	
DBP (mm Hg)		82.32±1.06	80.00±3.73	0.56	
Adiponectin (μg/ml)		10.32±1.16	17.04±3.02	0.06	
LDL cholesterol (mg/dl)		187.53±12.38	110.10±12.69	0.02	
HDL cholesterol (mg/dl)		42.87±1.28	55.28±5.11	0.02	
Triglycerides (mg/dl)		208.45±15.19	134.88±32.39	0.07	
Genotype- n(%)	GG	44(41.90)	28(58.33)	GG/GT	0.057
	GT	51(48.57)	16(33.33)	GT/TT	0.74
	TT	10(9.52)	4 (8.33)	GG/TT	0.47

TABLE 1. Characteristics of patients and comparison to controls.

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, LDL – low density lipoprotein, HDL- high density lipoprotein

Parameter	Genotype			p
	GG (n=44)	GT (n=51)	TT (n=10)	
Age (years)	61.24±1.61	64.88±1.23	62.60±3.39	0.20
Sex n (%male)	26 (59.09)	26 (50.98)	5 (50.00)	0.40
Metabolic syndrome n (%)	36 (81.82)	43 (84.31)	7 (70.00)	0.98
BMI (kg/m2)	31.46±1.07	31.51±0.96	29.51±1.86	0.69
Waist circumference (cm)	108.57±2.53	110.31±1.90	101.00±3.93	0.20
SBP (mmHg)	139.76±2.98	145.33±2.77	140.00±5.20	0.41
DBP (mmHg)	79.88±1.54	84.13±1.60	84.44±2.94	0.17
Adiponectin (μg/ml)	8.71±1.60	9.96±1.76	19.03±3.46	0.003[b,c]
LDL cholesterol (mg/dl)	193.66±19.49	186.24±18.77	167.00±24.28	0.44
HDL cholesterol (mg/dl)	41.57±1.97	45.26±1.78	36.44±4.40	0.10
Triglycerides (mg/dl)	229.32±26.93	198.32±20.25	167.30±24.79	0.47
Diabetic retinopathy (%)	17 (38.63)	28 (54.90)	3 (30.00)	0.26
Glycated hemoglobin (%)	7.18±0.21	7.73±0.19	6.87±0.39	0.04[b]
Diabetes duration (years)	9.88±1.33	10.57±1.10	6.06±1.82	0.25

TABLE 2. Comparison of diabetic patients according to according to genotype.

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, HDL- high density lipoprotein, LDL – low density lipoprotein, significant difference between [a]GG/GT, [b]GT/TT, [c]GG/TT carriers

Parameter	Nondiabetics			p
	Genotype			
	GG n=28	GT n=16	TT n=4	
Age (years)	49.73±2.97	51.13±4.56	46.67±11.35	0.90
Sex n (%male)	13 (46.42)	5 (31.25)	1 (25.00)	0.27
Metabolic syndrome (%)	7 (25)	5 (31.25)	0 (0.00)	0.99
BMI (kg/m^2)	25.21±0.94	24.26±1.36	25.24±3.38	0.84
Waist circumference (cm)	90.92±2.94	89.93±3.94	87.75±7.23	0.92
SBP (mmHg)	138.75±3.15	133.75±5.54	131.32±2.78	0.24
DBP (mmHg)	85.00±5.00	80.00±4.08	62.02±2.84	0.20
Adiponectin (µg/ml)	15.42±3.61	18.28±4.95	23.41±19.42	0.73
LDL cholesterol (mg/dl)	104.25±19.20	132.40±13.36	66.60±26.68	0.32
HDL cholesterol (mg/dl)	59.50±9.07	45.93±1.77	66.40±5.74	0.39
Triglycerides (mg/dl)	136.00±53.25	155.67±56.19	68.00±34.02	0.81

TABLE 3. Comparison of nondiabetic patients according to genotype.

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, LDL – low density lipoprotein. HDL- high density lipoprotein, significant difference between [a]GG/GT, [b]GT/TT, [c]GG/TT carrierrs.

significantly linked to the prevalence of T2 D in Chinese subjects. In other words not genotype but adiponectinemia is the main predictor for the development of diabetes; plasma levels of adiponectin being likely regulated by other confounding factors like inflammation and body fat and not only by genotype. We acknowledge that our study was nevertheless powered to assess influence of genotype on plasma adiponectin and not on the prevalence of diabetes; a higher number of patients might be necessary to prove an influence on the prevalence of diabetes. ❑

CONCLUSION

Our data suggest that in T2D patients, presence of the TT allele at the 276 locus of the ADIPOQ gene is associated to higher plasma adiponectin levels. Plasma adiponectin but not genotype at the 276 locus might be predictive for the presence of T2D.

ACKNOWLEDGEMENTS

This study was possible thanks to the grant idei 1167/208 of CNCSIS Romania.

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