

T45G and G276T Adiponectin Gene Polymorphisms in Primary Aldosteronism and Healthy Controls in an East Slovak Population

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Summary

Metabolic complications are frequent in primary aldosteronism (PA) and adiponectin gene polymorphisms seem to confer a genetic risk for metabolic alterations. Aim of the study was to evaluate the prevalence of metabolic symptoms in patients with PA compared to controls and the prevalence of two single nucleotide polymorphisms (SNPs), T45G and G276T, in the adiponectin gene and their relationship to metabolic syndrome (MS). The study involved 47 patients with PA and 90 controls selected from general population. Body mass index (BMI), and selected biochemical parameters were examined, and the mentioned SNPs were genotyped in all subjects. PA patients had a significantly higher BMI ($p < 0.0001$), blood glucose level ($p < 0.01$), and triglycerides ($p < 0.0005$) compared to controls. There were no significant differences in the prevalence of the studied genotypes of adiponectin gene polymorphisms. The 276GT genotype was linked with lower levels of triglycerides ($p \leq 0.05$), while 276GG was related to higher levels of triglycerides ($p = 0.01$). A similar but non-significant tendency was observed in relation to cholesterol levels. We can conclude that PA patients with the 276GT genotype have lower triglycerides levels, but there are not significant differences in the distribution of genotypes and alleles among PA patients and controls in an East Slovak population.

Key words

Primary aldosteronism • Adiponectin gene polymorphism • Metabolic syndrome • Triglycerides

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Introduction

Metabolic abnormalities, such as alterations in glucose homeostasis, dyslipidemia and obesity, are very common clinical features in patients with essential hypertension (Cuspidi *et al.* 2004). Previous studies confirmed that they are also associated with primary aldosteronism (PA), the most common form of secondary hypertension (Rossi 2010, for review, see Boscaro *et al.* 2008), with the prevalence of 5 to 10 % in hypertensive patients (Hannemann and Wallaschofski 2012).

Aldosterone excess is crucial for the development of cardiac and metabolic complications. Many authors documented a higher prevalence of metabolic symptoms in patients with PA compared to those with essential hypertension (EH) (Fallo *et al.* 2006, Iacobellis *et al.* 2010, Ronconi *et al.* 2010).

Adipose tissue secretes a number of adipocytokines, which can be involved in the etiology of obesity and MS (Ehrhart-Bornstein *et al.* 2004, Inadera 2008). The most studied adipocytokine is adiponectin, a protein with generally positive metabolic effects. It exerts insulin sensitising, anti-inflammatory, and anti-apoptotic actions in cells (Ohashi *et al.* 2012, Turer and Scherer 2012) and low plasma levels of circulating adiponectin are considered a biomarker of metabolic syndrome (Weyer *et al.* 2001, Li *et al.* 2009).

Adiponectin is an 18.5 kb protein encoded by the ADIPOQ gene located on chromosome 3q27 consisting of 3 exons. To date, several studies reported association of various single nucleotide polymorphisms (SNPs) in the ADIPOQ gene with MS, obesity, and glucose metabolism

disorders (Schwarz *et al.* 2006, Menzaghi *et al.* 2007, Mtiraoui *et al.* 2012). Two of the most commonly studied SNPs in the ADIPOQ gene are a silent T to G substitution in exon 2 (T45G, rs2241766) and a G to T substitution in intron 2 (G276T, rs1501299). In the T45G SNP, the G allele is associated with several negative effects that include lower adiponectin levels, higher BMI, lower insulin sensitivity, higher risk for developing hyperglycemia and T2DM, and higher levels of blood lipids. Therefore, the T allele seems to have a protective function for its carriers (reviewed by Enns *et al.* 2011). The G allele of G276T SNP was reported to be associated with lower insulin sensitivity, increased risk of T2DM, higher levels of blood lipids, and lower levels of circulating adiponectin (Hara *et al.* 2002, Gonzalez-Sanchez *et al.* 2005, Huang *et al.* 2010). Reports on the significance of the T allele of SNP G276T are controversial, some authors demonstrated its association with higher levels of circulating adiponectin (Menzaghi *et al.* 2004, Kyriakou *et al.* 2008), while others documented its association with obesity and lower adiponectin levels (Mousavinasab *et al.* 2006). There are only poor data regarding the association of these polymorphisms in PA, which is also associated with MS. To the best of our knowledge the only study by Ronconi *et al.* (2010) aimed at establishing a relationship between adiponectin gene polymorphisms and MS in PA patients. They observed an association of the 276TT genotype of the adiponectin gene with worse metabolic profile in PA and EH patients and the 45GG+GT genotypes of the adiponectin gene seemed to have a protective role for metabolic complications.

The aims of the present study were to evaluate the prevalence of metabolic symptoms in patients with PA compared to controls selected from general population from Eastern Slovakia and to examine whether there is a higher prevalence of mutant genotypes of two common SNPs (T45G and G276T) of the ADIPOQ gene in patients with PA and metabolic complications. We presume the higher prevalence of metabolic complications in PA patients compared to controls and that there is a relationship between metabolic disturbances in PA patients and the mutant genotypes of studied gene polymorphisms.

Subjects and Methods

137 subjects living in the same geographic area, the East Slovak region, were genotyped. The first group

consisted of 47 unrelated adults with a diagnosis of PA (27 males, 20 females) of mean age 58±9 years (range 32-76, median 59). The second group comprised 90 unrelated ethnically matched subjects who were randomly selected from community volunteers in the same district (32 males, 58 females) of mean age 55±15, (range 31-81, median 57.5 years). In controls we ruled out only PA diagnosis. All study participants gave separate written consent for the study and the collection of DNA.

Diagnosis of PA was established in all patients by laboratory evaluations including aldosterone renin ratio (ARR, >40 ng/dl per ng/ml/h), lack of suppressibility of plasma aldosterone after saline infusion (plasma aldosterone >7ng/dl after saline infusion). Adrenal venous sampling was used for detection of side lateralization in all patients older than 40 years and those under 40 years with negative CT scan (Funder *et al.* 2008).

Among 47 patients with PA 21 (54±8.5 years, range 32-66, median 54 years) were diagnosed to have aldosterone producing adenoma (APA) and 26 (62±8.1 years, range 41-76, median 64 years) had idiopathic aldosteronism (IHA).

All PA patients were treated with spironolactone before surgery. 30 (59.57 %) patients were diagnosed with the MS, 13 (6.11 %) had T2DM. In the control group 8 (8.89 %) subjects fulfilled criteria of MS, the other 11 (9.9 %) subjects had dyslipidemia, and 23 (20.7 %) subjects were treated for hypertension. Diet and statins were used for the treatment of lipid metabolism disorder in both groups. Medical treatment of hypertension most frequently included ACE inhibitors, beta and alpha-blockers, and diuretics, respectively.

MS was diagnosed based on Adult Treatment Panel III criteria (Executive summary 2001) requiring the presence of at least 3 of the following abnormalities: waist circumference greater than 88 cm in women and greater than 102 cm in men; fasting plasma glucose ≥110 mg/dl (6.1 mmol/l); fasting plasma TGs ≥150 mg/dl (1.69 mmol/l); HDL cholesterol <40 mg/dl in men and <50 mg/dl (1.29 mmol/l) in women; and blood pressure ≥130/85 mm Hg. The diagnosis of MS was postulated retrospectively based on previous data from documentation in each subject included in the study.

Body mass index (BMI), blood glucose, total cholesterol, and triglycerides (TG) levels were examined in all subjects. These parameters were evaluated using routine biochemical methods: plasma renin activity and plasma aldosterone were examined by the RIA method using kits from Immunotech France.

Genotyping

Genomic DNA was extracted from lymphocytes using a Wizard Genomic DNA Extraction Kit (Promega), and was resuspended in water to the bring concentration to approximately 20 ng/ μ l. Recently a rapid and unexpansive closed tube assay using small amplicon melting analysis after real-time polymerase chain reaction was described. This approach was adapted for genotyping T45G and G276T single nucleotide polymorphisms in the adiponectin gene in our laboratory. Heterozygotes were identified by a change in melting curve shape, and different homozygotes were distinguished by a change in melting temperature. The PCR was performed in glass capillaries on a LightScanner32 instrument (Idaho Technology Inc. Salt Lake City, USA) in a 15 μ l reaction volume. Master mix contained: 1x BioThermAB™ buffer (GeneCraft, Munster, Germany), 1x LCGreen^{Plus+} (Idaho Technology Inc.), 250 μ M dNTP (Jena Bioscience, Jena, Germany), 0.6 μ M forward primer, 0.6 μ M reverse primer, 3 mM MgCl₂ (Idaho Technology Inc.), 250 μ g/ml BSA (Fermentas, Burlington, Canada), 1U BioThermAB™ polymerase (GeneCraft) and approx. 20 ng DNA. For the SNP T45G, a 50 bp DNA fragment was amplified using the forward primer 5'-GCTGTTCTACTGCTATTAGCTC-3' and reverse primer 5'-GTCGTGGTTTCCTGGTCA-3'. For the G276T SNP, a 71 bp DNA fragment was amplified using the forward primer 5'-CTTTCATCACAGACCTCCTACACAGA-3' and reverse primer 5'-GTGTCTAGGCCTTAGTTAATAATGAATG-3'. The mismatch in the forward primer for SNP G276T was introduced to cover another polymorphism in the amplicon that could interfere with the melting analysis. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 60 cycles at 95 °C for 10 s, 54 °C for 20 s and 72 °C for 20 s. Amplification was immediately followed by melting analysis with a denaturation at 95 °C for 1 min and renaturation at 40 °C for 1 min. Data were acquired over the 65-90 °C range at a thermal transition rate of 0.05 °C/s.

Statistical analysis

Data are expressed as mean \pm SD (standard deviation). The central tendencies of the different groups were compared by unpaired two-tailed Student t-test or Mann-Whitney test for non-normally distributed parameters. To compare discrete variables, the χ^2 test and Fisher exact test were used as appropriate. The frequencies of the alleles and genotypes in case patients

Table 1. Clinical and biochemical characteristics in controls and PA patients.

	PA (n=47)	Controls (n=90)	Significance
<i>Sex (M/F)</i>	27/20	32/58	
<i>Age (years)</i>	58 \pm 9	55 \pm 15	ns
<i>BMI (kg/m²)</i>	29.86 \pm 4.57	25.76 \pm 4.47	0.0001
<i>Glucose (mmol/l, 3.0-6.1)</i>	5.32 \pm 1.48	4.84 \pm 0.90	0.010
<i>Cholesterol (mmol/l, 2.57-5.2)</i>	4.68 \pm 0.93	4.89 \pm 1.08	ns
<i>Triglycerides (mmol/l, 0.45-1.75)</i>	1.54 \pm 0.95	1.16 \pm 0.47	0.0005

Table 2. Genotypes and alleles frequencies in PA patients and controls.

	PA (n=47)	Controls (n=90)	Significance
T45G			
<i>T alleles</i>	88 (94 %)	157 (87 %)	
<i>G alleles</i>	6 (6 %)	23 (13 %)	ns
<i>TT</i>	41(87.23 %)	69 (76.67 %)	ns
<i>TG</i>	6 (12.77 %)	19 (21.11 %)	ns
<i>GG</i>	0 (0.00 %)	2 (2.22 %)	ns
<i>Hardy-Weinberg equilibrium</i>	0.64	0.62	
G276T			
<i>G alleles</i>	64 (68 %)	129 (72 %)	
<i>T alleles</i>	30 (32 %)	51 (28 %)	ns
<i>GG</i>	21(44.68 %)	45(50.00 %)	ns
<i>GT</i>	22(46.81 %)	39(43.33 %)	ns
<i>TT</i>	4(8.51 %)	6(6.67 %)	ns
<i>Hardy-Weinberg equilibrium</i>	0.597	0.525	

and controls were determined by direct count and compared by the χ^2 test with the values predicted assuming Hardy-Weinberg equilibrium. A p-value of less than 0.05 indicated statistical significance. The differences between continuous parameters divided into more than two subgroups were tested using one way ANOVA. Log transformation was performed for data

Table 3. Biochemical and clinical characteristics depending on T45G and G276T gene polymorphisms in PA patients and controls.

	45TT	45TG+GG	Significance	276GG	276GT	276TT	Significance
Patients	(n=41)	(n=6)		(n=21)	(n=22)	(n=4)	
BMI, kg/m ²	30.08±4.55	28.30±4.81	ns	31.12±4.72	28.88±4.41	28.55±3.80	ns
Glucose	5.39±1.56	4.88±0.55	ns	4.96±0.64	5.64±2.04	5.47±0.39	ns
Cholesterol	4.60±0.86	5.25±1.22	ns	4.81±1.04	4.57±0.86	4.66±0.76	ns
Triglycerides	1.56±1.00	1.44±0.37	ns	1.74±1.23	1.28±0.49	1.92±0.96	ns
Controls	(n=69)	(n=21)		(n=45)	(n=39)	(n=6)	
BMI, kg/m ²	25.68±5.00	26.03±3.46	ns	25.60±4.14	26.04±5.36	25.14±4.22	ns
Glucose	4.80±0.86	5.00±1.03	ns	4.84±0.98	4.84±0.81	4.95±1.04	ns
Cholesterol	4.88±1.08	4.95±1.08	ns	4.93±1.12	4.93±1.08	4.39±0.82	ns
Triglycerides	1.16±0.48	1.18±0.44	ns	1.23±0.51	1.12±0.44	0.93±0.24	ns

Table 4. Genotypes and alleles frequencies depending on BMI value in controls and PA patients.

	Patients				Controls			
	BMI≤25 (n=8)	BMI 25.1-29.9 (n=15)	BMI>30 (n=22)	Significance	BMI≤25 (n=52)	BMI 25.1-29.9 (n=37)	BMI>30 (n=7)	Significance
T45G								
T alleles	14(88 %)	28(93 %)	42(95 %)		91(88 %)	63(85 %)	13(93 %)	
G alleles	2(12 %)	2(7 %)	2(5 %)	ns	13(12 %)	11(15 %)	1(7 %)	ns
TT	6(75 %)	13(86.67 %)	20(90.91 %)	ns	42(80.77 %)	26(70.27 %)	6(85.71 %)	ns
TG+GG	2(25 %)	2(13.33 %)	2(9.09 %)	ns	10(19.23 %)	11(29.73 %)	1(14.29 %)	ns
G276T								
G alleles	9(56 %)	19(63 %)	32(73 %)		75(72 %)	51(69 %)	10(71 %)	
T alleles	7(44 %)	11(37 %)	12(27 %)	ns	29(28 %)	23(31 %)	4(29 %)	ns
GG	2(25 %)	5(33.33 %)	12(54.55 %)	ns	28(53.85 %)	18(48.65 %)	3(42.86 %)	ns
GT	5(62.50 %)	9(60.00 %)	8(36.36 %)	ns	19(36.54 %)	15(40.54 %)	4(57.14 %)	ns
TT	1(12.50 %)	1(6.67 %)	2(9.09 %)	ns	5(9.62 %)	4(10.81 %)	0(0.00 %)	ns
GT+TT	6(75 %)	10(66.67 %)	10(45.45 %)	ns	24(46.16 %)	19(51.35 %)	4(57.14 %)	ns

with non-normal distribution. If variances of compared subgroups were heterogenous, Welch's ANOVA was used. Statistical analysis was performed using SPSS software (version 15.0).

Results

In the group of PA patients, 30 (59.57 %) were diagnosed to have a MS, and among the control group 8 subjects (8.89 %) fulfilled the criteria of MS.

PA patients had significantly higher BMI in comparison with the control group ($p<0.0001$), and they also had significantly higher blood glucose levels

($p=0.01$) and TGs as well ($p=0.0005$). There were no significant differences in serum cholesterol levels among both groups (Table 1).

Frequencies of genotypes and alleles in patients and controls are shown in Table 2.

There were no significant differences in the prevalence of the TT, TG, and GG genotypes of the T45G adiponectin gene polymorphism between both groups. In addition, there were no significant differences in the prevalence of the GG, GT, and TT genotypes of the G276T adiponectin gene polymorphism between PA and control groups.

There were no significant differences in the BMI

Table 5. Genotypes and alleles frequencies depending on cholesterol and TAG values in PA patients.

Patients	Cholesterol ≤5.2 (n=35)	Cholesterol >5.2 (n=12)	Significance	Triglycerides ≤1.2 (n=18)	Triglycerides >1.2 (n=29)	Significance
T45G						
<i>T alleles</i>	63(95.00 %)	21(88 %)		32(94 %)	52(93 %)	
<i>G alleles</i>	3(5 %)	3(13 %)	ns	2(6 %)	4(7 %)	ns
<i>TT</i>	30 (90.91 %)	9(75.00 %)	ns	15(88.24 %)	24(85.71 %)	ns
<i>TG+GG</i>	3(9.09 %)	3(25.00 %)	ns	2(11.76 %)	4(14.29 %)	ns
G276T						
<i>G alleles</i>	45(64 %)	19(79 %)		21(58 %)	43(74 %)	
<i>T alleles</i>	25(36 %)	5(21 %)		15(42 %)	15(26 %)	
<i>GG</i>	13(37.14 %)	8(66.67 %)	0.07	4(22.22 %)	17(58.62 %)	0.01
<i>GT</i>	19(54.29 %)	3(25.00 %)	0.08	13(72.22 %)	9(31.03 %)	0.006
<i>TT</i>	3(8.57 %)	1(8.33 %)	ns	1(5.56 %)	3(10.34 %)	ns
<i>GT+TT</i>	22(62.86 %)	4(33.33 %)	0.08	14(77.78 %)	12(41.37 %)	0.015

and metabolic parameters in PA patients and controls according to the genotypes of the T45G adiponectin gene polymorphism (Table 3).

Moreover, there were no significant differences in BMI and biochemical metabolic parameters between PA patients and controls with the 276GG genotype and the 276GT+TT genotypes (Table 3).

We also were not able to detect the significant differences in the prevalence of alleles and genotypes in patients and controls in relation to BMI, patients with normal weight had similar frequencies of alleles and genotypes as compared with overweight and obese patients and control subjects (Table 4).

However, patients with PA and higher TG had a significantly higher frequency of the GG genotype of the G276T polymorphism ($p=0.01$), and those with lower TG levels had a higher frequency of GT+TT ($p=0.015$). A similar tendency was observed also in relation to cholesterol levels; however the differences in the frequency of the GG and GT genotypes did not reach statistical significance (Table 5).

Discussion

Our present study confirmed a significantly higher prevalence of metabolic symptoms in PA patients compared to controls selected from general population matched for age and BMI. Moreover, we did not find significant differences in the distribution of genotypes and alleles of both T45G and G276T polymorphisms of the adiponectin gene. This study also demonstrated that

GG genotype of the G276T ADIPOQ gene polymorphism may be responsible for a worse metabolic profile in patients with PA.

The underlying mechanisms of the development of metabolic complications in PA patients have not been completely clarified, but the role of aldosterone overproduction seems to be crucial. The adipocyte mineralocorticoid receptors may mediate the proadipogenic effect of both aldosterone (Ehrhart-Bornstein *et al.* 2004) and glucocorticoids (Krug and Ehrhart-Bornstein 2008) and, on the other hand, adipocytokine and lipid release from adipose tissue seems to be involved in the regulation of aldosterone secretion (Jang *et al.* 2005). Our study is consistent with previous findings demonstrating a higher prevalence of metabolic abnormalities in PA compared to controls.

We also assessed whether adiponectin gene polymorphisms can contribute to worse metabolic parameters in PA patients; unfortunately we did not find significant differences in the distribution of genotypes and alleles of the T45G and G276T ADIPOQ gene SNPs in patients and controls. This finding is not consistent with the study of Ronconi *et al.* (2010), they observed a significantly different distribution for the SNP G276T between PA patients and EH. In contrast to their study we compared PA patients with the general population, not subjects with EH.

We did not find significant differences in BMI or plasma levels of glucose, cholesterol, and TGs among different genotypes of the T45G and G276T adiponectin gene polymorphisms in PA patients and controls.

However, the 276GG genotype in PA patients was linked to higher TG levels and a significantly higher frequency of 276GT+TT genotypes in patients was related to normal levels of TG. We observed a similar tendency also in relation to cholesterol level, however the differences in the frequency of the GG and GT genotypes did not reach statistical significance. Ronconi *et al.* (2010) reported a better metabolic profile in patients with the 276GG and GT genotypes compared to patients with the 276TT genotype. They also observed an association between the 45GG+TG genotypes and a better metabolic profile and suggested a protective role of the 45G allele against central obesity and insulin resistance in PA patients; however we were not able to document this relationship in an East Slovak population with PA. We did not evaluate differences in lipid profile and genotypes between APA and IHA, but it could be a topic of interest in the future, because metabolic differences and differences in frequency of end-organ damage were demonstrated between patients with IHA and APA in a recent study (Šomlóová *et al.* 2012).

In the current literature, the results of studies on the ADIPOQ gene G276T and T45G polymorphisms and their association with MS are controversial. The G allele of the G276T polymorphism was associated with lower insulin sensitivity, lower levels of adiponectin, and increased levels of blood lipids (Hara *et al.* 2002, Gonzalez-Sanchez *et al.* 2005), and the T allele was found in subjects with higher levels of adiponectin and lower BMI (Menzaghi *et al.* 2004, Kyriakou *et al.* 2008). By contrast, two studies (Beebe-Dimmer *et al.* 2010, Huang *et al.* 2010) reported an association of the G276T T allele with obesity. Association of the adipokine genes with obesity-related metabolic disease has been explained by Enns *et al.* (2011). Authors concluded that the magnitude of change in adiponectin levels varies considerably between studies, since these studies examined populations very different in ethnicity, age, and health condition. Conversely, several studies did not find any association of the G276T SNP with metabolic disorders, obesity, or adiponectin levels (Iwashima *et al.* 2004, Lee *et al.* 2005, Vojarova de Courten *et al.* 2005), which is particularly in agreement with our study.

The G allele in T45G SNP is strongly associated with lower adiponectin levels, higher BMI, lower insulin sensitivity, higher risk of T2DM, and dyslipidemia, which is in part in agreement with our study.

Unfortunately we did not observe any association of the T45G SNP of the ADIPOQ gene with metabolic disturbance. However, we did not examine the plasma adiponectin levels, which is the main limitation of this study. On the other side adiponectin level in majority of studies was not related to above mentioned allele and genotypes of the T45G and G276T polymorphisms. Moreover, the plasma levels of adiponectin may vary in various conditions, therefore plasma adiponectin evaluation did not seem to be crucial in relation to these polymorphisms. In several studies the relationship between T45G and G276T adiponectin gene polymorphisms and serum adiponectin levels was not observed (Demirci *et al.* 2010, Al-Daghri *et al.* 2012, Tsuzaki *et al.* 2012). In our study we focused especially on metabolic symptoms as a part of MS. Second limitation of the study is a relatively small group of patients with PA and a different gender distribution between controls and patients, although to our best knowledge this is the second largest study regarding the T45G and G276T adiponectin gene polymorphisms in PA patients.

In conclusion, our results document a higher prevalence of some metabolic disturbances in patients with PA compared to an age-matched control group, at least partially due to aldosterone overproduction. There are no significant differences in the genotypes and allele distribution of the G276T and T45G SNPs of the adiponectin gene among PA patients and controls in an East Slovakian population. In PA patients with normal TG levels we observed a higher frequency of the 276GT genotype and a lower frequency of the 276GG genotype, indicating that the T allele may be protective for the development of hyperlipidemia in primary aldosteronism. However, this association needs to be confirmed by studies on larger cohorts of subjects.

Conflict of Interest

There is no conflict of interest.

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