Renalase gene polymorphisms in patients with type 2 diabetes mellitus with and without hypertension

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Background
Blood pressure (BP) is acutely regulated by the sympathetic nervous system through the action of vasoactive hormones (epinephrine, norepinephrine, and dopamine). Renalase, a recently discovered enzyme with monoamine oxidase activity is implicated in the degradation of catecholamines with a possible role in BP maintenance and cardiac protection against hypertension (HTN) and cardiovascular (CV) events.

Objectives
The aim of this study was to identify the potential involvement of renalase gene polymorphisms in patients with type 2 diabetes mellitus (T2DM) with or without HTN in the absence of diabetic nephropathy and to illustrate the role of renalase gene single-nucleotide polymorphisms (rs2576178 and rs10887800) in CV events.

Study design
This was a cross-sectional study.

Patients and methods
A total of 180 patients with T2DM attending the diabetes and cardiology clinics of Mansoura Hospital were recruited in the study: 100 patients with T2DM with HTN and 80 patients with T2DM who were normotensive. Further, 50 apparently healthy individuals matched in age and sex were included as a reference group. Clinical and laboratory examinations stressing on symptoms and signs of diabetes and HTN complications and ECG and Holter ECG monitoring stressing on QTc and QTd were performed; BMI, lipograms, microalbumin levels, and serum creatinine levels were also determined. Patients with renal disease, hepatic disease, and heart failure, those with previous or present renal or suprarenal lesions or endocrinopathies, and those with secondary HTN were excluded from the study. Genotype determination for two single-nucleotide polymorphisms (rs2576178 and rs10887800) in the renalase gene was carried out using the PCR method.

Results
The frequency of the GG allele of rs2576178 and rs10887800 was insignificantly higher in the diabetic hypertensive group than in the diabetic normotensive group. Both diabetic groups showed higher levels of GG alleles than the control group. The frequency of the GA allele of rs2576178 was significantly higher in the diabetic hypertensive group in comparison with the normotensive diabetic group. The allele frequency of G and A alleles of both studied renalases was also higher in the diabetic hypertensive group in comparison with the diabetic normotensive group; however, the differences were insignificant. The genotype distribution and allele frequencies did not show any statistically significant association with BMI, neuropathy, retinopathy, myocardial ischemia, QTc, or QTd.

Conclusion
The renalase gene can be potentially involved in BP regulation in T2DM. Further large-scale studies on the relationship between renalase and acute coronary syndrome and CV events are warranted.

Keywords:
cardiovascular events, hypertension, renalase gene polymorphism

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Introduction

Approximately 70% of all cases of mortality in diabetic patients are secondary to a coexisting cardiovascular disease (CVD) [1]. Hypertension (HTN) is a major risk factor for various CVDs including coronary heart disease and stroke [2]. Blood pressure (BP) is acutely regulated by the sympathetic nervous system through the action of vasoactive hormones (epinephrine, norepinephrine, and dopamine). Renalase is a novel soluble monoamine oxidase that regulates BP and cardiac functions by degrading catecholamines and probably other unknown

substrates [3,4]. Renalase is secreted by the kidney and by metabolizing circulating catecholamines participates in the regulation of cardiovascular (CV) functions with a dose-dependent reduction in BP, heart rate, myocardial contractility, and vascular tone in experimental animals [5–7]. Renalase activity is markedly augmented by an increase in plasma catecholamines (mainly epinephrine), thus playing a role in the minute-to-minute regulation of BP [3,5].

Renalase deficiency, even in the absence of kidney disease, is associated with HTN; in renalase knockout mice, the heart rate and BP were increased both during activity and rest, despite normal renal functions, kidney histology, and plasma aldosterone levels [4]. In humans, Zhao *et al.* [8] found that rs2576178 and rs2296545 were associated with HTN. Farzaneh-Far *et al.* [9] found the single-nucleotide polymorphism (SNP) rs2296545 to be associated with cardiac hypertrophy, cardiac dysfunction, and ischemia in patients with stable coronary artery disease (CAD).

Recombinant renalase has a protective effect on the myocardium during acute ischemia and decreases the myocardium infarction size by nearly 50% [7]. During heart failure, the increased level of catecholamines is probably secondary to the impaired synthesis of renalase by the kidneys [6,7].

Human renalase is encoded by a gene on chromosome 10 and has 10 exons [10]; it is considered a novel gene for type 2 diabetes mellitus (T2DM) [11].

Diabetes mellitus (DM) is a vascular disease; mortality in T2DM is usually attributed to CVD, and elevated BP is a major CV risk factor [2]. However, the etiopathogenic relationship between HTN and DM has not been elucidated completely. The severity of HTN depends on an inherited predisposition combined with environmental factors [12]. Identification of factors for a genetic risk of HTN is thus essential for risk prediction of CVD. Several genes have shown associations with essential HTN, but the results have often been discordant [13–15].

Aim

The aim of this preliminary study was to investigate the potential involvement of renalase gene polymorphisms in patients with T2DM with or without associated HTN in the absence of diabetic nephropathy and to illustrate the role of renalase in the observed CV changes.

Design

This was a cross-sectional study.

Patients and methods

The study comprised 180 patients with T2DM attending the diabetes and cardiology clinics of Mansoura Hospital for follow-up of their diabetes and BP state. In all, 100 T2DM patients with HTN and 80 patients with normotensive T2DM were included in the study. Further, 50 apparently healthy individuals matched in age and sex served as a reference group. After approval of the scientific committee, informed consent was obtained from all participants. The patients were subjected to thorough clinical examinations stressing on the duration and complications of DM and HTN and the family history of DM and HTN in first-degree relatives. Laboratory assessments stressing on lipograms, microalbumin levels, and serum creatinine levels were performed. ECG and Holter monitoring for ischemia, QTc, and QTd were also performed. DM was defined according to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [16]. HTN ($\geq$140 systolic, $\geq$90 diastolic) was defined according to the current diagnostic criteria [17] and/or as being under antidiabetic or antihypertensive treatment. Both QT dispersions (calculated as the mean difference between the QT maximum and the QT minimum intervals in all ECG leads) were calculated [18]. QTc is defined as the corrected QT intervals in successive cardiac beats. Prolongation of QTc greater than 460 ms is considered as prolongation of the QTc interval [$QTc = QT/\sqrt{R-R}$ (Bazett's formula)] (Tables 1 and 2).

Patients with secondary HTN, renal disease, hepatic disease, heart failure, and endocrinopathies and smokers were excluded.

The genotype was determined using PCR. Genomic DNA was extracted from peripheral blood leukocytes. Template DNA was then amplified using two pairs of oligonucleotide primers (Table 3) for detection of renalase gene polymorphisms (10 ml of each primer). The PCR mixture contained 20 ng genomic DNA, 3 mU $MgCl_2$, 50 mU KCl, 10 mU Tris HCl, pH 8.4, 5% dimethyl sulfoxide, each of 0.5 mU dNTPs, and 1 U of Taq

Table 1 Demographic and clinical profile of the studied groups

	T2DM with HTN (100 patients) (group 1)	T2DM without HTN (80 patients) (group 2)	Control (50 patients) (group 3)	P_1–P_3	P_2–P_3	P_1–P_2
Age (years)	41.1 ± 7.2	41 ± 6.9	41 ± 6.8	0.933	1	0.924
Duration of DM (years)	5 ± 0.3	5 ± 0.3	0	<0.001	<0.001	0.175
SBP (mmHg)	170 ± 10.2	130.1 ± 4.1	126.2 ± 5.2	<0.001	<0.001	<0.001
BMI (kg/m^2)	26.2 ± 2.2	25.9 ± 2.1	24.2 ± 1.3	<0.001	<0.001	0.352
Diabetic retinopathy	18 patients	6 patients	0	0.001	0.048	0.040
Diabetic neuropathy	20 patients	7 patients	0	0.001	0.032	0.036
FH of DM (first-degree relatives)	36%	34%	0	<0.001	<0.001	0.375
FH of HTN (first-degree relatives)	25%	20%	0	<0.001	<0.001	1

DM, diabetes mellitus; FH, family history; HTN, hypertension; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

polymerase (Pharmacia, Uppsala, Sweden) in a final volume of 50 µl. Amplification was performed by denaturation at 94°C for 1 mm annealing. Nucleotide perimeter attachment at 58°C for 1 min and extension (DNA elongation) at 72°C for 2 min as many as 30 cycles followed by a final extension at 72°C for 4 min.

The instrument used in the study was PCR thermal (Thermocyclers, Biometra, Germany). The products of the PCR assay were separated by electrophoresis on a *2%* agarose gel that had been enriched with ethidium bromide (0.1%). The gel was then visualized under ultraviolent light and the results were documented using a gel doc apparatus (Thermocyclers, Biometra).

The PCR products were digested with appropriate restrictive endonucleases at 37°C for 6–10 h. The reaction products were separated by electrophoresis on a 1.5–2.5% agarose gel. The quality of genotyping was controlled using blind DNA duplicates for same samples.

Statistical analysis

Statistical analysis was performed using SPSS version 16 (USA). Qualitative data were presented as frequency and percentages. Quantitative data were examined using the Kolmogorov–Smirnov test to test for normal distribution of data, and when the distribution was parametric, data were expressed as mean and SD. The Student *t*-test was used to test for differences in normally distributed quantitative data between the two groups. The Mann–Whitney *U*-test was used for comparisons between two groups when data were not normally distributed. Significance was considered when the *P* value was less than 0.05.

Results

The examined groups comprised 100 T2DM patients with HTN (group 1) who were identical to T2DM patients without HTN (group 2) as regards age, duration of DM, and family history for DM and HTN in the first-degree relatives; thus, there were statistically insignificant differences between the two studied groups with respect to these parameters.

The BMI was significantly elevated in T2DM patients compared with controls; however, there were insignificant differences between the hypertensive and normotensive groups.

Diabetic retinopathy and diabetic neuropathy showed a significantly higher prevalence among T2DM patients with HTN.

Table 2 Biochemical characteristics of the studied groups

	T2DM with HTN (100 patients) (group 1)	T2DM without HTN (80 patients) (group 2)	Control (50 patients) (group 3)	P_1–P_3	P_2–P_3	P_1–P_2
FPG (mg/dl)	177.2 ± 3.5	176.2 ± 7.02	90.9 ± 4.1	<0.001	<0.001	0.246
PPPG (mg/dl)	220.4 ± 4.6	216.1 ± 6.4	130.1 ± 4.8	<0.001	<0.001	0.128
HbA1-c (%)	8.2 ± 0.2	8.1 ± 0.3	5.1 ± 0.5	<0.001	<0.001	0.154
Plasma cholesterol (mg/dl)	188.1 ± 6.4	186.1 ± 7.5	140.2 ± 10.1	<0.001	<0.001	0.144
Serum TG (mg/dl)	160.6 ± 2.2	158.8 ± 4.1	110.1 ± 5.8	<0.001	<0.001	0.555
Serum creatinine	1 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	<0.001	0.112	0.07
24-h urinary albumin (mg)	26 ± 1.1	25.8 ± 1.2	15 ± 2.1	<0.001	<0.001	0.250

FPG, fasting plasma glucose; HTN, hypertension; PPPG, postprandial plasma glucose; T2DM, type 2 diabetes mellitus; TG, triglyceride.

Table 3 Primers for detection of polymerase

Renalase polymorphism	Sequence of primers	Type of restriction	AA genotype	GG genotype
rs2576178	Sense: 5′-AGCAGAGAAGCAGCTTAACCT-3′ Antisense: 5′-TATCTGCAAGTCAGCGTAAC-3′	*Msp*I	525 bp	423 + 102 bp
rs10887800	Sense: 5′-CAGGAAAGAAAGAGTTGACAT-3′ Antisense: 5′-AAGTTGTTCCAGCTACTGT-3′	*Pst*I	554 bp	415 + 139 bp

Table 4 Resting ECG, ambulatory ECG, echocardiography, and cIMT

	T2DM with HTN (100 patients) (group 1)	T2DM without HTN (80 patients) (group 2)	Control (50 patients) (group 3)	P_1–P_3	P_2–P_3	P_1–P_2
Resting ECG						
Flat T wave	10 patients	8 patients	0	0.021	0.021	1
ST segment depression (<1 mm)	9 patients	8 patients	0	0.029	0.021	0.820
LVH (Sv 1–2 + Rv 5–6) (>35 ms)	40 patients (40%)	16 patients (20%)	0	<0.001	<0.001	0.004
Ambulatory Holter						
Evidences of ischemia	20 patients	10 patients	0	<0.001	0.048	0.180
QTc prolonged (≥460 ms)	22 patients (22%)	6 patients	0	<0.001	0.048	0.016
QTd (>10 ms)	22 patients (22%)	6 patients	0	<0.001	0.048	0.016

HTN, hypertension; LVH, left ventricular hypertrophy; T2DM, type 2 diabetes mellitus.

Plasma cholesterol and serum triglyceride levels were significantly elevated in both hypertensive and normotensive diabetic groups compared with the control group but with insignificant differences.

The serum creatinine level was significantly elevated in the hypertensive diabetic group compared with the control group but showed insignificant differences on comparing the hypertensive diabetic group with the diabetic normotensive group.

In the present study, myocardial ischemia was considered clinically and electrographically present when flat or inverted T waves and/or ST segment deviations were observed. There was no significant ischemia between the hypertensive and normotensive diabetic groups; hence, the correlations were not stressed upon (Table 4).

Left ventricular hypertrophy was considered according to Sokolow's criteria [QRS amplitude > 35 ms in pericardial leads (Sv 1–2 + Rv 5–6)] (Table 4).

The frequency of the GG allele of rs2576178 was higher in the diabetic hypertensive group than in the diabetic normotensive group. Both groups had a higher frequency of the GG allele of rs2576178 than the control group (Tables 5 and 6). Moreover, the frequency of the GG allele of rs10887800 was higher in the hypertensive T2DM group when compared with the normotensive group (Table 7); the frequency of this allele in both groups was however higher than that in the control group (Table 6), but the calculated χ^2 value was insignificant. However, the frequency of the GA allele of rs2576178 was higher in the diabetic hypertensive group in comparison with the normotensive group, and the calculated χ^2 value was significant (Table 5).

The genotype distribution of the AA allele of rs2576178 was lower in the diabetic hypertensive group than in the normotensive T2DM and control groups, but the differences were insignificant. The frequency of the G and A alleles of both rs2576178 and rs10887800 was higher in the diabetic hypertensive group in comparison with the diabetic normotensive group; however, the differences were insignificant. The only significant differences were in the higher frequency distribution of the GA allele of rs2576178 among the T2DM with HTN group.

Table 5 Genotype distribution and allele frequency of rs2576178

Genotype	GG	GA	AA	Total
T2DM HTN (100 patients)	55	30	15	100
T2DM normotensive (80 patients)	48	12	20	80
P	0.501	0.01	0.09	
Allele		G	A	Total
T2DM HTN		140	60	200
T2DM normotensive		54	26	80
P		0.628	0.628	

HTN, hypertension; T2DM, type 2 diabetes mellitus.

Table 6 Renalase gene polymorphisms in the control group

Genotype	GG	GA	AA
rs2576178	8	12	30
rs10887800	6	15	29

Discussion

HTN is a common comorbidity in individuals with T2DM and a major risk factor for macrovascular and microvascular complications [19,20].

DM is a vascular disease [20]. However, the etiopathogenic relationship between HTN and DM has not been elucidated completely. Several genes have shown an association with essential HTN, but the results have been discordant [13–15].

The renalase gene is a potential marker in T2DM [11]. Zhao *et al.* [8] reported an association between gene polymorphisms and essential HTN.

The aim of this preliminary study was to investigate the potential involvement of some renalase genes polymorphisms in patients with T2DM with or without HTN and to identify the magnitude of renalase gene polymorphisms and the allele frequency of rs2576178 and rs10887800 among them.

The occurrence of a positive family history among the first-degree relatives of T2DM patients was significantly higher in the hypertensive diabetic group. Both DM and HTN are known to be genetically determined disorders [13,21]. An increased BMI is a common finding in T2DM and is usually attributed to insulin resistance in both obesity and T2DM with or without HTN [19,22].

Diabetic complications were significantly higher among the diabetic hypertensive group; this is in line with the findings of Bakris *et al.* [23], who focused on controlling BP and reported that it is as significant as hyperglycemic control, irrespective of the type of drug therapy.

It is known that diabetic microangiopathic and macroangiopathic complications are genetically determined [14]. However, in our study, the relationship between renalase gene polymorphisms and the studied complications was insignificant. This is possibly because of the small size of the population studied.

The plasma cholesterol and serum triglyceride levels were significantly elevated in both HTN and normotensive T2DM groups compared with the control group but with insignificant differences between the groups. Hypertriglyceridemia is one of the components of insulin resistance syndrome [24].

Myocardial ischemia evidenced by ECG and Holter monitoring showed insignificant differences between the diabetic hypertensive and diabetic normotensive groups, although it was significantly evident in relation

Table 7 Genotype distribution and allele frequency of rs10887800

Genotype	GG	GA	AA	Total
T2DM HTN (100 patients)	28	60	12	100
T2DM normotensive (80 patients)	16	58	6	80
P	0.215	0.080	0.31	
Allele		G	A	Total
T2DM HTN		116	82	198
T2DM normotensive		46	36	82
P		0.701	0.701	

HTN, hypertension; T2DM, type 2 diabetes mellitus.

to the control group. QTc and QTd prolongations were significantly more evident in the diabetic hypertensive group (Table 4); this could help identify myocardial insults in T2DM with or without HTN. However, studying the relationships between the alleles of rs2576178 and rs10887800 and T2DM revealed insignificant differences. This is in contrast to the findings of Wu *et al.* [7], who identified the protective effect of renalase in ischemia and on the myocardium and its role in decreasing the in infarct size.

Significant left ventricular hypertrophy was observed in the diabetic group but with no relation to renalase genes. Farzaneh-Far *et al.* [9] reported some rs SNPs to be associated with cardiac hypertrophy, cardiac dysfunction, and ischemia in patients with stable CAD.

The frequency of the GG and GA alleles of rs2576178 and rs10887800 was higher in the diabetic hypertensive group in comparison with the diabetic normotensive and control groups. Moreover, the frequency of GG, GA, and AA alleles of rs10887800 in the diabetic HTN group was higher than that in the T2DM without HTN group, but the calculated χ^2 value was insignificant. This is in contrast to the findings of Rampersaud *et al.* [11], who reported that renalase gene polymorphisms can be potential markers in T2DM. However, our results are in accordance with those of Zhao *et al.* [8], who reported an association between gene polymorphisms rs2576178 and rs2296545 and essential HTN. Reaven [24] reported that renalase polymorphisms did not show any effect on the BP level or HTN prevalence. The frequency of occurrence of GG and GA alleles of rs2576178 SNP was higher; the latter was significantly higher in the studied diabetic hypertensive patients than in controls. This may suggest an association between the renalase gene SNP and DM. Rampersaud *et al.* [11] considered renalase to be a novel contributor gene for T2DM. Large-scale studies are needed to illustrate renalase gene polymorphisms in patients with macrovascular (CAD) and microvascular complications of DM, together with their role in acute coronary syndrome and HTN.

In this preliminary study, renalase gene polymorphisms that revealed significantly higher levels in the diabetic hypertensive group in comparison with the diabetic normotensive group could play a role in the progression of diabetic nephropathy and acute coronary syndrome.

Conclusion

The renalase gene can be potentially involved in BP regulation in T2DM and in the ischemic and dysrhythmogenic myocardial changes of T2DM with or without HTN.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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